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Marcinkowskiego 2–6
50-368 Wrocław, Poland
Tel.: +48 71 784 12 05
E-mail: dental@umw.edu.pl

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Editorial

Journal Impact Factor and highly cited papers: The beginning of a new era in *Dental and Medical Problems*

Mieszko Więckiewicz[1,A–F], Helena Martynowicz[2,A–F]

[1] Department of Experimental Dentistry, Wroclaw Medical University, Poland
[2] Department and Clinic of Internal Medicine, Occupational Diseases, Hypertension, and Clinical Oncology, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Mieszko Więckiewicz
E-mail: mieszko.wieckiewicz@umw.edu.pl

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Keywords: Impact Factor, Journal Impact Factor, citation, scientometrics, *Dental and Medical Problems*

We are delighted to announce that *Dental and Medical Problems* is the first dentistry-focused scholarly journal in Poland and Eastern Europe to achieve a Journal Impact Factor (JIF) in the latest 2023 release of the Journal Citation Reports™!

After 3 years of dedicated efforts by the Editorial Office, all members of the Editorial Board, Editors, Authors, Reviewers, and Readers, we are pleased to announce the successful achievement of our intended goal. *Dental and Medical Problems* (*Dent Med Probl*) now stands as the first dentistry-focused scholarly journal in Poland and Eastern Europe to be granted a Journal Impact Factor (JIF) in the most recent 2023 release of the Journal Citation Reports™! The Impact Factor (IF) or Journal Impact Factor (JIF) of an academic journal is a scientometric index calculated by Clarivate™ that reflects the average number of citations of the articles published in the last 2 years in a given journal, as indexed by Clarivate's Web of Science.[1,2] In terms of categories, *Dent Med Probl* currently holds the following ranking positions in the Web of Science: 72/322 (Q1) in the category "Medicine, General & Internal"; and 70/156 (Q2) in the category "Dentistry, Oral Surgery & Medicine". This remarkable position is owed, among other factors, to our Authors, particularly those who have contributed highly cited papers to our Journal in the last 2 years. Therefore, we take this opportune moment to showcase their articles once again as a tribute to their hard work, and express our gratitude for choosing our Journal to publish their research.

In recent years, the articles most frequently cited in *Dent Med Probl* have centered around the coronavirus disease 2019 (COVID-19) pandemic, reflecting the profound significance of this global phenomenon. COVID-19 has impacted diverse aspects of oral health, such as saliva, oral mucosa, taste, bruxism, oral behaviors, and orofacial pain.[3–8] Additionally, it has influenced the psycho-emotional state and stress levels of oral healthcare providers, dental students, and patients, as well as the education of dental students.[9–11]

Over the past 2 years, the most frequently cited article has been a multinational study examining the knowledge, attitudes, and practices of dental practitioners regarding the COVID-19 pandemic, published by Kamate et al.[12]

This study utilized an online-based survey distributed among dentists worldwide, employing a combination of convenience and snowball sampling. The total number of responses received (860) was categorized by continent (Asia, Americas – North and South, Europe, Africa, and others – Australia and Antarctica). Dentists demonstrated commendable knowledge and practice scores, underscoring the importance of their preparedness in combating COVID-19.[12]

Another highly cited paper, authored by AbuBakr et al., aimed to shed light on the oral manifestations observed in mild-to-moderate cases of COVID-19.[4] This study, conducted through an online survey, involved 573 participants. The findings revealed that 71.7% of COVID-19 patients exhibited oral manifestations of varying incidence and significance, including oral or dental pain (23%), pain in jaw bones or joints (12.0%), halitosis (10.5%), ulcerations (20.4%), and xerostomia (47.6%). Additionally, some patients (28.3%) displayed 2 or 3 manifestations simultaneously.[4]

The following 2 highly cited articles were mini-reviews published by Paradowska-Stolarz[5] and Emodi-Perlman and Eli,[6] focusing on the oral manifestations of COVID-19 infection, and reviewing the initial existing literature on temporomandibular disorders (TMD), bruxism and orofacial pain during the COVID-19 pandemic, respectively.[5,6]

The short communication published by Nuvvula and Mallineni, addressing the teledentistry model in pediatric dentistry and providing guidance as to how to manage children with dental problems during the COVID-19 pandemic, has also garnered significant reader interest.[13]

The prevalence and popularity of articles related to COVID-19 align with global trends and should not be surprising, given the novelty and immense importance of the topic for human life and health.[14–16]

On this occasion, we would like to reiterate our gratitude to all members of the Editorial Board, as well as to all Authors, Reviewers, Editors, and Readers for their substantial contribution to the development of our journal. It is thanks to your diligent work and dedication that we have attained a standard of excellence.

Starting next year, *Dent Med Probl* will transition to a bimonthly publication schedule. Additionally, new article types, including perspective, comment, research highlights, statement, clinical/scientific guidelines, and research letters, will be introduced. We want to assure all Authors and Readers that maintaining superior publishing standards remains our top priority. We strongly encourage all scientists to submit manuscripts of high-quality content related to oral health and general medicine to our journal.

ORCID iDs

Mieszko Więckiewicz https://orcid.org/0000-0003-4953-7143
Helena Martynowicz https://orcid.org/0000-0003-1283-8460

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Original paper

Bacterial species and factors influencing the contamination of the inner and outer layers of face masks used in dentistry

Dong-Eun Lee[A–F]

Department of Dental Hygiene, Ulsan College, South Korea

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Address for correspondence
Dong-Eun Lee
E-mail: rhie1010@naver.com

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Abstract

Background. Many patients are clinically consulted in dental clinics, where aerosol-generating procedures are widely used. In our previous study, we evaluated the temperature, humidity and contamination rates on the inner layer of masks according to the mask-wearing time. However, it is important to assess the contamination rates on the outer layer of masks used in dentistry as well. Previously, while examining the contamination rates, we only identified the associated bacteria; no detailed analysis of bacterial species depending on the mask-wearing time was conducted. Furthermore, we did not evaluate factors that could contribute to the contamination of masks.

Objectives. The present study was intended to supplement the limitations of our previous study.

Material and methods. The used masks were collected. Thereafter, colony forming unit (CFU) quantification and 16S rDNA sequencing were performed to calculate the contamination rates and identify bacterial species. Data on the participants' medical and dental history was collected. The participants filled out a questionnaire and underwent saliva tests.

Results. On the inner and outer layers of the masks, 3.3×10^8 and 8.5×10^8 CFUs were found, respectively. The contamination rates of the masks increased with the increasing mask-wearing time. There was no correlation between the contamination rate on the inner layer and other factors, such as the probing depth (PD) $\geq$4 mm, the bleeding rate, the calculus rate, and saliva characteristics. The inner layer contamination rate increased as the number of treated teeth increased, and as the saliva buffering capacity decreased. The outer layer contamination rate increased with the number of times the mask was touched.

Conclusions. The contamination rates were higher on the outer layer than on the inner one, and the CFU count increased with the mask-wearing time. The following bacterial species were found on the masks: *Staphylococcus epidermidis* (*S. epidermidis*); *Staphylococcus aureus* (*S. aureus*); *Staphylococcus capitis* (*S. capitis*); *Streptococcus oralis* (*S. oralis*); and *Streptococcus koreensis* (*S. koreensis*). Oral health conditions may have affected the contamination of the inner layer. In addition, the number of times the mask was touched may have affected the contamination of the outer layer.

Keywords: bacterial contamination, mask contamination, mask contamination factors

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Introduction

Wearing a mask is crucial for protecting the individual's health in a dental clinic. It has become an essential safety measure during the coronavirus disease 2019 (COVID-19) pandemic.[1] Particularly, in dental clinics, clinicians have to consult many patients and aerosol-generating procedures are widely used. Consequently, the mask contamination rates may increase in such settings.[2] However, hospital guidelines for infection prevention lack evidence for mask replacement cycles.[3] Hence, in our previous study, we evaluated the temperature, humidity and contamination rates on the inner layer of masks according to the mask-wearing time.[4] Yet, it is important to assess the contamination rates on the outer layer of masks used in dentistry as well. No studies have referred to this issue so far. In addition, in our previous study, we only identified the associated bacteria; no detailed analysis of bacterial species depending on the mask-wearing time was conducted. Furthermore, we did not evaluate factors that could contribute to the contamination of masks.

Therefore, the present study was intended to supplement the limitations of our previous study.

Material and methods

Participants

This study was approved by the Ethics Committee of Ulsan College, South Korea (No. 1044363-A-2022-002). The power analysis was performed for the study (effect size: $d = 0.5$, an alpha (α) error of 0.05 and a power of 0.95) with the use of G*Power, v. 3.1.9.7 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The total sample size required to obtain conclusive results was 54, and a sample size of 100 was selected to compensate for possible error-related dropouts. Then, 100 participants who provided written informed consent after the study purpose had been explained to them were recruited. Finally, 91 participants (mean age: 28.3 ±0.3 years, no medical history, free of medications) were included for further analysis.

Sample collection

Clinicians, including dentists, dental hygienists and nursing assistants, were selected as participants in this study. The participants responded to a questionnaire including questions about the mask-wearing time, whether they chewed gum, coughed or sneezed while wearing the mask, and whether they washed their faces, brushed their teeth or put on makeup before using the mask. We also recorded behavioral factors, such as the number of times the mask was touched. After completing the survey, the masks were collected, placed in sterile zipper bags and sent to the laboratory. Sample numbers were indicated on the questionnaires and outside the zipper bags to ensure the anonymity of the participants.

Quantification of colony forming units (CFUs)

After collecting the masks, a square shape (1 cm × 1 cm) in the center of each mask was cut out, and the inner (inside) and outer layers (outside) were separated using tweezers. The separated pieces were placed in Eppendorf tubes containing 1 mL of the Luria–Bertani medium, vortexed for 1 min and incubated at 37°C for 24 h. After dilution (from 10^{-1} to 10^{-6}), aliquots of 100 µL were inoculated onto plate count agar (PCA) plates. After overnight incubation at 37°C, CFUs were counted.

Identification of bacteria

The colonies on the PCA plates were picked randomly and subcultured 3 times. Polymerase chain reaction (PCR) was implemented using a paired primer set (forward primer: 27F: AGA GTT TGA TCM TGG CTC AG; reverse primer: 1492R: GGT TAC CTT GTT ACG ACT TC). The reaction mixtures were subjected to 30 cycles of denaturation and annealing at 50°C in an automated thermal cycler (SimpliAmp™; Thermo Fisher Scientific, Waltham, USA). The PCR products were resolved by electrophoresis on 1% agarose gel containing ethidium bromide (EtBr). The final PCR products were purified using a DNA purification kit (LaboPass™; Cosmo Genetech Co., Ltd., Seoul, South Korea). Finally, the PCR products were analyzed with automated sequencing, using a cycle sequencing kit (BigDye™ Terminator, v. 3.1; Thermo Fisher Scientific), a sequencing machine (ABI 3730XL; Thermo Fisher Scientific) and the Sequencher DNA sequence analysis software, v. 5.2 (Gene Codes Corp., Ann Arbor, USA). The newly aligned 16S rDNA sequences were compared with the bacterial genes deposited in the GenBank® database (National Center for Biotechnology Information (NCBI), Bethesda, USA), and the bacterial strains with a match of 99% were searched using the Basic Local Alignment Search Tool (BLAST) for nucleotides (NCBI).[5] If alignment with the 27F and 1492R primers failed, additional experiments were conducted using an internal primer (800R: TAC CAG GGT ATC TAA TCC; 785F: GGA TTA GAT ACC CTG GTA), and if this alignment failed as well, 5.8S rRNA sequencing (ITS1: TCC GTA GGT GAA CCT GCG G; ITS4: TCC TCC GCT TAT TGA TAT GC) and 25–28S rRNA sequencing (NL1: GCA TAT CAA TAA GCG GAG GAA AAG; NL4: GGT CCG TGT TTC AAG ACG G) were performed additionally.

Medical and dental history taking

All participants underwent intraoral examinations, and one investigator collected data on the participants' medical

and dental history, medication, and progressive oral diseases, such as dental caries. The investigator also measured attrition and abrasion, the probing depth (PD), the bleeding rate and the calculus rate.

Saliva test

Five minutes after the participants brushed their teeth, their saliva was collected into 15-milliliter conical tubes for 5 min while sitting upright and comfortably on a chair. After that, the amount and pH of saliva were measured using a pH meter (PH-200, HM Digital, Inc., Carson, USA). After chewing gum for 30 s, the saliva secreted for 5 min was collected into a measuring cup to determine its amount. The secretion rates of unstimulated and stimulated saliva were recorded. The saliva buffering capacity was measured using a saliva-check buffer kit (GC Corp., Tokyo, Japan). After dropping the collected saliva onto the tester, the color change was read. The determination of the color change was conducted by 3 independent investigators, and the average was calculated.

Statistical analysis

The SPSS for Windows software, v.12.0 (SPSS Inc., Chicago, USA), was used for statistical analysis. The data was evaluated with the one-way analysis of variance (ANOVA) followed by the paired *t* test. The results of the experiments are represented as mean and standard error ($M \pm SE$). A p-value <0.05 was considered statistically significant.

Results

The average CFU count on the inner and outer layers of the masks was 3.3×10^8 and 8.5×10^8, respectively, and the difference was statistically significant ($p = 0.009$) (Fig. 1A). On both sides of the masks, the following bacterial species were found: *Staphylococcus epidermidis* (*S. epidermidis*) (inside: 48.7%; outside: 44.2%); *Staphylococcus aureus* (*S. aureus*) (inside: 10.5%; outside: 9.3%); *Staphylococcus capitis* (*S. capitis*) (inside: 2.6%; outside: 2.3%); *Streptococcus oralis* (*S. oralis*) (inside: 18.4%; outside: 9.3%); and *Streptococcus koreensis* (*S. koreensis*) (inside: 1.3%; outside: 1.3%) (Fig. 1B). Furthermore, *Streptococcus salivarius* (*S. salivarius*) (7.9%), *Staphylococcus lugdunensis* (*S. lugdunensis*) (2.8%), *Streptococcus infantis* (*S. infantis*) (1.3%), *Streptococcus rubneri* (*S. rubneri*) (1.3%), *Streptococcus australis* (*S. australis*) (1.3%), *Streptococcus sanguinis* (*S. sanguinis*) (1.3%), *Streptococcus parasanguinis* (*S. parasanguinis*) (1.3%), and *Moraxella osloensis* (*M. osloensis*) (1.3%) were found on the inner layer of the masks only (Fig. 1B, 'others' in the inside group). In contrast, *Staphylococcus warneri* (*S. warneri*) (18.6%), *Staphylococcus caprae* (*S. caprae*)

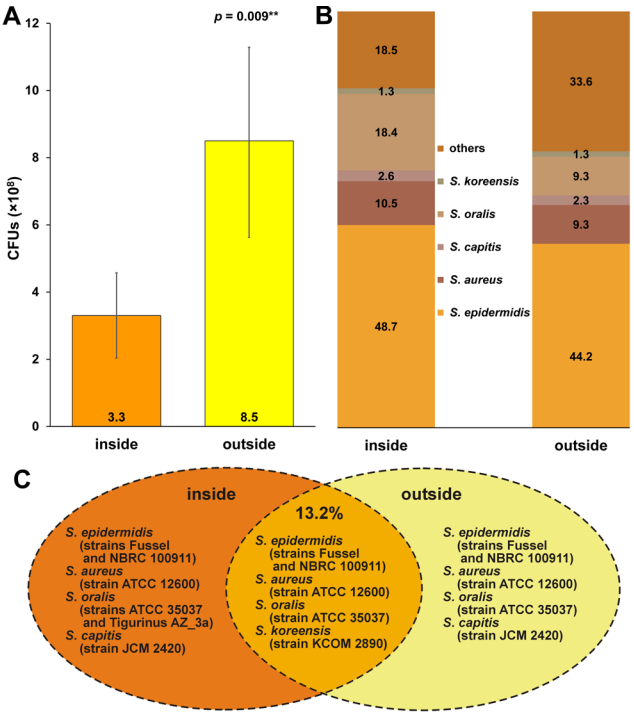

Fig. 1. Average colony forming unit (CFU) counts (A), and the distribution of bacterial species [%] (B) and bacterial strains (C) on the inner and outer layers of the masks

S. epidermidis – *Staphylococcus epidermidis*; *S. aureus* – *Staphylococcus aureus*; *S. capitis* – *Staphylococcus capitis*; *S. oralis* – *Streptococcus oralis*; *S. koreensis* – *Streptococcus koreensis*; ** statistically significant at $p < 0.01$.

(2.5%), *Streptococcus mitis* (*S. mitis*) (2.5%), *Enterococcus faecium* (*E. faecium*) (2.5%), *Psychrobacillus lasiicapitis* (*P. lasiicapitis*) (2.5%), *Bacillus thioparans* (*B. thioparans*) (2.5%), and *Lactobacillus plantarum* (*L. plantarum*) (2.5%) were found on the outer layer of the masks only (Fig. 1B, 'others' in the outside group). On the inner and outer layers, 13.2% of the bacteria found were of the same strains (*S. epidermidis*: strains Fussel and NBRC (National Institute of Technology and Evaluation (NITE) Biological Resource Center) 100911; *S. aureus*: strain ATCC (American Type Culture Collection) 12600; *S. oralis*: strain ATCC 35037; and *S. koreensis*: strain KCOM (Korean Collection for Oral Microbiology) 28901), while for the rest of the bacteria (*S. epidermis*, *S. aureus*, *S. oralis*, and *S. capitis*), the strains were different, even though the species were the same (Fig. 1C).

The contamination rates on the inner layer increased as the mask-wearing time increased (<4 h: 0.7×10^8 CFUs; 4–8 h: 2.2×10^8 CFUs; and >8 h: 7.1×10^8 CFUs) (Fig. 2A). When the mask-wearing time was less than 4 h, most bacteria found were *S. epidermidis* (66.7%). *Staphylococcus epidermidis* (41.1%), *Streptococcus aureus* (11.0%), *S. capitis* (3.5%), *S. oralis* (22.3%), and *S. koreensis* (1.6%) were discovered on the masks worn for 4–8 h. In addition, *S. epidermidis* (60.0%), *S. aureus* (6.7%) and *S. oralis* (13.3%) were found on the inner layer of the masks worn for more than 8 h; however, *S. capitis* and *S. koreensis* were not detected (Fig. 2B).

Furthermore, the contamination rates on the outer layer of the masks also increased as the mask-wearing time increased (<4 h: 0.8 × 10^8 CFUs; 4–8 h: 6.2 × 10^8 CFUs; and >8 h: 15.2 × 10^8 CFUs) (Fig. 2C). When the mask-wearing time was less than 4 h, half of the bacteria found were *S. oralis* (50.0%). On the masks worn for 4–8 h, *S. epidermidis* (41.2%), *S. aureus* (9.2%), *S. capitis* (2.7%), *S. oralis* (9.2%), and *S. koreensis* (2.7%) were discovered. Additionally, *S. epidermidis* (60.0%) and *S. aureus* (10.0%) were found on the outer layer of the masks worn for more than 8 h; however, *S. capitis*, *S. oralis* and *S. koreensis* were not detected (Fig. 2D).

Intraoral examinations were conducted to evaluate the factors affecting mask contamination, and the correlations between these factors and the mask contamination rate on the inner layer (in CFUs) were analyzed (Table 1). The participants had 27.3 ±1.6 teeth on average, and 15.1% of the participants had semi-erupted teeth (1.9 ±0.8). The participants who had undergone preventive dental treatment, such as coating with a dental sealant, constituted 58.5% of the total sample. Among them, the average number of teeth covered with a sealant was 3.9 ±2.7. In addition, 62.3% of the participants had been provided with preservation and/or prosthetic treatment, such as resin fillings or gold crowns (6.6 ±3.8 teeth on average). Furthermore, 56.6% of the participants had caries. Among them, the average number of teeth with dental caries was 3.0 ±2.3, and 9.4% and 11.3% of the participants had attritions (1.8 ±1.3) and abrasions (8.0 ±6.7), respectively. It was also observed that 11.3% of the participants had PD > 4 mm (4.9 ±3.2 mm; $r = -0.109$), 56.6% experienced bleeding (bleeding rate: 3.2 ±3.6%; $r = 0.008$) and 77.4% had calculus (calculus rate: 8.9 ±13.3%; $r = -0.117$). Of the total participants, 5.7% were undergoing orthodontic treatment, and 20.8% were equipped with retainers after the completion of orthodontic treatment. Table 1 shows the correlations between particular factors and the CFU count, as well as sample percentages, and the average values for teeth or the indices examined.

Saliva tests were conducted to analyze the correlations between saliva characteristics and mask contamination on the inner layer (Table 2). The unstimulated saliva secretion rate was 0.5 ±0.3 mL/min, the initial saliva pH was 6.8 ±0.3, the stimulated saliva secretion rate was 1.1 ±0.5 mL/min, and the saliva buffering capacity was 9.2 ±2.0.

All factors were graphically analyzed. The inner layer contamination rate increased (from 3.1 × 10^6 to 5.2 × 10^8 CFUs) as the number of treated teeth increased, but the differences were not statistically significant (Fig. 3A). In addition, as the saliva buffering capacity decreased (from 9.9 to 8.9), the contamination rate on the inner layer of the masks increased. These differences were not statistically significant, either (Fig. 3B).

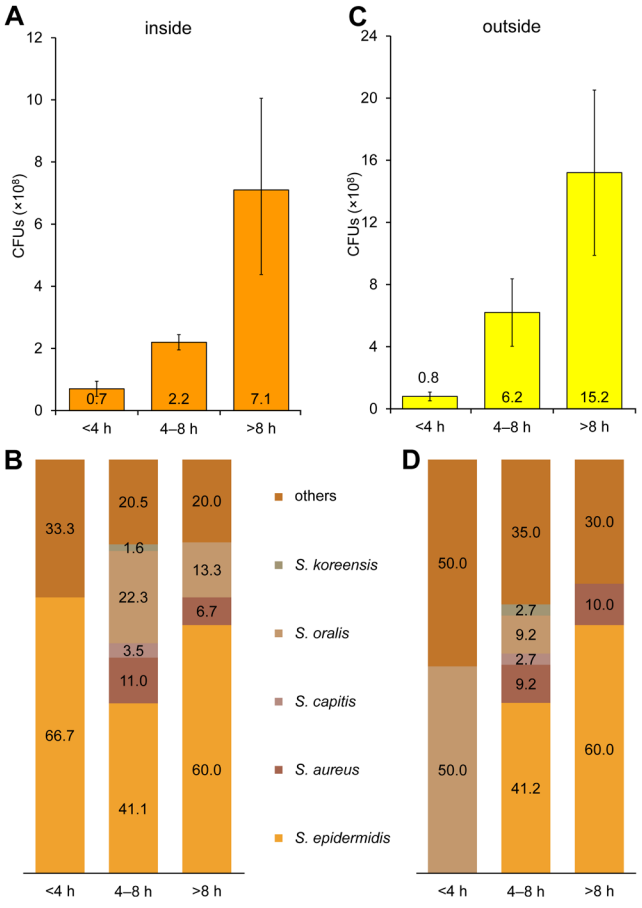

Fig. 2. Identification of bacterial species according to the mask-wearing time

A,C – contamination rates on the inner and outer layers of the masks, respectively; B,D – distribution of bacterial species according to the mask-wearing time on the inner and outer layers of the masks, respectively. Data presented as percentage (%). CFU – colony forming unit.

Table 1. Correlations between oral health conditions and the mask contamination rate

Factor	*r* (correlation with CFUs inside the mask)	Percentage of participants [%]	*M* ±*SE*
Number of teeth	–	–	27.3 ±1.6 teeth
Semi-erupted teeth	–	15.1	1.9 ±0.8 teeth
Preventive dental treatment	–	58.5	3.9 ±2.7 sealed teeth
Preservation and/or prosthetic treatment	–	62.3	6.6 ±3.8 teeth
Caries	–	56.6	3.0 ±2.3 teeth
Attrition	–	9.4	1.8 ±1.3 teeth
Abrasion	–	11.3	8.0 ±6.7 teeth
PD > 4 mm [mm]	–0.109	11.3	4.9 ±3.2
Bleeding rate [%]	0.008	56.6	3.2 ±3.6
Calculus rate [%]	–0.117	77.4	8.9 ±13.3
Orthodontic treatment	–	5.7	–
Retainers	–	20.8	–

r – Pearson correlation coefficient; *M* – mean; *SE* – standard error; PD – probing depth.

We hypothesized that there could be a correlation between the number of times the mask was touched and mask contamination on the outer layer; hence, behavioral factors were analyzed. The participants touched the mask approx. 10.7 ±9.9 times, and the outer layer contamination rate increased (from 10.5×10^6 to 11.1×10^8 CFUs) with the number of times the mask was touched. Yet, the differences were not statistically significant (Fig. 4).

Table 2. Correlations between saliva characteristics and the mask contamination rate

Factor	*r* (correlation with CFUs inside the mask)	*M* ±*SE*
Unstimulated saliva secretion rate [mL/min]	–0.186	0.5 ±0.3
Saliva initial pH	–0.247	6.8 ±0.3
Stimulated saliva secretion rate [mL/min]	–0.082	1.1 ±0.5
Saliva buffering capacity	–0.128	9.2 ±2.0

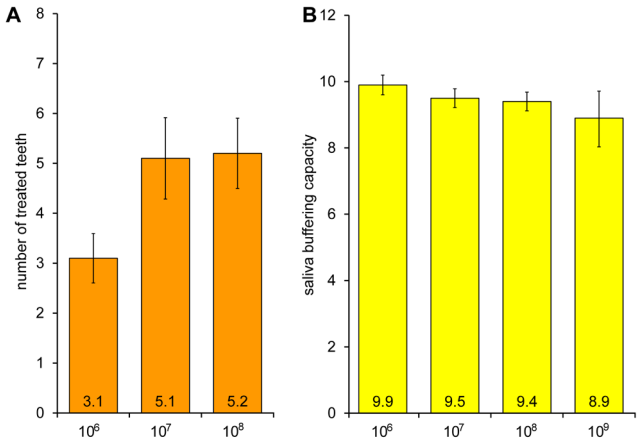

Fig. 3. Correlation between the colony forming unit (CFU) count on the inner layer and the number of treated teeth (A) and the saliva buffering capacity (B)

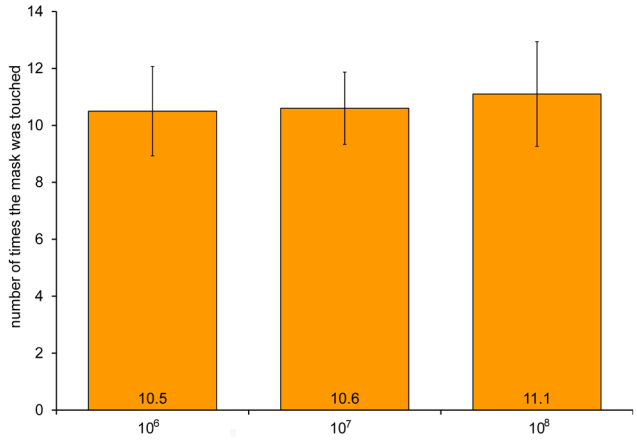

Fig. 4. Correlation between the colony forming unit (CFU) count on the outer layer and the number of times the mask was touched

Discussion

The results of studies on mask contamination differ. In some articles, contamination was higher on the inner layer of masks,[6] while in others, contamination was higher on the outer layer.[7,8] These varying outcomes are probably due to the environment in which the participants wore masks. The contamination outside the mask is higher in dental clinics, where aerosol-generating procedures occur.[7,8] However, under strict control over contamination conditions (e.g., in operating rooms), the contamination rate is higher inside the mask.[6] Due to the generation of aerosols, air pollution in dental clinics is relatively higher than in other environments. Consequently, in the present study, the contamination rate on the outer layer of the masks was higher than on the inner layer in most samples (Fig. 1A).

We performed 16S rDNA sequencing to identify bacterial species. The bacteria detected on both layers were classified as the *Staphylococcus* and *Streptococcus* genera, which are Gram-positive, facultative anaerobic bacteria. They are also part of the normal human flora, typically the skin flora and, less commonly, the mucosal flora. The normal flora may be easily found on the used masks, as wearing a mask involves close contact with the human skin (Fig. 1B).[4,9]

Some bacteria belonging to the *Staphylococcus*, *Streptococcus* and *Moraxella* genera were observed only on the inner layer of the masks. For example, *S. salivarius* is commonly found in the oral cavity,[10] and *S. infantis*,[11] *S. rubneri*,[12] *S. australis*,[13] *S. sanguinis*,[14] and *S. parasanguinis*[15] can be detected in the oral cavity and the throat. In addition, *S. lugdunensis* is pathogenic and can cause various infections.[16] *Moraxella osloensis* is a commensal Gram-negative bacterium that causes invasive infections, mainly in immunocompromised individuals.[17] However, if infectious bacteria were found inside the mask, we could not ascertain whether the mask wearer was also infected. It is necessary to conduct a future study that would assess the relationship between the bacteria found on the inner layer of masks and the participants' medical history.

Some bacteria belonging to the *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Psychrobacillus*, *Bacillus*, and *Lactobacillus* genera were observed only on the outer layer of the masks. *Streptococcus mitis*[18] and *E. faecium*[19] are commonly found in the human oral cavity or gastrointestinal tract. Therefore, they can be easily observed on the used masks. *Staphylococcus warneri*[20] and *S. caprae*[21] can cause opportunistic infectious diseases in immunocompromised individuals, but they are also found in the normal flora. Interestingly, some bacteria rarely found in humans, such as *P. lasiicapitis*, *B. thioparans* and *L. plantarum*, were found on the outer layer of the masks. This was probably due to the contamination of the mask through an unexpected route. Future research on whether these bacteria can be found in humans is needed.

On the inner and outer layers of the masks, 13.2% of bacteria were of the same species and strain. Thus, there is a possibility that the contamination inside the mask due to human-derived bacteria may spread to the outer layer, or the contamination outside the mask due to external air pollution may spread to the inner layer (Fig. 1C).

In our previous study, the CFU count on the inner layer of masks increased with the mask-wearing time.[4] Similar to our previous results, the mask contamination rate increased with the increasing mask-wearing time (Fig. 2A,C). In the previous study, the mask contamination rate in mask samples was 0.35×10^8 CFUs and 0.95×10^8 CFUs after 2 h and 4 h of wearing, respectively.[4] Similarly, the present study showed that the mask contamination rate on the inner layer after 4 h of wearing was 0.7×10^8 CFUs, which is within the range of the previous results.

Bacteria such as *S. epidermidis* and *S. aureus* are commonly found on the skin, and they survive relatively well even in dry environments; hence, they could survive for more than 8 h on the masks (Fig. 2B,D).[9]

Some bacteria, including *S. capitis*, *S. oralis* and *S. koreensis*, were only found on both the inner and outer layers of the masks worn for 4–8 h (Fig. 2B,D). *Staphylococcus capitis* is part of the normal flora of the human skin[20] and can survive well in dry environments; however, it was not detected on the masks worn for more than 8 h. This might be due to the fact that the ability to withstand dry environments for a long time varies among bacterial species, even if they belong to the same group of the normal human flora. *Streptococcus oralis* was observed on the masks worn for more than 4 h (Fig. 2B,D). The bacterium is easily found in the oral cavity.[22] The mask is not contaminated with *S. oralis* immediately after it is put on, but through conversation or breathing, as the mask-wearing time increases. Although *S. koreensis* was found on the masks worn for 4–8 h, further research is needed for accurate interpretation.

We hypothesized that oral health conditions and saliva characteristics could cause the contamination on the inner layer of the masks. Hence, intraoral examinations and saliva tests were performed. However, no correlations were found between those factors and the CFU count (Tables 1,2).

The contamination rate on the inner layer of the masks increased as the tooth treatment rate increased. In addition, as the saliva buffering capacity rate decreased, the contamination rate of the inner layer of the masks increased as well. However, these differences were not statistically significant (Fig. 3). Reduced saliva buffering capacity and a decreased pH maintenance ability of saliva increase the possibility of oral diseases, such as dental caries.[23] It was expected that poor oral health conditions might increase the contamination of the inner layer of the masks; yet, their impact was not pronounced.

Finally, it was hypothesized that the contamination rate on the outer layer of the masks would increase with the number of times the mask was touched. Indeed, the contamination rates increased, but the differences were not statistically significant (Fig. 4), perhaps due to the complex source of contamination of the outer layer of the masks.

Due to the risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, wearing a mask has become more important. Several previous studies revealed that saliva could serve for the detection of SARS-CoV-2.[24–26] Thus, wearing a mask is essential for protecting not only mask wearers, but also individuals around them from the risk of infection. Patients must take off their masks to receive dental treatment; hence, it is essential for clinicians to wear masks. According to the infection control guidelines, the sterilization of equipment is essential. However, the masks worn by clinicians do not undergo a separate sterilization process. This suggests that mask contamination through an unexpected route may cause secondary infection. Therefore, it is recommended that masks that have undergone a photosterilization process[27] with the use of ultraviolet (UV) irradiation,[28] which is widely used in dentistry and does not cause damage to the mask or filter, be used.

Limitations

Anaerobic bacteria were not investigated in this experiment. Besides, the individual respiratory rates varied among the participants.

Conclusions

The contamination rates were higher on the outer layer than on the inner one. Furthermore, the CFU count on the inner and outer layers of the masks increased with the mask-wearing time. *Staphylococcus epidermidis*, *S. aureus*, *S. capitis*, *S. oralis*, and *S. koreensis* were found on the used masks. Oral health conditions may have affected the contamination of the inner layer, but their influence was not significant. The number of times the mask was touched may have affected the contamination of the outer layer; yet, this influence was insignificant, either.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Ulsan College, South Korea (No. 1044363-A-2022-002). All participants provided written informed consent.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Dong-Eun Lee https://orcid.org/0000-0002-3510-3152

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Original paper

Influence of the frameworks of implant-supported prostheses and implant connections on stress distribution

Bruna Santos Honório Tonin[1,B–D], Raniel Fernandes Peixoto[2,A–C], Jing Fu[3,C,E], Maria da Gloria Chiarello de Mattos [1,A], Ana Paula Macedo[1,A–C,F]

[1] Department of Dental Materials and Prosthesis, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
[2] Department of Restorative Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Brazil
[3] Department of Prosthodontics, the Affiliated Hospital of Qingdao University, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Bruna Santos Honório Tonin
E-mail: brunahtonin@gmail.com

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Abstract

Background. The maintenance of marginal bone integrity around dental implants continues to be a clinical challenge. It is still unclear whether loading multiple implant-supported prostheses that have different implant connections influences bone resorption.

Objectives. The aim of this in vitro study was to compare stress distribution around residual edentulous ridges supported by external hexagon (EH) and Morse taper (MT) implants with screw-retained frameworks obtained with the use of different methods.

Material and methods. Three-element implant-supported prostheses with distal cantilevers were manufactured according to different techniques of obtaining the framework: LAS – framework sectioned and welded with a laser; TIG – framework sectioned and welded with tungsten inert gas (TIG); and CCS – framework obtained using a computer-aided design/computer-aided manufacturing (CAD/CAM) system. Occlusal and punctual loading (150 N) was applied to the cantilevers. In the photoelastic stress analysis, the fringe orders (n) were quanitified using the Tardy method, which calculates the maximum shear stress value (τ) at each selected point.

Results. High stress around the implants and tightening were observed in the TIG group, mainly in the crestal bone region for the EH and MT implant connections. The LAS and CCS frameworks exhibited lower stress for the MT connection under occlusal and punctual loading.

Conclusions. The comparative analysis of the models showed that the MT connection type associated with the laser-welded or CAD/CAM frameworks resulted in lower stress values in the crestal bone area, suggesting the preservation of bone tissue in this region.

Keywords: CAD/CAM, dental prosthesis, dental stress analysis, dental implant

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Introduction

Residual edentulous ridges may need augmentation procedures before or during implant placement. Some methods are associated with additional surgical intervention, cost, surgical time, and morbidity.[1] Another alternative is rehabilitation with an implant-supported prosthesis with a cantilever. All the force applied in the posterior region of the cantilever is transmitted to the implants, and consequently to the adjacent bone, which causes some concerns regarding stress distribution.[2] Previous studies compared different implant systems in terms of stress distribution with regard to the bone.[3,4] However, these studies used only one element or multiple elements splinted to implant-retained restorations without a cantilever. Thus, research is still required to make this method safer.

To minimize distortion in the framework, and consequently reduce the stress transmitted to the implant-supported system, it is possible to section and weld one-piece frameworks obtained by conventional casting.[5] Different welding techniques can be applied, such as conventional welding, gas-torch brazing, laser welding, and tungsten inert gas (TIG) welding. The TIG method has been associated with good flexural strength, and even with the resistance and adaptation values superior to laser welding for different metal alloys.[6,7] Laser welding has been introduced to dental laboratory procedures as an alternative method to soldering, brazing or TIG welding.[8] Yttrium aluminum garnet (YAG) doped with neodymium (Nd) crystals is used to emit laser beams (the Nd:YAG laser) to weld dental alloys.[9] As the laser energy can be concentrated on a small area, minimal heating or oxidation effects occur in the region surrounding the welded spot; however, this method presents variable resistance results.[10]

Computer-aided design/computer-aided manufacturing (CAD/CAM) can also be used to fabricate implant-supported frameworks.[11,12] This technique has provided significant improvement in the marginal adaption of frameworks,[13] and may result in better stress distribution as compared to frameworks manufactured using traditional laboratory procedures. Although the CAD/CAM technology eliminates several steps, it introduces others, such as scanning, intricate software utilization, design, and machining, which also depend on the experience of the operator and the equipment used.[14]

With regard to the geometry of implant connections, some studies have reported that the Morse taper (MT) connections provide joint stability and higher frictional resistance against rotational and lateral movements under vertical loading than external connections.[15] The external hexagon (EH) connections have been reported to be advantageous in terms of their anti-rotational mechanism and compatibility with different implant systems.[16]

To evaluate biomechanical behavior, photoelasticity has been used. This stress analysis enables the visualization and quantification of stress distribution in its entirety. It shows how polarized light is affected when passing through a plastic model under experimental loading. The method reveals full-field stress patterns.[17] Photoelastic models have been successfully used to explain differences between various kinds of prosthetic treatment, revealing stress behavior of implant-supported prostheses in the peri-implant bone tissue, and to assess the impact of compromised conditions through comparative stress-related outcome analyses.[17–20] In addition to stress location, its intensity and concentration can be interpreted on the basis of the color, number and closeness of the emerging fringes.[17,21]

The purpose of the present study was to use the photoelastic method to analyze stress distribution around implants supporting a cantilever fixed partial denture (FPD) under both occlusal and punctual loading conditions. The effects of different implant–abutment connections and frameworks, whole and welded, were compared.

Material and methods

Six photoelastic models were produced from the master polycarbonate models (rectangular block format: 50 mm × 30 mm × 15 mm; 4 mm of height was removed at the posterior region of the model to simulate the resorption of the posterior region of the mandible). The implants (Ø 3.75 mm × 9 mm EH (Ti Titamax; Neodent, Curitiba, Brazil) and Ø 3.75 mm × 9 mm MT (CM Titamax; Neodent)) and a resin tooth were positioned into the models. The implants corresponded to the second premolar and the first molar, and a resin tooth replica (Protemp™ 4; 3M ESPE, St. Paul, USA) replaced the first premolar. The distance between the center of the tooth and the center of the implant was 7.5 mm; the distance between the centers of the implants was 9.5 mm, according to the odontometric parameters described in previous studies.[21–23] The root of the resin tooth received a 0.3-millimeter layer of polyether (Impregum™ Soft; 3M ESPE) to simulate the periodontal ligament (PDL).[24]

A 3-unit implant-retained prosthesis with a distal cantilever was waxed up on the copings of screw-retained abutments (EH and MT abutments, cobalt-chromium (Co-Cr) copings; Neodent). The prosthesis was duplicated (silicone azul; Polglass, Ribeirão Preto, Brazil) to standardize the specimens in all groups. To obtain frameworks, the waxed 3-unit implant-retained prostheses were reduced by 2 mm, and another silicone mold was made. For the fabrication of CAD/CAM frameworks, the previously reduced frameworks were scanned using a D700 scanner (3Shape, Copenhagen, Denmark) and machined in Co-Cr using a milling machine (Ceramill Motion 2; Amman Girrbach, Koblach, Austria).

The frameworks were fabricated using a Co-Cr alloy (Fit Cast Cobalto; Talmax, Curitiba, Brazil) and separated into the following 3 groups: LAS – conventional cast framework sectioned and welded with a laser; TIG – conventional

cast framework sectioned and welded with TIG; and CCS – framework produced with the CAD/CAM system. Using a 0.5-millimeter-thick, sharp stainless steel blade, the LAS and TIG frameworks were sectioned to be welded after conventional casting. The spruing, investing, burnout, and casting techniques were standardized. Subsequently, the frameworks were carefully removed by using glass microspheres (Polidental Indústria e Comércio, São Paulo, Brazil) of 100 μm granulation at a pressure of 60 lbf/in^2. Small nodules were removed with high-speed rotary tungsten carbide burs under constant cooling.

The laser welding machine (desktop Compact; Dentaurum, Nova Lianka, São Paulo, Brazil) was set at 310 V and the pulse was fixed at 9 ms. Laser welding was performed at diametrically opposed points until the entire diameter of the section in the framework received the welding points to minimize distortion. Tungsten inert gas welding was performed using a plasma welding machine (NTY 60 K; Kernit, Indaiatuba, Brazil) according to the adapted methodology.[25] In an argon gas environment, the tungsten (W) electrode was positioned 3–6 mm from the infrastructure, with the settings of 4 A and 0.15 s. Similarly to laser welding, TIG welding was executed at diametrically opposed points on the section of the framework.

All the steps of pressing the infrastructures with the IPS InLine PoM ceramic (Ivoclar Vivadent, Schaan, Liechtenstein) strictly followed the manufacturer's recommendations.

Photoelastic stress analysis

The abutments were tightened to the implants, and the prosthesis frameworks were tightened on the abutments. Before each analysis, the models were heated to 50°C for 10 min to release the stress induced within the model. Subsequently, the models were cooled at approx. 23°C for 10 min and the absence of residual stresses was confirmed with a polariscope (FL 200; G.U.N.T. Gerätebau, Hamburg, Germany).[20] The cantilevers were subjected to a simulated occlusal axial load and a single-point load cell (both of 150 N).[26]

To perform the qualitative analysis, the polariscope was adjusted to the circular polarization mode. Stress intensity, represented by respective fringe orders (n – number of fringes), and location were compared subjectively. A greater number of fringes indicated greater intensity of tension, and the closer the fringes were to each other, the greater the stress concentration was.[27,28] Interpretation was performed using the following scale: (1) low stress – 1 fringe or less; (2) moderate stress – between 2 and 3 fringes; or (3) high stress – more than 3 fringes.[29]

To perform the quantitative analysis, the polariscope was adjusted to the circular mode. Stress distribution with regard to isochromatic fringes at 6 points of interest (3 in the cervical region of the implants, near the simulated crestal bone, 1 in the apical region of each implant, and 1 in the apical region of the first premolar) (Fig. 1) was analyzed using the photoelastic model. The isochromatic fringe values (n) for each of the reading points were measured using the Tardy method of compensation.[30] The individual shear stress value (τ) for each point was determined using the stress-optic law, as follows (Equation 1):

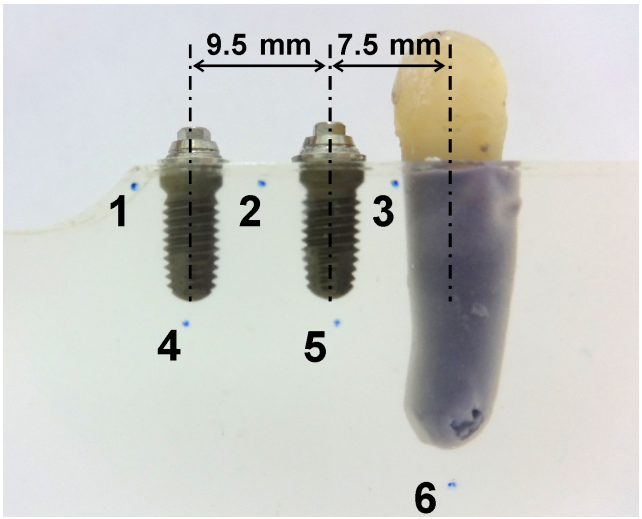

Fig. 1. Distance between the implants and the tooth, and points selected for the quantitative analysis

$$\tau = \frac{K_\sigma \times n}{2 \times b} \quad (1)$$

where:

τ – maximum shear stress [kPa];

n – value of the fringe order at the analyzed point; and

b – thickness of the model [mm] (15 mm).

The optical constant of the photoelastic material $K_\sigma = 3.56$ was predetermined in a calibration procedure.[29]

Results

The photoelastic stress analysis, as showed in Fig. 2 and corroborated by the shear stress values in Table 1, indicated varying stress levels across different scenarios. In the LAS-EH configuration, the observed stress levels were as follows: (1) high between the implants (P2; fringe order 4); (2) moderate between the implant and the tooth (P3, fringe order 2); and (3) moderate in the apical region of the implants (P4 and P5; fringe order 3). The TIG-EH and CCS-EH configurations demonstrated low to moderate stress with a fringe order of 1 at the dental apex (P6) and a fringe order of 2 in the crestal bone region below the cantilever (P1). With regard to the MT connection, TIG-MT exhibited the following stress levels: (1) moderate in the region below the cantilever (P1; fringe order 3); (2) moderate between the implants, and between the implant and the tooth (P2 and P3; fringe order 3); and (3) high in the apical region of the implants

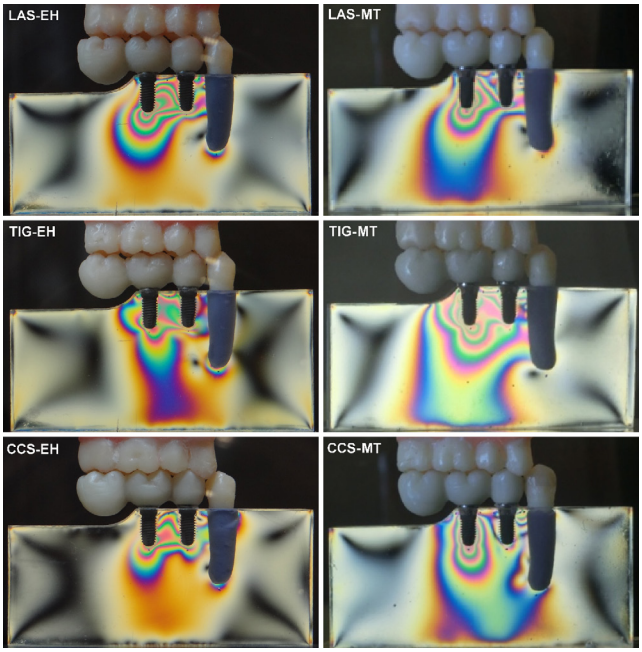

Fig. 2. Stress caused by the application of an occlusal load

LAS – conventional cast framework sectioned and welded with a laser; TIG – conventional cast framework sectioned and welded with tungsten inert gas (TIG); CCS – framework produced with the computer-aided design/computer-aided manufacturing (CAD/CAM) system; EH – external hexagon implant; MT – Morse taper implant.

Table 1. Maximum shear stress values (τ) [kPa] for each point with regard to the external hexagon (EH) and Morse taper (MT) implants under occlusal loading

Point	EH			MT		
	LAS	TIG	CCS	LAS	TIG	CCS
P1	114.9	239.1	110.7	219.8	674.3	456.2
P2	207.9	249.3	304.9	238.9	297.5	186.0
P3	182.5	146.0	127.8	63.6	299.2	77.7
P4	538.4	414.9	401.5	861.9	790.0	429.7
P5	380.2	134.3	249.4	151.2	362.0	49.8
P6	112.5	202.3	132.3	41.3	52.1	58.7

P1 – below the cantilever; P2 – between the implants; P3 – between the implant and the tooth; P4, P5 – in the apical region of the implants; P6 – at the dental apex.

(P4 and P5, fringe order 4). The LAS-MT and CCS-MT configurations showed fringe order fringe order 1 below the cantilever (P1) and between the implants (P2), and fringe orders 3 and 2 at the apical region of the implants (P4 and P5, respectively).

Regarding the differences in stress distribution between the implant connections under occlusal loading, the LAS-EH configuration exhibited a greater number of fringes between the implants and in the apical regions of the implants. The TIG-MT framework showed the highest stress below the cantilever, in the apical region of the implants, and between the implant and the tooth. Comparing the frameworks fabricated using the CAD/ CAM system, the EH system presented higher stress at all points (except for P1 and P4) as compared to the MT connection.

Under a punctual load of 150 N applied to the cantilever, the photoelastic stress analysis indicated varied stress concentration for the EH and MT implant connections (Fig. 3, Table 2). For the EH connection, the LAS group exhibited a fringe order of 4 at P4, corresponding to a maximum shear stress value of 643.8 kPa. The TIG group showed a fringe order of 3 at points P1, P4, and P5, with notably high shear stress values at P1 (660.7 kPa) and P4 (589.8 kPa). The CCS group demonstrated similar stress patterns, with a fringe order of 3 at P4 and a maximum shear stress value of 637.1 kPa. Other points under the EH connection mostly showed a fringe order of 2, indicating moderate stress levels. For the MT connection, the LAS group presented a high fringe order of 4 at point P4, where the shear stress reached 704.9 kPa. Tungsten inert gas welding on the MT connection produced the highest shear stress across most points, with the peak at P4 (788.4 kPa), followed by P1 (666.9 kPa) and P5 (600.3 kPa). The CCS-MT configuration also showed high stress at P4, with a fringe order of 3 and a shear stress value of 483.4 kPa. Fringe order 2 was observed in the remaining points for all MT groups, suggesting lower stress concentration.

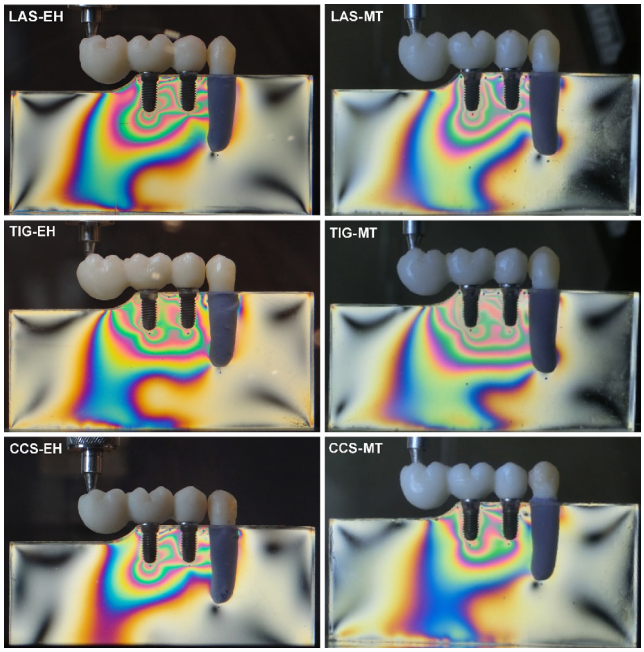

Fig. 3. Stress caused by the application of a punctual load

Table 2. Maximum shear stress values (τ) [kPa] for each point with regard to the external hexagon (EH) and Morse taper (MT) implants under punctual loading

Point	EH			MT		
	LAS	TIG	CCS	LAS	TIG	CCS
P1	354.0	660.7	419.1	176.8	666.9	392.5
P2	212.9	353.8	196.9	259.3	446.3	266.5
P3	401.3	151.3	316.0	148.8	315.7	100.0
P4	643.8	589.8	637.1	704.9	788.4	483.4
P5	432.6	539.9	335.4	322.3	600.3	358.7
P6	45.6	87.9	138.6	44.6	61.2	38.8

The comparative analysis of the implant connections revealed that the EH system generally exhibited higher shear stress values than the MT connection in certain points within the LAS and CCS groups. Specifically, the LAS-EH configuration showed higher stress at points P1 (354.0 kPa), P3 (401.3 kPa), P5 (432.6 kPa), and P6 (45.6 kPa), while the CCS-EH configuration demonstrated higher values at P1 (419.1 kPa), P3 (316.0 kPa), P4 (637.1 kPa), and P6 (138.6 kPa).

Discussion

Under occlusal loading, laser welding exhibited the highest stress pattern at the apices of the EH implants, between the implants, and between the implant and the tooth. However, TIG welding presented the highest shear stress in critical areas, such as the crestal bone, for both implant connections. The lost-wax fabrication process of frameworks is related to a high coefficient of thermal expansion of the wax, and its dimensional stability is subject to air temperature; however, a combination of distortions in different dimensions can cause a significant misfit at the prosthesis–abutment interface. Consequently, it may result in the overload of the bone.[31] Although the distortions are difficult to eliminate, they can be minimized by sectioning and welding frameworks.

Laser welding promotes significant mechanical longevity of the framework due to its high precision level, biocompatibility and minimal side effects. Furthermore, this technique yields a framework with reasonable hardness and minimizes the heat-affected zone, which is crucial for maintaining the integrity of the material.[32] De Castro et al. evaluated stress distribution in Co-Cr frameworks with the use of laser welding and TIG welding, and concluded that the stress generated around the implants was similar for both techniques.[25] In the present study, although laser welding presented high stress between the implants, TIG welding showed the highest shear stress values in the crestal bone region at 3 key points, as well as in the apical region of the implants for both the EH and MT connections. This was consistent across all measured points, except for P3 and P4 in the TIG-EH configuration, where the stress was not the highest as compared to other groups, as detailed in Table 2.

In the case of cantilevers, careful planning is necessary to preserve the bone around the implants, mainly because of the distribution of the stress transmitted to the marginal area of the bone during chewing. The concentration of high-intensity fringes at the distal implant under punctual loading on the cantilever, as exhibited by TIG welding in both implant systems, increases the possibility of crestal bone resorption. Some studies have reported an increase in the flexural strength demonstrated by TIG-welded frameworks.[6,7] Several factors may influence the mechanical strength. Tungsten inert gas welding correlates with resistance because of the welding penetration, consequently resulting in fewer pores, cracks and flaws; thus, high stress to the bone in the crestal bone area can be associated with the high mechanical strength provided by TIG welding.[7]

The study found that the frameworks fabricated using the CAD/CAM technique generally exhibited lower stress values under occlusal and punctual loading. However, this was not consistent across all cases. A detailed examination of Tables 1 and 2 reveals exceptions, where the CAD/CAM frameworks did not result in the lowest stress values as compared to other techniques. Although the CAD/CAM method has improved the fit of frameworks, distortions can still be present when the procedures employed to apply the ceramic involve the lost-wax process followed by heat pressing. Some studies have reported the superiority of the CAD/CAM technique in terms of fit accuracy of implant-supported FDP as compared to the conventional cast frameworks.[11,13] However, these reports compared CAD/CAM with conventional casting, without taking into consideration the copings for the screw-retained abutments, as in the present study. The overcasting technique uses cylinders with premachined metal straps to avoid casting the cylinder base and to minimize distortions.[18] This situation may explain the same stress pattern observed for the laser-welded and CAD/CAM frameworks for the MT implants under occlusal and punctual loading on the cantilever.

According to the results, the type of the implant–abutment connection influenced the distribution of stress to the bone. The MT system produced a lower number of fringes and lower shear stress values under both types of loading, except when the framework of the TIG group was tightened. However, a study by Goiato et al. showed a more stable inferface for internal connections due to the intimate contact between the internal part of the implant and the external part of the abutment,[33] which favors load distribution.[34] Moreover, the cited study did not use copings for the screw-retained abutments, and only a punctual load was applied to the cantilever. Sousa et al. evaluated the EH and MT connections by using the finite element analysis (FEA), and proved that the MT connection significantly decreased the strain levels in the peri-implant bone.[35] This result is in partial agreement with the findings of the present study, since the TIG group showed the highest shear stress values, especially for the MT connection, which can be attributed to the high flexural strength resulting from the welding penetration.

Limitations

The photoelastic stress analysis and other in vitro methods present certain limitations, and the results should be considered with caution when extrapolating them to clinical situations. These limitations relate to the different elasticity moduli of oral tissues, and the inability of solid,

isotropic photoelastic models to differentiate between the cortical and cancellous bones.[21,36]

Despite the differences, photoelasticity is regarded as a fairly accurate method for assessing stress patterns, as the fundamental stress concentration trends are typically consistent with those observed clinically.[37]

For a more comprehensive understanding, future research could explore three-dimensional (3D) photoelastic stress analysis. This advanced approach would accommodate the complexity of the oral environment more effectively by including shear stress as part of the loading conditions, thereby providing a simulation that would more representative of clinical situations.[19]

Conclusions

Within the limitations of this study, we conclude that the use of laser-welded and CAD/CAM frameworks with MT implants results in lower stress values in the crestal bone area. Although there was high stress associated with TIG welding on the MT system, the EH system exhibited more stress in other groups in comparison with the MT system.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Bruna Santos Honório Tonin https://orcid.org/0000-0001-7949-9980
Raniel Fernandes Peixoto https://orcid.org/0000-0002-6845-0767
Jing Fu https://orcid.org/0000-0001-6876-6166
Maria da Gloria Chiarello de Mattos https://orcid.org/0000-0002-1939-7998
Ana Paula Macedo https://orcid.org/0000-0002-7716-106X

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Original paper

Comparison of gray-level detectability on computer monitors among several dental specialties: A web-based study

Burçin Arıcan Alpay[1,A–F], Cansu Büyük[2,A–F], Ayfer Atav Ateş[3,A–F]

[1] Department of Endodontics, School of Dental Medicine, Bahçeşehir University, Istanbul, Turkey
[2] Department of Dentomaxillofacial Radiology, Faculty of Dentistry, Istanbul Okan University, Turkey
[3] Department of Endodontics, Faculty of Dentistry, Istinye University, Istanbul, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Burçin Arıcan Alpay
E-mail: dr.burcinarican@gmail.com

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Abstract

Background. Diagnosis in dentistry begins with the correct reading and interpreting of the dental radiograph.

Objectives. The aim of the present study was to examine the effects of the imaging technique used, the dentistry specialty and the years of experience on the gray-level perception among dentists.

Material and methods. A custom web application was developed. Dentomaxillofacial radiologists (DentRads), endodontists (Ends) and general dental practitioners (GDPs) were invited via e-mail to participate in the study. A total of 46 participants met the requirements of the test. The test comprised 2 webpages. On the 1st page, the participants were asked for information such as gender, specialty, the years of experience, and the imaging techniques they used. Then, on the 2nd page, they were welcomed with instructions and directions, and asked to rearrange 85 gray color tones represented by square bars of equal dimensions. These mixed gray bars were placed in 4 rows according to the principles of the Farnsworth–Munsell 100-hue test (FM). Each clinician's test results were recorded in a database. The individual's level of recognition of gray tones was evaluated through the total error score (TES), which was calculated using a web-based independent scoring software program. Lower TES values were a desirable result, indicating fewer misplacement, while higher scores indicated more misplacements of gray tones. The testing time (TT) was recorded automatically.

Results. The years of the participants' experience as dentists or specialists did not affect TES or TT. The dentists who used the charge-coupled device-complementary metal oxide semiconductor (CCD-CMOS) had lower TES values than those who used analog radiographs ($p < 0.05$).

Conclusions. While the specialty and the years of experience did not affect the clinicians' ability to recognize gray tones, the digital imaging techniques (photostimulable phosphor (PSP) and CCD/CMOS) could improve the clinicians' gray-level perception.

Keywords: grayscale, dental radiology, contrast resolution

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Introduction

Dental radiology procedures are among the most necessary and common ones in modern dentistry; they are used for the diagnosis of apical and periapical abnormalities, treatment plans, supporting treatment, the evaluation of care, and forensic situations.[1] Therefore, radiographic image interpretation plays an important role at the forefront in all branches of dentistry before, during and after treatment. The ability to identify subtle differences in grayscale images, combined with good knowledge of anatomy, often leads to a successful diagnosis.

Contrast resolution is defined as the number of possible shades of gray that a pixel can carry, which also expresses the bit depth. Digital images have a bit depth from 8 (256 shades of gray) to 16 (65,536 shades of gray).[2] However, even if the monitor has a wide contrast range, the observer may not be able to perceive details.[3] One of the most recognized methods in medical imaging used to measure the limits of the human visual system (HVS) is Barten's model.[4] It comprises a series of experiments at different luminance levels that characterize the contrast sensitivity of HVS. Based on Barten's model, a calibration standard has been described for medical imaging monitors. The standard, called Digital Imaging and Communication in Medicine (DICOM®) Grayscale Standard Display Function (GSDF), states the precise display luminance that should be produced for a given input value.[4]

The GSDF provides that different monitors can be set to have the same grayscale response, resulting in the improvement of the perceptual linearity of observers.[3] While GSDF calibration in medical-grade grayscale monitors is accomplished during the manufacturing process, for consumer-grade monitors, the calibration may be achieved with medical application software.[5] However, considering that clinical dental practice requires relatively high room illuminance, and that medical-grade monitors and the additional software are costly, dental radiographs are most often interpreted on consumer-grade monitors.[6] Furthermore, most of the affordable and practical imaging monitors support only 8 bits.[4] Therefore, GSDF has not been greatly implemented.

In dentomaxillofacial radiology, contrast resolution refers to the dental image quality in terms of clear differences in gray shades with regard to enamel and dentin, root canal morphology, the pulp system, the marginal bone levels, air, and the trabecular bone pattern.[7,8] Diagnosing root fractures, resorptions or fractured root canal instruments, and determining the working length or accessory canals are some of the challenging radiological tasks for dentists, requiring the ability to differentiate fine details. In addition, the correct interpretation of the root canal system in terms of canal morphology and number of canals is fundamental for the successful outcome of endodontic therapies.[8,9] Thus, contrast sensitivity is an important competence when evaluating dental radiological images.[10] Ganesan et al. concluded that the observer's experience has a great impact on their visual search pattern.[11]

To evaluate the quality of the radiographic imaging system, there are some suggested methods, such as the perceptibility curve tests or the receiver operating characteristic (ROC) curve tests.[12] However, when it comes to evaluating the perception of the observer, there is no quantitative study in the literature that assesses the ability of dentists to differentiate gray tones. The Farnsworth–Munsell 100-hue test (FM) is widely used for color discrimination, including 85 colored caps.[13] The accuracy of the observer in arranging the caps (forming a gradual transition in chroma) is gauged with the total error score (TES). The TES increases with the number of misplacements. Starting from this point, the authors of the present study claim that FM, which provides a numerical score, might be modified for the discrimination of gray tones.

Diagnosis in dentistry begins with the correct reading and interpretation of the dental radiograph.[14] All dentists, whether specialists or not, use radiographs in their clinical routines. However, some branches of dentistry use radiographs not only for diagnosis, but also in all process steps. Endodontists (Ends) use radiographs pre-, during and post-treatment. Dentomaxillofacial radiologists (DentRads) routinely focus on normal and pathological differences on the scans obtained with cone-beam computed tomography (CBCT) and all other radiographic imaging systems in daily clinical practice. To the best of the authors' knowledge, no studies have evaluated the ability of dentists to interpret contrast resolution. Therefore, the present study aimed to examine the influence of the imaging technique used, the dentistry specialty and the years of experience on the ability to perceive gray tones among dental professionals, as well as the testing time (TT). The null hypotheses were as follows:

1. There is no difference in the perception of gray tones and TT between the dentistry branches.
2. Neither the years of experience nor the imaging techniques used by clinicians have an effect on the interpretation of contrast resolution.

Material and methods

This laboratory study was conducted according to the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines.[15] The study was approved by the institutional ethics committee at Istanbul Okan University, Turkey (approval No. 21.10.2020/24).

This research was a web-based study. Prior to the main study, 3 pilot studies were carried out. In the 1st and 2nd pilot studies, the participants were asked to rearrange 32 or 64 gray color bars from white to black. It was shown that they all reordered whitish and blackish colors almost faultlessly, but had problems arranging light to dark gray

colors. Based on the outcomes of the pilot studies, the main study was reconstructed focusing on gray tones, which were a major problem for dentists. Then, the 3rd pilot study was applied to 20 people under the conditions of the main study, using 85 gray tones selected consequently (22–106) from a 128-tone (7 bits/pixel) grayscale. Afterward, the web page was blocked, and the pilot study was concluded. A reliability test was performed. Considering the coefficient of correlation between the TES and TT values as a result of the 3rd pilot study ($\rho = -0.581$), the minimum number of participants to be included, with 95% confidence ($1-\alpha$) and 95% test strength ($1-\beta$), was determined to be 27.

In light of these data, the individuals who participated in the pilot studies were not included in the main study, and other DentRads, Ends, and general dental practitioners (GDPs) were invited to participate. Invitations were sent to randomly selected members of relevant associations in Turkey via e-mail. A reminder e-mail was automatically sent to those who did not respond to the first e-mail within 10 days. Then, after 20 days, the system was closed.

The inclusion criteria for the respondents were as follows:

– the participant should be a GDP, End or DentRad;
– the participant should have had an eye examination in the last 18 months;
– the participant should not have any eye defects, or if they did, they should have been fixed with glasses or contact lenses;
– the participant should be using a MacBook Air laptop or be familiar with it.

The exclusion criteria for the respondents were as follows:

– participants who did not visit the website after the 2 e-mails;
– participants who had an eye defect (glaucoma, cataract, etc.);
– participants who refused to use a MacBook Air laptop;
– participants who had been diagnosed with attention deficit hyperactivity disorder (ADHD).

A custom web application was developed by a web developer using HTML, CSS, JavaScript, and PHP. The application consisted of 2 webpages. On the 1st page, the participants were asked to provide personal information, including gender, specialty, the years of experience, and the imaging techniques (i.e., charge-coupled device-complementary metal oxide semiconductor (CCD-CMOS), photostimulable phosphor (PSP), analog) used in their daily clinical routines (Fig. 1A). In addition, they were directed to calibrate their monitors, using the link provided (macOS User Guide; https://support.apple.com/en-hk/guide/mac-help/mchlp1109/mac). The participants were not allowed to proceed to the 2nd page without confirming that the necessary calibration was performed. After providing informed consent to participate in the study, the participants were directed to the 2nd page.

The 2nd page included test instructions and directions (Fig. 1B). Initially, the participants were informed about the contents of the test. According to the instructions, the test was to be taken on a MacBook Air laptop (Apple Inc., California, USA) at a distance of approx. 60 cm from the monitor (3 spans). The participants were offered a MacBook Air laptop to use if they did not have one. The test design was inspired by FM. Eighty-five gray color tones represented with square bars of equal length and width were randomly placed in 4 rows according to the principles of FM, as follows: 22 bars in the 1st row (white-like gray) and 21 bars in the 2nd (light gray), 3rd (dark gray) and 4th (black-like gray) rows. These gray tones were selected consequently (22–106) from a 128-tone (7 bits/pixel) grayscale (Fig. 1B). The lightest (1–21) and darkest (107–128) gray tones were excluded. Each tone was numbered from 1 to 85, but the enumeration was not visible to the participants. On this page, the participants were asked to rearrange the bars from lightest to darkest in the correct order. They were only allowed to drag and change the bars in the same row.

The participants who got acquainted with the instructions were asked to start the test by clicking the "start" button. Then, when they completed the test, they were asked to click the "submit" button.

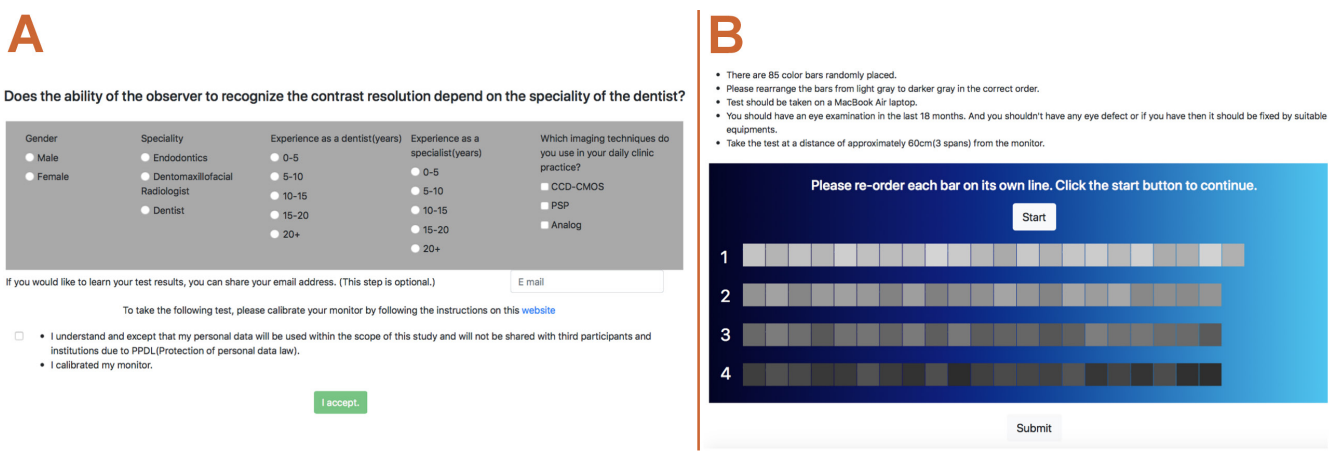

Fig. 1. First (A) and second (B) webpages of the application

The test results of each clinician were recorded in a PHP database. The individual's level of recognition of gray tones was evaluated through TES. The score was calculated using a web-based independent scoring software program for FM (the classical method), which was coded by Béla Török (https://www.torok.info/colorvision/fm100.htm) (Fig. 2). Lower TES values were a desirable result, indicating fewer misplacements, while higher scores indicated more misplacements of gray tones. The testing time was recorded automatically.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA). The data was assessed for normality using the Shapiro–Wilk test. The one-way analysis of variance (ANOVA) was used to compare normally distributed data. The Mann–Whitney *U* test and the Kruskal–Wallis test compared non-normally distributed data. The results were presented as mean and standard deviation ($M \pm SD$) for normally distributed data, and as median (minimum–maximum) (*Me* (min–max)) for non-normally distributed data. The significance level was set at $p < 0.05$.

Forty-six participants completed the study, and the power of the test (99.9%) was obtained as a result of the post-hoc power analysis.

Results

Sixty-eight dentists visited the website within 30 days. Sixteen visitors did not complete the test, 3 visitors submitted their tests in less than 15 s, and the TES values of 3 other visitors were higher than 500. Therefore, the results of these 22 participants were discarded from the study. A total of 46 individuals met the requirements of the test. The numbers of respondents by dentistry branch were 16 GDPs, 15 Ends and 15 DentRads. Eighteen participants were male, and 28 were female. The TT values according to gender, branch, the years of experience, and the radiographic method used by the participants are shown in Table 1. The time to complete the test ranged from 280 s to 1,228 s. There was no correlation between TT and gender, the years of experience or the radiographic method used ($p > 0.05$). However, DentRads spent significantly more time than GDPs to complete the test ($p < 0.05$).

The mean TES values for the participants are shown in Table 2. Higher TES values represented more misplacements of the gray bars. The TES results ranged from 3 to 435. The TES results of the specialty groups were GDPs > Ends > DentRads, but the differences were not statistically significant ($p > 0.05$).

The participants used various imaging techniques in their clinical practice. The respondents were allowed to choose more than one answer to the question regarding

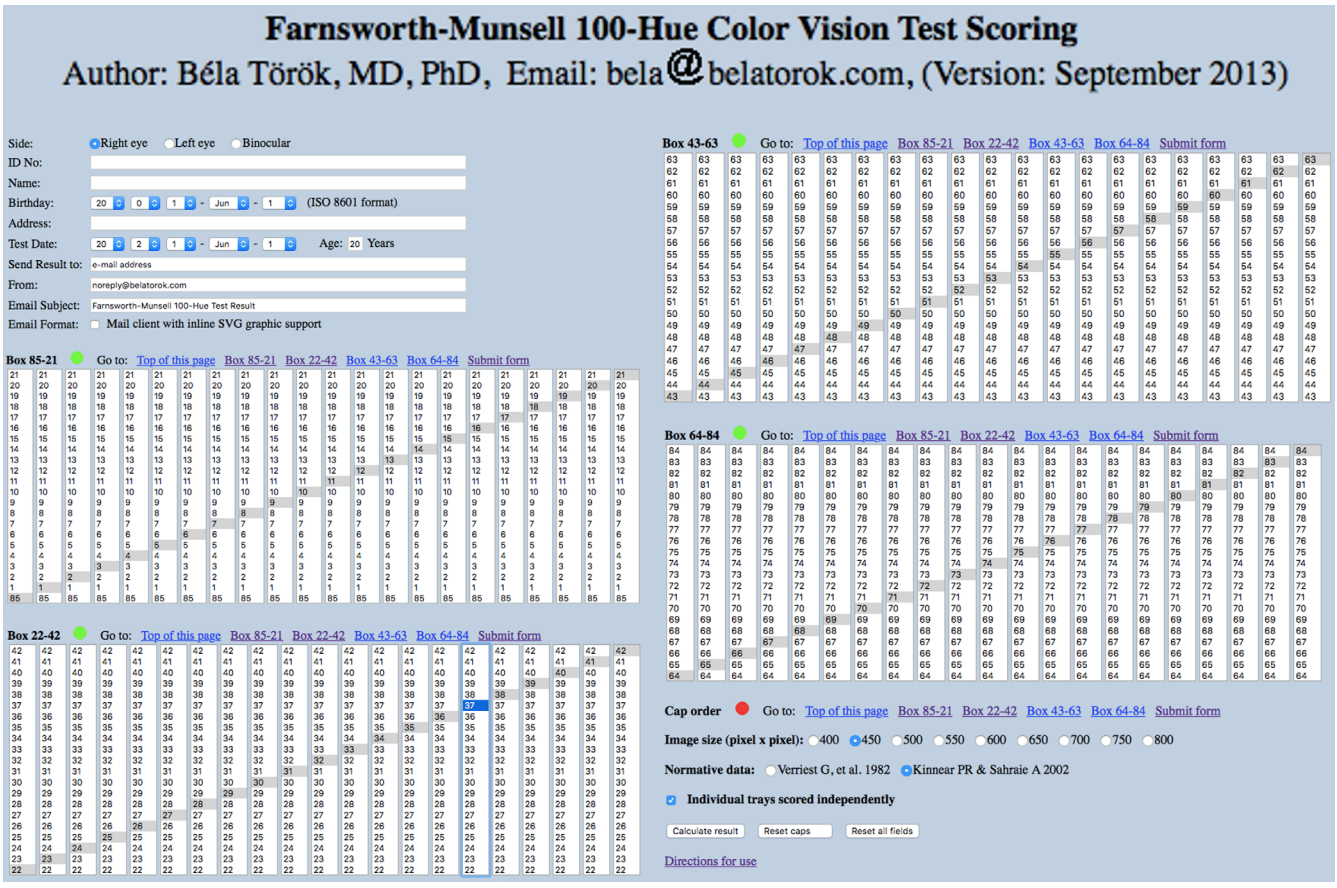

Fig. 2. Data entry page of a web-based independent scoring software program coded by Béla Török (https://www.torok.info/colorvision/fm100.htm)

Table 1. Intergroup comparisons of the testing time (TT) [s] at 95% confidence interval (*CI*)

Group		TT [s]			
		M ±*SD*	*Me* (min–max)	test statistic	*p*-value
Gender	male (*n* = 18)	571.7 ±270.1	480.5 (280–1,064)	*U* = 213.5	0.386
	female (*n* = 28)	630.6 ±256.7	596.5 (281–1,228)		
Branch	GDPs (*n* = 16)	535.4 ±260.9	435 (280–1,068)[a]	χ^2 = 7.438	0.024
	Ends (*n* = 15)	532.3 ±227.4	446 (286–1,074)[ab]		
	DentRads (*n* = 15)	759.7 ±238.5	769 (281–1,228)[b]		
Radiographic imaging technique	analog (*n* = 9)	478.1 ±221.0	403 (280–983)	χ^2 = 4.513	0.211
	CCD-CMOS (*n* = 10)	595.4 ±196.7	630.5 (286–843)		
	PSP (*n* = 22)	688.1 ±292.4	701 (281–1,228)		
	hybrid (*n* = 5)	510.2 ±212.3	450 (286–831)		
Work experience as a dentist [years]	0–5 (*n* = 10)	647.7 ±261.9	677 (297–1,068)	F = 1.810	0.160
	5–10 (*n* = 21)	676.2 ±290.6	750 (280–1,228)		
	10–15 (*n* = 9)	465.7 ±163.3	433 (286–831)		
	15–20 (*n* = 6)	513.0 ±193.9	476 (281–843)		
Work experience as a specialist [years]	0–5 (*n* = 18)	687.9 ±274.1	729 (281–1,228)	F = 0.585	0.564
	5–10 (*n* = 8)	579.8 ±252.9	519 (286–1,074)		
	>10 (*n* = 4)	590.0 ±186.8	557 (403–843)		

M – mean; *SD* – standard deviation; *Me* – median; min – minimum; max – maximum; GDP – general dental practitioner; End – endodontist; DentRad – dentomaxillofacial radiologist; CCD-CMOS – charge-coupled device-complementary metal oxide semiconductor; PSP – photostimulable phosphor; *U* – Mann–Whitney *U* test; χ^2 – Kruskal–Wallis test; F – ANOVA; the values marked with different superscript letters were significantly different ($p < 0.05$).

Table 2. Intergroup comparisons of the total error score (TES) values at 95% confidence interval (*CI*)

Group		TES			
		M ±*SD*	*Me* (min–max)	test statistic	*p*-value
Gender	male (*n* = 18)	132.4 ±112.8	100 (6–435)	*U* = 279.5	0.536
	female (*n* = 28)	110.5 ±88.8	97.5 (3–309)		
Branch	GDPs (*n* = 16)	151.2 ±125.2	143 (3–435)	F = 2.251	0.118
	Ends (*n* = 15)	124.9 ±88.3	113 (6–309)		
	DentRads (*n* = 15)	78.9 ±59.2	53 (6–188)		
Radiographic imaging technique	analog (*n* = 9)	196.9 ±82.7	190 (82–324)[b]	χ^2 = 10.268	0.016
	CCD-CMOS (*n* = 10)	91.5 ±130.8	35 (6–435)[a]		
	PSP (*n* = 22)	108.3 ±82.0	100 (3–309)[ab]		
	hybrid (*n* = 5)	81.4 ±57.0	85 (6–155)[ab]		
Work experience as a dentist [years]	0–5 (*n* = 10)	83.1 ±86.9	69 (3–280)	χ^2 = 4.583	0.205
	5–10 (*n* = 21)	124.7 ±117.4	85 (16–435)		
	10–15 (*n* = 9)	118.1 ±74.2	115 (6–251)		
	15–20 (*n* = 6)	160.8 ±67.4	171.5 (35–218)		
Work experience as a specialist [years]	0–5 (*n* = 18)	88.5 ±71.6	66 (6–309)	χ^2 = 2.138	0.343
	5–10 (*n* = 8)	108.6 ±88.9	112 (6–267)		
	>10 (*n* = 4)	149.0 ±80.2	171.5 (35–218)		

The values marked with different superscript letters were significantly different ($p < 0.05$).

the imaging methods. Five participants reported using more than one technique, which included PSP and CCD-CMOS (the hybrid group). While the first choice of Ends (*n* = 6; 40.00%) and DentRads (*n* = 13; 86.67%) was PSP, the first choice of GDPs (*n* = 7; 43.75%) was analog radiography. In terms of TES, there were no significant differences between the PSP and hybrid groups as compared to the other groups. The dentists who used CCD-CMOS had significantly lower TES values than those who used analog radiographs ($p < 0.05$) (Table 2).

Although the lowest TES values were obtained by professionals with 0–5 years of experience, the years of the

participants' experience as dentists (Experience 1) or specialists (Experience 2) did not affect the TES results ($p > 0.05$) (Table 2). Also, no statistically significant differences were observed between specialists (DentRads and Ends) and GDPs regarding both the TES and TT values ($p < 0.05$) (Table 3).

Table 3. Intergroup comparison of the testing time (TT) and total error score (TES) values

Group		TT [s]	TES
GDPs ($n = 16$)	*M* ±*SD*	535.4 ±260.9	151.2 ±125.2
	Me (min–max)	435 (280–1,068)	143 (3–435)
Specialists ($n = 30$) Ends ($n = 15$) DentRads ($n = 15$)	*M* ±*SD*	646.0 ±256.5	101.9 ±77.5
	Me (min–max)	606 (281–1,228)	91.5 (6–309)
Test statistic		$U = 172.0$	$U = 195.0$
p-value		0.117	0.299

The order of gray tones entered into the software can also be visualized by a ring chart (Fig. 3). The misplaced gray bars are shown in red. Accordingly, the upper left part of the diagram represents the 1st row, the lower left part – the 2nd row, the lower right part – the 3rd row, and the upper right part – the 4th row. The participants often misplaced the bars representing the white-like gray and light gray colors. The 4th row (black-like gray) had the highest rate of correct placement.

Discussion

It is well known that radiographs are the main adjunct to the clinical examination, and dentists need them in both operative and preventive dentistry. Dentomaxillofacial radiologists and endodontists are the main groups that use radiographic imaging techniques in diagnosis, follow-up sections, in pre-treatment, treatment and post-treatment procedures. On the other hand, GDPs constitute the dominant group of dentists, dealing with all fields of dentistry, and they use all types of radiographs in their clinical routine. Identifying and improving the ability of dentists to detect gray tones, as well as determining the factors affecting this ability, would shorten the diagnostic process, reduce costs and positively affect the prognosis of treatment.[16] Therefore, the present study aimed to compare the contrast resolution recognition abilities of the above-mentioned 3 groups of dentists. According to the results, there was a difference between the dentistry branches with regard to TT, as DentRads spent significantly more time than GDPs to complete the test. The years of experience had no effect on TES or TT. However, the dentists who used CCD-CMOS had significantly lower TES values than those who used analog radiographs ($p < 0.05$). For this reason, the 1st null hypothesis was partially rejected, and the 2nd hypothesis was also partially rejected.

When the TES and TT values were analyzed, it turned out that DentRads spent the longest time completing the

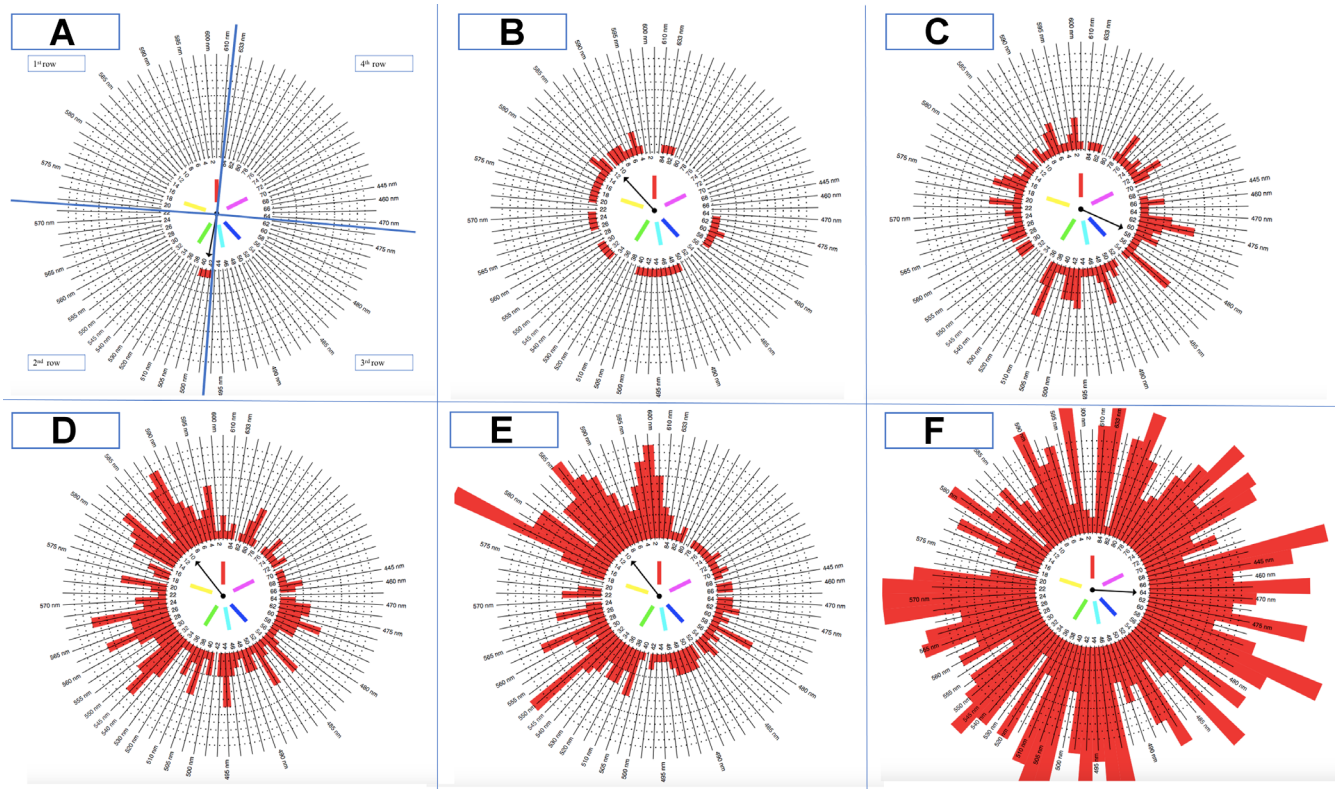

Fig. 3. Ring charts of the participants whose TES values were 3 (A), 53 (B), 151 (C), 280 (D), 324 (E), and 1,035 (F) (discarded from the study)
The red bars show the incorrect placements and their accumulations. For example, in Fig. 3A, gray tones numbered 40, 41 and 42 in the 2nd row were misplaced, and this area was shown as a single red row.

test, but no statistically significant differences were observed between this group and the other groups with regard to the TES values. Given that radiologists often view radiographs on larger screens in dark and private rooms, it is understandable that the focusing time will be longer on a 13-inch screen. In addition, it has been shown that the computer vision syndrome (CVS), which is defined as an eye and vision problem due to computer use,[17] manifested mainly as difficulty with focusing, is quite common among oral radiologists.[18–20] This situation and the prolonged focusing time may affect their work performance.

The lowest TES values were obtained in the group with 0–5 years of experience for both Experience 1 (as a dentist) and Experience 2 (as a specialist). Considering that the number of years of experience is directly proportional to the age of the person, it can be concluded that older participants had more difficulty with distinguishing and ranking gray tones. In addition, this computer-based study may have attracted more attention from the younger generation. Another possibility is that young participants may follow new developments and use digital systems more intensively. However, one of the limitations of this study may be that the participants were asked about their years of experience, not their age.

Radiographic interpretation could be influenced by both objective[21,22] and subjective factors.[23,24] Kamburoğlu et al. compared observer agreement between Ends and DentRads in the evaluation of endodontically and non-endodontically treated teeth for the presence of periapical lesions by using CBCT with 2 different voxel sizes.[25] They reported that the voxel size and the specialty affected intra- and inter-observer reliability. The DentRads group showed better intra- and inter-observer agreement under all conditions.[25] In this study, in which the contrast resolution perception ability of the participants was compared using the TES values, no significant differences were found between the dentistry branches. However, the DentRads group showed better results and lower *SD* values. The reason for this difference between these 2 studies may be the number of specialists participating in the study. While only 2 DentRads and 2 Ends participated in the study by Kamburoğlu et al., the number of participants in our study was at least 15 for each group examined. In addition, since this study measured the dentists' ability to recognize contrast resolution and not their radiographic diagnostic ability, the participants' expertise may not have affected the outcomes.

There were several limitations to this study. The study was conducted amid the coronavirus disease 2019 (COVID-19) pandemic, when the world had come to a standstill. People all around the world had to provide online services from their homes, including many professions that one may not have expected. Although this study was primarily meant as a face-to-face project, unfortunately, this design could not be implemented under COVID-19 conditions. In addition, considering a previous study that stated there were no differences between a computer-based FM (CBFM) and a manual FM (MFM) regarding TES and TT, and also suggested that CBFM was a very reliable, cost-effective and rapid method,[26] our online study could also provide valuable outcomes.

The most challenging problem for these kinds of studies is to design a test that can be standardizable and applicable using virtual methods. Ophthalmologists use various methods, such as the Spaeth/Richman contrast sensitivity test (SPARCS) and the Pelli–Robson (PR) chart test, in the assessment of contrast sensitivity, which are difficult to monitor and translate to the dentistry field.[27] The FM test, which assesses HVS, is used for the detection of color blindness. It could be performed either via the Internet or by using physical derivatives. The data obtained from the test proposes several quantitative pieces of information. In this study, only the application protocol and design of the FM test were adapted.

To obtain standardization, several precautions were taken, including screen calibration, detailed guidance for the test environment and the use of the same brand of computer. Based on a study by Koenderink et al.,[28] the distance from the monitor was determined as approx. 60 cm. The order of the gray bars in each row was established using www.random.org, and each participant saw the same random sequence. In this way, inter-participant standardization was ensured. The participants were also directed to a webpage for screen calibration. This webpage link was placed with a button for the ease of use on the 1st page of the application. Digital zoom was also automatically forbidden by the system. All participants were instructed to use laptops of the same model to standardize the factors related to the monitor and display resolution. MacBook Air laptops were provided to 5 participants who did not have one.

In the present study, the participants used MacBook Air laptop monitors rather than medical monitors or desktop monitors. Medical monitors, which have complex technology, are excellent pathfinders for both medical and dental radiologists during diagnostic processes. However, physicians cannot access these functional monitors in their clinical practice because of their high cost. It was not preferred to use desktop monitor models, since they may have different resolution characteristics and cause calibration problems, and it was not possible to standardize monitor brands. The MacBook Air laptop with the Display Calibrator Assistant enables the control of screen calibration. It has support for millions of colors and a native resolution of 2,560 × 1,600 at a density of 227 pixels per inch. It automatically adjusts the white point of the user's display to the color temperature of the environment for a more natural viewing experience. Due to their display features, MacBook Air laptops were used in the present study.

The main concern of the researchers was to supply suitable ambient light in the evaluation room for the participants.

Although a high ambient light level (>1,000 lux) is almost always present in dental clinics, a low ambient light level (<50 lux) is recommended to enhance the dentist's performance during the detection of caries.[29,30] It has been shown that reporting rooms should have good ambient light. It should be adjustable, as bright as the computer screen, and neither too bright nor too dark.[19] Baltacıoğlu et al. reported that different ambient light levels did not affect the diagnostic ability of observers.[31] In light of this information, ambient light was ignored in this study. However, the participants were instructed in the e-mail invitation to take the test under dim light conditions.

The respondents were asked about the imaging techniques they were using in their clinical routines, and the relationship between the method used and the TES result was assessed. It is broadly accepted that PSP plates have a wider dynamic range than CCD and CMOS sensors.[32] Moreover, both digital systems (PSP plates and CCD/CMOS sensors) offer better contrast resolution than analog systems. On the other hand, the view box of analog films provides better luminance levels than typical digital monitors.[32] Since the study was designed to assess the arrangement of digital data on the monitor, the lower TES results of the digital system users as compared to the analog system users were consistent.

Dental radiographs play a fundamental role in each step of treatment. However, it is not known whether the development of digital radiography technology has improved clinicians' ability to prepare a proper treatment plan.[23] This is because we interpret digital radiographs rather than read them.[33] Goldman et al. reported that many factors, such as the experience of the observer, the conditions in which radiographs are examined and the settings of the X-ray systems, could influence this interpretation.[33] From this point of view, although the years of the participants' experience were evaluated and the examining conditions were standardized in the current study, the results may not be generalizable when considering both observational and technical factors. Interpreting radiographic images poses challenges, particularly when discerning anatomical malformations within the teeth. The fine details of dental structures may not be easily discernible from radiographs. Additionally, the presence of hard tissues can lead to superimpositions on the images, complicating the interpretation of the results. The designed test cannot represent these factors completely. Additionally, dentists normally do not calibrate their personal monitors every time they use them. For this reason, the present study may be a pathfinder for further studies that will focus on contrast resolution under clinical conditions.

Conlusions

Within the limitations of the present study, while the specialty and the years of experience did not affect the clinicians' ability to recognize gray tones, the digital imaging techniques (PSP and CCD/CMOS) could improve the clinicians' gray-level perception. Although the contrast sensitivity of the observer plays a fundamental role when evaluating dental radiographs, it is not the only factor that determines the outcomes. Further studies of the contrast resolution recognition ability of clinicians, supported by clinical cases, are needed.

Ethics approval and consent to participate

The study was approved by the institutional ethics committee at Istanbul Okan University, Turkey (approval No. 21.10.2020/24). All participants provided informed consent.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Burçin Arıcan Alpay https://orcid.org/0000-0001-5757-0571
Cansu Büyük https://orcid.org/0000-0001-8126-0928
Ayfer Atav Ateş https://orcid.org/0000-0003-0270-8646

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Original paper

Association between drinking water fluoride and the serum alkaline phosphatase and phosphate levels in pregnant women and newborn infants

Thippeswamy Honne Manjunathappa[1,A,C], Devananda Devegowda[2,B,F], Nanditha Kumar Mysore[1,D,E], Prashanth Vishwanath[2,B,E], Prashanth Sathya Narayana[3,B,F]

[1] JSS Dental College and Hospital, JSS Academy of Higher Education and Research, Mysore, India
[2] Department of Biochemistry, JSS Medical College, JSS Academy of Higher Education and Research, Mysore, India
[3] Department of Pediatrics, JSS Medical College, JSS Academy of Higher Education and Research, Mysore, India

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Nanditha Kumar Mysore
E-mail: nanditha10@gmail.com

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Abstract

Background. Endemic fluorosis (skeletal and dental) is a serious public health problem in many parts of the world, especially in India. Age, sex, dietary calcium (Ca), the hormonal status, the dose and duration of the fluoride intake, and renal efficiency in handling fluoride all influence fluoride metabolism.

Objectives. The aim of the study was to evaluate the effect of the fluoride present in drinking water on the serum alkaline phosphatase (ALP) and phosphate levels in pregnant women and newborn infants.

Material and methods. In the present cross-sectional study, the participants were categorized into 2 groups based on a fluoride concentration in their drinking water: the low/optimum-fluoride group (<1 ppm); and the high-fluoride group (≥1 ppm). Each group was comprised of 90 pregnant women who were recruited from the hospital at the time of admission for delivery. Fluoride was measured in their drinking water, urine, maternal serum, and cord blood. The ALP and phosphate levels were measured in serum using a fully automated analyzer.

Results. The drinking water consumed by the pregnant women contained fluoride, which was significantly positively correlated with the urine and blood serum fluoride levels. There were significant differences in the ALP levels between the 2 groups in both maternal serum and cord blood. The level of phosphate in maternal serum was significantly higher in the high-fluoride group. The results of both simple and multivariate regression analyses revealed that the fluoride content in drinking water was significantly associated with the ALP level in cord blood and the phosphate level in maternal serum.

Conclusions. The ALP levels were negatively associated with drinking water fluoride concentrations in both maternal serum and cord blood. The phosphate levels in maternal serum were positively associated with drinking water fluoride concentrations.

Keywords: pregnancy, cord blood, alkaline phosphatase, fluoride, serum phosphate

Introduction

Fluorine (F) is the 13th most common element in the Earth's crust, and is widely distributed as fluorspar, fluorapatite and cryolite. These minerals are easily soluble in water and are present in groundwater, which contains high levels of fluoride.[1] The public health benefits associated with fluoridated dental products and optimally fluoridated drinking water are widely cited.[2] Besides the benefits, long-term fluoride exposure can also cause adverse effects, such as dental fluorosis and skeletal fluorosis.[2] Endemic fluorosis (skeletal and dental) is a serious public health problem in many parts of the world, especially in India. Age, sex, dietary calcium (Ca), the hormonal status, the dose and duration of the fluoride intake, and renal efficiency in handling fluoride all influence fluoride metabolism.[3] Fluoride is known to cross the placental barrier. In vitro studies have shown that it accumulates in brain regions involved in learning and memory, and alters proteins and neurotransmitters in the central nervous system (CNS).[4] Hence, it can be presumed that fluoride can also adversely affect CNS.

Alkaline phosphatase (ALP) is an enzyme that occurs in all tissues of the human body. A high concentration of ALP is found in bone, liver, kidney, intestinal, and placental tissue. During pregnancy, ALP is known to gradually increase, reaching a peak in the 3rd trimester that is approx. twice its pregestational value.[5] Serum bone-specific ALP is one of the most specific markers of bone formation.[6]

Phosphorus (P) is important in DNA synthesis and acts as a mitogen. Dietary P deficiency is rare, since most foods contain this element. The recommended dietary allowance (RDA) for P is 700 mg/day for both pregnant and non-pregnant women, and no additional supplementation is recommended for pregnant women based on the current knowledge.[7]

A literature search revealed no studies associating fluoride in drinking water with pregnant women's serum ALP and phosphate levels. Therefore, the present study hypothesized that the fluoride present in drinking water is associated with the serum ALP and phosphate levels in pregnant women.

Methodology

Based on a fluoride concentration in their drinking water, pregnant women were categorized into 2 groups. One group was considered low/optimum-fluoride (concentration below 1 ppm) and the other group was considered high-fluoride (concentration equal to or above 1 ppm) based on the World Health Organization (WHO) criteria.[8] Ninety pregnant women from each group were included in the study. Before the commencement of the study, ethical clearance was obtained from the institutional ethics committee at the JSS Dental College and Hospital, Mysore, India (No. JSS/DC/Ethical/2014-15).

All subjects were recruited from the Department of Gynecology of the JSS Hospital, Mysore, India, during a prenatal visit approx. 1 month prior to their due date. Subjects who provided written informed consent were included in the study. Data on any prediagnosed endocrine disorders, serious pregnancy complications and/or bone disorders was obtained from medical records. If any of these conditions were present, the subject was excluded from the study. All data collection was performed during 1 year (July 2019–June 2020). At the time of recruitment, the mother's age, socioeconomic status (SES), educational level, and medical history, as well as the type of drinking water, were recorded. The socioeconomic status was categorized based on the modified version of the Kuppuswamy classification.[9]

The sample size was calculated based on a power of 80% and a 95% confidence interval (*CI*) according to previous study findings, assuming a mean difference (*MD*) of 40 and a standard deviation (*SD*) of 92 in the ALP levels. The records pertaining to the infant's length, weight and head circumference were assessed by a well-trained staff member using a standardized protocol. The duration of the pregnancy was also recorded.

Fluoride analysis

The study participants were asked to obtain samples of the water they consumed during the course of their pregnancy. A fluoride concentration in drinking water, urine, maternal serum, and cord blood was assessed according to the American Public Health Association (APHA) guidelines, using a 9609BNWP fluoride electrode (Orion™; Thermo Scientific, Mumbai, India). The electrode was calibrated daily before it was used to measure the fluoride concentration. Fasting urine and serum samples before delivery, and cord blood after delivery were used to assess fluoride concentrations.

Laboratory measurements

First, the blood samples collected from the pregnant women for routine investigation before delivery were used to assess the fluoride, ALP and phosphate levels. Then, after delivery, these parameters were assessed in the cord blood samples. The blood samples were collected and immediately placed in tubes, labeled and submitted to the hospital's clinical analysis laboratory, where they were centrifuged and transported under refrigeration. The ALP and phosphate levels were analyzed using a fully automated chemistry analyzer (TBA-120FR™; Toshiba, Tokyo, Japan). All samples were stored at –20°C.

Data management and statistical analysis

All the collected data was entered into a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, USA).

The IBM SPSS Statistics for Windows, v. 23.0 (IBM Corp., Armonk, USA), was used for data analysis. For descriptive data analysis, mean and standard deviation ($M \pm SD$) or frequency and percentage (n (%)) were used. For inferential data analysis, the unpaired two-sample t test was used to compare data with normal distribution, and the non-parametric Mann–Whitney U test was used to compare data with skewed distribution. The normality of data was tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The frequencies and percentages were compared using Pearson's χ^2 test. Associations for both groups were determined using Spearman's correlation test.

To determine the one-to-one association, a simple linear regression model was used. In the model, the drinking water fluoride content was considered an independent variable, and the serum ALP and phosphate levels were entered as dependent variables. After the consideration of covariates, a multivariate linear regression analysis was performed. Covariates that showed significant differences between the low/optimum-fluoride and high-fluoride groups in inferential statistics were considered covariates in the multivariate regression analysis. The covariates which exhibited significant differences were SES and maternal education. The multivariate regression analysis model equation was used to predict the serum ALP and phosphate levels, with an increase by 1 ppm of fluoride in drinking water. A p-value <0.05 was considered statistically significant.

Results

There were 2 groups examined. One group had a low/optimum fluoride concentration in their drinking water (0.50 ±0.28 ppm) and the other had a high fluoride concentration in their drinking water (2.65 ±1.29 ppm). The mean age of the pregnant women in the low/optimum-fluoride group was 23.88 ±3.57 years, while it was 24.13 ±3.85 years in the high-fluoride group. Other baseline characteristics considered were SES, maternal education, the duration of pregnancy, the maternal height, weight and body mass index (BMI), and the birth weight of the newborn. The 2 groups differed significantly in terms of SES and maternal education. Fluoride concentrations in urine, maternal serum and cord blood were 0.200 ±0.240 ppm, 0.014 ±0.014 ppm and 0.011 ±0.011 ppm in the low/optimum-fluoride group, and 1.920 ±1.190 ppm, 0.153 ±0.113 ppm and 0.110 ±0.100 ppm in the high-fluoride group, respectively. The comparison of the values of all parameters between the low/optimum-fluoride and high-fluoride groups showed statistically significant differences ($p < 0.001$) (Table 1).

Table 2 presents the serum ALP and phosphate levels in the 2 groups. Significantly lower levels of ALP were observed in both maternal serum and cord blood in the high-fluoride group. Conversely, the levels of phosphate in maternal serum were significantly higher in the high-fluoride group as compared to the low/optimum-fluoride group.

Table 1. Baseline demographic and clinical characteristics of the study population

Variable		Group		*p*-value
		low/optimum-fluoride $n = 90$	high-fluoride $n = 90$	
Age [years]		23.88 ±3.57	24.13 ±3.85	0.645
SES	class I	13 (14.4)	3 (3.3)	0.000**
	class II	26 (28.9)	18 (20.0)	
	class III	31 (34.4)	23 (25.6)	
	class IV	15 (16.7)	27 (30.0)	
	class V	5 (5.6)	19 (21.1)	
Maternal education	illiterate	4 (4.4)	10 (11.1)	0.000**
	primary (1–4 years)	10 (11.1)	23 (25.6)	
	middle (5–7 years)	16 (17.8)	26 (28.9)	
	high school and PUC	32 (35.6)	23 (25.6)	
	degree and diploma	28 (31.1)	8 (8.9)	
Maternal height [ft]		5.14 ±0.98	5.15 ±0.87	0.763
Maternal weight [kg]		58.26 ±4.26	57.39 ±4.39	0.253
BMI [kg/m^2]		23.87 ±2.79	23.48 ±2.67	0.282
Duration of pregnancy [weeks]		38.31 ±1.13	38.47 ±0.97	0.321
Birth weight of the newborn [kg]		2.69 ±0.57	2.60 ±0.56	0.274
Drinking water fluoride concentration [ppm]		0.500 ±0.280	2.650 ±1.290	0.000**
Urine fluoride concentration [ppm]		0.200 ±0.240	1.920 ±1.190	0.000**
Maternal serum fluoride concentration [ppm]		0.014 ±0.014	0.153 ±0.113	0.000**
Cord blood fluoride concentration [ppm]		0.011 ±0.011	0.110 ±0.100	0.000**

Data presented as mean ± standard deviation ($M \pm SD$) or as frequency (percentage) (n (%)). SES – socioeconomic status; BMI – body mass index; PUC – pre-university course; ** highly statistically significant.

Table 3 shows the strength of the association between the amount of fluoride present in drinking water, urine, maternal serum, and cord blood and the serum ALP and phosphate levels. The ALP levels were negatively associated with fluoride concentrations, while the maternal serum phosphate levels were positively associated with fluoride concentrations (Fig. 1,2).

Table 4 presents the results of the simple linear regression analysis and the selected covariates for the multivariate regression analysis. In the simple linear regression analysis, the serum ALP and phosphate levels were considered dependent variables, and the drinking water fluoride concentration was considered an independent variable. In the multivariate regression analysis, SES and maternal education were considered. The results of both simple and multivariate regression analyses revealed that the fluoride content in drinking water was significantly associated with the ALP level in cord blood and the phosphate level in maternal serum.

Discussion

This study was designed to determine fluoride concentrations in pregnant mothers' drinking water, urine and blood before delivery, and in cord blood after delivery. Another objective of the study was to investigate the associations between the fluoride content and the ALP and phosphate levels in maternal serum right before delivery and in cord blood after delivery. To the best of our knowledge, this is the first study to evaluate the effects of fluoride on the aforementioned parameters at both low/optimum and high levels of fluoride in the drinking water consumed by pregnant women.

This study investigated the association between the urine and serum fluoride levels in pregnant mothers right before delivery and the cord blood fluoride levels after delivery. The findings of the study reveal that as the fluoride concentration in drinking water increased,

Table 2. Comparison of the serum alkaline phosphatase (ALP) and phosphate levels in pregnant women consuming low/optimum-fluoride and high-fluoride drinking water

Variable			Group		*p*-value
			low/optimum-fluoride *n* = 90	high-fluoride *n* = 90	
ALP [IU/L]		maternal serum	326.78 ±135.69	279.72 ±136.89	0.018*
		cord blood	254.14 ±121.95	195.79 ±107.94	0.001**
Phosphate [mg/dL]		maternal serum	3.47 ±2.65	4.47 ±3.43	0.001**
		cord blood	4.49 ±2.98	4.12 ±2.26	0.285
ALP [IU/L]	maternal serum	<37 (deficient)	0 (0.0)	0 (0.0)	0.130
		37–306 (normal)	47 (52.2)	58 (64.4)	
		>306 (excess)	43 (47.8)	32 (35.6)	
	cord blood	<37 (deficient)	0 (0.0)	0 (0.0)	0.009*
		37–306 (normal)	64 (71.1)	79 (87.8)	
		>306 (excess)	26 (28.9)	11 (12.2)	
Phosphate [mg/dL]	maternal serum	<2.8 (deficient)	33 (36.7)	11 (12.2)	0.001**
		2.8–4 (normal)	47 (52.2)	53 (58.9)	
		>4 (excess)	10 (11.1)	26 (28.9)	
	cord blood	<2.8 (deficient)	18 (20.0)	21 (23.3)	0.734
		2.8–4 (normal)	31 (34.4)	33 (36.7)	
		>4 (excess)	41 (45.6)	36 (40.0)	

Data presented as *M* ±*SD* or as *n* (%). * statistically significant; ** highly statistically significant.

Table 3. Spearman's correlation between fluoride concentrations in drinking water, urine, maternal serum, and cord blood and the alkaline phosphatase (ALP) and phosphate levels in maternal serum and cord blood

Correlation	ALP in maternal serum	ALP in cord blood	Phosphate in maternal serum	Phosphate in cord blood
Drinking water fluoride concentration	–0.148 ($p < 0.048$*)	–0.248 ($p < 0.001$**)	0.287 ($p < 0.000$**)	–0.043 ($p < 0.571$)
Urinary fluoride concentration	–0.113 ($p < 0.131$)	–0.207 ($p < 0.005$**)	0.260 ($p < 0.000$**)	–0.042 ($p < 0.574$)
Maternal serum fluoride concentration	–0.187 ($p < 0.012$*)	–0.300 ($p < 0.000$**)	0.236 ($p < 0.001$**)	–0.104 ($p < 0.165$)
Cord blood fluoride concentration	–0.159 ($p < 0.033$*)	–0.313 ($p < 0.000$**)	0.175 ($p < 0.019$*)	–0.148 ($p < 0.470$)

* statistically significant; ** highly statistically significant.

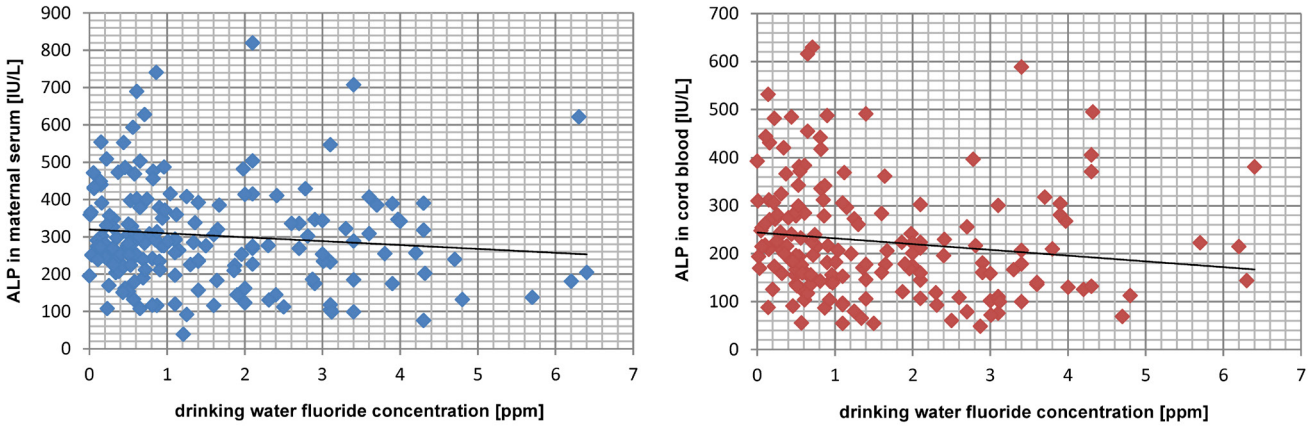

Fig. 1. Correlation between the drinking water fluoride concentration and the alkaline phosphatase (ALP) level in maternal serum and cord blood

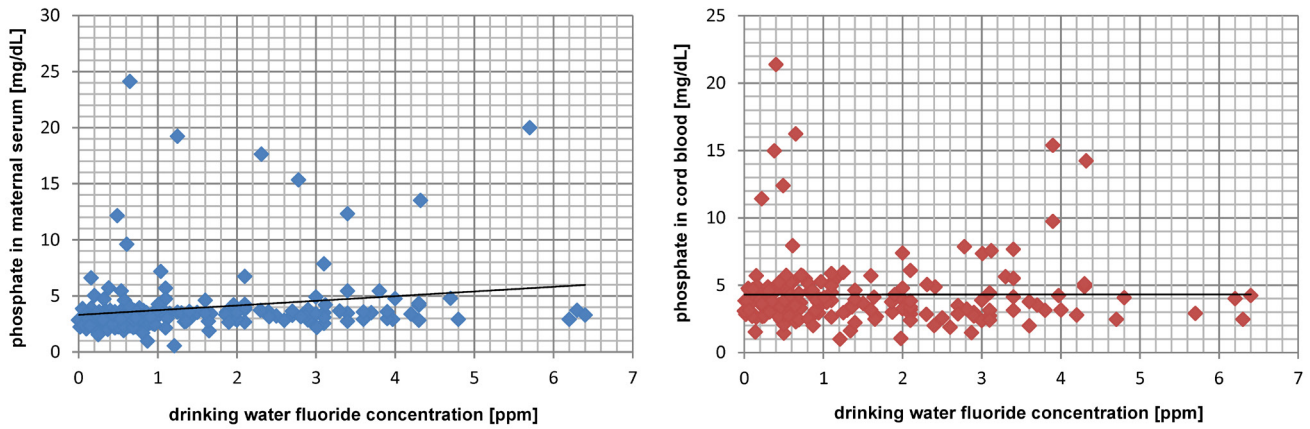

Fig. 2. Correlation between the drinking water fluoride concentration and the phosphate level in maternal serum and cord blood

Table 4. Unadjusted and adjusted associations estimated based on the linear regression models between the drinking water fluoride concentration and the alkaline phosphatase (ALP) and phosphate levels in maternal serum and cord blood

Parameter			Constant	B	*SE*	*p*-value
ALP	maternal serum	†	319.664	–10.403	7.189	0.150
		‡	300.318	–9.620	7.621	0.208
	cord blood	†	243.936	–12.022	6.147	0.050*
		‡	206.358	–13.085	6.521	0.046*
Phosphate	maternal serum	†	3.309	0.417	0.160	0.010**
		‡	2.876	0.394	0.169	0.021*

SE – standard error; † simple linear regression analysis without adjusting any variables (the drinking water fluoride concentration was considered as an independent variable, and the serum ALP and phosphate levels were considered as dependent variables); ‡ multivariate linear regression analysis after adjusting for SES and maternal education; * statistically significant; ** highly statistically significant.

the fluoride concentration in maternal serum and cord blood also increased. According to the obtained results, the role of the placenta in blocking fluoride is very minimal. These results are consistent with previous studies by Ahmed et al.[10] and Opydo-Szymaczek and Borysewicz-Lewicka.[11]

Alkaline phosphatase has been identified as an early and vital indicator for the estimation of bone formation and bone turnover. There is no research pertaining to the effects of high and low/optimum fluoride concentrations in drinking water on the bone marker ALP levels among pregnant women and in cord blood. In the present study, the mean ALP levels among pregnant women were higher than the cord blood ALP levels. This result is in accordance with a study by Verity et al.,[12] but contradictory to Yamaga et al.'s study.[13]

Some previous studies reported a weak inverse correlation between the maternal serum vitamin D3 and ALP levels.[14–16] A few studies found an insignificant association between the vitamin D3 and ALP levels.[17–19]

In the present study, lower ALP levels were noted in the high-fluoride group, which might be due to variations in the vitamin D3 levels. Those consuming high-fluoride drinking water had significantly lower ALP levels in maternal serum and cord blood than the low/optimum-fluoride group. Liu et al. showed that when the concentration of fluoride in drinking water was 0.58–1.59 mg/L, the ALP levels were higher, while fluoride concentrations of 1.60–3.37 mg/L were associated with lower ALP levels.[20] In the present study, the majority of samples that belonged to the high-fluoride group had fluoride concentrations of more than 1.5 mg/L and this group subjects exhibited lower ALP levels. These results are in accordance with the Liu et al.'s study.[20] Liu et al. showed that ALP activity was elevated in the low-fluoride group due to the direct stimulation of F, whereas in the high-fluoride group, fluoride could directly inhibit the enzyme activity or osteoblast activity.[20] Therefore, we consider that the ALP level may be one of the reference indicators for fluoride exposure.

In the present study, significant negative correlations were found between the fluoride content in drinking water, urine, maternal serum, and cord blood and the ALP levels. We found that the higher the fluoride level in maternal serum and cord blood were noted, the more decreased levels of ALP were observed. Further studies could be conducted to evaluate bone activity in pregnant women who consume high-fluoride drinking water.

Both Ca and P are essential inorganic elements for cell growth and proliferation. Phosphorus is important for DNA synthesis and for inducing mitogenesis. Due to higher bone turnover in the fetus, the values of cord blood Ca, P and bone metabolic markers are higher than the maternal serum ones.[21] In this study, the low/optimum-fluoride group had higher phosphate values for cord blood than maternal serum. However, in the high-fluoride group, a reverse result was observed. The results also showed that the high-fluoride group had significantly higher phosphate values for maternal serum than the low/optimum-fluoride group. In cord blood, a reverse result was observed. This observation may be due to a lower secretion of parathyroid hormone (PTH) into maternal blood within the high-fluoride group as compared to the low/optimum-fluoride group.[22] Previous studies showed that the secretion of PTH is directly linked to the P level. The uptake of phosphate from the intestine and bones into blood depends on the secretion of PTH. When a breakdown of bone occurs, more Ca than P is released into the bone. An increase in activated vitamin D mediates the absorption of both Ca and P in the intestine. The end result of the release of PTH is a slight decrease in the serum concentration of phosphate.[23]

Limitations

There are limitations to this study that should be addressed. Firstly, fluoride was assessed only in drinking water and not in other sources. Secondly, the study was conducted in a single center and had a small sample size. Thirdly, the cause-and-effect relationship could not be inferred with certainty, as this was a cross-sectional study.

Conclusions

The findings of this cross-sectional study demonstrated that a high fluoride content in drinking water was associated with the serum ALP and phosphate levels. We recommend multicenter studies with larger populations to establish the cause-and-effect relationship. Our data highlights that in high-fluoride areas, continuous screening of pregnant women should be performed to evaluate the ALP and phosphate levels. The government should take initiatives to create awareness regarding the effects of high fluoride levels in drinking water.

Ethics approval and consent to participate

The study was approved by the institutional ethics committee at the JSS Dental College and Hospital, Mysore, India (No. JSS/DC/Ethical/2014-15). All participants provided written informed consent.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Thippeswamy Honne Manjunathappa https://orcid.org/0000-0003-0358-6521
Devananda Devegowda https://orcid.org/0000-0003-0577-4622
Nanditha Kumar Mysore https://orcid.org/0000-0002-5355-2840
Prashanth Vishwanath https://orcid.org/0000-0003-1582-8057
Prashanth Sathya Narayana https://orcid.org/0000-0002-2520-7310

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Original paper

Relationship between the salivary concentrations of proteinase-3 and interleukin-8 and severe early childhood caries

Mina Biria[1,A,C,E,F], Mandana Sattari[2,A,C,E], Parastoo Iranparvar[1,D–F], Leila Eftekhar[3,B–D]

[1] Department of Pediatric Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Department of Pediatric Dentistry, Faculty of Dentistry, Zanjan University of Medical Sciences, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Leila Eftekhar
E-mail: Leila.eftekhar.a@gmail.com

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Conflict of interest
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Abstract

Background. Severe early childhood caries (S-ECC) is a multifactorial transmissible infectious disease continuing to affect infants and toddlers worldwide. Saliva plays a modulatory role in the pathogenesis of dental caries.

Objectives. The present study aimed to assess the salivary levels of proteinase-3 (PR3) and interleukin-8 (IL-8) as pro-inflammatory cytokines related to the function of neutrophils in association with S-ECC and its treatment.

Material and methods. Fifty children aged 36–60 months were recruited (25 caries-free controls and 25 S-ECC patients). Saliva sampling was performed in all participants. In the S-ECC group, sampling was repeated 6–8 weeks after restorative treatment. The salivary concentrations of PR3 and IL-8 were determined using the enzyme-linked immunosorbent assay (ELISA). The χ^2 test, Fisher's exact test, the independent *t* test, and the paired *t* test were applied at $p < 0.05$.

Results. The baseline salivary concentrations of PR3 and IL-8 in the S-ECC group were significantly higher than in the caries-free group ($p < 0.001$). A significant reduction occurred in the levels of these cytokines following restorative treatment in the S-ECC group ($p < 0.001$), although they were still significantly higher than in the caries-free group ($p < 0.05$).

Conclusions. The salivary levels of PR3 and IL-8 were significantly affected by the presence of dental caries in children, implying their potential efficiency as non-invasive indicators in the determination of the caries risk and treatment effectiveness.

Keywords: cytokines, dental caries, saliva, biomarker, interleukin-8

Introduction

Early childhood caries (ECC) is a major public health problem worldwide, particularly in developing countries.[1] It is defined as the presence of one or more decayed (cavitated or non-cavitated), missed (due to caries) or filled surfaces (dmfs) in any primary tooth of children aged ≤72 months. Severe ECC (S-ECC) in 3–5-year-old children is defined as the presence of one or more decayed, missing (due to caries) or filled smooth surfaces in primary maxillary anterior teeth, or a dmfs score ≥4 at the age of 3 years, ≥5 at the age of 4, or ≥6 at the age of 5.[2] Early childhood caries negatively affects the oral health-related quality of life (OHRQoL) of children and is associated with several complications, such as toothache, dental abscesses, the loss of appetite, malnutrition, and an increased risk of caries in primary and permanent dentition. Moreover, it imposes a high economic burden on the family as well as the public health system.[3,4] Therefore, the determination of its pathogenesis can influence caries preventive strategies.

Saliva plays a significant regulatory role in the pathogenesis of dental caries. The properties of saliva, such as its viscosity, buffering capacity, remineralization potential, antimicrobial activity, and the presence of immunological factors in its composition, affect the development of caries.[5] Saliva sampling is a simple, economical and non-invasive method for oral health examinations, with a minimum risk of sample contamination. The analysis of salivary biomarkers is an appropriate method for the early diagnosis of oral diseases, as well as for the determination of prognosis and treatment success.[6]

The first line of body defense and the immune response to dental caries consists in the activity of neutrophils present in saliva, which control bacterial infections through chemotaxis, opsonization, endothelial cell migration, and phagocytosis.[7] Neutrophils are also considered one of the primary sources of lysozyme, the salivary enzyme responsible for the direct antibacterial action of saliva.[8]

Interleukin-8 (IL-8) is a neutrophil chemotactic factor secreted by various cells, such as macrophages, T cells, fibroblasts, neutrophils, and vascular endothelial cells. It encourages the accumulation of neutrophils and the subsequent increase in the secretion of lysozyme against bacteria.[9]

Neutrophil defensins (human neutrophil peptides (HNPs) 1–4) also play a critical role in the defense mechanisms of saliva and microbial homeostasis. They act by opsonizing bacteria, causing their non-oxidative death and improving phagocytosis.[10] The precursor of HNPs 1–4 is eliminated by proteinase-3 (PR3) during the differentiation and maturation of neutrophils, and active HNPs 1–4 are reserved in the azurophilic granules of neutrophils to be released in the presence of inflammation.[11] Proteinase-3 is a serine protease that is involved in the inflammatory reactions associated with several infectious and non-infectious diseases; however, little is known regarding its role in the process of dental caries.[12]

The association between salivary immunological biomarkers and ECC has been evaluated in some previous studies[2,8]; however, few of them have shown the relationship between S-ECC and the salivary levels of IL-8 and PR3. The confirmation of this relationship by well-designed clinical studies could make the analysis of these salivary cytokines a useful tool for determining the severity of dental caries and the dental pulp status,[13] evaluating the efficacy of caries prevention protocols, and assessing children's susceptibility to dental caries.[2,12,14,15]

The present study aimed to assess the relationship between S-ECC and its treatment and the salivary concentrations of PR3 and IL-8 as pro-inflammatory cytokines related to the function of neutrophils. In addition, the possible risk factors associated with S-ECC were determined.

Material and methods

Participants

The present study was conducted at the Department of Pediatric Dentistry of the Shahid Beheshti University of Medical Sciences, Tehran, Iran, between April 2018 and December 2018. Children aged 36–60 months were recruited using the convenience sampling method and divided into 2 groups: S-ECC patients; and caries-free children who served as controls. The participants were included in the study according to the following criteria: complete physical and mental health; no confounding history of systemic diseases; no consumption of local or systemic drugs during the past 2 months; the absence of any gingival inflammation or periodontal disease; and the absence of any exfoliating primary teeth or erupting permanent teeth at the time of this study. The sample size was calculated based on previous studies to be 25 in each group (α = 5% and a power of 90%)[16]; however, in the S-ECC group, 35 patients were initially recruited to compensate for the possible sample loss during the follow-up period and to improve the validity of the study.

Each participant was enrolled in the study after reading, understanding and completing the written informed consent document by their parent. Furthermore, a questionnaire was completed by the parents in order to assess their educational status and to determine their children's dietary habits.

The research was designed and performed in accordance with the Declaration of Helsinki, and was approved by the institutional committee for ethics in research (IR.SBMU.DRC.REC.1397.036).

Clinical examinations

Caries-free children (i.e., the control group) were selected according to the clinical screening examinations performed at 5 randomly selected kindergartens in Tehran, Iran. For improved visibility, the teeth were dried with sterile gauze and examined under adequate artificial lighting, using disposable explorers and dental mirrors (Asia Dental, Tehran, Iran). In order to ensure the absence of interproximal caries, only children with open tooth contacts were enrolled.

Children affected with S-ECC were selected from among the patients referred to the Department of Pediatric Dentistry of the Shahid Beheshti University of Medical Sciences, following clinical and radiographic examinations. Participants were selected according to the definition of S-ECC by the American Academy of Pediatric Dentistry (AAPD), i.e., the presence of one or more decayed, missing (due to caries) or filled smooth surfaces in primary maxillary anterior teeth, or a dmfs score ≥4 at the age of 3 years, ≥5 at the age of 4, or ≥6 at the age of 5.[2] The dmfs score of each child was recorded. The plaque index (PI) of each patient was also determined using the Greene and Vermillion's simplified oral hygiene index (OHIS), which consists of the simplified debris and calculus indices (DI-S and CI-S).[17,18] The modified version of this index for primary dentition, introduced by Miglani et al., was utilized in this study; it evaluates the buccal surfaces of the second primary molars and central incisors in the upper right and lower left quadrants.[17]

Saliva sampling

In both groups, approx. 1 mL of unstimulated whole resting saliva was collected by passive drooling for 5 min.[19] Saliva samples were all collected between 9 a.m. and 11 a.m. to minimize the effect of the circadian rhythm on the composition of saliva.[2] The children were requested to refrain from eating, drinking, toothbrushing, and using dental floss for 2 h before sampling.[9] The samples were collected into capped, sterile, pre-chilled microtubes, and coded. The microtubes were placed on dry ice to prevent the hydrolysis of salivary proteins, and were immediately transferred to the Laboratory of Immunology. The saliva samples were then centrifuged (Eppendorf® centrifuge, model 5415; Eppendorf, Hamburg, Germany) for 10 min at 6,000 rpm. The supernatant from each sample was carefully transferred to a new microtube by using a sampler and stored at –70°C until further use. Subsequently, the patients in the S-ECC group received all the required restorative treatment, in addition to preventive procedures, such as oral hygiene instruction, nutritional counseling, and professional prophylaxis and fluoride therapy. All procedures were performed by an experienced postgraduate student of pediatric dentistry. Post-treatment saliva sampling was performed 6–8 weeks after the completion of restorative treatment, and the samples were stored under the same conditions as mentioned above.

Investigating the salivary concentrations of PR3 and IL-8

The salivary concentrations of IL-8 and PR3 were determined through the enzyme-linked immunosorbent assay (ELISA). For this purpose, the samples were kept at room temperature to thaw, and the ELISA test was performed according to the instructions provided in the ELISA kits of human IL-8 (Human Interleukin-8 ELISA Kit; MyBioSource, San Diego, USA; item code: MBS772139) and PR3 (Human Proteinase-3 Antibody ELISA Kit; MyBioSource; item code: MBS773125). The plates containing the saliva samples were transferred to the ELISA microplate reader (Anthos 2020; Biochrom Ltd., Waterbeach, UK) for spectrophotometric analysis at a wavelength of 450 nm. All analyses were performed by 2 experienced immunologists who were blinded to the sample groups. Inter-examiner reliability was evaluated using Cohen's kappa coefficient ($\kappa = 0.8$).

The optical density (OD) values were converted to the concentration levels according to the respective standard curve provided by the manufacturer. Then, the salivary levels of IL-8 and PR3 were quantified and reported in picograms per milliliter (pg/mL).

Statistical analysis

Data was analyzed using the IBM SPSS Statistics for Windows software, v. 21.0 (IBM Corp., Armonk, USA). Numerical data was presented as mean and standard deviation ($M \pm SD$). The Kolmogorov–Smirnov test was used to assess the normality of distribution of the salivary cytokine concentrations. The independent t test, the χ^2 test and Fisher's exact test were used to compare the 2 groups. The paired t test was also used to evaluate the treatment effect in the S-ECC group. The potential risk factors related to S-ECC were determined using the multiple logistic regression model. The level of significance was set at 0.05.

Results

A total number of 60 children were initially included in the study – 35 S-ECC-affected and 25 caries-free controls. However, 10 patients in the S-ECC group dropped out of the study because of uncooperative behavior or not being available for post-treatment sampling. Therefore, the data gathered from 25 children in each group was subjected to statistical analysis.

Table 1 presents the patients' background information on age, gender, the dmfs score, PI, maternal education, and the nighttime breast/formula feeding duration and type. The independent t test revealed a significant difference between the 2 groups in terms of PI ($p < 0.001$). Furthermore, the mean values for the patient age were

significantly higher in the S-ECC group than in the control group (p = 0.006). Conversely, there was no significant difference in gender distribution between the 2 groups, as revealed by the χ^2 test (p = 0.569). The results of the χ^2 test also showed that the maternal educational levels were significantly higher in the control group than in the S-ECC group (p = 0.009); however, such a significant difference was not observed among fathers (p = 0.061). The mean duration of nighttime breast/formula feeding in the S-ECC group was approx. 5.6 months longer as compared to the caries-free group, which was considered a significant difference (p = 0.002). However, the type of nighttime feeding (i.e., breast vs. formula) did not differ significantly between the groups (p = 0.479).

Table 2 presents the mean salivary concentrations of IL-8 and PR3 in the 2 groups. Due to the normal distribution of the salivary cytokine levels in each group at both time points shown by the Kolmogorov–Smirnov test ($p > 0.05$), the independent t test and the paired t test were used for further comparisons. The initial mean salivary concentrations of IL-8 and PR3 in the S-ECC group were significantly higher than in the caries-free group ($p < 0.001$). After controlling for the confounding effect of age with the use of the analysis of covariance (ANCOVA), the 2 groups still showed significant differences regarding the salivary levels of IL-8 and PR3 ($p < 0.001$).

As shown in Table 3, in the S-ECC group, a significant reduction occurred in the cytokine levels following restorative treatment as compared to the baseline values ($p < 0.001$), although the levels were still significantly higher than in the caries-free group (p = 0.030 for IL-8, and p = 0.002 for PR3).

The determination of the possible risk factors associated with S-ECC with the use of multiple logistic regression models revealed that PI (p = 0.018) and the duration of nighttime breast/formula feeding (p = 0.021) had a significant influence on the development of S-ECC. The odds ratio (*OD*) for the duration of nighttime feeding was calculated to be 1.3, meaning that each further month increased the incidence of S-ECC by 30% (Table 4).

Table 3. Pre- and post-treatment concentrations of interleukin-8 (IL-8) and protease-3 (PR3) [pg/mL] in the severe early childhood caries (S-ECC) group

Cytokine concentration [pg/mL]	Time point	*M* ±*SD*	*SE*	*p*-value
IL-8	pre-treatment	35.84 ±4.39	0.87	<0.001*
	post-treatment	24.68 ±4.92	0.98	
PR3	pre-treatment	242.48 ±54.33	10.86	<0.001*
	post-treatment	184.72 ±37.86	7.57	

* statistically significant.

Table 4. Possible risk factors for severe early childhood caries (S-ECC)

Risk factors	Wald χ^2 statistic	*p*-value	*OR*
Age	3.495	0.062	1.17
Gender	0.702	0.402	0.44
PI	5.611	0.018*	32.72
Maternal educational level	0.701	0.704	–
Paternal educational level	0.013	0.994	–
Nighttime feeding duration	5.337	0.021*	1.26
Nighttime feeding type (breast/formula)	0.900	0.956	–

OR – odds ratio; * statistically significant.

Table 1. Patients' background information

Group	Age [months] *M* ±*SD*	Gender *n* (%)		dmfs score *M* ±*SD*	PI *M* ±*SD*	Maternal educational level *n* (%)			Nighttime feeding duration [months] *M* ±*SD*	Nighttime feeding type *n* (%)		
		boys	girls			high school diploma	bachelor's degree	master's degree or higher		breast	formula	both
S-ECC (*n* = 25)	53.9 ±7.2	13 (52)	12 (48)	11.8 ±3.2	1.44 ±0.46	14 (56)	5 (20)	6 (24)	18.1 ±6.9	15 (60)	2 (8)	8 (32)
Caries-free (*n* = 25)	40.5 ±6.3	15 (60)	10 (40)	0	0.93 ±0.34	4 (16)	12 (48)	9 (36)	12.5 ±4.6	18 (72)	0	7 (28)
p-value	0.006*	0.569		<0.05*	<0.001*	0.009*			0.002*	0.475		

M – mean; *SD* – standard deviation; dmfs – decayed, missed or filled surfaces in primary dentition; PI – plaque index; S-ECC – severe early childhood caries; * statistically significant.

Table 2. Initial concentrations of interleukin-8 (IL-8) and protease-3 (PR3) [pg/mL] in the study groups

Cytokine concentration [pg/mL]	Group	*M* ±*SD*	*SE*	*p*-value	F (ANCOVA)	*p*-value (ANCOVA)
IL-8	S-ECC (*n* = 25)	35.84 ±4.39	0.87	<0.001*	122.347	<0.001*
	caries-free (*n* = 25)	20.00 ±3.88	0.77			
PR3	S-ECC (*n* = 25)	242.48 ±54.33	10.86	<0.001*	122.347	<0.001*
	caries-free (*n* = 25)	158.73 ±32.92	6.58			

SE – standard error; * statistically significant.

Discussion

Saliva plays an essential role in the development and progression of dental caries owing to its continuous and direct contact with the teeth, as well as the presence of various immune-related factors.[20] The analysis of salivary cytokines is considered an appropriate, non-invasive method for monitoring oral conditions.[9] When performed before and after the treatment of dental caries, the cytokine levels can be regarded as a suitable caries assessment tool and prognostic biomarkers.[2]

The present study aimed to assess the salivary concentrations of PR3 and IL-8 as pro-inflammatory cytokines related to the neutrophil function in caries-free and S-ECC-affected children, and revealed significantly higher salivary levels of IL-8 and PR3 in the S-ECC group in comparison with the control group. Restoration treatment in the S-ECC group resulted in a significant decrease in the salivary concentrations of these cytokines, although their levels were still higher as compared to the caries-free group, indicating the continuation of inflammatory stimulation.

Few clinical studies have investigated the relationship between IL-8 and dental caries. Gornowicz et al. reported significantly higher levels of pro-inflammatory cytokines, i.a., IL-8, in adolescents having dental caries as compared to caries-free ones, confirming the role of IL-8 as an essential chemokine in neutrophil chemotaxis.[9] Zhao et al. similarly showed a significantly higher concentration of IL-8 in patients having active carious lesions.[19] In another case–control study by Sharma et al., the salivary level of IL-8 was shown to be significantly higher in ECC-affected children as compared to caries-free controls.[2] Although a significant reduction in the IL-8 levels occurred following dental restorations, the concentrations were still significantly higher than the corresponding values in the caries-free group.[2] The results of these investigations are consistent with those of the present study.

On the other hand, Seyedmajidi et al. found no significant differences in the salivary levels of IL-8 among caries-free, ECC- and S-ECC-affected children.[8] The controversy between their results and the present study could be attributed to the differences in the age or mean dmfs score of the patients enrolled, and the utilization of non-parametric tests for statistical analysis as opposed to the less conservative parametric tests used in the present study.

The reduction in the levels of IL-8 pro-inflammatory mediator following a decrease in the microbial load may be due to the fact that cariogenic microorganisms are mainly Gram-positive, and their products, such as lipoteichoic acid (LTA), which is abundantly found in cariogenic streptococci, stimulate toll-like receptor 2 (TLR2) and nucleotide-binding oligomerization domain (NOD) proteins, leading to the considerable production of IL-8.[21,22] It is worth noting that due to the incapability of the complement system to eliminate Gram-positive bacteria because of their thick cell walls, the involvement of neutrophils as the primary phagocytes of the immune system is imperative.

Limited studies have been conducted regarding the relationship between the salivary levels of PR3 and dental caries. Yang et al. reported lower levels of PR3 in patients having dental caries as compared to the caries-free group, and found an inverse relationship between the salivary concentration of PR3 and the severity of caries,[12] which is in contrast to our results. This difference can be justified by a lower mean dmfs score of patients in their study (i.e., 6.3 vs. 11.8 in the present study). One of the main tasks of PR3 is the production and activation of antimicrobial peptides.[23] Thus, as long as dental caries is present as a source of infectious bacteria, higher salivary levels and activity of PR3 are expected. Similar findings were reported in previous studies regarding periodontitis.[24,25] Since PR3 serves as a regulator of the immune system and inflammatory reactions, its increased level in the S-ECC group can be considered a mechanism of host immunity to confront bacteria and prevent uncontrolled inflammatory responses.

To the best of our knowledge, the effect of treating carious lesions on the salivary levels of PR3 has not been investigated in any previous studies. Considering the post-treatment reduction in the salivary levels of IL-8 as a neutrophil chemotactic factor in the present study, the subsequent reduction in the PR3 levels can be attributed to decreased migration of neutrophils, as the primary source of PR3, to the oral cavity. Furthermore, the post-treatment care employed in the present study, including oral hygiene instruction, nutritional counseling, and professional prophylaxis and fluoride therapy, may be responsible for further reduction of the IL-8 and PR3 levels in the S-ECC group following restorative treatment. In the present study, the time interval between pre- and post-treatment sampling was decided to be 6–8 weeks. This decision was made according to a recommendation by Sharma et al. for using longer periods in future studies, since the concentrations of salivary markers are affected by this time interval, and elevated cytokine levels are expected with more extended duration.[2]

Considering the limitations of the present study and the small number of clinical studies evaluating the relationship between IL-8 and PR3 and dental caries, further studies with larger sample sizes and longer follow-up duration are required to obtain more definitive conclusions regarding this issue.

Conclusions

The salivary levels of IL-8 and PR3 were significantly affected by the presence of dental caries in children. Therefore, the analysis of these salivary cytokines can be considered a suitable, non-invasive method for the determination of the caries risk and treatment effectiveness.

Ethics approval and consent to participate

The research was designed and performed in accordance with the Declaration of Helsinki, and was approved by the institutional committee for ethics in research (IR.SBMU.DRC.REC.1397.036). Each participant was enrolled in the study after reading, understanding and completing the written informed consent document by their parent.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Mina Biria https://orcid.org/0000-0002-0126-8020
Mandana Sattari https://orcid.org/0000-0002-5852-9615
Parastoo Iranparvar https://orcid.org/0000-0001-7071-2124
Leila Eftekhar https://orcid.org/0000-0001-6285-1511

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Original paper

Efficacy of root coverage with the use of the conventional versus laser-assisted flap technique with platelet-rich fibrin in class I and class II gingival recession: A randomized clinical trial

Manisha Bommala[A–F], Rekha Rani Koduganti[A–F], Veerendranath Reddy Panthula[A–F], Surya Prasanna Jammula[A–F], Himabindu Gireddy[A–F], Manasa Ambati[A–F], Bharathchandra Ganachari[A–F]

Department of Periodontics, Panineeya Institute of Dental Sciences & Research Centre, Hyderabad, India

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Address for correspondence
Rekha Rani Koduganti
E-mail: rekhasarat2304@gmail.com

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Abstract

Background. The coronally advanced flap (CAF) technique is the preferred option in treating recession defects, especially when the attached gingiva is adequate. A laser-assisted vestibular releasing incision after surgery could enhance the outcome. Platelets, when used as adjunctive treatment, have shown good results. However, laser biostimulation post-surgery has not been studied.

Objectives. The present study compared the benefits of using the conventional and laser-assisted flap technique with platelet-rich fibrin (PRF) in the treatment of class I and class II gingival recession.

Material and methods. The study included 24 subjects, both males and females. The participants, diagnosed with Miller's class I and II gingival recession, were categorized into 2 groups: group A ($n = 12$) treated with CAF and PRF; and group B ($n = 12$) treated with laser-assisted CAF and PRF. Root coverage (RC), the probing depth (PD), the clinical attachment loss (CAL), and the keratinized tissue width (KTW) were assessed pre- and 6 months postoperatively. The wound healing index (WHI) and the visual analog scale (VAS) scores were assessed 1 week post-surgery.

Results. Most clinical parameters improved significantly within the groups at 6 months postoperatively as compared to baseline ($p < 0.05$), except for PD and percentage root coverage (PRC). However, when intergroup comparisons were made, it was observed that both groups performed equally well and the differences between them were not significant.

Conclusions. Both treatment modalities improved the clinical parameters post-surgery. However, further trials are warranted to affirm the benefits of the laser-assisted CAF technique.

Keywords: platelet-rich fibrin, gingival recession, coronally advanced flap, laser biostimulation

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Introduction

Gingival recession is a highly prevalent problem among adults. It can cause esthetic impairment, dentin hypersensitivity, root abrasion, and root caries.[1–3] The key reason for this condition is inflammation within the marginal gingival connective tissue; others include traumatic toothbrushing, the frenal pull and the malposition of the teeth.[4] It is always important to correct the malposition of the teeth orthodontically before attempting recession coverage; otherwise, the treatment will not be successful. Many classifications are available to grade gingival recession, and it has been observed that the prognosis for grade 1 and grade 2 gingival recession is good. Free autogenous grafts and pedicle grafts, such as coronally advanced flaps (CAFs) and semilunar flaps, have been employed to treat recession. Among these techniques, CAF is the commonly used procedure to reposition the gingiva in the coronal direction, providing good clinical outcomes.[5–7] Acellular dermal matrices have also been used for root coverage (RC) with good results.[8] Various platelet concentrates have been used in dentistry, including pure platelet-rich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP) and platelet-rich fibrin (PRF), which in turn comprises pure platelet-rich fibrin (P-PRF), leukocyte- and platelet-rich fibrin (L-PRF) and injectable platelet-rich fibrin (I-PRF).[9]

Platelet-rich fibrin plays a vital role in the regeneration of the lost bone and soft tissues. This biomimetic agent has better healing properties as compared to other platelet concentrates, which justifies its use in mucogingival procedures and the implant therapy.[10,11] A laser-assisted releasing incision in the vestibule after surgery aids in decreasing tension on the flap and promotes better healing of the surgical site. Mucogingival procedures are very technique-sensitive, and patients often experience pain after surgery. The low-level laser therapy (LLLT) alleviates gingival inflammation, decreases pain and promotes wound healing.[12] Hence, the present study compared the efficacy of conventional and laser-assisted CAF with PRF in RC.

Material and methods

Preliminary plan and ethics statement

The study was designed as a randomized, parallel-arm clinical trial comparing the efficacy of RC with the use of the conventional vs. laser-assisted flap technique with PRF in the treatment of class I and class II gingival recession. It included 24 patients and was conducted at the outpatient ward of a tertiary referral care center in Hyderabad, India. The study was carried out from May 2019 to October 2020. It complied with the ethical standards established by the World Medical Association (WMA) in the Declaration of Helsinki, and was approved by the institutional ethics committee at the Panineeya Institute of Dental Sciences & Research Centre, Hyderabad, India (PMVIDS&RC/IEC/PERIO/DN/0218-2018). All participants were given a detailed verbal and written description of the study, and a signed consent form was obtained from each of them. The flowchart of the study design is presented in Fig. 1.

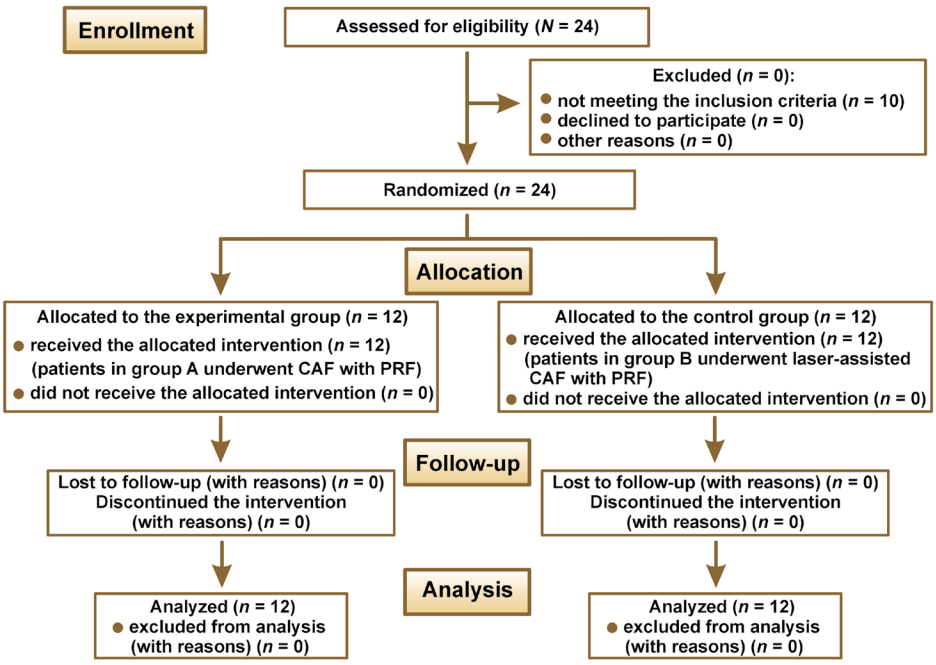

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) 2010 flowchart of the study
CAF – coronally advanced flap; PRF – platelet-rich fibrin.

Inclusion and exclusion criteria

Patients with Miller's class I and II recession defects on the anterior teeth, with the probing depth (PD) >3 mm, the clinical attachment loss (CAL) >5 mm and the keratinized tissue width (KTW) >2 mm, were included in the study. Pregnant and lactating women, smokers, systemically compromised patients, subjects who had undergone the periodontal therapy in the last 6 months, and those who were on antibiotics 3 months prior to commencing the study (confirmed while recording the case history) were excluded from the study.

Sample size calculation

As per a statistician's suggestion, to obtain a difference in complete root coverage (CRC) between the groups with a power of 80% and a 95% confidence interval (*CI*), 12 patients had to be included in each group. The primary outcome variables assessed were the recession depth (RD), the gingival thickness (GT) and percentage root coverage (PRC), whereas PD, CAL, KTW, the visual analog scale (VAS) score, and the wound healing index (WHI) were the secondary outcomes measured.

Estimation of the clinical parameters

The recession depth, GT, PD, CAL, and KTW were assessed using a Williams probe at baseline (D_0), 3 months (D_3) and 6 months (D_6) post-surgery, whereas PRC was assessed at 1 month (D_1) and 6 months(D_6) post-surgery. The VAS scores and WHI were estimated 1 week after surgery.

Randomization

One investigator (RRK) assigned the cases by randomly picking them out from the sealed envelopes, and the other investigator (MB) performed the surgeries for both groups. Both the patients and the statistician were blinded to the assignment.

Groups

Group A comprised 12 patients who underwent surgery to treat their denuded roots by CAF and PRF application (Fig. 2).

Fig. 2. Recession depth (RD) measurement at baseline in group A

Group B comprised 12 patients who underwent laser-assisted RC by CAF and PRF application (Fig. 3).

Fig. 3. Recession depth (RD) measurement at baseline in group B

Presurgical procedure

The patients initially received a comprehensive periodontal examination and a complete plaque control program, including oral hygiene, to eliminate the habits related to the etiology of recession. Scaling, root planing and occlusal adjustments were performed 1 month before the surgical protocol was implemented.

Surgical procedure

The patient was seated comfortably on a dental chair, and then asked to rinse their mouth with a 1:1 ratio of 0.2% chlorhexidine digluconate solution. The operative site was anesthetized with 2% lignocaine hydrochloride with adrenaline (1:80,000), using the block and infiltration techniques.

Preparation of the platelet-rich fibrin membrane

Platelet-rich fibri was harvested from a simple blood sample (2 mL) drawn from the patient's antecubital vein at the time of the surgical procedure. It was then treated with single centrifugation at 2,700 rpm for 12 min. After the centrifugation procedure, 3 distinct layers were formed, of which the intermediate layer was that of a dense PRF clot. The fibrin clot was easily separated from the red blood cell (RBC) base by using sterile tweezers and scissors, with the preservation of the 2 other RBC layers. This dense PRF clot was used as a membrane.

Group A

A full-thickness submarginal trapezoidal flap was raised with the use of blade No. 15 on the labial aspect of the tooth being treated, through an intrasulcular incision extending horizontally to dissect the labial aspect of the adjacent papilla. Two vertical incisions were made – one at the distal gingival line angle, and the other at the mesial line angle of the subject's affected tooth. The submarginal horizontal incisions connected with the vertical incisions were extended up to the mucogingival junction (MGJ) to provide the proper displacement of the flap. The flap

was raised through sharp dissection. The papillae were de-epithelialized. Before placing the pedicle flap on the denuded root, thorough root planing was performed using curettes, and the prepared PRF membrane was placed and sutured using 4–0 resorbable sutures. The pedicle flap was then sutured 1 mm coronal to the cementoenamel junction (CEJ) of the affected tooth, using 4–0 resorbable sutures. A COE-PACK™ dressing was applied (Fig. 4).

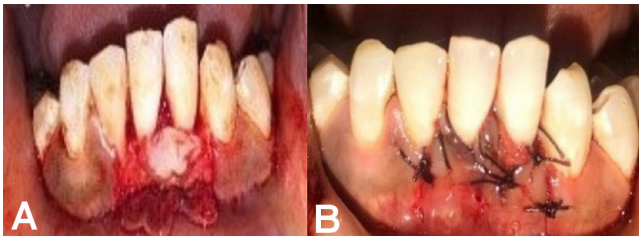

Fig. 4. Surgical procedure in group A
A – placement of PRF; B – suturing of CAF.

Group B

A similar surgical protocol was followed in group B, but an additional vestibular releasing incision was made using an optical-fiber diode laser at a power of 3 W in the continuous mode (Fig. 5). This procedure helped prevent muscle reattachments that could hinder the outcome of the treatment. Photobiostimulation was performed at the end of the RC procedure and on day 7 with a 980 nm diode laser at a power of 1 W in the continuous mode for 60 s (Fig. 6).

Postoperative protocol

The patients were advised to take an analgesic (aceclofenac 100 mg, 3 times a day for 5 days) and an antibiotic (amoxicillin 500 mg, 3 times a day for 5 days) post-surgery, and instructed to refrain from toothbrushing and flossing until the removal of the sutures. They were also instructed to rinse their mouth with chlorhexidine mouthwash (0.12%) twice daily for a period of 1 month. The sutures were removed 7 days post-surgery (Fig. 7 and 8).

Each patient was reinstructed about proper oral hygiene measures, and was recalled after 1 week and thereafter monthly until the end of the 6th month. Scaling and oral hygiene reinforcement were provided at each follow-up visit whenever indicated until the 6th month.

Fig. 6. Laser biostimulation 1 week post-op in group B

Fig. 7. Post-op follow-up after 6 months in group A

Fig. 8. Post-op follow-up after 6 months in group B

Fig. 5. Surgical procedure in group B
A – placement of PRF; B – suturing of CAF; C – laser vestibular releasing incision.

Statistical analysis

The data was analyzed using the Microsoft Excel and GraphPad Prism software. the continuous data was summarized as mean and standard deviation (*M* ±*SD*). The intragroup comparisons were performed using the repeated one-way analysis of variance (ANOVA) for continuous data, followed by Bonferroni's multiple comparison test. The intergroup comparisons were performed using the repeated two-way ANOVA for continuous data. All *p*-values less than 0.05 were considered statistically significant.

Results

Group A

The mean RD at D_0, D_3 and D_6 was 2.54 mm, 1.96 mm and 1.70 mm, respectively. It was observed that the RD values decreased significantly at D_6 as compared to D_0 ($p < 0.001$). The mean CAL at D_0, D_3 and D_6 was 4.19 mm, 3.49 mm and 3.19 mm, respectively, and the values also decreased significantly between D_0 and D_6 ($p < 0.001$). The mean GT at D_0, D_3 and D_6 was 1.43 mm, 1.53 mm and 1.57 mm, respectively, and the GT values increased significantly from D_0 to D_3 and from D_3 to D_6 ($p < 0.05$). The mean KTW at D_0, D_3 and D_6 was 1.97 mm, 2.65 mm and 2.93 mm, respectively. There was a significant increase in the KTW values from D_0 to D_6 ($p < 0.05$). The mean PD at D_0, D_3 and D_6 was 1.65 mm, 1.43 mm and 1.47 mm, respectively. There was no significant change from D_0 to D_6 for the PD values ($p = 0.127$). The mean PRC at D_3 and D_6 was 22.63% and 34.00%, respectively. There was no significant change in the PRC values between D_3 and D_6. Therefore, there was improvement at different time points for the parameters RD, GT, CAL, and KTW, but the PD and PRC values did not improve (Table 1).

Group B

The mean RD at D_0, D_3 and D_6 was 2.30 mm, 1.91 mm and 1.63, respectively. There was a significant decrease at D_6 as compared to D_0 ($p < 0.05$). The mean GT at D_0, D_3 and D_6 was 1.42 mm, 1.50 mm and 1.65 mm, respectively. There was significant improvement in GT from D_0 to D_6 ($p < 0.05$). The mean PD at D_0, D_3 and D_6 was 1.96 mm, 1.60 mm and 1.69, respectively. There was a significant change in the PD values from D_0 to D_6 ($p < 0.05$). The mean KTW at D_0, D_3 and D_6 was 2.06 mm, 2.66 mm and 2.85 mm, respectively. There was a significant increase in the KTW values from D_0 to D_6 ($p < 0.05$). The mean CAL at D_0, D_3 and D_6 was 4.30 mm, 3.45 mm and 3.26 mm, respectively. There was a significant decrease in CAL from D_0 to D_6 ($p < 0.05$). The mean PRC at D_3 and D_6 was 30.69% and 39.37%, respectively. There was no significant change in the PRC values between D_3 and D_6. Therefore, there was improvement at different time points for the parameters RD, PD, GT, CAL, and KTW, but the PRC values did not improve (Table 2).

Table 1. Intragroup comparison of the clinical parameters at different time points in group A (repeated one-way ANOVA and Bonferroni's test)

Parameter	Time point	*n*	minimum	maximum	*M* ±*SD*	*p*-value
RD [mm]	D_0	12	1.50	3.17	2.54 ±0.62	0.0003**
	D_3	12	0.00	3.00	1.96 ±0.83	
	D_6	12	0.00	3.00	1.70 ±0.80	
GT [mm]	D_0	12	1.00	2.00	1.43 ±0.42	0.0270*
	D_3	12	1.00	2.00	1.53 ±0.46	
	D_6	12	1.00	2.00	1.57 ±0.43	
PD [mm]	D_0	12	1.00	2.33	1.65 ±0.42	0.1270
	D_3	12	1.00	2.33	1.43 ±0.47	
	D_6	12	1.00	2.33	1.47 ±0.50	
CAL [mm]	D_0	12	2.83	5.00	4.19 ±0.73	0.0004**
	D_3	12	2.00	5.00	3.49 ±0.96	
	D_6	12	2.00	5.00	3.19 ±0.89	
KTW [mm]	D_0	12	0.00	3.67	1.97 ±0.98	0.0060*
	D_3	12	1.00	5.00	2.65 ±1.01	
	D_6	12	1.67	4.00	2.93 ±0.77	
PRC [%]	D_3	12	0.00	100.00	22.63 ±27.61	0.1310
	D_6	12	−5.57	100.00	34.00 ±32.38	

M – mean; *SD* – standard deviation; RD – recession depth; GT – gingival thickness; PD – probing depth; CAL – clinical attachment loss; KTW – keratinized tissue width; PRC – percentage root coverage; time points: D_0 – at baseline; D_3 – at 3 months post-op; D_6 – at 6 months post-op; * statistically significant ($p < 0.05$); ** highly statistically significant ($p < 0.001$).

Table 2. Intragroup comparison of the clinical parameters at different time points in group B (repeated one-way ANOVA and Bonferroni's test)

Parameter	Time point	*n*	minimum	maximum	*M* ±*SD*	*p*-value
RD [mm]	D_0	12	1.00	5.00	2.30 ±1.16	0.0020*
	D_3	12	0.17	5.00	1.91 ±1.38	
	D_6	12	0.00	5.00	1.63 ±1.41	
GT [mm]	D_0	12	0.75	2.00	1.42 ±0.41	0.0090*
	D_3	12	1.00	2.00	1.50 ±0.40	
	D_6	12	1.25	2.00	1.65 ±0.28	
PD [mm]	D_0	12	1.00	3.00	1.96 ±0.45	0.0190*
	D_3	12	1.00	3.00	1.60 ±0.62	
	D_6	12	1.00	3.00	1.69 ±0.63	
CAL [mm]	D_0	12	2.33	7.00	4.30 ±1.31	0.0004**
	D_3	12	1.17	7.00	3.45 ±1.77	
	D_6	12	1.00	7.00	3.26 ±1.75	
KTW [mm]	D_0	12	0.00	3.67	2.06 ±1.14	0.0020*
	D_3	12	0.00	5.00	2.66 ±1.60	
	D_6	12	0.00	4.67	2.85 ±1.47	
PRC [%]	D_3	12	−12.50	87.50	30.69 ±33.88	0.1450
	D_6	12	0.00	100.00	39.37 ±37.40	

* statistically significant ($p < 0.05$).

Intergroup comparison of RD, GT and PD in groups A and B

The mean RD in group A at D_0, D_3 and D_6 was 2.54 mm, 1.96 mm and 1.70 mm, respectively, and in group B, it was 2.30 mm, 1.91 mm and 1.63 mm, respectively. Upon intergroup comparison, the differences in results were not significant. The mean GT in group A at D_0, D_3 and D_6 was 1.43 mm, 1.53 mm and 1.57 mm, respectively, and in group B, it was 1.42 mm, 1.50 mm and 1.65 mm, respectively, showing no significant differences upon intergroup comparison The mean PD in group A at D_0, D_3 and D_6 was 1.65 mm, 1.43 mm and 1.47, respectively, and in group B, it was 1.96 mm, 1.60 mm and 1.69 mm, respectively, showing no significant differences between the groups (Table 3).

Intergroup comparison of CAL, KTW and PRC in groups A and B

The mean CAL in group A at D_0, D_3 and D_6 was 4.19 mm, 3.49 mm and 3.19 mm, respectively, and in group B, it was 4.30 mm, 3.45 mm and 3.26, respectively, with no significant differences between the groups. The mean KTW in group A at D_0, D_3 and D_6 was 1.97 mm, 2.65 mm and 2.93 mm, respectively, and in group B, it was 2.06 mm, 2.66 mm and 2.85 mm, respectively, showing no significant differences. The mean PRC in group A and group B at D_3 was 22.63% and 30.69%, respectively, and at D_6, it was 34.00 and 39.37%, respectively. Thus, the CAL, KTW and PRC values also did not show statistically significant differences upon intergroup comparison (Table 4).

Intergroup comparison of the VAS scores and WHI in groups A and B

The mean VAS score at 1 week for group A was 2.29, and for group B, it was 2.17. The mean WHI score at 1 week for group A was 3.13, and for group B, it was 3.42. There were no significant differences between the groups for either of these parameters (Table 5).

Discussion

Gingival recession is a very common problem associated with esthetic and functional impairment. Thus, the achievement of CRC is the goal of every clinician.[13]

The CAF technique as a treatment option was introduced by researchers nearly a century ago. Thereafter, other modalities of RC were implemented. The proper case selection is vital for obtaining a successful outcome. The parameters to be assessed include RD, the recession width (RW), the vestibular depth, KTW, the width and height of the interdental soft tissue, and the insertion of the frenulum.[14,15]

The present study was conducted to evaluate the efficacy of the conventional CAF with PRF vs. the laser-assisted CAF with PRF in the treatment of class I and class II gingival recessions. Laser biomodulation was implemented after the laser-assisted CAF; this study is among the few studies that have examined RC, employing this principle.

The CAF technique as a treatment option has yielded very good results pertaining to PRC, as well as color matching to the adjacent tissues.[16] Moreover, it has been

Table 3. Intergroup comparison of the recession depth (RD), the gingival thickness (GT) and the probing depth (PD) at different time points (repeated two-way ANOVA)

Parameter	Group	Time point	*n*	minimum	maximum	*M* ±*SD*	*p*-value
RD [mm]	conventional	D_0	12	1.50	3.17	2.54 ±0.62	0.539
	laser		12	1.00	5.00	2.30 ±1.16	
	conventional	D_3	12	0.00	3.00	1.96 ±0.83	0.918
	laser		12	0.17	5.00	1.91 ±1.38	
	conventional	D_6	12	0.00	3.00	1.70 ±0.80	0.882
	laser		12	0.00	5.00	1.63 ±1.41	
GT [mm]	conventional	D_0	12	1.00	2.00	1.43 ±0.42	0.958
	laser		12	0.75	2.00	1.42 ±0.41	
	conventional	D_3	12	1.00	2.00	1.53 ±0.46	0.862
	laser		12	1.00	2.00	1.50 ±0.40	
	conventional	D_6	12	1.00	2.00	1.57 ±0.43	0.607
	laser		12	1.25	2.00	1.65 ±0.28	
PD [mm]	conventional	D_0	12	1.00	2.33	1.65 ±0.42	0.100
	laser		12	1.00	3.00	1.96 ±0.45	
	conventional	D_3	12	1.00	2.33	1.43 ±0.47	0.444
	laser		12	1.00	3.00	1.60 ±0.62	
	conventional	D_6	12	1.00	2.33	1.47 ±0.50	0.362
	laser		12	1.00	3.00	1.69 ±0.63	

Table 4. Intergroup comparison of the clinical attachment loss (CAL), the keratinized tissue width (KTW) and percentage root coverage (PRC) at different time points (repeated two-way ANOVA)

Parameter	Group	Time point	*n*	minimum	maximum	*M* ±*SD*	*p*-value
CAL [mm]	conventional	D_0	12	2.83	5.00	4.19 ±0.73	0.798
	laser		12	2.33	7.00	4.30 ±1.31	
	conventional	D_3	12	2.00	5.00	3.49 ±0.96	0.954
	laser		12	1.17	7.00	3.45 ±1.77	
	conventional	D_6	12	2.00	5.00	3.19 ±0.89	0.906
	laser		12	1.00	7.00	3.26 ±1.75	
KTW [mm]	conventional	D_0	12	0.00	3.67	1.97 ±0.98	0.849
	laser		12	0.00	3.67	2.06 ±1.14	
	conventional	D_3	12	1.00	5.00	2.65 ±1.01	0.989
	laser		12	0.00	5.00	2.66 ±1.60	
	conventional	D_6	12	1.67	4.00	2.93 ±0.77	0.722
	laser		12	0.00	4.67	2.85 ±1.47	
PRC [%]	conventional	D_3	12	0.00	100.0	22.63 ±27.61	0.530
	laser		12	−12.50	87.50	30.69 ±33.88	
	conventional	D_6	12	−5.57	100.00	34.00 ±32.38	0.711
	laser		12	0.00	100.00	39.37 ±37.40	

Table 5. Intergroup comparison of the visual analog scale (VAS) and wound healing index (WHI) scores at 1 week (D_1) post-op (repeated two-way ANOVA)

Parameter	Group	Time point	*n*	minimum	maximum	*M* ±*SD*	*p*-value
VAS	conventional	D_1	12	1.00	3.00	2.29 ±0.69	0.715
	laser		12	1.00	4.00	2.17 ±1.03	
WHI	conventional	D_1	12	2.00	4.00	3.13 ±0.61	0.362
	laser		12	2.00	5.00	3.42 ±0.90	

proven to be efficacious in the treatment of multiple gingival recessions, with good esthetic results.[17]

Platelet-rich fibrin helps increase the efficacy of CAF, as PRF is rich in growth factors that are released within 7–28 days. The membrane acts as a barrier that prevents the ingress of gingival epithelial cells into the defect. Furthermore, it plays a direct role in increasing angiogenesis and modulating tissue healing, and helps ward off inflammation.[18,19]

Pioneers in research related to PRF conducted a biological assay.[20] They performed a comparative study to evaluate the roles of transforming growth factor beta 1 (TGFβ-1), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF-BB) in the platelet-poor plasma (PPP) supernatant, and the PRF clot exudate serum. The study revealed that PRF slowly polymerized fibrin, leading to the incorporation of glycanic chains and platelet concentrates in the fibrin mesh. Hence, PRF has the added advantage of continuous slow release of cytokines during tissue remodeling, thus accelerating its wound healing properties.[20]

Eren and Atilla conducted a split-mouth trial wherein 22 patients with class I or class II gingival recession participated.[21] Forty-four defects were evaluated after receiving either CAF with PRF (test group: 22 defects) or CAF with the subepithelial connective tissue graft (SCTG) (control group: 22 defects). All the clinical parameters pertaining to RC were assessed preoperatively and 6 months postoperatively. The RD, RW, recession area (RA), and KTW were calculated on standardized photographs, using digital image analysis software. The results indicated that both groups performed equally well, with no significant differences between them, prompting the use of PRF as an alternative to SCTG in localized gingival recessions.[21]

Another clinical study was conducted to ascertain the potential benefits of using PRF with the modified CAF for the treatment of gingival recession.[22] For this split-mouth research, 12 patients with Miller's class I and class II gingival recession in 2 non-adjacent anterior teeth were chosen. One tooth with gingival recession was subjected to modified CAF, while the other was treated with CAF with PRF. The changes in parameters (RD, RW, GT, and CAL) from 1 month to 3 months and 6 months within the groups were statistically non-significant. Upon intergroup comparison, only the change in GT was found to be statistically significant in the CAF + PRF group.[22]

Potey et al. conducted a split-mouth trial to evaluate and compare the effectiveness of CAF with or without the use of PRF membrane in the treatment of multiple adjacent recession defects (MARD) clinically and by means of cone-beam computed tomography (CBCT).[23] Twenty healthy patients having 75 MARD were allocated

randomly to the CAF + orthodontic button group (CAFB) or the CAFB + PRF membrane group (CAFB + PRF). No notable differences were observed between the groups regarding PRC, RD, PD, or CAL. The use of PRF resulted in a highly significantly increased GT at 6 months, indicating that CAFB can be successfully used to treat MARD with predictable outcomes.[23]

Bhattacharya et al. performed a study to assess the efficacy of PRF collected in silica (SiO_2) tubes or titanium (Ti)-coated tubes, using immunohistochemistry (IHC).[24] The results showed that in the Ti group, the IHC staining was better, with more T cells, B lymphocytes and platelets. It was concluded that Ti PRF was better than silica PRF in periodontal regeneration.[24]

In the current study, the use of PRF resulted in improved RD, GT, PD, CAL, and KTW in both groups.

The laser-assisted CAF procedure was used in the patients allocated to group B. A laser-assisted vestibular releasing incision was made (980 nm diode laser at a power of 3 W). A relieving incision was made superficially, 7 mm apical to the gingival margin, which minimized tension on the flap, and also prevented muscle reattachments during the tissue remodeling phase. Due to the dual advantages of this technique, it is anticipated that RC would be better and more stable.[25] However, no documented studies are available to report the benefits of the laser vestibular releasing incision approach.

It was observed in the current study that the patients in group B performed equally well as those allocated to group A.

The benefits of using LLLT to facilitate wound healing have been reported in many studies. The therapy consists in biostimulation or biomodulation, and is based on the principle that irradiation at a specific wavelength is able to alter cellular behavior. The mitochondrial cellular respiratory chain is stimulated and the membrane calcium (Ca) channels are activated, thus enhancing tissue metabolism and proliferation.[26]

The major changes observed in the wounds treated with LLLT include increased granulation synthesis, enhanced neovascularization of tissue, and increased fibroblast proliferation, maturation, attachment, and matrix synthesis. The tensile strength of flap margins after LLLT has been reported to increase, preventing the collapse of the healing wound, and thus minimizing soft tissue recession.[27]

In another study comparing outcomes when using an additional external vestibular releasing incision made with a diode laser or a scalpel along with a laterally positioned flap, the authors reported reduced patient discomfort in the laser incision group.[28]

A systematic review conducted by Yan et al. showed that after applying light irradiation to gingival recession, the outcomes of CRC ranged from 70% to 90%.[29] Thus, a higher CRC was found in the experimental group as compared to the group that did not undergo laser treatment following surgery. However, the pooled analysis from this meta-analysis related to CRC found that there was no significant difference between the 2 groups based on the 6- and 12-month results (p = 0.300 and p = 0.160, respectively).[29] In accordance with the results of the review, CRC in the present study was similar between the 2 groups at 6 months postoperatively. A trend regarding the shift of gingival margins over time has been observed in previous studies.[30,31]

Aleksić et al. conducted a study to evaluate WHI with regard to the use of PRF and connective tissue graft (CTG) in the treatment of recession defects.[32] It was observed that there was less patient discomfort and better acceptance with faster healing in the PRF group as compared to the CTG group.[32] In the present study, the mean WHI scores in the conventional and laser groups at 1 week were 3.13 and 3.42, respectively, showing no significant difference. The results could be due to the use of the PRF membrane, which is known to release various proinflammatory cytokines, such as interferon gamma (IFγ), tumor necrosis factor alpha (TNFα), interleukin 1 beta (IL-1β), and IL-6, which help in T cell differentiation, and growth factors, such as vascular endothelial growth factor (VEGF), PDGF, fibroblast growth factor (FGF), TGF, and IGF, which act as anti-inflammatory agents and promote faster healing.[33]

Pain control following the operation is a necessary part of periodontal treatment. This pain results from tissue trauma and the release of inflammatory mediators, and reaches its highest peak following the cessation of local anesthesia. The analgesic mechanism of low-level laser is not yet clear. However, several studies have pointed out that light interference may be the cause of physiological changes in numerous cell types. Low-level laser can modify the inflammatory process in a dose-related manner; hence, it can reduce inflammatory pain. In acute pain, the optimal outcome is reached when low-level laser is prescribed within the first 72 h following the operation.[34]

Another split-mouth randomized clinical trial (RCT) evaluated the benefits of laser application in 12 patients after palatal graft harvesting.[35] In the test group, following the free gingival graft procedure, a 660 nm diode laser with a power of 200 mW was applied to the target site for 32 s. This was repeated on days 1, 2, 4, and 7 postoperatively. In the control group, sham laser was used in the same way. To evaluate the amount of epithelialization and for clinical repair observations, photographic images were used. The amount of sedative drugs taken was recorded to assess the pain scale on day 14. The palatal wound in the laser-applied group was significantly better healed than in the control group regarding clinical repair and epithelialization. Then, on day 21, the amount of epithelialization was significantly better in the laser-applied group than in the control group. However, the 2 groups showed no significant differences in the amount of sedative drug used and bleeding. The authors concluded that low-level laser might heal the wound in the palatal graft site.[35]

In the current study, the VAS scores were assessed in both group A and group B 1 week postoperatively. It was observed that in group A, the score was 2.29, and in group B, it was 2.17, showing no significant difference between the groups.

Hence, the present study showed improvement in RD, GT, CAL, and KTW, but the differences in the values of these parameters between the groups were not statistically significant. The results obtained were in accordance with earlier studies.[36,37] However, the PRC values did not show significant improvement in this study, which was also observed in previous research.[38]

Limitations

The follow-up period for the groups in this study was 6 months. The results could have been more significant if the follow-up had been carried out for 9 months.

Conclusions

This study showed improvement in RD, GT, CAL, and KTW, although both groups performed equally well. Perhaps the validity of the study could have been strengthened by increasing the sample size as well as conducting the study for a longer period of time. The benefits of using laser for both the vestibular releasing incision and biomodulation have to be confirmed by conducting more clinical trials.

Ethics approval and consent to participate

The research was approved by the institutional ethics committee at the Panineeya Institute of Dental Sciences & Research Centre, Hyderabad, India (PMVIDS&RC/IEC/PERIO/DN/0218-2018). Each participant provided written informed consent to participate in the study.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Manisha Bommala https://orcid.org/0000-0002-8973-9981
Rekha Rani Koduganti https://orcid.org/0000-0003-0524-9668
Veerendranath Reddy Panthula https://orcid.org/0000-0002-0247-2357
Surya Prasanna Jammula https://orcid.org/0000-0003-3773-3673
Himabindu Gireddy https://orcid.org/0000-0002-2198-9538
Manasa Ambati https://orcid.org/0000-0002-2387-6561
Bharathchandra Ganachari https://orcid.org/0000-0001-8905-2621

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Original paper

Evaluation of knowledge and awareness about teledentistry among dentists and patients living in Turkey

Neslihan Özveren[1,A-F], Büşra Sevinç[1,B–D], Ayça Sarıalioğlu Güngör[2,A–C], Ezgi Baltacı[1,B,D], Gözde Serindere[3,D–F], Özge Özgür[1,B,D]

[1] Department of Pediatric Dentistry, Faculty of Dentistry, Trakya University, Edirne, Turkey
[2] Department of Restorative Dentistry, Faculty of Dentistry, Bezmialem Vakıf University, Istanbul, Turkey
[3] Department of Dentomaxillofacial Radiology, Faculty of Dentistry, Mustafa Kemal University, Hatay, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Address for correspondence
Neslihan Özveren
E-mail: neslihanozveren@trakya.edu.tr

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Abstract

Background. Teledentistry is a field of telemedicine that combines digital technology and clinical dentistry, enabling remote communication between dentists and patients.

Objectives. The aim of the present study was to evaluate the knowledge and awareness of dentists and patients about teledentistry in Turkey.

Material and methods. This cross-sectional study was conducted among general and specialist dentists in Turkey, and dental patients in Edirne, Turkey. A questionnaire prepared in Google Docs was shared virtually among Turkish dentists, as well as administered to the patients referred to the university dental clinic in Edirne.

Results. Among the 336 dentists participating in the study, 69.9% were female, 39.6% were working in the profession for 1–5 years, and 48.5% were specialist dentists. A total of 86.9% of dentists stated they would like to use teledentistry for radiological examinations, some follow-up examinations, and for follow-up during holidays. There were 21.1% of dentists who thought that teledentistry practices could be the new standard of oral healthcare, and only 34.0% were willing to try such practices. Among the 447 patients in the study, 49.9% were female, 79.0% were aged 20–44 years, and 54.4% had middle income. There were 74.5% of patients who stated that it would be easier to communicate with the dentist via a teledentistry application, 80.3% of patients in the underserved regions stated it would facilitate access to the dentist, 76.3% of patients stated that it would reduce costs, and 88.8% of patients thought that this method could help overcome the problem of isolation during the coronavirus disease 2019 (COVID-2019) pandemic period.

Conclusions. Based on the results, it can be said that the teledentistry approach would provide convenience for both dentists and patients in terms of dentist–patient communication, cost and efficiency of dental care.

Keywords: oral health, questionnaire, awareness, teledentistry

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Introduction

The rapid development of communication technologies over the years has led to a different dimension of the service exchange between healthcare professionals and patients in the healthcare system. Telehealth encompasses all medical activities, such as diagnosis, treatment, disease monitoring and prevention, continuing the education of healthcare providers and consumers, and research and evaluation.[1] The term telemedicine refers to health services that are provided in the form of synchronous (voice or video calls) or asynchronous (store-and-forward) interactions, using electronic communication technologies instead of the patient and doctor meeting in person.[2,3] Teledentistry is a branch of telemedicine that can be applied in all fields of dentistry, such as oral and dental care, consultation, and remote patient monitoring and treatment planning, in order to improve oral and dental health. It uses health information technology and telecommunication systems.[4,5]

Teledentistry as a novel approach was widely used during the coronavirus disease 2019 (COVID-19) pandemic.[6] It involves several issues, such as consulting potential new patients, reviewing the results of treatment, pre-interviewing emergency patients, and it also can be used as an initial screening tool. The advantages of teledentistry include the remote control of fast and recordable data, simultaneous communication with many people, and the reduction of hospital costs and patient waiting time.[7,8] Through teledentistry applications, shortening the time to reach a specialist can prevent delays in the diagnosis and treatment of diseases.[9,10] Teledentistry also has the potential to address disparities in oral healthcare between rural and urban communities.[11,12] Thus, it can contribute to the improvement of patients' quality of life.

Research has shown that teledentistry has a high level of sensitivity and specificity.[13,14] Queyroux et al. found much consistency between the clinical examination, which is the gold standard, and teledentistry in their study.[15] It has also been reported that the positive predictive value and the accuracy rate in teledentistry, as well as the sensitivity of teledentistry applications, are quite high.[16,17]

Concerns about data quality and security, the reimbursement procedures, necessary technical infrastructure, the licensing of the programs to be used, the risk of violation of the patient's privacy, appropriate and secure Internet support for data, image and voice transmission, and the inherent risk of improper diagnosis are the issues to be considered with regard to the increasing use of teledentistry practices.[18–20] One of the obstacles to the adoption of teledentistry practices by patients and physicians may be the lack of information on this subject.[21]

The aim of the present study was to evaluate the knowledge and awareness of dentists and patients about teledentistry in Turkey.

Material and methods

Methods

Ethics approval for the study was obtained from the medical research ethics committee at the Faculty of Medicine of Trakya University, Edirne, Turkey (TUFT-BAEK 2020/319). The study was conducted in accordance with the tenets of the Declaration of Helsinki. Informed written consent was obtained from the patients and dentists, and the confidentiality of the answers was assured.

This cross-sectional study was conducted among general and specialist dentists in Turkey, and dental patients in Edirne. After a questionnaire (Table 1) was prepared in Google Forms, we collected the emails of dentists in Turkey and shared the questionnaire virtually with them. We also asked patients who had been referred to the university dental clinic in Edirne between November 11, 2020 and April 2, 2021 to complete the survey (Table 2). The inclusion criteria for dentists were as follows: residing in Turkey; working within the borders of Turkey; having graduated from any faculty of dentistry in Turkey; and having access to electronic media. The inclusion criterion for pa-

Table 1. Questionnaire for dentists

No.	Questions for dentists
1.	Gender
2.	Years of occupation
3.	Employment institution
4.	Professional specialty
5.	Have you heard of teledentistry applications before?
6.	I know the concept of teledentistry.
7.	I think that teledentistry practices can be useful.
8.	In which situations would you prefer to use a teledentistry application?
9.	Teledentistry practices may violate the patient's privacy.
10.	Talking to the patient in person combined with teledentistry practices can be helpful.
11.	Teledentistry practices could be time-saving.
12.	Teledentistry practices could be profitable.
13.	My follow-up appointments could be better with the use a teledentistry application.
14.	Teledentistry practices could be the standard way of providing oral healthcare.
15.	Teledentistry can make it easier for me to communicate with the patient.
16.	If I were using a teledentistry application, I could not always rely on the accuracy of the patient information entered into the system.
17.	At some point in my professional life, I would like to provide health services with the use of a teledentistry application.
18.	I would like the address information of the institution I work for to be shared with the patient through a teledentistry application.
19.	I would like to share information about the patient's treatment needs with other physicians or to guide the patient through a teledentistry application.

Table 2. Questionnaire for patients

No.	Questions for patients
1.	Gender
2.	Age
3.	Educational level
4.	Monthly income
5.	How satisfied would you be if there was an online (teledentistry) remote examination for dental patients?
6.	The teledentistry program can make it easier for me to communicate with the dentist.
7.	Using teledentistry, my dentist can do my check-ups well enough.
8.	Teledentistry can contribute to regular dental examinations.
9.	A dental examination with teledentistry is as accurate as during a face-to-face meeting.
10.	Teledentistry can reduce the cost of dental services.
11.	Teledentistry can be a time saver for me.
12.	Teledentistry can improve access to specialists in rural and underserved communities.
13.	Considering the COVID-19 pandemic, teledentistry, which reduces contact with the patient, can help overcome the problem of isolation.
14.	Can teledentistry violate your privacy?

COVID-19 – coronavirus disease 2019.

tients was having been examined at the Trakya University Oral and Dental Health Practice and Research Center.

Sample size

According to the Turkish Dental Association and the Turkish Statistical Institute 2019 report, 38,948 dentists were living within the borders of Turkey, and the population of Edirne was 413,903. With a 95% confidence interval (*CI*) and a margin of error of 5%, at least 320 dentists and 394 patients were found to be required as the sample size. Considering the missing data, 370 dentists and 450 patients were planned to participate in the study.

Results

The distribution of the 336 dentists participating in the study regarding gender, the duration of employment, and the institutions they were working for is summarized in Table 3. The distribution of dentists according to their specialties is shown in Fig. 1. Among the dentists participating in the study, 69.9% were female, 39.6% were working in the profession for 1–5 years, and 48.5% were specialist dentists.

There were 86.9% of dentists who stated that they would like to use teledentistry for radiological examinations, some follow-up examinations, and for follow-up during holidays; 82.4% stated they would like to discuss patients with other dentists or use the function of asking for opinions through teledentistry applications. However, 66.1% of dentists thought that follow-up with a teledentistry application would not be sufficient, 85.4% thought they would have a problem of trust in the patient information obtained in this way, and 46.4% thought they might experience patient confidentiality violation.

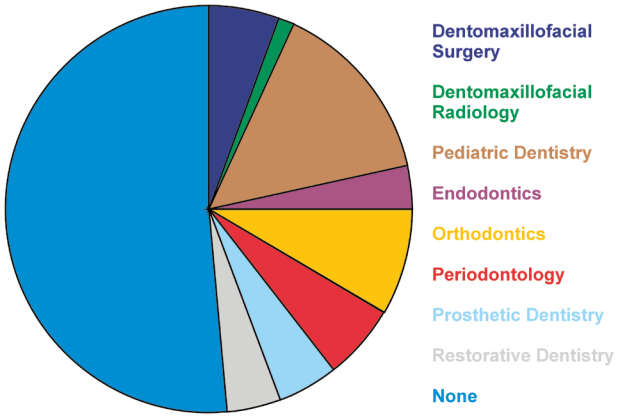

Fig. 1. Distribution of the dentists according to their specialties

As a result, 21.1% of dentists thought that teledentistry practices could be the new standard of oral healthcare, and only 34.0% were willing to try these practices.

The effects of gender, years of experience, institution, and specialization on the dentists' awareness of teledentistry practices, their willingness to try teledentistry practices, as well as the possibility of employing teledentistry as a standard practice in oral healthcare were evaluated. The effects of these factors are summarized in Table 3.

Accordingly, while there was no difference between genders in terms of being aware, it was observed that those who had worked longer, were specialist dentists,

Table 3. Distribution of the dentists included in the study by gender, years of occupation and the employement institution, and the effects of these factors on the awareness of teledentistry

Factors		Percentage of all dentists [%]	I am aware of teledentistry [%]	*p*-value
Gender	male	30.1	30.7	0.654
	female	69.9	33.2	
Years of occupation	1–5	39.6	20.3	0.002*
	6–10	19.9	43.3	
	11–15	10.4	45.7	
	16–20	6.8	43.5	
	>20	23.3	34.6	
Employment institution	university	31.3	42.9	0.010*
	PHDODHC	10.1	41.2	
	polyclinic	29.8	22.0	
	private clinic	27.1	27.5	
	other	1.7	50.0	
Specialty	yes	48.5	41.1	0.001*
	no	51.5	24.3	

PHDODHC – Provincial Health Directorate Oral and Dental Health Center; * statistically significant ($p < 0.05$).

or were working at a university or the Provincial Health Directorate Oral and Dental Health Center (PHDODHC) were more likely to be aware of teledentistry practices.

It was determined that the same factors did not have a significant effect on the dentists' views regarding the possibility of teledentistry becoming a standard practice in oral healthcare and their willingness to try teledentistry practices ($p > 0.05$).

The distribution of the 447 patients who participated in the study regarding gender, age, the educational status, and the income status is summarized in Table 4. There were 49.9% of participants in the study who were female, 79.0% were aged 20–44 years, and 54.4% had middle income. The educational status of the patients is shown in Fig. 2.

Among the patients, 74.5% stated that it would be easier to communicate with dentists via a teledentistry application, 80.3% of patients in underserved regions responded that it would facilitate access to the dentist, 71.4% of patients stated that teledentistry practices would contribute to regular dental examinations, 79.0% of patients indicated that teledentistry could save time, 76.3% of patients stated that it would reduce costs, and 88.8% of the patients thought that this method could help overcome the problem of isolation during the COVID-19 pandemic period. However, 51.0% of patients stated that follow-up with the use of teledentistry would not be sufficient, 62.2% of patients indicated that the interviews conducted through a teledentistry application would not be as accurate as face-to-face interviews, and 24.8% of patients marked that they might experience personal information privacy violation. As a result, 38.9% of patients reported that they would be satisfied with the use of teledentistry applications.

Table 4. Distribution of the patients included in the study by gender, age, educational level, and income level, and the effects of these factors on a positive attitude toward teledentistry practices

Factors		Percentage of all patients [%]	I would be satisfied with the use of teledentistry by dentists [%]	*p*-value
Gender	male	50.1	43.8	0.036*
	female	49.9	34.1	
Age [years]	20–44	79.0	38.0	0.019*
	45–54	14.5	32.3	
	55–64	4.5	65.0	
	>64	2.0	66.7	
Educational level	uneducated	1.8	37.5	0.126
	primary school	24.2	47.2	
	high school	33.6	34.0	
	university	32.9	35.4	
	PhD/master's degree	7.5	50.0	
Income level	low	19.5	40.2	0.001*
	middle	54.4	39.9	
	high	26.1	38.9	

* statistically significant ($p < 0.05$).

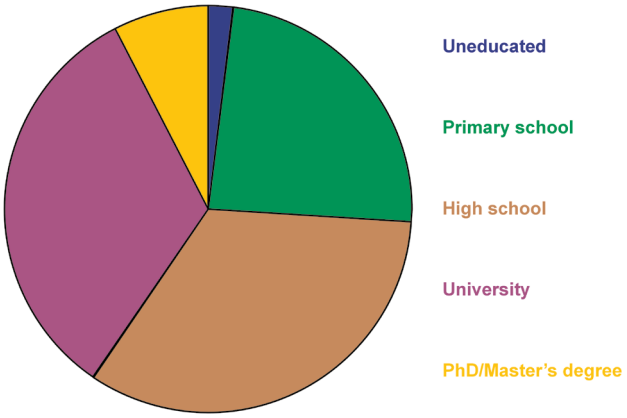

Fig. 2. Educational status of the patients

The effects of gender, age, educational level, and income level on the positive views of patients regarding teledentistry practices were examined. The effects of these factors are summarized in Table 4.

Accordingly, different income and education levels did not significantly affect the attitude toward teledentistry; however, it was observed that males and those who were relatively older had a more positive perspective.

Discussion

To the best of our knowledge, this is the first study to evaluate the perceptions of patients and clinicians in Turkey regarding teledentistry. It provides insight into the level of knowledge, awareness and experience of teledentistry among Turkish dentists and patients. This questionnaire study included 336 dentists from different regions of the country and 447 patients from one city in Turkey.

Teledentistry is a novel method of health service delivery that enables dentists to triage patients and conduct follow-up,[21] as well as to give advice and reassurance when necessary.[22] Consistent with other studies in the literature,[22–27] our results show positive and promising results for the future of teledentistry, as both patients and dentists alike seem to find merits in this service.

In the present study, 33.2% of the participating female dentists and 30.7% of the male dentists reported that they were aware of teledentistry. There were 86.9% of dentists who stated that they would like to use teledentistry for radiological examinations, some follow-up examinations, and for follow-up during holidays. Among the dentists, 66.1% thought that follow-up with a teledentistry application would not be sufficient, 85.4% thought they would have a problem of trust in the patient information obtained in this way, and 46.4% thought they might

experience patient confidentiality violation. There were 21.1% of dentists who thought that teledentistry practices could be the new standard of oral healthcare, and only 34% were willing to try these practices. Among specialists, 41.1% were aware of teledentistry.

In a study by Subhan et al., it was observed that 62.5% of dentists did not know about teledentistry before COVID-19, but most of them (68.6%) now have knowledge about it.[24] Almazrooa et al. reported that 83.0% of dentists were sure that teledentistry could improve daily dental practice.[25] Al-Khalifa and AlSheikh reported that more than 70.0% of respondents agreed that teledentistry would improve dental practice by increasing peer communication, mentoring and the referral of new patients.[26] There were 80.0% of respondents who were concerned about confidentiality.[26] In a study by Abbas et al., 72.4% of dentists reported that they were aware of teledentistry, and 76.0% reported that teledentistry could be really helpful in health education.[28] According to the opinion of 35.9% of dentists, teledentistry could be used in every branch of dentistry, and 88.2% of respondents responded that teledentistry could be useful in improving access to oral health services. Raucci-Neto et al. reported that 89.5% and 81.4% of general practitioners and specialists, respectively, had no previous experience with teledentistry.[29] There were 38.9% of dentists who also had superficial information. Among the dentists, 37.7% believed that there would be difficulties when using teledentistry, and 48.9% thought that teledentistry would not be fully effective.[29] In a study by Pradhan et al., 96.1% of postgraduate students were aware of teledentistry.[30] There were 58.4% of respondents who thought that teledentistry helped with consulting a specialist about a particular patient's problem.[30]

When evaluated in terms of patients, the results of the current study were as follows: 74.5% of patients mentioned that it would be easier to communicate with the dentist via a teledentistry application, 80.3% of the patients in the underserved regions responded that it would facilitate access to the dentist, 71.4% of patients said that teledentistry practices would contribute to regular dental examinations, 79.0% of patients indicated that teledentistry practices could save time, 76.3% of patients stated that it would reduce costs, and 88.8% of patients thought that this method could help overcome the problem of isolation during the COVID-19 pandemic period. However, 51.0% of patients stated that follow-up with teledentistry would not be sufficient, 62.2% of patients indicated that the interviews conducted through a teledentistry application would not be as accurate as face-to-face interviews, and 24.8% of patients marked that they might experience personal information privacy violation. There were 38.9% of patients who reported that they would be satisfied with the use of teledentistry applications.

In a study by Byrne and Watkinson, 76.0% of patients said that remote consultation was more convenient than face-to-face consultation, and 66.0% stated that they would like more such appointments to be made in the future, if appropriate.[31] In a study by Menhadji et al., the majority of patients (83.4%) agreed that it was easy to make an appointment online, and 84.9% of patients found it easy to follow appointment instructions.[23] The majority of patients (70.7%) strongly agreed that their video consultation was uneventful. Only a small number of patients (2.1%) strongly disagreed about a better understanding of their dental condition. The majority (59.2%) strongly agreed that the dentist told the patient everything he needed to know, and thus answered all questions. Among patients, 64.0% thought that the consultation was comprehensive.[23] Rahman et al. stated that patients using the virtual clinic and telephone consultation were satisfied with their experience at 97% and 94%, respectively.[27] All participants agreed with the statement that teledentistry would be very useful in saving time, and a significant proportion (96.0%) would use it again in light of COVID-19.[27]

As a result of the literature review, different results were found among studies. The reason for this situation may be that the studies were carried out in different countries, under different conditions (infrastructure, dental education curriculum, etc.), and with different characteristics of the participants included in the study (the educational status, the income status, etc.).

Based on the results of the present study, the use of the teledentistry approach can enable earlier access to healthcare, provide specialist care, minimize work time, and reduce traveling long distances for consultation.[26] For healthcare providers, it has the potential to eliminate inappropriate referrals[31] and reduce long waiting lists.[32] Thanks to teledentistry applications, whci allow remote communication, the risk of infection, specifically cross-infection between patients, healthcare workers and other people, can be reduced.[33] However, there are some challenges, limitations and legal issues to be considered with regard to teledentistry, as there are concerns about reimbursement, logistics, licensing regulations, costs, the lack of a physical examination, and data quality and security according to each country's rules.[1,34,35] While applying teledentistry, it is necessary to comply with state laws and the principles of practice.[34] It is very important to establish a well-equipped technological infrastructure system to ensure adequate service and security regarding the exchange of patient data.[35–38]

Limitations

The strengths of this study are the large sample size with different employment institutions, a high response rate, and the recording of opinions for both dentists and patients. However, there are also some limitations associated with the characteristics of survey studies. This study provided information about the teledentistry knowledge and awareness of patients living in Edirne. Thus, our findings

cannot be generalized to the whole population of Turkey. This survey shows a positive attitude toward teledentistry as a general concept. Specific research is needed to target specific aspects, such as the use of patient screening, diagnosis, referral, or other implementations.

Conclusions

It is important to conduct future comprehensive studies on teledentistry with a larger number of participants to try to minimize difficulties in practice, strengthen the infrastructure technologically, and increase the knowledge level of dentists by including this subject in dentistry undergraduate and postgraduate education in Turkey.

Ethics approval and consent to participate

Ethics approval for the study was obtained from the medical research ethics committee at the Faculty of Medicine of Trakya University, Edirne, Turkey (TUFT-BAEK 2020/319). Informed written consent was obtained from all the participants.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Neslihan Ozveren https://orcid.org/0000-0002-1090-5415
Büşra Sevinç https://orcid.org/0000-0003-4081-823X
Ayça Sarıalioğlu Güngör https://orcid.org/0000-0002-8779-2949
Ezgi Baltacı https://orcid.org/0000-0001-8553-3858
Gözde Serindere https://orcid.org/0000-0001-7439-3554
Özge Özgür https://orcid.org/0000-0003-2413-1550

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Original paper

Investigation of the relationship between probable sleep bruxism, awake bruxism and temporomandibular disorders using the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD)

Basak Cigdem Karacay[1,A,B,D–F], Tugba Sahbaz[2,A,C,D]

[1] Department of Physical Medicine and Rehabilitation, Kirsehir Ahi Evran University, Turkey
[2] Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Beykent University, Istanbul, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Basak Cigdem Karacay
E-mail: basakcigdem@hotmail.com

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Abstract

Background. The causal relationship between bruxism and temporomandibular disorders (TMD) is not clear.

Objectives. The present study investigated which TMD are associated with probable sleep bruxism (SB) and awake bruxism (AB) according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). The study further evaluated the association between probable SB and AB and TMD.

Material and methods. A total of 143 patients were selected – bruxers (SB and AB) and non-bruxers. A diagnosis of probable bruxism was made after a physical examination and when the symptoms were detected. The patients were evaluated using DC/TMD. From among Axis I assessment instruments, the TMD Pain Screener, the Symptom Questionnaire and the Clinical Examination Form were used. Moreover, the Graded Chronic Pain Scale (GCPS) (v. 2), the Jaw Functional Limitation Scale-8 (JFLS-8), the Patient Health Questionnaire-4 (PHQ-4), and the Oral Behaviors Checklist (OBC) were applied within the scope of Axis II.

Results. Diagnoses of muscle disorders and disk displacement with reduction were significantly more frequent in the SB and AB groups than in non-bruxers. A diagnosis of arthralgia was significantly more prevalent in the AB group than in non-bruxers. The JFLS-8 scores and the TMD Pain Screener scores were higher in the AB group than in the SB group and in non-bruxers. Distress levels, and the GCPS and OBC scores were higher in the SB and AB groups as compared to non-bruxers. The results of binary logistic regression analysis showed that only the OBC score was significantly higher in the TMD subgroup (*OR* (odds ratio) = 1.228; 95% *CI* (confidence interval): 1.014–1.488).

Conclusions. Both SB and AB were associated with pain-related TMD and intra-articular joint disorders. The muscle disorders and disk displacement with reduction subtypes were associated with SB and AB. Unlike SB, AB was also associated with arthralgia. Bruxers (both SB and AB) displayed parafunctional habits. However, AB was associated with greater functional limitation of the jaw as compared to SB.

Keywords: pain, temporomandibular disorders, jaw, sleep bruxism, awake bruxism

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Introduction

Temporomandibular disorders (TMD) occur because of problems with the temporomandibular joints and the masticatory muscles.[1] The etiology of TMD is multifactorial and numerous risk factors have been reported in the literature. Trauma, anatomical differences, genetic predisposition, the psychological status, and parafunctional habits are among the TMD risk factors.[2–6] Activities such as chewing gum, yawning, squeezing objects between the teeth, playing wind instruments, supporting the jaw with the hands, and chewing on one side are all considered to be parafunctional habits.[7] Such parafunctional habits lead to the overuse of the temporomandibular joints and the surrounding muscles.

The etiology of bruxism is unclear, though recent studies reported genetic predisposition for sleep bruxism (SB).[8,9] Additionally, low plasma sodium concentration and inflammatory markers have been associated with SB.[10,11] Moreover, SB has been linked to the motor activity of the jaw muscles.[12,13]

International consensus on the evaluation of bruxism states that bruxism should be examined separately as SB and awake bruxism (AB).[14] Both SB and AB are associated with mastication muscle activity. In SB, phasic and tonic activity is observed during sleep. Meanwhile, AB is characterized by such activities as the continuous contact of the teeth and/or pushing the mandible during wakefulness.[14] Bruxism is diagnosed through symptoms, examination findings and quantitative methods.[14] Quantitative methods include electromyography and polysomnography.[14] Lobbezoo et al. graded bruxism into 3 sub-categories, including possible bruxism, probable bruxism and definite bruxism.[14] A diagnosis of possible SB/AB is symptom-based only, probable SB/AB is diagnosed based on clinical findings, whereas definite SB/AB is diagnosed based on instrumental evaluation.[14]

In a recent review, the relationship between bruxism and TMD was not clearly established.[15] Moreover, a recent polysomnographic study that focused on the relationship between TMD and SB reported that SB was not a risk factor for the development of TMD.[16] Additionally, the distribution of TMD was equal in SB and non-bruxer patients.[16] One study also reported no relationship between SB and TMD-related pain.[17] However, AB and myofascial TMD have been associated with painful conditions, such as headaches.[18] In light of this information, and since SB and AB are considered 2 different types of bruxism, it appears that their effects on TMD are different. The present study evaluated the effects of SB and AB on TMD by comparing them with a non-bruxer group.

Bruxism is not evaluated through a separate questionnaire in the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) tools. Instead, bruxism is examined within the Oral Behaviors Checklist (OBC) of the DC/TMD Axis II.[19] The DC/TMD were created based on the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD).

The DC/TMD are valuable tools, since they allow investigators to examine TMD by dividing them into diagnostic subgroups. According to DC/TMD, TMD are stratified into 4 different groups, which are pain-related TMD and headaches, intra-articular joint disorders, degenerative joint disorders, and subluxation.[20]

Some studies investigating the relationship between bruxism and TMD questioned about TMD symptoms and pain. Most of them used RDC/TMD to classify the diagnosis of TMD.[15,21,22] The present study aimed to examine the relationship between bruxism and TMD using the current DC/TMD criteria.

The primary purpose of our study was to demonstrate which TMD types are associated with probable SB and AB according to DC/TMD. The secondary aim was to determine the relationship between probable SB and AB and TMD.

Material and methods

Compliance with ethical standards and ethical approval

The present study was conducted according to the 1964 Declaration of Helsinki, and all participants provided voluntary informed written consent before being enrolled in the study. Approval was granted by the Clinical Research Ethics Committee at Yozgat Bozok University, Turkey (approval No.: 2017-KAEK-189_2021.03.10_19). The study was registered at ClinicalTrials.gov (NCT04866849).

Participants

This observational cross-sectional study involved 143 healthy volunteers, aged 18–65 years. The study was conducted between May 2021 and August 2021. The participants were selected from among the relatives of the patients who reported to the outpatient clinic at Yerkoy State Hospital, Yozgat, Turkey. All participants were evaluated by the same physiatrist (B.C.K.), who is experienced in diagnosing and treating patients with TMD. After a physical examination, the participants were split into 3 groups – SB, AB or non-bruxers – according to their diagnosis of bruxism. Accordingly, 25 participants were determined to have SB, 42 participants were determined to have AB, and 76 participants were determined to be non-bruxers. The exclusion criteria were congenital temporomandibular joint disease, and the history of previous temporomandibular region trauma or surgery. Participants treated with oral analgesics or muscle relaxants in the past week, participants who used immunosuppressive drugs, and those with cancer or systemic inflammatory disease were also excluded from the study. Patient recruitment was terminated when the appropriate sample size was reached.

Diagnosis of bruxism

Bruxism was evaluated as probable SB or probable AB by questioning the participants about symptoms, and also based on the findings of the physical examination. The DC/TMD OBC question 1 was used to ask about SB symptoms, and questions 3 and 4 were used to ask about AB symptoms. The participants were asked to fill out the OBC questionnaire before the physical examination.

The abnormal wear of the teeth, the presence of tooth marks in the buccal region, the presence of tooth marks on the tongue, and the hypertrophic appearance of the masseter muscle were investigated during the physical examination. The presence of at least one of the above 4 findings was sought in the investigation of SB/AB.

Lobbezoo et al. stated that a physical examination and symptoms should be used for the diagnosis of probable bruxism.[14] Therefore, in this study, the participants had to be diagnosed with bruxism based on symptoms and physical examination findings to be considered a bruxer (SB/AB).

Participants not diagnosed with bruxism and those who did not undergo a physical examination were considered non-bruxers, and were included in the control group. Patients who did meet these criteria were excluded from the study.

Measurements

All participants were evaluated according to the DC/TMD Axis I and Axis II findings.[19] While classifying TMD, symptoms such as jaw sounds, the presence of locking as well as headaches were examined using the Symptom Questionnaire. Pain was assessed using the TMD Pain Screener. Additionally, the Clinical Examination Form was used for the standard examination of the patients. The Symptom Questionnaire, the TMD Pain Screener and the Clinical Examination Form were included in the DC/TMD Axis I assessment tools.[19]

The patients were evaluated the using DC/TMD Axis II assessment tools – the Graded Chronic Pain Scale (GCPS) (v. 2), the Jaw Functional Limitation Scale-8 (JFLS-8), the Patient Health Questionnaire-4 (PHQ-4), and OBC. The participants were asked to open their mouths widely so that the maximum mouth opening could be measured. With the help of a ruler, the distance between the front teeth was also measured and recorded.[23] Additionally, symptom duration, age, gender, the education level, and the body mass index (BMI) were recorded.

The Symptom Questionnaire is a tool that collects information about pain, headaches, temporomandibular joint sounds, and jaw locking in patients with TMD.[19,24] The TMD Pain Screener evaluates the stiffness of the jaw, the presence of pain during various activities and the persistence of pain in patients with TMD. Moreover, the TMD Pain Screener checks for pain in the last 30 days.[24] The OBC assesses parafunctional habits through self-reporting. The OBC checks if the patient overuses the jaw joints and the surrounding muscles.[19,24] The JFLS is available in 2 forms: short (JFLS-8), consisting of 8 items; and long (JFLS-12), consisting of 12 items. In the present study, JFLS-8 was used to evaluate the functional limitation of the jaw. It was also used to assess the functional limitation experienced by the patient while performing various activities, with a 10-point Likert scale used for each item.[19,24] The GCPS assesses the severity of chronic pain and pain-related disability in patients with TMD.[19,24] The PHQ was developed to assess mood states, such as depression and anxiety, as well as concentration problems and energy in TMD patients. The questionnaire occurs in 2 different forms: PHQ-4; and PHQ-9. The PHQ-4 form, which mostly assesses anxiety and depression, was used in this study.[19,24]

Sample size calculation

Study sample size calculations were performed with the G*Power software, v. 3.1.9.4 (Heinrich Heine University of Düsseldorf, Germany; https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The effect size was 0.3 for the χ^2 tests with $\alpha = 0.05$ and power of 0.80. The total sample size was estimated at 143.

Statistical analysis

The IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA) was used for data analysis. Descriptive statistics were used to summarize the participant data as frequency or as mean and standard deviation ($M \pm SD$). The data distribution was evaluated with the Kolmogorov–Smirnov test. The χ^2 test and Fisher's exact test were used to compare categorical data between 2 groups. The independent sample t test and the Mann–Whitney U test were used to compare quantitative variables. The independent variables with a significance value of $p < 0.20$ for the difference between them were analyzed by binary logistic regression. Odds ratios (*OR*s) and their respective 95% confidence intervals (*CI*s) were also estimated. Moreover, $p < 0.05$ was used to define statistical significance.

Results

A total of 143 participants were selected: 76 were non-bruxers; 25 were in the SB group; and 42 were in the AB group. The mean age of the participants was 44.23 ±10.20 years, with a BMI of 26.69 ±4.67. When the demographic data was analyzed in the 3 groups, no statistical difference was identified in terms of age, gender, BMI, education, occupation, or the marital status ($p > 0.05$). The prevalence of smoking, chewing gum, nail biting, and cheek/lip biting was higher in the SB and AB groups than in the control group ($p < 0.05$) (Table 1).

Table 1. Demographic characteristics of the participants

Variable		Control group $n = 76$	SB group $n = 25$	AB group $n = 42$	*p*-value
Age [years] *M* ±*SD*		43.19 ±10.86	44.12 ±8.95	46.50 ±9.54	0.254
Gender M/F		26/50	6/19	9/33	0.289
BMI [kg/m^2] *M* ±*SD*		26.26 ±4.32	28.34 ±3.96	27.66 ±4.25	0.058
Education *n* (%)	elementary	25 (32.9)	11 (44.0)	16 (38.1)	0.105
	secondary	24 (31.6)	9 (36.0)	20 (47.6)	
	university	27 (35.5)	5 (20.0)	6 (14.3)	
Occupation *n* (%)	unemployed	25 (32.9)	4 (16.0)	10 (23.8)	0.332
	desk worker	30 (39.5)	15 (60.0)	18 (42.9)	
	physically demanding	21 (27.6)	6 (24.0)	14 (33.3)	
Marital status *n* (%)	married	60 (78.9)	20 (80.0)	37 (88.1)	0.452
	unmarried	16 (21.1)	5 (20.0)	5 (11.9)	
Habits *n* (%)	smoking	7 (9.2)	11 (44.0)	15 (35.7)	<0.001[a,b]
	alcohol	13 (17.1)	4 (16.0)	7 (16.7)	0.992
	chewing gum	14 (18.4)	11 (44.0)	23 (54.8)	<0.001[a,b]
	nail biting	12 (15.8)	9 (36.0)	18 (42.9)	0.002[a,b]
	pen biting	5 (6.6)	3 (12.0)	7 (16.7)	0.203
	cheek/lip biting	15 (19.7)	12 (48.0)	20 (47.6)	0.002[a,b]

SB – sleep bruxism; AB – awake bruxism; *M* – mean; *SD* – standard deviation; M – male; F – female; BMI – body mass index; statistically significant difference: [a] between the control and SB groups; [b] between the control and AB groups.

Table 2. Comparison of the assessment results between the groups

Variable		Control group $n = 76$	SB group $n = 25$	AB group $n = 42$	*p*-value
Mouth opening [mm] *M* ±*SD*		42.74 ±5.12	41.75 +5.21	40.07 ±4.09	0.045[b]
TMD Pain Screener score *M* ±*SD*		0.43 ±0.86	2.88 +0.92	4.85 ±1.04	<0.001[a,b,c]
JFLS-8 score *M* ±*SD*		0.20 ±0.52	1.68 +0.68	2.58 ±0.56	<0.001[a,b,c]
OBC score *M* ±*SD*		4.39 ±4.16	16.28 +5.24	20.71 ±5.39	<0.001[a,b]
PHQ-4 score *M* ±*SD*		0.89 ±1.20	4.72 +2.09	6.07 ±1.20	<0.001[a,b]
GCPS score *n* (%)	0	65 (85.5)	–	–	<0.001[a,b]
	1	9 (11.8)	12 (48.0)	8 (19.0)	
	2	2 (2.6)	10 (40.0)	18 (42.9)	
	3	–	2 (8.0)	13 (31.0)	
	4	–	1 (4.0)	3 (7.1)	

TMD – temporomandibular disorders; JFLS-8 – Jaw Functional Limitation Scale-8; OBC – Oral Behaviors Checklist; PHQ-4 – Patient Health Questionnaire-4; GCPS – Graded Chronic Pain Scale; statistically significant difference: [a] between the control and SB groups; [b] between the control and AB groups; [c] between the SB and AB groups.

Mouth opening was decreased in the AB group (40.07 ±4.09 mm) as compared to non-bruxers (42.74 ±5.12 mm). The JFLS-8 scores and the TMD Pain Screener scores were higher in the AB group than in both the SB group and non-bruxers. Additionally, the JFLS-8 scores and the TMD Pain Screener scores were higher in the SB group than in non-bruxers. Distress levels, and the GCPS and OBC scores were increased in the SB and AB groups as compared to non-bruxers ($p < 0.001$) (Table 2).

The post-examination diagnoses of the participants were compared. Diagnoses of muscle disorders and disk displacement with reduction were significantly

more frequent in the SB and AB groups than in non-bruxers. Additionally, the arthralgia diagnosis was significantly more prevalent in the AB group than in non-bruxers ($p < 0.05$) (Table 3). All participants were divided into 2 subgroups – diagnosed with TMD or not diagnosed with TMD. The factors affecting the diagnosis of TMD were then examined. The results of binary logistic regression analysis showed that only the OBC score was significantly higher in the TMD subgroup (*OR* = 1.228; 95% *CI*: 1.014–1.488) (Table 4).

Table 3. Comparison of the participants' diagnoses

Diagnosis		Control group *n* = 76	SB group *n* = 25	AB group *n* = 42	*p*-value
Normal		64 (84.2)	0 (0)	0 (0)	–
Pain-related TMD and headaches	muscle disorders	5 (6.6)	10 (40.0)	22 (52.4)	<0.001[a,b]
	arthralgia	1 (1.3)	2 (8.0)	5 (11.9)	0.021[b]
	headaches attributed to TMD	1 (1.3)	2 (8.0)	1 (2.4)	0.231
	total	7 (9.2)	14 (56.0)	28 (66.7)	<0.001[a,b]
Intra-articular joint disorders	DD with reduction	3 (3.9)	7 (28.0)	7 (16.7)	0.003[a,b]
	DD with reduction, with intermittent locking	1 (1.3)	2 (8.0)	3 (7.1)	0.077
	DD without reduction, without limited opening	0 (0)	1 (4.0)	2 (4.8)	0.140
	DD without reduction, with limited opening	0 (0)	0 (0)	1 (2.4)	0.441
	degenerative joint disorders	1 (1.3)	1 (4.0)	1 (2.4)	0.797
	total	5 (6.6)	11 (44.0)	14 (33.3)	<0.001[a,b]

Data presented as number (percentage) (*n* (%)). DD – disk displacement; statistically significant difference: [a] between the control and SB groups; [b] between the control and AB groups; [c] between the SB and AB groups.

Table 4. Univariate logistic regression analysis of the factors affecting temporomandibular disorders (TMD)

Factor		TMD no *n* = 64	TMD yes *n* = 79	*p*-value	*OR*	95% *CI* (lower–upper)	*p*-value
Age [years] *M* ±*SD*		41.03 ±11.90	44.67 ±9.59	0.045	0.976	0.900–1.058	0.239
Gender M/F		22/42	19/60	0.175	1.025	0.864–1.855	0.554
BMI [kg/m2] *M* ±*SD*		25.97 ±3.87	27.57 ±4.63	0.030	1.025	0.864–1.216	0.076
Education *n* (%)	elementary	23 (35.9)	29 (36.7)	0.467	–	–	–
	secondary	21 (32.8)	32 (40.5)		–	–	
	university	20 (31.3)	18 (22.8)		–	–	
Occupation *n* (%)	unemployed	26 (40.6)	37 (46.8)	0.100	ref.	–	0.100
	desk worker	15 (23.4)	26 (32.9)		3.746	0.566–24.801	
	physically demanding	23 (35.9)	16 (20.3)		0.555	0.055–5.651	
Marital status *n* (%)	married	46 (71.9)	11 (13.9)	0.036	0.689	0.164–2.895	0.611
	unmarried	18 (28.1)	68 (86.1)				
SB *n* (%)	yes	–	25 (31.6)	<0.001	0.000	0.000	0.997
	no	64 (100)	54 (68.4)				
AB *n* (%)	yes	–	42 (53.2)	<0.001	0.000	0.000	0.998
	no	64 (100)	37 (46.8)				
OBC score *M* ±*SD*		3.74 ±3.26	17.32 ±6.82	<0.001	1.228	1.014–1.488	0.036*
PHQ-4 score *M* ±*SD*		0.78 ±1.09	4.94 ±2.67	<0.001	1.154	0.609–2.189	0.660

OR – odds ratio; *CI* – confidence interval; ref. – reference; * statistically significant.

Discussion

The results showed that SB and AB were associated with muscle disorders and disk displacement with reduction. Unlike SB, AB was also associated with arthralgia. However, it has been previously reported that no relationship exists between SB and TMD,[16] or between SB and TMD-related pain.[17] Those studies evaluated SB using polysomnography,[16,17] and the discrepancy could also be due to the fact that in the current study, probable SB was evaluated. Nonetheless, in a study in which SB was evaluated clinically without using polysomnography, a relationship between SB and painful TMD was found, similar to the results of the present study.[22] Reissmann et al. also reported that both SB and AB were associated with painful TMD.[25] Meanwhile, AB has been associated with myofascial TMD in the literature.[18] In another study, self-reported bruxism was associated with painful TMD.[26] Our results also support these findings. Commisso et al. reported that bruxism could cause joint damage by increasing frictional stress on the disk,[27] which was supported by another recent study.[28] The latter study reported that disk displacement and joint-related pathologies might occur in bruxers.[28] Thus, our results show that intra-articular joint disorders are associated with both SB and AB.

Although previous studies reported AB to be associated with pain-related TMD, bruxism may be associated with intra-articular joint disorders, degenerative joint disorders, and subluxation. Our results also support this hypothesis by showing that SB and AB are associated with pain-related TMD and intra-articular joint disorders. We also found SB and AB to be associated with muscle disorders and disk displacement with reduction, using DC/TMD. Thus, in patients with bruxism, apart from increased muscle activity or muscle-related causes, the temporomandibular joint is also affected. The presence of bruxism also increases the severity of TMD.[29] Therefore, in clinical practice, the investigation and management of bruxism are important in the intra-articular joint disorder subtypes, such as disk displacement with reduction.

Silva et al. found a relationship between AB and headache.[18] However, no relationship was found between bruxism (both SB and AB) and TMD-related headache in the current study, though SB and AB were associated with painful TMD. Similar to our results, previous studies discovered no relationship between headache and bruxism, and the presence of bruxism did not increase the risk of headache.[30] A recent study demonstrated that AB increased the risk of tension-type headache.[31] In the current study, only TMD-related headache was examined according to DC/TMD, and headache types such as migraine and tension-type headache were not investigated. Thus, the data should be interpreted with caution.

Parafunctional habits result in the overuse of the muscles in the orofacial region. Thus, changes in muscle length and muscle dysfunction can cause myofascial pain.[30] In this study, smoking, alcohol consumption, gum chewing, nail biting, pen biting, and cheek/lip biting were evaluated as parafunctional habits. The results suggest that people with bruxism (both SB and AB) also develop parafunctional habits. Although the causal relationship between bruxism and TMD is unclear, studies have reported that parafunctional habits are a risk factor for TMD,[15,32] which is consistent with our findings.

Cumulative smoking and tobacco use are associated with bruxism. Indeed, nicotine can increase bruxism, and can also cause the excessive use of the jaw joints and the surrounding muscles. Thus, a link may exist between nicotine intake and bruxism.[33] We found that smoking habits were more common in bruxers (both SB and AB) than in healthy subjects. We attributed the greater prevalence of smoking in bruxers to the relationship between nicotine and bruxism, which shows that smoking is a parafunctional habit similar to bruxism.

A recent study that examined TMD-related factors using DC/TMD reported that the female gender and increased stress levels were associated with TMD severity.[29] Our study revealed that stress was not a risk factor for TMD, although the bruxism groups (both SB and AB) reported more distress and chronic pain. In a meta-analysis, TMD patients were reported to be more sensitive to pain than healthy subjects.[34] Therefore, the relationship between bruxism and chronic pain might be related to the coexistence of painful TMD and bruxism. It may also be associated with the complex nature of bruxism and central hypersensitivity. The relationships between bruxism, TMD, pain, and distress also show that in the case of bruxism, multidisciplinary management strategies are vital. In our study, the female gender was not considered a risk factor for TMD, which is consistent with the results of other studies that examined TMD risk factors.[32,35]

We revealed that a minimal decrease was found in the maximum mouth opening measurement in the AB group in comparison with healthy subjects. However, the measurements were within the functional mouth-opening range in both bruxers and healthy subjects.[23] We also found a relationship between bruxism (both SB and AB) and the functional limitation of the jaw. Additionally, AB was associated with greater functional limitation of the jaw as compared to SB. This correlation may be due to the association of bruxism with TMD. The correlation may also be due to the fact that bruxism can affect the masticatory muscles, which limits jaw functions.

Limitations and strengths

A major limitation of our study is that bruxism could not be measured quantitatively. Electromyography or polysomnography are necessary for the diagnosis of definite SB/AB.[14] However, it has been reported that the diagnosis of probable SB/AB through a physical examination

and symptom questioning may be sufficient, especially in cases where the sample size is large.[14]

A relationship has been noted between SB and obstructive sleep apnea syndrome and simple snoring.[36,37] However, these confounding factors could not be distinguished, since a polysomnographic examination was not performed in this study. Moreover, the subject's symptoms fluctuated because of the nature of bruxism and TMD, which can also be considered a limitation of this study. Since the sample consisted of a healthy population, this issue should be considered when generalizing our findings. Despite all of these limitations, this study used the current and valid DC/TMD tools. The diagnostic classification of the patients and the physical examination were performed by the same evaluator, which are the strengths of the study. Another strength is that probable SB and probable AB were evaluated separately.

Conclusions

Both SB and AB were associated with pain-related TMDs and intra-articular joint disorders. The muscle disorders and disk displacement with reduction subtypes were associated with SB and AB. Unlike SB, AB was also associated with arthralgia. Bruxers (both SB and AB) displayed parafunctional habits, and a relationship was noted between parafunctional habits and TMD. Although the bruxism groups (both SB and AB) reported more distress and chronic pain, no relationship was found between stress and TMD. Awake bruxism was associated with greater functional limitation of the jaw as compared to SB.

Trial registration

The study was registered at ClinicalTrials.gov (NCT04866849).

Ethics approval and consent to participate

The present study was conducted according to the 1964 Declaration of Helsinki, and all participants provided voluntary informed written consent before being enrolled in the study. Approval was granted by the Clinical Research Ethics Committee at Yozgat Bozok University, Turkey (approval No.: 2017-KAEK-189_2021.03.10_19).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Basak Cigdem Karacay https://orcid.org/0000-0001-6422-5450
Tugba Sahbaz https://orcid.org/0000-0002-5974-8991

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Original paper

Relationship between pain severity, satisfaction with life and the quality of sleep in Polish adults with temporomandibular disorders

Piotr Seweryn[1,B–D,F], Sylwia Magdalena Orzeszek[1,B–D,F], Marta Waliszewska-Prosół[2,B,C,F], Andrej Jenča[3,C,E,F], Magdalena Osiewicz[4,C,D,F], Anna Paradowska-Stolarz[5,A,C,E,F], Orit Winocur-Arias[6,C,E,F], Marek Ziętek[1,C,E,F], Wojciech Bombała[7,C,D,F], Mieszko Więckiewicz[1,A–D,F]

[1] Department of Experimental Dentistry, Wroclaw Medical University, Poland
[2] Department of Neurology, Wroclaw Medical University, Poland
[3] Department of Stomatology and Maxillofacial Surgery, Faculty of Medicine, Pavol Jozef Šafárik University in Košice and Akadémia Košice, Slovakia
[4] Department of Integrated Dentistry, Dental Institute, Faculty of Medicine, Jagiellonian University Medical College, Cracow, Poland
[5] Department of Maxillofacial Orthopedics and Orthodontics, Wroclaw Medical University, Poland
[6] Department of Oral Pathology and Oral Medicine, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Israel
[7] Statistical Analysis Center, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Sylwia Magdalena Orzeszek
E-mail: sylwia.winiewska87@gmail.com

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Abstract

Background. Temporomandibular disorders (TMD) pose a serious health problem that can have a negative effect on patients' lives, impair work performance, and result in work absences and restrictions in daily activities.

Objectives. The aim of this observational, cross-sectional study was to evaluate the level of satisfaction with life among Polish patients with TMD and to assess the influence of pain severity on this parameter. A secondary goal was to investigate sleep quality within this patient group and explore its relationship with pain.

Material and methods. A total of 219 patients from the Outpatient Clinic for Temporomandibular Disorders at the University Dental Polyclinic in Wroclaw, Poland, participated in this study. These individuals underwent a clinical examination using the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) protocol and completed 2 validated questionnaires, namely the Satisfaction With Life Scale (SWLS) and the Pittsburgh Sleep Quality Index (PSQI). Furthermore, the patients were assessed for the severity of masseter muscle pain (MMP) and temporal muscle pain (TMP), and the average pain in these muscles (AMP) was calculated. Subsequently, a statistical analysis was performed on the collected data.

Results. The group of patients with average satisfaction with life exhibited significantly higher levels of MMP ($p = 0.025$) and AMP ($p = 0.044$) as compared to the high-satisfaction group. Regarding sleep quality, 50.23% of the patients experienced poor sleep quality. Poor sleep quality was found to be statistically associated with higher levels of TMP ($p = 0.032$) and AMP ($p = 0.028$). Moreover, women demonstrated significantly worse sleep quality as compared to men ($p = 0.002$). The findings indicate that PSQI has a greater impact on SWLS than vice versa.

Conclusions. Due to a large number of TMD patients experiencing poor sleep quality and the associated reduced life satisfaction, these parameters should be considered as influential factors that modify the management of patients with TMD.

Keywords: satisfaction with life, sleep quality, temporomandibular disorders, orofacial pain, masticatory muscle pain

Introduction

"Temporomandibular disorders" (TMD) is an umbrella term that refers to a group of conditions involving the masticatory muscles, the temporomandibular joints (TMJs) and the associated structures.[1] The typical manifestations of TMD include masticatory muscle pain or TMJ pain, limited jaw movement, joint sounds, such as clicking or crepitus, myofascial pain, and headaches.[2] Temporomandibular disorders are a pervasive issue with significant implications for the quality of life, characterized by chronic pain and restrictions in daily activities. Moreover, the presence of elevated levels of anxiety, perceived stress, and even depression, commonly associated with TMD, poses a serious health problem that can impair work performance and result in work absences.[3] It is considered the 2nd most common cause of oral region pain, following odontalgic pain.[4] Additionally, it ranks as the 2nd main cause of musculoskeletal pain after chronic low-back pain.[5]

The prevalence of TMD is estimated to be between 5% and 12% in the general population,[6] with a higher incidence of up to 30% among young adults.[7] In the Polish population, approx. 55.9% of individuals experience at least one symptom of TMD.[8] Gender differences are apparent, with women more commonly affected by TMD in the Polish population.[8–10]

The etiology of TMD is multifactorial and often unclear.[2] The current biopsychosocial model encompasses various conditions and factors that may contribute to the occurrence of TMD.[9] It incorporates psychosocial aspects, such as stress reactions, environmental factors, emotions, and cognition, with biological components, including structural and functional abnormalities.[1] Numerous studies have demonstrated an association between TMD and psychological distress.[11–15] A recent study conducted on a Polish population of TMD patients revealed a high prevalence of stress, anxiety and depression within the study group.[13] Other studies indicate that these psychological factors can be risk factors for the development of TMD.[14] Chronic TMD are also known to coexist with psychosocial impairments, such as depression and somatization.[16] Importantly, some TMD patients may exhibit reduced treatment responsiveness due to their mental state. Studies suggest that patients with high levels of catastrophizing thinking are less likely to respond to treatment unless psychotherapy is utilized.[17] The same applies to patients with depression and anxiety.[13,18] As a result, TMD patients are at risk of experiencing a significant reduction in their quality of life and social functioning.[3,15]

Many studies indicate that TMD may have a negative effect on patients' lives, manifested as chronic pain, the loss of energy, activity restriction (inability) due to physical ailments, emotional disorders, anxiety/depression, taste changes, discomfort when eating, and absence from work due to chronic pain.[3] Consequently, the present study aimed to assess the level of satisfaction with life in Polish patients with TMD and to investigate the influence of TMD-related pain on this parameter. Additionally, since there is evidence suggesting an association between TMD and sleep quality,[19–23] our secondary goal was to assess the relationship between sleep quality and masticatory muscle pain in Polish patients with TMD. The reason for undertaking this research is the lack of studies pertaining to the adult Polish population with TMD, using reliable methodological tools. The existing number of studies conducted with rigorous methodologies is limited, thus necessitating high-quality research.

Material and methods

Participants and the study design

The study group comprised 219 adults who sought treatment at the Outpatient Clinic for Temporomandibular Disorders at the University Dental Polyclinic in Wroclaw, Poland. The study adhered to the principles outlined in the Declaration of Helsinki, and the research protocol obtained approval from the Bioethical Committee of Wroclaw Medical University (No. KB-165/2021). Furthermore, this observational, cross-sectional study was retrospectively registered in the ClinicalTrials.gov database on January 6, 2022, and received the registration number NCT 05183503.

Inclusion and exclusion criteria

The following inclusion criteria were established for the study group: a diagnosis of TMD based on the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) examination[24]; and an age of 18 years or older.

The exclusion criteria for the study were as follows:

- neurological conditions, such as cerebrovascular and neurodegenerative diseases, post-toxic changes, and inflammatory changes; systemic diseases, including rheumatoid arthritis, systemic lupus erythematosus and primary Sjögren's syndrome; toxic or traumatic diseases related to head or craniofacial trauma;
- chronic use of medications that can affect neuromuscular function or the activity of the central nervous system (CNS), such as neuroleptics, analgesics, antiepileptic drugs, psychostimulants, steroids, sedatives, and hypnotics;
- history of psychiatric diseases, such as anxiety, depression, attention deficit hyperactivity disorder (ADHD), autism, and schizophrenia, as well as sleep disorders;
- cognitive impairment;
- active oncological disease;
- underweight (body mass index (BMI) <18.5 kg/m^2) or obesity with BMI ≥ 35 kg/m^2; and
- pregnant women, mothers of infants, and lactating women.

Data collection

Data was collected from November 2018 to December 2020; during that period, the patients were requested to complete questionnaires. Following the completion of the questionnaires, clinical examinations of TMJs and the masticatory muscles were conducted. The questionnaires administered to the patients included the Satisfaction With Life Scale (SWLS) and the Pittsburgh Sleep Quality Index (PSQI). Detailed descriptions of these questionnaires are provided later in the text. A dentist with a minimum of 5 years of practice and trained in the DC/TMD protocol conducted the clinical examinations. The patient diagnoses were assigned separately for each side, and multiple diagnoses were possible for each side.[24]

Clinical examination of the masticatory muscles and the temporomandibular joints (DC/TMD)

The DC/TMD protocol is a standardized tool utilized worldwide for assessing disorders related to the masticatory muscles and TMJs. It consists of 2 axes – Axis I focuses on diagnosing biological factors through physical examinations, while Axis II involves the assessment of psychosocial factors by using validated questionnaires. The protocol structure allows a comprehensive evaluation of patients, aligning with the biopsychosocial model.

Following the completion of the questionnaires, the participants underwent evaluation by experienced dentists with a minimum of 5 years of practice. All the examiners performing the assessments underwent training and calibration according to the protocol provided on the official website of the International Network for Orofacial Pain and Related Disorders Methodology (https://ubwp.buffalo.edu/rdc-tmdinternational). The training was conducted by a clinician with 10 years of experience in managing TMD. The diagnoses were assigned independently for each side, adhering to the official DC/TMD taxonomy.[24,25]

Assessment of the masticatory muscle pain severity

During the clinical examination, each patient was requested to provide a rating for the perceived pain experienced during the palpation of the masseter and temporal muscles on each side individually. Subsequently, the average of these ratings was calculated. The numeric rating scale (NRS), a widely utilized and well-validated tool for measuring pain intensity, was employed for this purpose. The scale ranges from 0 to 10, with 0 indicating no pain and 10 representing the worst imaginable pain.[26] In his study, the severity of masseter muscle pain (MMP) and temporal muscle pain (TMP), as well as the average muscle pain (AMP) were calculated.

Questionnaires

Satisfaction With Life Scale

This questionnaire serves as a simple yet reliable instrument for assessing life attitudes. It consists of 5 questions, and patients assign a score ranging from 1 to 7 for each question. A score of 1 signifies 'strongly disagree', while a score of 7 represents 'strongly agree'. The total score is obtained by summing the scores for all questions. The lowest possible score is 5, while the maximum score is 35. In this questionnaire, the scores were categorized into STEN groups: STEN 1–4 (5–17 points) indicates low life satisfaction; STEN 5–6 (18–23 points) indicates average life satisfaction; and STEN 7–10 (24–35 points) indicates high life satisfaction.[27]

Pittsburgh Sleep Quality Index

This questionnaire serves as a self-report tool for assessing sleep quality over a 1-month interval. It comprises 19 items creating 7 components that measure various aspects of sleep, including subjective sleep quality, sleep latency, sleep disturbances, and sleep duration, among others. Each component is rated on a scale from 0 to 3. The scores for all components are summed, resulting in a maximum score of 21. A cutoff point of 5 was established, where higher scores indicated poorer sleep quality. The questionnaire used has demonstrated reliability as a tool for sleep assessment.[28]

Sample power and size calculation

The sample size was calculated using G*Power, v. 3.1.9.7 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The determination aimed for a statistical power of 0.8, an effect size of 0.22 for correlations between SWLS and NRS AMP and an effect size of 0.40 for correlations between PSQI and NRS AMP. It was ascertained that the sample size needed was 207 people for the 1st dependency and 208 people for the 2nd one. Therefore, 219 patients were qualified for the study to be able to detect significant differences and correlations between the studied parameters. The process of recruiting patients for the study is presented in Fig. 1.

Statistical analysis

Statistical analysis was conducted using the Statistica software, v. 13 (StatSoft Polska, Cracow, Poland), with a significance level set at 0.05. The distribution of data was assessed using the Shapiro–Wilk test. Categorical data were presented as number and percentage (*n* (%)). The NRS values for the masseter and temporal muscles were computed as the average of the values obtained from

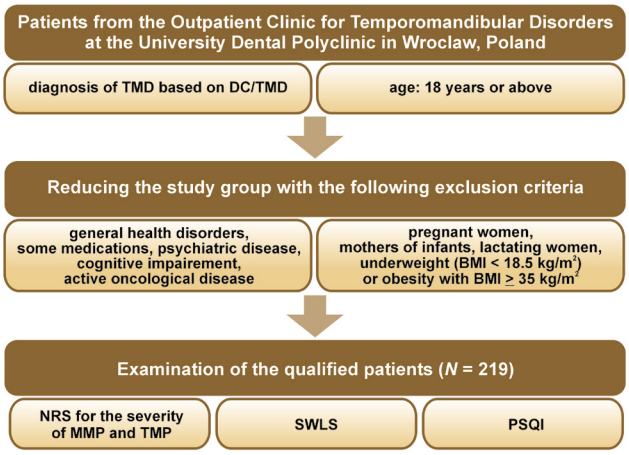

Fig. 1. Flowchart of the recruitment process

TMD – temporomandibular disorders; DC/TMD – Diagnostic Criteria for Temporomandibular Disorders; BMI – body mass index; NRS – numeric rating scale; MMP – masseter muscle pain; TMP – temporal muscle pain; SWLS – Satisfaction With Life Scale; PSQI – Pittsburgh Sleep Quality Index.

the right and left sides. Consequently, the relationship between the NRS values and the TMD diagnosis was examined using the Mann–Whitney *U* test. The same approach was employed to analyze the association between the pain levels and gender.

Results

Sample characteristics

The study involved a sample of 219 Caucasian Polish adults diagnosed with TMD. Among the participants, 163 (74%) were women and 56 (26%) were men. The age range of the participants was 18–80 years, with a mean age of 40.06 ±16.37 years. Most of the group was comprised of women, with a female-to-male ratio of 3:1. Statistically significant differences were observed between women and men in terms of age. The mean age for women was 42 ±16.95 years, while the mean age for men was 34 ±13.17 years.

Distribution of temporomandibular disorders

The distribution of diagnoses among the participants was as follows: myalgia was the most common diagnosis, being predominant in both women and men; the 2nd most frequent diagnosis was disk displacement with reduction, which was also dominant in both genders; and the 3rd most common disorder was arthralgia. It is worth noting that a single patient could have multiple DC/TMD diagnoses. More specific information regarding the distribution of diagnoses by gender can be found in Table 1.

In terms of correlation between the DC/TMD diagnosis and the age of the patients, no statistically significant relationships were observed ($p > 0.05$).

Table 1. Distribution of temporomandibular disorders (TMD) diagnoses by gender

TMD diagnosis	Women (*n* = 163)	Men (*n* = 56)	Total (*N* = 219)
Myalgia	120 (73.62)	31 (55.36)	151 (68.95)
Myofascial pain	21 (12.88)	3 (5.36)	24 (10.96)
Disk displacement with reduction	60 (36.81)	13 (23.21)	73 (33.33)
Disk displacement without reduction	4 (2.45)	2 (3.57)	6 (2.74)
Arthralgia	38 (23.31)	10 (17.86)	48 (21.92)
Osteoarthritis and other degenerative changes	13 (7.98)	2 (3.57)	15 (6.85)
Subluxation	4 (2.45)	2 (3.57)	6 (2.74)

Data presented as number (percentage) (*n* (%)).

Pain intensity (NRS)

The results of the Mann–Whitney *U* test indicated that women had significantly higher pain values as compared to men in all assessed parameters ($p < 0.05$). These findings are illustrated in Fig. 2–4.

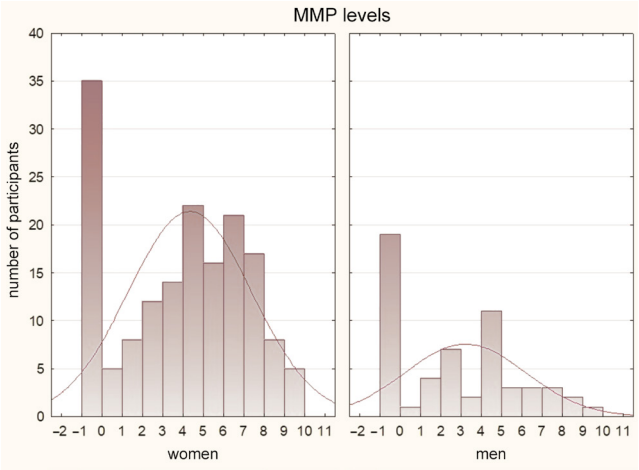

Fig. 2. Distribution of the levels of pain in the masseter muscle by gender

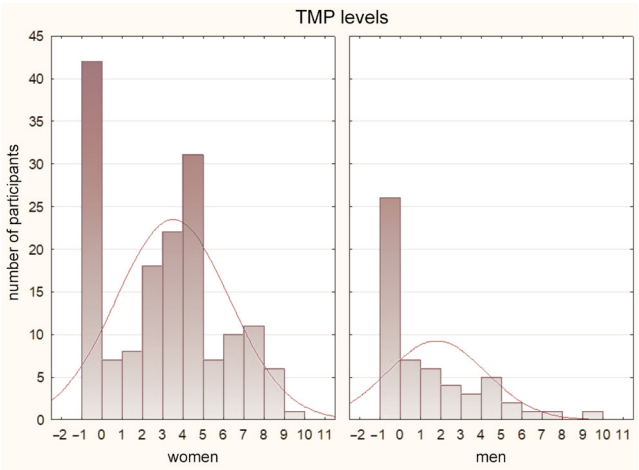

Fig. 3. Distribution of the levels of pain in the temporal muscle by gender

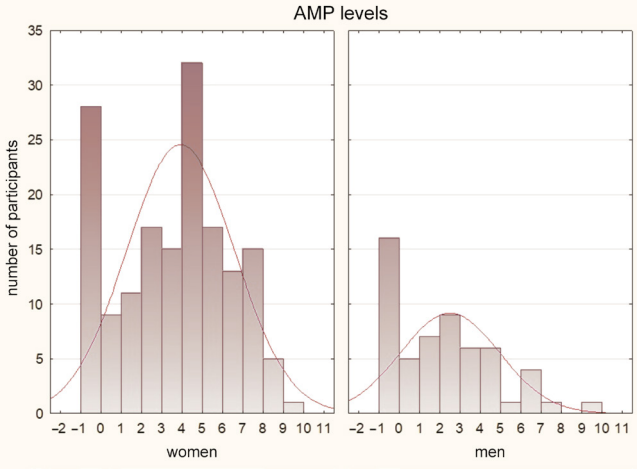

Fig. 4. Distribution of the average muscle pain (AMP) with regard to the masseter and temporal muscles by gender

Regarding the analysis of the relationship between age and pain, the Kendall τ test was conducted, revealing no significant correlation between these 2 variables ($p > 0.05$).

In terms of DC/TMD diagnosis, several significant results were obtained. The Mann–Whitney *U* test demonstrated significantly higher levels of pain in the diagnosis of myalgia for MMP, TMP and AMP. For the diagnosis of myofascial pain, significantly higher pain values were observed for TMP and AMP. In the case of disk displacement without reduction, significantly higher pain results were found for MMP and AMP. However, there were no significant differences in the pain values among the other diagnoses ($p > 0.05$). Detailed results of the statistical analysis can be found in Table 2.

Satisfaction With Life Scale

In this questionnaire, 60% of the participants ($n = 132$) achieved STEN 7–10 indicating high life satisfaction. In 28% of the participants ($n = 61$), STEN 5–6 was obtained, representing average satisfaction with life, while only 12% of the participants ($n = 26$) achieved STEN 1–4 indicating low life satisfaction.

Table 2. Statistical analysis (*p*-values) of the relationship between the DC/TMD diagnosis and the pain levels

TMD diagnosis	MMP	TMP	AMP
Myalgia	<0.0001*	<0.0001*	<0.0001*
Myofascial pain	0.076	0.006*	0.017*
Disk displacement with reduction	0.736	0.612	0.565
Disk displacement without reduction	0.031*	0.091	0.041*
Arthralgia	0.386	0.722	0.415
Osteoarthritis and other degenerative changes	0.745	0.918	0.849
Subluxation	0.911	0.062	0.278

DC/TMD – Diagnostic Criteria for Temporomandibular Disorders;
* significantly higher results.

To investigate the relationship between the SWLS scores and gender, Somers' *D* test was performed, revealing no significant correlation between these variables (d(X|Y) = 0.12664). Furthermore, the Kruskal–Wallis analysis of variance (ANOVA) was conducted and showed no significant differences between the STEN groups ($p = 0.529$).

To assess the correlation between the specific TMD diagnoses and the SWLS scores, Somers' *D* test was conducted. This test, being asymmetrical, enables the analysis of the influence of the TMD diagnosis on the SWLS score and vice versa. However, no dependencies were found in either direction. None of the results exceeded the cutoff value of 0.4, which was considered significant.

The statistical analysis regarding pain was performed using the Kruskal–Wallis ANOVA. This analysis revealed a significant difference between the STEN 5–6 and STEN 7–10 groups in terms of MMP ($p = 0.025$) and AMP ($p = 0.044$). In both cases, the STEN 5–6 group exhibited higher pain values. However, no significant differences were observed between the STEN 1–4 group and the other groups ($p > 0.05$). It is worth noting that the lack of significance may be attributed to the small number of participants in this section ($n = 26$).

Pittsburgh Sleep Quality Index

Out of the study group, 217 participants completed the questionnaire correctly. Regarding the cutoff point, the participants were almost evenly split. A score below 5 was obtained by 108 patients (49.77%), while a score above 5 was acquired by 109 patients (50.23%). This indicates that over half of TMD patients experience poor sleep quality.

To analyze the relationship between age and the PSQI results, the Mann–Whitney *U* test was employed. However, no significant differences between the groups was found ($p = 0.226$). In terms of gender, the χ^2 test was conducted, revealing that women scored significantly higher in PSQI as compared to men ($p = 0.002$). Figure 5 illustrates this difference, using a box-whisker chart.

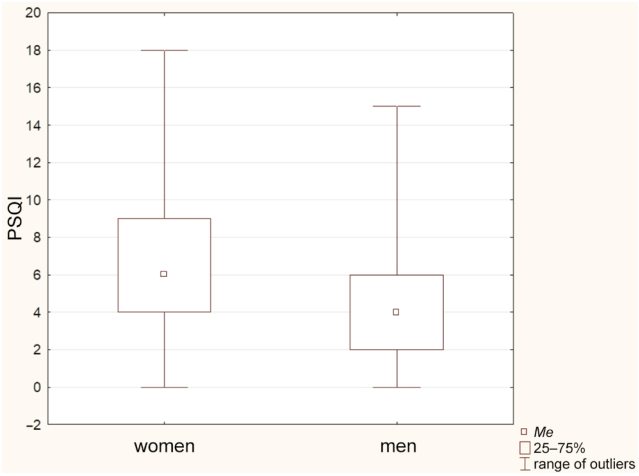

Fig. 5. Box-whisker chart of the Pittsburgh Sleep Quality Index (PSQI) results by gender

Me – median.

To assess the relationship between the specific TMD diagnoses and the PSQI scores, the χ^2 test was employed. However, no significant correlation was found for any of the TMD diagnoses ($p > 0.05$).

In terms of relationship between pain and the PSQI scores, the Mann–Whitney U test was conducted. The results demonstrated that participants with a PSQI score above 5, indicating worse sleep quality, exhibited higher levels of TMP ($p = 0.032$) and AMP ($p = 0.028$). Although not statistically significant, there was a close approach to significance for MMP ($p = 0.059$).

Satisfaction With Life Scale vs. Pittsburgh Sleep Quality Index

To examine the relationship between PSQI and SWLS in the study group, Somers' D test was conducted. The analysis revealed a significant correlation between these 2 variables. The relationship was negative and moderate ($X|Y = -0.4150$), indicating that worse sleep quality was associated with lower scores on SWLS. As Somers' D test is asymmetrical, it was possible to determine the reverse relationship as well. However, a lesser correlation was obtained ($Y|X = -0.3807$), which did not exceed the level of significance. These results suggest that the impact of PSQI, representing the quality of sleep reported by the patient, on the level of life satisfaction is greater than the reverse relationship.

These findings confirm the earlier conclusions drawn from the relationships between the pain levels, sleep quality and satisfaction with life. It can be concluded that the level of satisfaction with life in TMD patients is influenced by various factors, including the experienced pain levels and factors such as sleep quality. This is particularly important, as it explains why the level of pain experienced is not always the sole determinant of the treatment response. Other accompanying symptoms, such as sleep disorders, stress, anxiety, and depression, may play a role in the patient's response to therapeutic interventions. Therefore, additional approaches, such as psychotherapy, may be beneficial for patients in this group. The distribution of the SWLS levels with regard to PSQI is presented in Fig. 6.

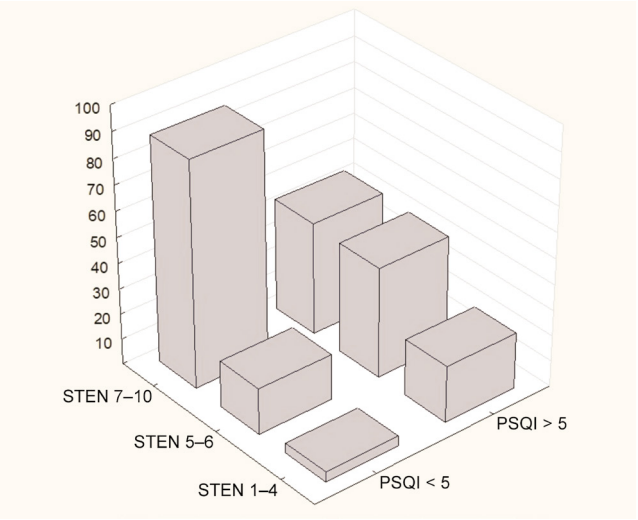

Fig. 6. Two-dimensional chart of the distribution of the Satisfaction With Life Scale (SWLS) and Pittsburgh Sleep Quality Index (PSQI) scores

Discussion

This study aimed to investigate the level of satisfaction with life and sleep quality in adult patients with TMD. The clinical examination of the patients was based on the standardized DC/TMD protocol, which is considered the gold standard in TMD assessment. To measure life satisfaction and sleep quality, self-report questionnaires were utilized, namely SWLS and PSQI. These questionnaires have been widely and successfully used in previous studies.[19,29,30]

In this study group comprising TMD patients, most of the participants were female, which aligns with findings from other studies on TMD.[31,32] Statistical analysis revealed that women reported significantly higher pain levels as compared to men. This observation may be attributed to the fact that myalgia, which is the most common type of TMD pain, is also more prevalent in women than in men.[8] Notably, myalgia was also the most frequently diagnosed condition in this study group. Previous research by Tuuliainen et al. emphasized the association between muscle pain and psychological distress.[33] Studies have shown that women are more likely to have higher levels of depression and anxiety, placing them at a higher risk for muscle-related TMD.[13,34,35] Hormone fluctuations, biological differences and greater pain sensitivity in women may contribute to the higher prevalence of various TMD types among females as compared to males.[6,36]

It is indeed interesting that despite myalgia being the most prevalent pain-related TMD type in this study, no correlation with satisfaction of life was observed. This finding contrasts with a different study, which indicated a significantly higher presence of myalgia in patients with depression.[13] Although different questionnaires are used to measure these parameters, depression is generally associated with lower satisfaction with life. Another study by Boggero et al. observed significantly lower satisfaction with life in patients with myogenic TMD in Kentucky, USA.[19] Interestingly, including the pain levels in the analysis weakened, but did not eliminate the effect, suggesting that a decrease in satisfaction with life in TMD patients may be connected not only to the pain levels.

On the other hand, in the present study, the levels of pain in the masticatory muscles were significantly lower in the STEN 7–10 group as compared to the STEN 5–6 group, indicating that the intensity of painful sensations in these structures influenced satisfaction with life. This suggests that the diagnosis itself may not have as much

impact on the quality of life as the level of pain experienced. The STEN 1–4 group did not show a significant difference as compared to the STEN 7–10 group, but as mentioned earlier, this group had only 26 participants. Since the methodology used in the abovementioned studies was similar, the observed differences may be attributed to regional and sociological factors within the study populations.[13,19]

The question arises as to why the relationship between the TMD diagnosis and satisfaction with life is not as straightforward as expected. Based on our research, it can be concluded that the level of satisfaction with life in TMD patients is influenced not only by the presence of a specific diagnosis and the intensity of pain experienced, but also by other factors, such as anxiety, depression, stress, and sleep quality. The previously cited studies lead to similar conclusions.[13,19]

Regarding sleep quality, the present study demonstrated that more than half of the participants experienced poor sleep quality. These findings are consistent with previous research on this topic.[37–42] Benoliel et al. observed that 43.3% of TMD patients reported poorer sleep quality as compared to 28.3% in the control group.[37] Additionally, Sanders et al. found that participants with poor sleep quality had approximately twice the incidence rate of TMD as compared to those with good sleep quality in their cohort study.[38]

The conclusion drawn from this study aligns with the results of previous research investigating sleep quality in TMD patients.[37–43] Sleep quality is an essential factor that, alongside pain and the psycho-emotional state of TMD patients, influences the reported complaints and modifies the treatment approach. This finding is of great importance for healthcare practitioners who treat TMD patients, as they should consider various comorbidities, including sleep disturbances.

Regarding specific TMD types, some authors have reported that patients with myofascial pain have poorer sleep quality as compared to patients with TMJ pain and controls.[44,45] In this study, no significant correlation was found between the specific TMD diagnoses and sleep quality. However, the group of patients experiencing more severe pain, particularly in the temporal muscle, demonstrated poorer quality of sleep. These results are consistent with the findings of Benoliel et al.[37] A conclusion that can be drawn from these findings is that the level of pain is the most significant factor influencing sleep quality, rather than the specific TMD diagnosis. Differences in outcomes may arise from the fact that most participants in this study were diagnosed with myalgia, which differs from myofascial pain (which Lei et al. focused on in their study[44]) in terms of pain radiation patterns.[24]

Yap et al. concluded that, in general, patients with pain-related TMD experienced decreased sleep quality.[29] Specifically, in their study, patients with myalgia demonstrated greater sleep impairment as compared to those with intra-articular disorders.[29] Other studies have also suggested that musculoskeletal pain, not only in the temporomandibular region, is associated with poorer sleep quality, and this association is complex and influenced by various factors.[41,46] Conversely, poorer sleep quality can provoke and exacerbate head and orofacial pain syndromes, as sleep deprivation is known to affect pain perception.[47] Psychological distress, including depression and anxiety, is considered a factor that influences sleep quality.[39]

Interestingly, in our previous research, we found that 54.8% of TMD patients experienced depression.[13] This is a similar percentage to the number of patients with poor sleep quality in the present study, indicating a potential link between depression, sleep disturbances and TMD. The role of depression as a contributing factor in sleep disturbances was also suggested by Dubrovsky et al.[42]

The results of the present study indicate that women are more likely to experience poorer sleep quality as compared to men. This finding is consistent with previous studies on TMD patients, which have also observed a higher prevalence of depressive symptoms in women as compared to men. Given that depression can affect both TMD and sleep quality, these results contribute to a comprehensive understanding of the interconnections between these factors.

The relationship between TMD and sleep quality is indeed complex and influenced by multiple factors, including muscle pain and the psycho-emotional state of individuals. A study by Machado de Resende et al. showed that the TMD management involving occlusal splints, physical therapy and counseling could lead to improvement in sleep quality and the overall quality of life, supporting the notion that addressing TMD-related factors can have a positive effect on sleep.[40]

These findings contribute to the growing body of literature highlighting the prevalence of poor sleep quality as a common symptom in TMD, emphasizing the importance of sleep assessment in the diagnostic process of TMD.[29,30,43]

In this study, a significant correlation between sleep quality and satisfaction with life was observed. This finding aligns with previous research indicating an interaction between these 2 parameters. A study by Steptoe et al. demonstrated that individuals with poorer sleep quality reported lower levels of life satisfaction and experienced more negative emotions daily, including emotional stress and anxiety.[46] In this study, we observed that sleep quality had a more significant impact on the perceived satisfaction with life than the other way round. However, it is important to note that only 12% of the patients in the study described their level of satisfaction with life as low. Most patients in the study group reported high (60%) or moderate (28%) life satisfaction.

Therefore, we can conclude that while pain is one component that affects the perceived satisfaction with life, it is

not the sole factor. Patients with moderate satisfaction with life may experience higher levels of MMP as compared to those with high satisfaction with life. However, life satisfaction is also influenced by other factors, such as the levels of the perceived stress, anxiety and depression. Hence, it is crucial to consider these additional modulating factors and implement a differentiated approach to TMD management for patients with lower life satisfaction.

Limitations

The existing literature suggests a connection between muscle pain and psychological distress. Hence, questionnaires focusing on the psycho-emotional well-being of participants, such as the Patient Health Questionnaire-9 (PHQ-9) or the Generalized Anxiety Disorder-7 (GAD-7) questionnaire, could serve as valuable tools for addressing this matter. In this study, muscle pain was evaluated using NRS, which involved applying pressure to the muscles. To enhance precision and objectivity, the muscle pain threshold could be measured using an algometer. Additionally, the absence of a control group could be perceived as a limitation of the study. Another limitation of the study is the fact that no instrumental analysis of sleep was performed, and patients with sleep deprivation, e.g., shift workers with circadian rhythm disorders, were not excluded.

Conclusions

The conclusions drawn from our research indicate that screening for sleep quality and assessing satisfaction with life is a very important part of clinical evaluations with regard to Polish adults with TMD. Considering these factors in the diagnosis and management protocols can have a significant impact on pain intensity, pain-related disability and the patient's response to TMD treatment. The questionnaires utilized in this study offer clinicians the means to identify patients with sleep disturbances or diminished life satisfaction, enabling specialized care and ensuring more effective assistance through multimodal management in this patient population.

Trial registration

The study was retrospectively registered in the ClinicalTrials.gov database on January 6, 2022, and received the registration number NCT 05183503.

Ethics approval and consent to participate

The research protocol obtained approval from the Bioethical Committee of Wroclaw Medical University, Poland (No. KB-165/2021). The patients provided their written informed consent to participate in the present study.

Data availability

The datasets generated and analyzed during the current study are not publicly available due to privacy and ethical restrictions. However, they can be obtained from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Piotr Seweryn https://orcid.org/0000-0002-0958-575X
Sylwia Magdalena Orzeszek https://orcid.org/0000-0002-3139-5696
Marta Waliszewska-Prosół https://orcid.org/0000-0002-9074-2803
Andrej Jenča https://orcid.org/0000-0002-9297-9134
Magdalena Osiewicz https://orcid.org/0000-0002-8108-4546
Anna Paradowska-Stolarz https://orcid.org/0000-0003-2817-1445
Orit Winocur-Arias https://orcid.org/0000-0002-9905-7263
Marek Ziętek https://orcid.org/0000-0002-3675-9268
Wojciech Bombała https://orcid.org/0009-0001-9989-8337
Mieszko Więckiewicz https://orcid.org/0000-0003-4953-7143

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Original paper

Comparison of the analgesic effects of single-dose 75 mg oral pregabalin versus single-dose 400 mg oral ibuprofen after impacted third mandibular molar surgery: A randomized, double-blind, split-mouth clinical trial

Fatemeh Latifi[1,A,B,E,F], Parsia Choobsaz[2,C,D], Amir-Ali Yousefi-Koma[2,A–F], Hannaneh Yousefi-Koma[3,C,E,F], Mohammad Hassan Mirtaleb[2,C,D]

[1] Department of Oral and Maxillofacial Surgery, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] School of Medicine, Tehran University of Medical Sciences, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Address for correspondence
Amir-Ali Yousefi-Koma
E-mail: amiraliykoma@gmail.com

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Abstract

Background. Pain is the most prevalent complication after dentoalveolar surgery. Failure in effective pain control could potentially lead to systemic sequels, such as tachycardia, hypertension, improper nutrition, and central sensitization. Pregabalin is a gamma-aminobutyric acid (GABA) analog with inhibitory and analgesic effects on the central nervous system (CNS). Prescribing gabapentinoids as complementary analgesics reduces the consumption of opioid and non-opioid analgesics, and consequently their side effects.

Objectives. The main purpose of the present study was to compare the analgesic effects of pregabalin (single-dose 75 mg) vs. ibuprofen (single-dose 400 mg) on patients' pain levels after impacted third mandibular molar surgery.

Material and methods. In this randomized, double-blind, split-mouth clinical trial, 24 patients aged 19–34 years volunteered for 2 consecutive (1 month apart) third mandibular molar surgeries (the contralateral teeth). The patients were randomly placed into 2 groups: group G1 ($n = 12$) was prescribed pregabalin (single-dose 75 mg) after the 1st surgery and ibuprofen (single-dose 400 mg) after the 2nd surgery; and group G2 ($n = 12$) was prescribed the exact opposite of the G1 arrangement. During the first 24 h post-surgery, the patients recorded the number of complementary analgesics they took (single-dose 400 mg ibuprofen) and their level of pain on a visual analog scale (VAS) every 2 h.

Results. The average level of pain at 2 h post-surgery (T1) was significantly lower when pregabalin was prescribed ($p < 0.05$). Most patients needed complementary analgesics at 4 h post-surgery (T2). However, during the first 24 h post-surgery, the patients required significantly more complementary analgesics when ibuprofen was prescribed.

Conclusions. In comparison with oral ibuprofen (single-dose 400 mg), oral pregabalin (single-dose 75 mg) had a stronger analgesic effect at 2 h after impacted third mandibular molar surgery ($p < 0.05$). Pregabalin resulted in a significantly lower consumption of complementary analgesics in the first 24 h post-surgery as compared to ibuprofen.

Keywords: pain, ibuprofen, pregabalin, third mandibular molar surgery

Introduction

Pain is the most prevalent complication after dentoalveolar surgery (~80%),[1] and the practitioner is normally expected to ensure pain control so that it could be acceptable. Failure in effective pain control not only results in patient dissatisfaction and reduced quality of life, but it may also lead to systemic sequels, such as tachycardia, hypertension, improper nutrition, and central sensitization, which develops resistance to the effects of most common analgesic drugs.[2] The injection of long-acting local anesthetics, prescribing nitrous oxide (N_2O),[3] as well as opioid and non-opioid oral analgesics, are some methods to control post-operation pain.[4] However, N_2O can cause nausea, vomiting, headache, drowsiness, and excessive sweating or shivering.[5] Opioids, such as naldemedine, can cause diarrhea,[6] while tramadol can potentially cause seizures.[7] Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, are the most recommended drugs for acute dentoalveolar pain, but they can cause nausea, diarrhea and excessive bleeding.[8]

Gabapentinoids (e.g., gabapentin and pregabalin) are gamma-aminobutyric acid (GABA) analogs with significant anticonvulsant and pain relief effects. Prescribing gabapentinoids as complementary pain relievers reduces the consumption of opioid and non-opioid analgesics, and consequently their side effects.[9]

Pregabalin was initially used to treat muscle spasms, but due to its effects on convulsions, allodynia and hyperalgesia, it has gradually received more attention.[10] Optimal effects on diabetic neuropathic pain, post-herpetic neuralgia and fibromyalgia make this drug more noticeable,[11] and due to its antianxiety effects, along with the prevention of sensitization and resistance to analgesics, pregabalin has become a considerable complementary drug after surgical procedures.[12,13] The post-operation usage of pregabalin is more efficient than pre-surgical prescription,[14] with its analgesic effects (300 mg) after third mandibular molar extraction verified. However, side effects, including dizziness, blurred vision, restlessness, headache, xerostomia, and drowsiness have been reported.[15–17] The preoperative prescription of single-dose pregabalin (75 mg) significantly decreases pain after septorhinoplasty.[18]

Fibromyalgia causes various musculoskeletal and soft tissue pain, as does third mandibular molar surgery. Pregabalin has shown remarkable results when used as a single drug in the treatment of fibromyalgia,[19] and also plays a noticeable role in controlling chronic pain after spinal surgery.[20] Furthermore, pregabalin brings positive effects in relieving post-mastectomy chronic pain (PMCP) and consequent anxiety.[10] However, studies have shown contradictory results with regard to analgesic effects and complications after the consumption of low-dose pregabalin.[14,16]

The main purpose of this randomized, double-blind, split-mouth clinical trial was to compare the efficiency and analgesic effects of oral pregabalin capsules (single-dose 75 mg) vs. oral ibuprofen capsules (single-dose 400 mg) after third mandibular molar surgery. Pregabalin and ibuprofen were both tested on each patient for their effects in reducing pain levels and the need to take supplementary analgesics.

Material and methods

Study design, participants and ethical practices

This randomized, double-blind, split-mouth clinical trial was designed and conducted according to the randomized, double-blind clinical trial protocol and the split-mouth design, and followed the Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines.[21] The Iranian Ministry of Health and Medical Education and the Research Ethics Committee of Dental Research Center at the Shahid Beheshti University of Medical Sciences, Tehran, Iran, approved the trial on February 18, 2017 (IR.SBMU.RIDS.REC.1395.392). The trial was also registered with the Iranian Registry of Clinical Trials (https://www.irct.ir) (IRCT2016122131501N1).

Between December 31, 2017, and December 30, 2020, 95 patients who were candidates for the extraction of their bilateral impacted third mandibular molars visited the Department of Oral and Maxillofacial Surgery of the School of Dentistry at the Shahid Beheshti University of Medical Sciences. All clinical evaluations and third mandibular molar surgical procedures were executed in the above-mentioned department by an oral and maxillofacial surgeon (OMFS). A complete and comprehensive medical history was collected from each patient and documented. Out of the 95 patients, 57 were eligible to take part in our clinical trial, and 24 (13 men and 11 women) volunteered. Figure 1 displays a complete and comprehensive CONSORT flow diagram of the patients' enrollment and allocation. All participants read and signed a patient consent form. The patients were fully informed that their personal information (e.g., first name, last name, phone number, home address, work address, and photographs) would not be reported and/or published anywhere and that they were allowed to leave the study whenever they wanted.

Inclusion criteria

The inclusion criteria were as follows:

- healthy participants classified according to the American Society of Anesthesiologists as class I or II (ASA I/II), candidates for bilateral impacted third mandibular molar extraction; and
- healthy bilateral impacted third mandibular molars with the same level of difficulty regarding the tooth angle, the impaction level and the relation to the anterior ramus border.

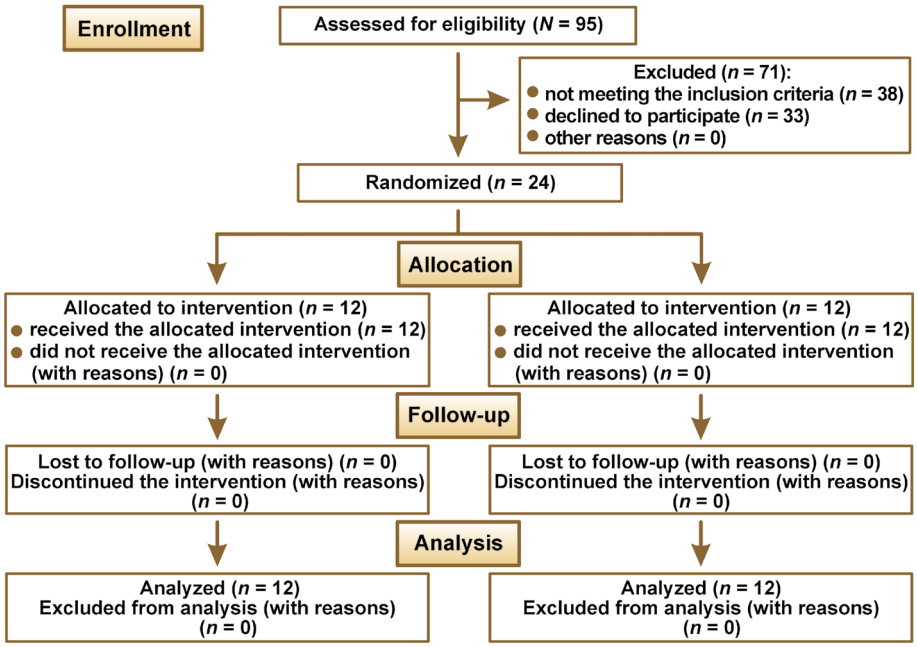

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) 2010 flow diagram

Exclusion criteria

The exclusion criteria were as follows:

– a current lesion surrounding bilateral impacted third mandibular molars;
– a history of chronic use of NSAIDs, pregabalin, sedative drugs, and/or corticosteroids;
– present or past addiction to analgesics;
– the usage of any analgesics 1 week before both surgeries;
– psychological disorders;
– menstruation (to prevent the distortion of the data on the female patients' pain levels in the 2nd half of the menstrual cycle (the luteal phase), the female patients were asked about the regularity and duration of their cycle; by doing so, we were able to schedule both of their surgeries in the 1st half of their cycle);
– a current bone disease;
– current orofacial pain;
– patients with epilepsy; and
– a history of allergies to local anesthesia.

Randomization, allocation concealment and blinding

A person not involved in this study in any way (neither a patient nor a clinician/researcher) was kindly asked to randomize all the 24 participants into 2 groups – group 1 (G1) and group 2 (G2), each with the same number of participants (n = 12) – using a computer-generated system (http://www.jerrydallal.com/random/randomize.htm; seed code: 25592). Group 1 was prescribed oral pregabalin (single-dose 75 mg) (Lyrica™; Pfizer Inc., New York, USA) after the 1st surgery and oral ibuprofen (single-dose 400 mg) (Advil Liqui-Gel™; Wyeth, Madison, USA) after the 2nd surgery. Group 2 was prescribed the exact opposite of the G1 arrangement, receiving oral ibuprofen (single-dose 400 mg) after the 1st surgery and oral pregabalin (single-dose 75 mg) after the 2nd surgery. All surgeons, clinicians and researchers responsible for the design and execution of this clinical trial, as well as all participants, were blinded to the name and type of the drug prescribed.

Interventions

All participants had a digital orthopantogram (OPG) panoramic view of their teeth and jaw taken 1 month before surgery (or more recently). All patients underwent the surgical extraction of impacted third mandibular molars, conducted by an OMFS under local anesthesia. The patients were asked to refrain from taking analgesics 1 week before both surgeries. All 48 surgeries were carried out according to the protocol of the clinical trial executed by Demirbas et al.,[22] with the inferior alveolar nerve block (IANB) (the Halsted technique) and the long buccal nerve block performed using 2 carpules (1.8 mL) of lidocaine (2%) and epinephrine (1:100,000) (Loghman Pharmaceutical and Hygienic Co., Tehran, Iran) for optimum local anesthesia. If the patients were still feeling pain during surgery, a third carpule (1.8 mL) of lidocaine (2%) with epinephrine (1:100,000) was used for another round of IANB. The bone surface was exposed through a sulcular incision with distal extension, using a #15 scalpel blade. The buccal and lingual flaps were displaced and retracted by using a #9 molt periosteal elevator, with the lingual flap retracted to protect the lingual nerve. Third mandibular molars were exposed after proper ostectomy and odontotomy with

the use of fissure and round-shaped burs on high-speed handpieces. Sterile saline was used as a coolant/irrigant throughout the whole surgery. Third mandibular molars were then sectioned using the fissure and round-shaped burs, and extracted using #79 (for lower wisdom teeth) or #222 forceps. The flaps were replaced and sutured in an interrupted style, using braided, non-absorbable 3-0 USP (the United States Pharmacopeia system) silk sutures (Hur Teb, Takestan, Iran) and a reverse cutting needle (19 mm, 3/8 circle).

After the completion of the surgery (T0), either oral pregabalin capsules (single-dose 75 mg) or oral ibuprofen capsules (single-dose 400 mg) (control) were prescribed based on the blinding principle and the randomization performed earlier (G1 and G2). One month after the 1st surgery, the 2nd surgery was performed on the contralateral teeth by the same operator using the same techniques and materials in exactly the same operation room as the 1st surgery. If the patients were prescribed ibuprofen after the 1st procedure, pregabalin was prescribed after the 2nd procedure, and vice versa.

Outcomes

A visual analog scale (VAS) questionnaire was designed to document the patients' post-operative pain severity (from 0 = no pain to 10 = unbearable pain). Identical questionnaires were used after both procedures. Time point T0 demonstrated the time of surgery completion, while T12 was the end point of the first 24 h post-surgery. During the first 24 h post-surgery, the patients recorded the number of complementary analgesics they took (single-dose 400 mg ibuprofen) and their level of pain on VAS every 2 h (T0, T1, T2, T3, T4, T5, T6, and T12). All surgeries were scheduled to be performed somewhere between 8 a.m. and 10 a.m. Therefore, at T6 (12 h post-surgery), most patients would have been asleep, and we asked them to go to bed between 10 p.m. and 11 p.m. so that the results could be comparable. Hence, we did not include the sleeping period (i.e., T7, T8, T9, T10, and T11) in our questionnaires. However, if the patients had any sleeping difficulties and/or unbearable pain that was preventing them from going to sleep, T7–T11 were also completed in their questionnaires. The patients were allowed to use complementary analgesics (single-dose 400 mg ibuprofen) if they experienced moderate to severe pain, which was grade 5 or higher on VAS. The time and amount of the analgesic intake were also recorded.

Statistical analysis

A repeated-measures analysis of variance (ANOVA) model was used to compare the pain distribution between the 2 surgeries for each patient. The level of significance set for the study was 0.05.

Results

Interventions

A single OMFS performed all 48 impacted third mandibular molar surgeries (24 patients, each with bilateral impacted third mandibular molar surgeries, 2 consecutive surgeries, 1 month apart) under local anesthesia. The 2 consecutive surgeries for each patient were performed exactly at a 1-month interval, as planned. None of the surgeries was delayed or performed before the completion of the 1-month gap. None of the patients needed any extra local anesthetics, and the initial 2 carpules (1.8 mL) of lidocaine (2%) with epinephrine (1:100,000) were efficient for all participants. There were no complications during and/or after any of the surgeries.

Participants

All 24 participants had both surgeries completed and were asked to visit the Department of Oral and Maxillofacial Surgery 1 week after each surgery for follow-up. The VAS questionnaires completed by the patients were also collected 1 week after each surgery. None of the patients had any sleeping difficulties, and all reported that they were asleep by 10–11 p.m. None of the patients experienced unbearable pain before, during or after their sleep.

Outcomes

Patients' pain severity

The average levels of the patients' post-operative pain (from 0 to 10 on VAS) at the specified time points (T0–T12) are shown in Table 1 (both surgeries included, G1 and G2 combined). According to the VAS questionnaire, the pain levels differed between the 2 groups at T1 post-surgery. The pregabalin group experienced significantly less pain at T1 ($p < 0.05$). At T1, the average pain levels were 0.52 ±0.87 and 0.80 ±1.12 when pregabalin and ibuprofen were prescribed, respectively. However, at all other time points (i.e., T0, T2, T3, T4, T5, T6, and T12), there were no significant differences between the 2 groups.

Supplementary analgesics

Table 2 shows the total number of supplemental analgesics (single-dose 400 mg ibuprofen) taken by all patients at each time point (both surgeries included, G1 and G2 combined). At 4 h post-surgery (T2), most of the patients experienced moderate to severe pain and took supplemental analgesics (single-dose 400 mg ibuprofen). At T2, 43 supplementary analgesics were taken – 20 when pregabalin (single-dose 75 mg) was prescribed and 23 when ibuprofen (single-dose 400 mg) was prescribed. At 6 h post-surgery (T3), 19 supplementary analgesics were

Table 1. Average levels of post-operative pain severity on a visual analog scale (VAS) in the participants after the completion of both surgeries

Time point	Pregabalin (single-dose 75 mg) (G1 and G2 combined)	Ibuprofen (single-dose 400 mg) (control) (G1 and G2 combined)	*p*-value
T0	0.24 ±0.66	0.28 ±0.74	0.714
T1	0.52 ±0.87	0.80 ±1.12	0.016*
T2	5.96 ±1.31	5.88 ±1.42	0.692
T3	4.12 ±1.51	3.92 ±1.15	0.446
T4	1.84 ±1.43	1.96 ±1.67	0.694
T5	0.64 ±0.76	0.64 ±0.76	1.000
T6	0.56 ±0.77	0.44 ±0.77	0.574
T12	0.36 ±0.49	0.32 ±0.63	0.802

Data presented as mean ± standard deviation (*M* ±*SD*). G1 – group 1 (the patients who were prescribed pregabalin after the 1st surgery and ibuprofen after the 2nd surgery); G2 – group 2 (the patients who were prescribed ibuprofen after the 1st surgery and pregabalin after the 2nd surgery); T0–T12 – immediately and every 2 h post-surgery; VAS scoring – from 0 = no pain to 10 = unbearable pain; * statistically significant.

Table 2. Total number of supplemental analgesics (single-dose 400 mg ibuprofen) taken by the participants after the completion of both surgeries

Time point	Pregabalin (G1 and G2 combined)	Ibuprofen (G1 and G2 combined)	*p*-value
T0	0	0	1.000
T1	0	0	1.000
T2	20	23	0.018*
T3	7	12	0.050*
T4	2	2	1.000
T5	0	0	1.000
T6	0	0	1.000
T12	0	0	1.000
Total	29	37	0.043*

* statistically significant.

taken, including 7 when pregabalin was prescribed and 12 when ibuprofen was prescribed. At 8 h post-surgery (T4), 4 supplementary analgesics were taken, with 2 taken when pregabalin was prescribed and 2 when ibuprofen was prescribed. None of the patients required supplemental analgesics at any other time point (i.e., T0, T1, T5, T6, and T12).

The patients who were prescribed ibuprofen (single-dose 400 mg) needed more complementary analgesics during the first 24 h post-operation as compared to those with the prescribed pregabalin (single-dose 75 mg) ($p < 0.05$). The number of supplemental analgesics taken at T2 and T3 was significantly lower for the pregabalin group than for the ibuprofen group. In total, the patients needed 29 supplemental analgesics after pregabalin and 37 after ibuprofen during the first 24 h post-surgery.

The data extracted from the patients' VAS questionnaires and the recorded documents showed that one of the patients did not need any supplemental analgesics at any time point after both surgeries. All patients declared that even if they needed to, they would take only one supplemental analgesic (single-dose 400 mg ibuprofen). Therefore, at all time points, the number of supplemental analgesics shows the exact number of patients that used them (Table 2).

Discussion

Post-surgical pain and discomfort affect patients' quality of life and satisfaction with treatment. Tissue trauma during surgery and tooth extraction causes cellular damage and inflammation.[23] Phospholipase A2 (PLA2) enzymes catalyze the hydrolysis of cell membrane phospholipids, resulting in cyclooxygenase-derived leukotrienes and lipoxygenase-generated prostaglandins.[24] Non-steroidal anti-inflammatory drugs have strong peripheral and anti-inflammatory effects, and can manage chronic pain perfectly. Ibuprofen, a member of the NSAID family, suppresses the synthesis of powerful inflammatory mediators, but also inhibits platelet attachment and extends the bleeding time.[25]

Gamma-aminobutyric acid analogs is a family of inhibitory neurotransmitters (e.g., gabapentin and pregabalin). Their exact mechanism of action is not clear, though pregabalin is known to diminish CNS irritability by affecting voltage-dependent calcium (Ca) channels and reducing the release of glutamate, noradrenaline and substance P. The drug is completely absorbed after oral administration, distributed to most tissues, and it crosses the blood–brain barrier (BBB). Pregabalin has a 6-hour half-life and minimum metabolism in the liver, resulting in a very low rate of drug interactions. More than 90% of pregabalin is excreted by the kidneys without any change.[26] Pregabalin exerts its analgesic effect by manipulating voltage-dependent Ca and potassium (K) channels, which guide inflammatory amino acids.[26]

This double-blind, randomized clinical trial was designed to examine differences between the analgesic effects of oral pregabalin capsules (single-dose 75 mg) and oral ibuprofen capsules (single-dose 400 mg) after third mandibular molar surgical extraction. When pregabalin was prescribed, the patients experienced significantly less pain only 2 h post-surgery (T1) as compared to ibuprofen ($p < 0.05$). However, during the remaining 22 h, no significant differences were observed between the 2 groups. Tiippana et al. reviewed the effects of gabapentin, pregabalin and narcotic drugs on post-surgical pain; their results corroborate our findings, though the optimum dosage was not reported.[17]

Generalized anxiety disorder (GAD) and CNS sensitivity exacerbate post-surgical pain. Clinical studies show that prescribing GABA analogs before surgery decreases the release of glutamate – an excitatory neurotransmitter – and reduces patients' anxiety. According to this, pregabalin declines pain intensity, opioid consumption and drug adverse effects after surgery.[12,13]

In many clinical cases, pregabalin has shown a better analgesic effect than NSAIDs.[26] In this clinical trial, we found that pregabalin produced better results during the first 2 h. Pereira-Santos et al. claimed that diminishing the anxiety of patients undergoing dentoalveolar surgery significantly decreased pain.[27] Meanwhile, Hill et al. discovered that 300 mg pregabalin resulted in remarkably less pain than 50 mg pregabalin in molar extraction; however, the side effects of high-dose pregabalin were not considered.[28]

Paech et al. declared that single-dose pregabalin (50 mg) had no significant analgesic effect on acute pain after minor obstetrics and gynecology (OB-GYN) surgeries.[29] Women undergoing OB-GYN surgeries can express various pain levels depending on their pathological backgrounds, allergies, sensitivity, and mental health. Additionally, the prescribed dosage of pregabalin in the abovementioned study[29] (50 mg) was lower than the conventional dose used in similar studies. Furthermore, pregabalin mainly has an analgesic effect on acute bone-derived pain and does not show a significant effect on visceral pain.[29] Controlled-release pregabalin can significantly reduce musculoskeletal pain in fibromyalgia patients.[30] Indeed, Pauer et al. demonstrated that 450 mg of pregabalin per day significantly reduced pain in fibromyalgia patients and improved their sleep quality.[31] In addition, Cheung et al. evaluated pain after third mandibular molar surgery in 40 patients and discovered that prescribing pregabalin (75 mg) post-surgery had a significantly better analgesic effect than pre-operative administration.[14]

Olmedo-Gaya et al. discovered that patients who received 2 doses of pregabalin (75 mg) before and after third molar surgery had a significantly lower demand for supplementary analgesics than the group that did not take pregabalin.[16] Both groups were prescribed acetaminophen (650 mg) every 8 h for the first 48 h.[16]

In this randomized clinical trial, we discovered that patients in both groups started consuming supplementary analgesics (single-dose 400 mg ibuprofen) 4 h after the operation. This time coordinates with the half-life of the local anesthetic drug administered and a significant decrease in blood lidocaine concentration.[16] There was a significant difference in supplementary analgesic demand, with the control group requiring more supplementary analgesics (single-dose 400 mg ibuprofen) in the first 24 h post-surgery.

Limitations

The study limitations are as follows:

– determining the pain level was a challenging process for some patients;
– the anxiety rates had a direct effect on the patients' perception of pain, and sometimes led to exaggeration and inaccuracy in the VAS records; and
– finding patients who had the same level of impaction in their third mandibular molars was time-consuming.

Recommendations

More randomized clinical trials should be designed to investigate these findings further.

We suggest designing supplementary studies with more participants in all study groups and an additional focus on patients' mental health. The patients' stress and anxiety levels caused by the fear of surgery significantly affected their perception of pain. A section of the questionnaire could be dedicated to patients' stress and anxiety levels.

Patients could be studied for an extended period of time, with pregabalin being prescribed for days after surgery.

The effect of pregabalin and its superiority/inferiority with regard to NSAIDs could be examined in other oral surgeries (e.g., dental implants, pathologies and soft tissue surgeries).

Pregabalin could be compared with other analgesics, including combinational drugs.

Conclusions

Oral pregabalin (single-dose 75 mg) had a better analgesic effect in the first 2 h after impacted third mandibular molar surgery than oral ibuprofen (single-dose 400 mg) ($p < 0.05$). Furthermore, pregabalin (single-dose 75 mg) significantly decreased the need for supplemental analgesics during the first 24 h post-surgery as compared to ibuprofen (single-dose 400 mg).

Trial registration

The trial was registered with the Iranian Registry of Clinical Trials (https://www.irct.ir) (IRCT2016122131501N1).

Ethics approval and consent to participate

The study was approved by the Iranian Ministry of Health and Medical Education and the Research Ethics Committee of Dental Research Center at the Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RIDS.REC.1395.392). All participants provided written informed consent.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Fatemeh Latifi https://orcid.org/0000-0001-9867-1643
Parsia Choobsaz https://orcid.org/0000-0002-9887-6769
Amir-Ali Yousefi-Koma https://orcid.org/0000-0002-0140-8097
Hannaneh Yousefi-Koma https://orcid.org/0000-0001-7498-301X
Mohammad Hassan Mirtaleb https://orcid.org/0000-0002-2386-5838

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Original paper

Novel association between single-nucleotide polymorphisms of *IKKβ* at rs17875746 and rs12676482 and periodontitis

Athraa Ali Mahmood[1,A–D], Raghad Fadhil Abbas[2,C,E,F], Hashim Mueen Hussein[3,E,F]

[1] Department of Oral Surgery and Periodontology, College of Dentistry, Al-Mustansiriyah University, Baghdad, Iraq
[2] Department of Periodontology, College of Dentistry, University of Baghdad, Iraq
[3] Department of Conservative Dentistry, College of Dentistry, Al-Mustansiriyah University, Baghdad, Iraq

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Address for correspondence
Hashim Mueen Hussein
E-mail: hashimmueenhussein@gmail.com;
hashimmueenhussein@uomustansiriyah.edu.iq

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Abstract

Background. Single-nucleotide polymorphisms (SNPs) in the *IKKβ* gene have been associated with susceptibility to various inflammatory illnesses, including periodontitis.

Objectives. The aim of the present study was to investigate the association between *IKKβ* SNPs (at rs17875746 and rs12676482) and periodontitis in an Iraqi Arab population.

Material and methods. In this case–control study, 94 Iraqi volunteers were split into 2 groups, with the case group including 62 periodontitis patients (37 men and 25 women) and the control group including 32 racially matched healthy people (19 men and 13 women). Periodontal parameters were recorded for each individual. Then, 2 mL of venous blood was taken from each participant to isolate their genomic DNA. In particular, the genotyping of rs17875746 and rs12676482 in *IKKβ* was performed with the use of the polymerase chain reaction (PCR) sequencing methods.

Results. The effect of the distribution of *IKKβ* SNPs on periodontitis was assessed by counting the odds ratio (*OR*), which was 5.264 for rs17875746 and 0.900 for rs12676482. Surprisingly, allele T revealed a significantly higher association with periodontitis for rs17875746 ($OR = 6.750$) than allele G ($p = 0.038$). Overall, the GT genotype in rs17875746 had a higher chance of developing the disease ($OR = 3.321$) as compared to other genotypes. Meanwhile, the GA genotype in rs12676482 had a higher chance of developing the disease ($OR = 1.242$) as compared to other genotypes. In addition, rs17875746 showed a significant positive association with tooth mobility, a family history, clinical attachment loss (CAL), and gingival recession (GR) in the study groups.

Conclusions. The *IKKβ* polymorphisms may increase genetic susceptibility to periodontitis in Iraqi Arab patients.

Keywords: *IKKβ*, periodontitis, single-nucleotide polymorphisms

Introduction

The breakdown of periodontal attachment and alveolar bone resorption are characteristic of the chronic inflammatory disease known as periodontitis. The disease is initiated by microbial plaque and exacerbated by risk factors, such as systemic diseases, genetic factors and environmental factors.[1] It has approx. 50% heritability, meaning that genetic variance influences people's susceptibility to this disease.[2] Specifically, genetic polymorphisms in the molecules involved in the pathogenesis of periodontitis have been linked to an increased risk of periodontitis at an individual level.[3,4]

The transcription factor nuclear factor-kappa B (NF-κB) plays a critical role in regulating the inflammatory processes affecting the gingiva and the alveolar bone in periodontitis.[5] Additionally, NF-κB is a heterodimer comprising the p65 and p50 proteins, which are associated with the inhibitor of NF-κB (IκB) existing in an inactive form inside the cytoplasm. Nuclear factor-kappa B is activated through the phosphorylation of IκB by IκB kinase (IKK) and causes the expression of several selected genes, including those for inflammatory cytokines, such as interleukin-6 (IL-6), IL-1β and tumor necrosis factor-alpha (TNF-α).[6–8] Three subunits make up the IKK complex – IKKα, IKKβ and IKKγ – and each is encoded by a different gene.[9]

IKKβ is essential for activating NF-κB and producing cytokines in response to inflammatory and innate immune stimuli, and is associated with the stimulation of malignancies, such as breast cancer, pancreatic cancer, melanoma, and acute myeloid leukemia. Additionally, it is a crucial mediator of inflammation-induced bone loss (as in periodontitis), a regulator of bone homeostasis, and it is necessary for in vivo osteoclastogenesis. However, the primary role of IKKβ in osteoclastogenesis is to prevent TNF-α from inducing the death of osteoclast precursors.[8–10]

Polymorphisms, most commonly single-base variations or single-nucleotide polymorphisms (SNPs), may affect gene expression or the protein synthesis rate, and influence the periodontium through the immunological reaction, thus increasing susceptibility to periodontitis.[11,12] The examples of such SNPs are *IKKβ* at rs17875746 and rs12676482. However, no previous study has reported correlations between *IKKβ* genetic defects or variants and periodontitis. Furthermore, no study has evaluated the potential genetic association between *IKKβ* polymorphisms and periodontitis. Hence, this novel study hypothesized that *IKKβ* SNPs are associated with periodontitis in Iraqi Arabs. Additionally, the rationale of this study was that immunogenetics has the potential to provide novel and valuable knowledge regarding susceptibility to periodontitis.

Additional research is required to validate and broaden our basic knowledge on the genetic associations between periodontitis and inflammatory biomarkers that substantially impact the disease. These associations and biomarkers are expected to play a significant role in periodontitis.

Material and methods

Study design and setting

The study used a case–control design.

Blood samples were collected from subjects admitted to the Department of Periodontology of the College of Dentistry at the University of Baghdad, Iraq, and the Iraqi National Centre for Blood Donation/Ministry of Health from March to August 2022.

Participants

Eligibility criteria

All participants (N = 94) were of the same ethnic background (Iraqi Arab), non-smokers, systemically healthy males and females, with ≥20 teeth. Any pregnant or lactating women, individuals with plaque retentive factors, such as orthodontic or prosthodontic appliances, and those receiving antibiotic therapy in the last 3 months or periodontal treatment in the previous 6 months were excluded (Fig. 1).

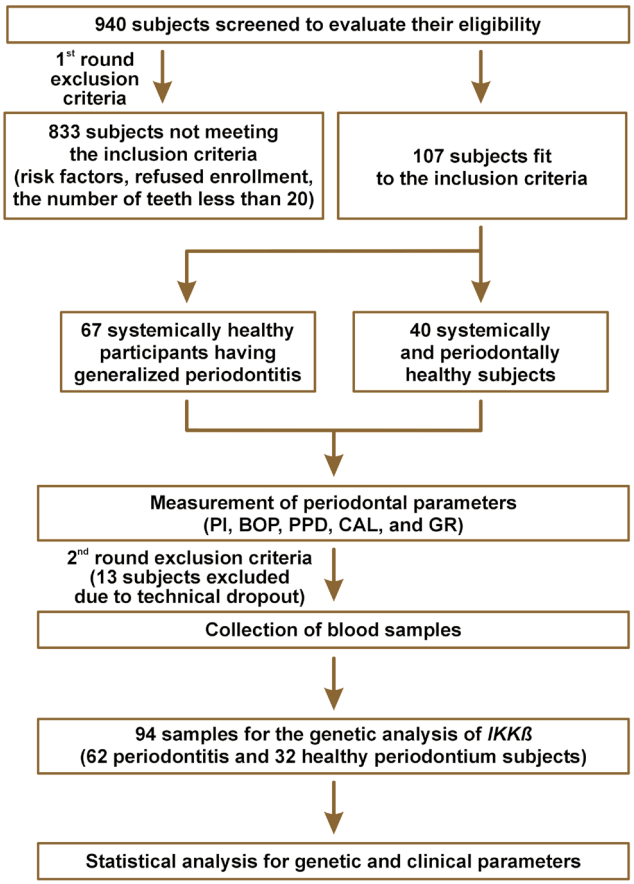

Fig. 1. STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) flowchart of the study

PI – plaque index; BOP – bleeding on probing; PPD – probing pocket depth; CAL – clinical attachment loss; GR – gingival recession.

Before blood specimens were collected, written informed consent was obtained from each patient. The Institutional Ethics Committee of the College of Dentistry approved the study (No. 453).

Allocation

The participants comprised 2 groups: the periodontally healthy control group (n = 32); and the periodontitis group (n = 62).

Variables

In particular, the criteria of the European Federation of Periodontology/American Academy of Periodontology (EFP/AAP) were used to diagnose periodontitis and determine which participants belonged to each group. Periodontitis must exhibit an unstable status, specifically, a probing pocket depth (PPD) ≥5 mm or PPD ≥ 4 mm with bleeding on probing (BOP), as well as the absence of the risk factors for periodontal disease (diabetes mellitus and smoking). The control group had the following characteristics: PPD ≤ 3 mm without clinical attachment loss (CAL); and BOP < 10%.[13,14]

Measurements

Clinical parameters, including the plaque index (PI), BOP, PPD, CAL, and gingival recession (GR), and the number of mobile and missing teeth were measured using a manual Michigan 0 periodontal probe and recorded by the same examiner.

Around 2–3 mL of venous blood was drawn from each individual and transferred into an ethylenediaminetetraacetic acid (EDTA) tube (1.5 mg/mL) for the genetic analysis of *IKKβ* by means of the polymerase chain reaction (PCR) sequencing methods. These methods included DNA extraction, PCR amplification, direct Sanger sequencing, and data analysis.

Bias

Inter-examiner and intra-examiner calibration were conducted on 5 subjects until an agreement level of >0.75 was reached for all clinical parameters. The inter-examiner and intra-examiner consistency for continuous data (PPD, CAL and GR) was determined using an interclass correlation coefficient (*ICC*). Meanwhile, for categorical data (PI and BOP), the κ test was used to examine whether the correlations were significant. Any differences were discussed, and the sessions were repeated until the desired agreement level was reached.

Sample size

A pilot study was conducted using the first 12 samples collected from each group, following an allocation ratio of 1:2 for financial reasons (i.e., 4 periodontally healthy samples and 8 periodontitis samples). The samples were analyzed using the PCR sequencing methods to validate the primers and calculate the sample size. The sample size was calculated with the use of an online calculator – EpiTools (https://ausvet.com.au) – at a 95% confidence interval (*CI*) and a power of 80%, using the odds ratio (*OR*) of genetic polymorphisms of *IKKβ* at rs17875746 between the control and periodontitis groups. The *OR* was 5.5 in the pilot study and thus, the sample size required for the current study was 87 (29 for the control group and 58 for cases per the allocation ratio of 1:2). This number was increased to 94 subjects to account for dropouts during the laboratory tests. The values obtained from the pilot study were added to the final data of the main study.

Quantitative variables

The DNA was isolated from the samples by using an ABIOpure™ extraction kit (Alliance Bio, Bothell, USA). Then, the extracted DNA was quantified using a Quantus™ fluorometer (Promega Corporation, Fitchburg, USA). All DNA was stored at −40°C until tested. The primers were created by extracting the *IKKβ* gene sequence from the National Center for Biotechnology Information (NCBI) and using PrimerQuest (Integrated DNA Technologies, San Diego, USA), which covered most of the *IKKβ* gene, to find any potential SNPs present in the study groups and compare them to the expected sequences.

The DNA samples were amplified using forward and reverse primers (TGTAAAACGACGGCCAGTGCTCTCATGGGTCATTCTTATG and CAGGAAACAGCTATGACCACCTTGCCGTCTGAAATAC) in a lyophilized form with a product size of 983 bp (Macrogen, Seoul, South Korea). The primers were optimized at 55°C, 58°C, 60°C, 63°C, and 65°C to assess the ideal temperature and increase the accuracy of PCR amplification. The PCR amplification procedure for DNA was performed with a new, fast and ready-to-use PCR Express thermal cycler (Bio-Rad Laboratories, Hercules, USA). The procedure included 32 cycles, with 1 cycle at 94°C for 4 min (for initial denaturation), 30 cycles at 94°C for 30 s (for denaturation), at 60°C for 30 s (for annealing) and at 72°C for 30 s (for extension), and then 1 cycle at 72°C for 7 min (for final extension). The steps were stopped throughout incubation for 10 min at 4°C.

The PCR product of each sample (5 µL) was added to agarose gel, and electrophoresis was performed using an agarose gel kit (Promega Corporation) to verify the existence of PCR amplification for 1 h (at 100 V and 50 A). After electrophoresis, the separated DNA bands in the gel were visualized under ultraviolet (UV) light, and the images obtained by using gel imaging equipment (Major Science Co., Ltd., Taoyuan City, Taiwan) were saved for further analysis (Fig. 2).

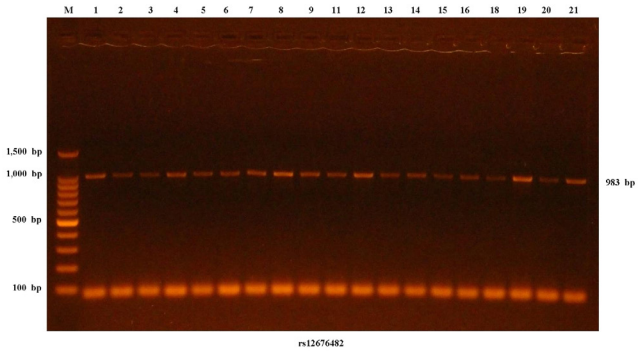

Fig. 2. Electrophoresis of the amplified *IKKβ* gene at 983 bp for some samples
M – 100-bp DNA ladder marker.

When gene fragments of the expected size appeared in PCR, the results were transmitted to Macrogen in South Korea, where the nucleotide sequences of these fragments were identified. The Basic Local Alignment Search Tool (BLAST), available from the NCBI site (http://blast.ncbi.nlm.nih.gov/Blast.cgi), was used to analyze the sequences.

Statistical analysis

All data collected in this study was analyzed using IBM SPSS Statistics for Windows, v. 28 (IBM Corp., Armonk, USA). The mean and standard deviation (*M* ±*SD*), median (*Me*), range, and number and percentage (*n* (%)) values are shown. The Mann–Whitney *U* test compared the 2 study groups, with the χ^2 test used to compare categorical variables. Meanwhile, Spearman's correlation coefficient (*r*) evaluated the association between polymorphisms and clinical variables.

The Hardy–Weinberg equilibrium (HWE) test was used to calculate the expected alleles from the observed genotypes. The association between a particular genotype and the disease risk was expressed with *OR*. Specifically, *OR* = 1 indicated no association, *OR* < 1 indicated a decreased risk and *OR* > 1 indicated an increased risk, while *p*-values <0.05 were considered significant.

Results

Descriptive data

Table 1 provides an overview of the findings and the distribution of the demographic and clinical variables of the groups. According to the Mann–Whitney *U* test, the periodontitis group had significantly greater PI and BOP than the control group, and significantly more mobile and missing teeth according to the χ^2 test ($p \leq 0.001$). Furthermore, the control group was free of PPD, CAL and GR.

Table 1. Demographic and clinical parameters of the study groups

Parameter		Control group (*n* = 32)	Periodontitis group (*n* = 62)	*p*-value
Age [years]	range	30–52	30–55	≥0.05†
	Me	37	40	
Sex	M (*n* (%))	19 (59)	37 (60)	≥0.05‡
	F (*n* (%))	13 (41)	25 (40)	
Family history	yes (*n* (%))	2 (6)	10 (16)	≥0.05‡
	no (*n* (%))	30 (94)	52 (84)	
PI [%]	*M* ±*SD*	16 ±10	65 ±23	≤0.001†***
	Me	12	68	
BOP [%]	*M* ±*SD*	6 ±4	48 ±19	≤0.001†***
	Me	6	45	
PPD [mm]	*M* ±*SD*	–	4.51 ±0.62	–
	Me	–	4.30	
CAL [mm]	*M* ±*SD*	–	3.46 ±0.99	–
	Me	–	3.30	
GR [mm]	*M* ±*SD*	–	2.24 ±1.16	–
	Me	–	2.23	
Teeth	mobile (*n* (%))	0 (0)	46 (3)	≤0.001‡***
	missing (*n* (%))	16 (2)	198 (13)	

The number of present teeth: 880 in the control group; 1,538 in the periodontitis group. *M* – mean; *SD* – standard deviation; *Me* – median; M – male; F – female; † Mann–Whitney *U* test; ‡ χ^2 test; *** statistically significant at $p \leq 0.001$.

Outcome data

After sequencing with the Sanger method, 2 polymorphisms were detected in the *IKKβ* gene at rs17875746 and rs12676482. Moreover, there was a substitution of guanine (G) with thymine (T) in the rs17875746 SNP, while in the rs12676482 SNP there was a substitution of G with adenine (A), as shown in Fig. 3 and Fig. 4, respectively.

The HWE tests for rs17875746 for each group and the total sample revealed significant differences between the observed and expected SNPs in the periodontitis group and the total sample ($p \leq 0.001$), while the results of the equilibrium for rs12676482 were non-significant, as shown in Table 2.

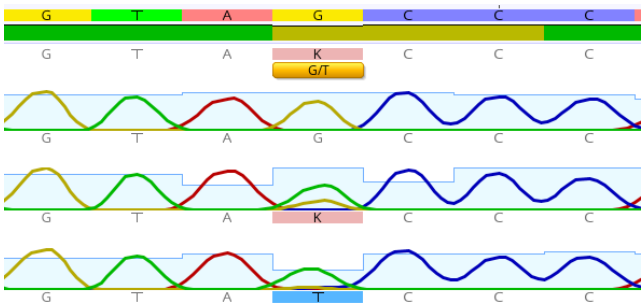

Fig. 3. Analysis of the rs17875746 single-nucleotide polymorphism (SNP) of the *IKKβ* gene by means of Sanger sequencing
The single 'G' peak indicates a G homozygous allele, the single 'T' peak indicates a T homozygous allele, while the presence of the 'G' and 'T' peaks indicates a G/T heterozygous allele.

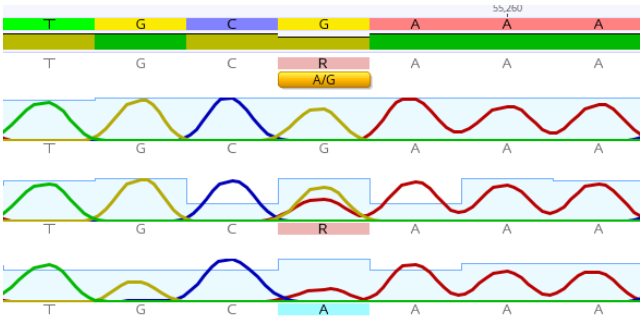

Fig. 4. Analysis of the rs12676482 single-nucleotide polymorphism (SNP) of the *IKKβ* gene by means of Sanger sequencing

The single 'G' peak indicates a G homozygous allele, the single 'A' peak indicates an A homozygous allele, while the presence of the 'G' and 'A' peaks indicates a G/A heterozygous allele.

Main results

The SNP type, number and distribution for both groups are illustrated in Table 3. All SNPs detected after the sequencing of the *IKKβ* gene were of a single-base substitution, as there were 9 transversion SNPs in the periodontitis group and only one in the control group that belonged to rs17875746, exhibiting a non-significant difference. Meanwhile, there were 18 transition SNPs in the periodontitis group for rs12676482 and 10 in the control group for the same SNP, which represented a non-significant difference. The effect of the distribution of *IKKβ* SNPs on periodontitis was assessed based on *OR* with 95% *CI*, which was 5.264 for rs17875746 and 0.900 for rs12676482.

The genetic analysis of *IKKβ* SNPs revealed the allele frequency for both SNPs in the study groups (Table 4). For rs17875746, there was a significantly higher frequency of allele G in the healthy group than in the periodontitis group. For rs12676482, the frequency of allele G was higher in the periodontitis group (83.9%) than in the healthy group (79.7%), while the control group showed a higher frequency of allele A (20.3%) than the periodontitis group (16.1%). However, the differences between the groups for alleles G and A for rs12676482 were non-significant ($p > 0.05$). Surprisingly, the frequency of allele T had a high association with periodontitis (*OR* = 6.750) for rs17875746. Moreover, the χ^2 test was conducted to determine the genotype frequency of all SNPs in *IKKβ*. The results showed non-significant differences in their distribution between the periodontitis and control groups (Table 4).

Other analyses

In a more detailed description, the results illustrate the impact of each genotype of *IKKβ* SNPs on the occurrence and prevention of the disease. The GT genotype in rs17875746 had a higher chance (*OR* = 3.321) for developing periodontitis than other genotypes. Meanwhile, the GA genotype in rs12676482 had a 1.242 chance of developing the disease, which was higher than for other genotypes.

Table 2. Hardy–Weinberg equilibrium (HWE) values in *IKKβ* single-nucleotide polymorphisms (SNPs) for the study groups

IKKβ		Both study groups		Control group		Periodontitis group	
rs	genotype	observed	expected	observed	expected	observed	expected
17875746	GG	84	81.4	31	31.0	53	50.6
	GT	7	12.1	1	1.0	6	10.8
	TT	3	0.4	0	0.0	3	0.6
	HWE	16.703		0.008		12.356	
	p-value	0.000***		0.928		0.000***	
12676482	GG	66	63.9	22	20.3	44	43.6
	GA	23	27.2	7	10.4	16	16.8
	AA	5	2.9	3	1.3	2	1.6
	HWE	2.247		3.365		0.132	
	p-value	0.133		0.066		0.716	

rs – reference SNP; G – guanine; T – thymine; A – adenine; *** statistically significant at $p \leq 0.001$.

Table 3. Types of *IKKβ* single-nucleotide polymorphisms (SNPs) in the study groups

IKKβ polymorphisms	Control group		Periodontitis group		*OR* (95% *CI*)	χ^2	*p*-value
	n	%	*n*	%			
rs17875746 (transversion)	1	3.1	9	14.5	5.264 (0.636–43.550)	2.881	0.090
rs12676482 (transition)	10	31.3	18	29.0	0.900 (0.356–2.274)	0.050	0.824

OR – odds ratio; *CI* – confidence interval.

Table 4. Genotype and allele distribution of *IKKβ* single-nucleotide polymorphisms (SNPs) in the study groups

IKKβ polymorphisms	Genotype/ allele	Control group		Periodontitis group		*OR* (95% *CI*)	χ^2	*p*-value	RR
		n	%	*n*	%				
rs17875746	GG	31	96.9	53	85.5	0.190 (0.023–1.572)	2.881	0.090	0.882
	GT	1	3.1	6	9.7	3.321 (0.382–28.85)	1.315	0.252	3.097
	TT	0	0.0	3	4.8	–	1.599	0.206	–
	G	63	98.4	112	90.3	0.148 (0.019–1.166)	4.319	0.038*	0.918
	T	1	1.6	12	9.7	6.750 (0.858–5.313)	4.319	0.038*	6.194
rs12676482	GG	22	68.8	44	71.0	1.111 (0.440–2.808)	0.050	0.824	1.032
	GA	7	21.9	16	25.8	1.242 (0.451–3.421)	0.177	0.674	1.180
	AA	3	9.4	2	3.2	0.322 (0.051–2.036)	1.585	0.208	0.344
	G	51	79.7	104	83.9	1.325 (0.611–2.876)	0.510	0.475	1.052
	A	13	20.3	20	16.1	0.754 (0.348–1.637)	0.510	0.475	0.794

RR – relative reduction; * statistically significant at $p \leq 0.05$.

The statistics presented in this study indicate a significant positive association between rs17875746 and tooth mobility, a family history, CAL ($p = 0.034$), and GR ($p = 0.011$) in the study groups, while the associations of rs17875746 with other parameters were non-significant ($p \geq 0.05$). In addition, the associations of rs12676482 with all parameters were non-significant ($p \geq 0.05$), as shown in Table 5. Furthermore, the results showed a non-significant correlation between the SNPs of *IKKβ* in the study groups, which is illustrated in Table 6.

Table 5. Associations of *IKKβ* single-nucleotide polymorphisms (SNPs) with demographic and clinical parameters

Parameter	rs17875746		rs12676482	
	r	*p*-value	*r*	*p*-value
Age	0.096	0.355	0.067	0.518
Sex	0.192	0.064	–0.086	0.412
Plaque control	0.070	0.504	0.001	0.992
Family history	0.279	0.006**	0.095	0.360
PI	0.110	0.291	0.038	0.718
BOP	0.095	0.361	–0.119	0.255
PPD	0.145	0.163	–0.112	0.281
CAL	0.219	0.034*	–0.042	0.686
GR	0.260	0.011*	0.046	0.656
Tooth mobility	0.215	0.037*	0.111	0.285
Tooth loss	0.156	0.133	–0.034	0.748

r – Spearman's correlation coefficient; * statistically significant at $p \leq 0.05$; ** statistically significant at $p \leq 0.01$.

Table 6. Correlation between *IKKβ* single-nucleotide polymorphisms (SNPs)

Correlation	rs17875746	
	r	*p*-value
rs12676482	0.130	0.213

Discussion

Key results

Periodontitis can be defined as a complex genetic infectious disease. Although periodontal pathogens are an initiating factor for periodontitis, other environmental and genetic risk factors play a critical role in its pathogenesis. Identifying these risk factors is crucial for the effective prevention and management of the disease,[15,16] which would influence the quality of life of adults in Iraq.[17,18] The significant differences in PI and BOP between the control and periodontitis groups shown in Table 1 can be explained by the fact that microbial plaque is the primary cause of periodontal disease.[3,15,19] In previous research, Nascimento de Macêdo et al. reported that a PI of 65% or more than 4 missing teeth were positively associated with periodontal tissue destruction[20]; this result is consistent with the findings of the current study.

The induction of IKKβ by bacterial endotoxins, such as lipopolysaccharides, during the immune response activates interferon regulatory factors (IRFs) and NF-κB in tissues.[8,21] Nuclear factor-kappa B plays a critical role as

a potent inflammatory mediator, and a stimulator of osteoclastogenesis and bone resorption during periodontitis. Thus, IKKβ is a crucial modulator of osteoclast preservation and a necessary component of inflammation-induced bone loss, unlike IKKα.[10] This finding is corroborated by the significant positive association between *IKKβ* rs17875746 and tooth mobility, CAL and GR.

Interpretation

Variations in the *IKKβ* gene have been linked to many inflammatory and proliferative diseases through the secretion of inflammatory cytokines, such as IL-1β and IL-6, which have substantial effects also on the pathogenesis of periodontitis.[8,22] Thus, the gene polymorphisms of *IKKβ* may influence the genetic susceptibility profile of periodontitis by influencing the inflammatory response. A study in mice by Kure et al. suggested that periodontal disease patients might benefit from an effective prevention or suppression method that would inhibit IKK via the down-regulation of NF-κB.[23]

The most common type of genetic polymorphism is SNP. In this case, a single nucleotide at a specific point is substituted by another nucleotide, and the structure and function of the gene are altered, which may consequently result in different diseases, like periodontal diseases and dental caries, especially in obese patients and those with poor oral hygiene.[24–28]

The genetic variants of *IKKβ* polymorphisms at rs12676482 have been evaluated for their role in various disease states, such as the tumor risk in nasopharyngeal carcinoma tissues[29] and the risk of systemic lupus erythematosus in the Chinese population.[30] However, another study on the Chinese population showed no genetic predisposition to systemic lupus erythematosus risk at rs12676482.[31] Owing to such inconsistent results, additional research is required to confirm the precise process.

Identifying genetic susceptibility to periodontitis could be very useful for developing individual treatment strategies, new diagnostic methods and more effective preventive measures, as well as for gaining a better understanding of periodontitis pathogenesis.[19] Thus, in the present study, 2 SNPs of the *IKKβ* gene at rs17875746 and rs12676482 were investigated and tested to determine their associations with the periodontitis risk in a sample of the Iraqi population. Unfortunately, no references or studies were available to make comparisons with the current results on *IKKβ* polymorphisms.

Complex diseases such as periodontitis are characterized by polygenic causes, and the genes involved in these diseases are regarded as susceptibility genes.[32] Many susceptibility genes are thought to be related to periodontitis, but the number and type of such genes in similar situations can vary among different forms of the disease and ethnicity.[33] Therefore, in this study, we observed patients of the same ethnic background (Iraqi Arab), with no significant differences in age and sex, to avoid any bias.

The results show the distribution of the SNP genotypes in an Iraqi population. Homozygous TT and heterozygous GT at rs17875746, and heterozygous GA at rs12676482 were the most prevalent genotypes in periodontitis cases in comparison with healthy controls (Table 4). However, the differences were not significant. Allele T (at rs17875746) and allele G (at rs12676482) were more prevalent in the periodontitis group as compared to controls; they were also associated with a higher disease risk, as indicated by the elevated *OR* (Table 4).

Our study has the obvious contribution of being the first to assess the possible association between SNPs in the *IKKβ* gene and susceptibility to periodontitis in an Iraqi Arab population. Furthermore, the main findings of the study specified that *IKKβ* polymorphisms at rs17875746 may result in greater susceptibility to periodontitis, which was supported by the elevated *OR*, as well as a positive association between SNPs and increased tooth mobility, CAL and GR in the periodontitis group.

Limitations

Our study has some limitations, with the sample size and the genetic heterogeneity of periodontitis being 2 restrictions that prevent the generalization of the findings. Furthermore, we did not detect the corresponding levels of the *IKKβ* gene and the IKKβ protein expression in each group. In this regard, the findings of this study require further investigation.

Conclusions

The present study revealed that polymorphisms at rs17875746 and rs12676482 of the *IKKβ* gene may be associated with genetic disease susceptibility in an Iraqi Arab population. Furthermore, a positive association between rs17875746 and tooth mobility, a family history, CAL, and GR indicates the potential role of the SNP in increasing the severity of periodontitis. Identifying genetic markers for periodontitis susceptibility would allow people at risk to be identified quickly, and might eventually aid periodontitis treatment through individualized therapy. However, more research is needed to evaluate the contribution of *IKKβ* to periodontitis in addition to other risk factors, i.e., pathological, genetic and environmental ones, because of its complicated nature.

Ethics approval and consent to participate

The study was approved by the Institutional Ethics Committee of the College of Dentistry at the University of Baghdad, Iraq (No. 453). Written informed consent was obtained from each patient.

Data availability

The data for *IKKβ* SNPs at rs17875746 and rs12676482 are deposited at the NCBI GenBank database under accession numbers LC738785, LC738786, LC738787, LC738788, LC738789, and LC738790.

Consent for publication

Not applicable.

ORCID iDs

Athraa Ali Mahmood https://orcid.org/0000-0003-3095-7092
Raghad Fadhil Abbas https://orcid.org/0000-0002-3750-4611
Hashim Mueen Hussein https://orcid.org/0000-0002-1981-2768

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Original paper

Prevalence of high blood pressure in periodontal patients: A pilot study

Neus Lanau[1,A–F], Javier Mareque-Bueno[1,A,C,E,F], Michel Zabalza[2,A,C–F]

[1] Department of Oral Medicine and Public Health, Faculty of Dentistry, Universitat Internacional de Catalunya, Barcelona, Spain
[2] Department of Oral Medicine and Public Health, Faculty of Dentistry and Faculty of Medicine, Universitat Internacional de Catalunya, Barcelona, Spain

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Address for correspondence
Neus Lanau
E-mail: neuslanau@uic.es

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Abstract

Background. Arterial hypertension and periodontal diseases are pathologies with a high prevalence worldwide. Recent evidence suggests a possible causal relationship between them. Patients with moderate or severe periodontitis tend to have higher blood pressure measurements and a 30% to 70% higher likelihood of developing hypertension.

Objectives. The aim of this cross-sectional pilot study was to ascertain the prevalence of high blood pressure in patients with periodontitis.

Material and methods. The study included 40 patients diagnosed with periodontitis who required non-surgical periodontal treatment. Demographic, periodontal and clinical characteristics, including blood pressure measurements, were registered.

Results. Fifteen percent of the patients were classified as hypertensive ($n = 6$), 67.5% as high-normal ($n = 27$) and 17.5% as normotensive ($n = 7$). Recent studies have estimated that the prevalence of high-normal blood pressure in the general population ranges between 30% and 50%. These findings suggest that patients with periodontal disease are more likely to have elevated blood pressure than patients with healthy periodontal tissues.

Conclusions. High-normal blood pressure is associated with a 3 times higher likelihood of developing hypertension, so early detection and prevention are crucial public health strategies. Despite the limitations of this pilot study, it highlights the role of dentists in the prevention, diagnosis and blood pressure control to improve health and cardiovascular risk of patients with periodontitis.

Keywords: periodontitis, blood pressure, hypertension, cardiovascular risk, high blood pressure

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Introduction

Arterial hypertension is a significant preventable cardiovascular risk factor that is associated with higher rates of vascular mortality and morbidity.[1] High blood pressure affects approx. 30–40% of the adult population worldwide.[2] It is estimated that 1.39 billion people had hypertension in 2010,[3] and it remains the leading cause of premature death in the world (10.4 million deaths per year).[4] Due to the aging population and a high prevalence of arterial hypertension, the global burden of complications from high blood pressure continues to rise. In this scenario, the control of blood pressure and identification of hypertensive patients is an essential public health goal.

The etiopathogenesis of hypertension involves multiple mechanisms, such as environmental and pathophysiological factors, which include autonomic and neurohormonal dysregulations, endothelial dysfunction, oxidative stress, mechanical changes, stiffness in the arterial wall, and local and systemic inflammation.[5,6]

Periodontal disease (gingivitis and periodontitis) is a multifactorial inflammatory condition caused by bacteria that affect soft and hard periodontal tissues. It is found in 20–50% of the world's population[7] and one of its etiological mechanisms is a chronic inflammation response catalyzed by multiple mediators.[8,9]

The high prevalence of hypertension and periodontitis as well as their etiological similarities have been the subject of increased research.[10] Recent articles suggest a possible causal relationship between the two conditions.[11] Patients with moderate or severe periodontitis tend to have higher blood pressure measurements, and there is a 30–70% higher possibility of developing hypertension in this population.[12–14] Furthermore, recent evidence suggests that the treatment of periodontitis could benefit blood pressure levels.[11,15]

This data emphasizes the importance of oral inflammation in high blood pressure and the role that dentists and dental treatments can play in the prevention, detection and management of high blood pressure. High blood pressure is a manageable risk factor, and its control directly affects the cardiovascular risk of patients.

The aim of this study was to ascertain the prevalence of high blood pressure in patients with periodontitis in clinical practice.

Material and methods

Definition of hypertension and high-normal blood pressure

Hypertension is defined as a systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg in adults older than 18 years diagnosed in the office or clinic.[2] There are 3 grades of hypertension: grade 1 hypertension (SBP: 140–159 mmHg and/or DBP: 90–99 mmHg); grade 2 hypertension (SBP: 160–179 mmHg and/or DBP: 100–109 mmHg); and grade 3 hypertension (SBP ≥ 180 mmHg and/or DBP ≥ 110 mmHg).

Ideally, hypertension should be diagnosed at more than one office visit (2–3 visits at 1- to 4-week intervals), and, if possible, the diagnosis should be confirmed by out-of-office blood pressure measurements. This protocol is intended to prevent white-coat hypertension, defined as elevated blood pressure in the clinic but not in out-of-office measurements. Patients with white-coat hypertension represent 10–30% of the population and are typically at intermediate cardiovascular risk between normotensive patients and those with sustained hypertension.[3,16]

Prehypertension, renamed high-normal blood pressure in the 2018 European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) guidelines,[2] is defined as SBP of 130–139 mmHg and/or DBP of 85–89 mmHg. In this classification,[2] normal blood pressure is defined as SBP of 120–129 mmHg and/or DBP of 80–84 mmHg. Finally, optimal blood pressure is defined as SBP < 120 mmHg and DBP < 80 mmHg.

Definition of periodontitis

According to the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions,[17,18] in the context of clinical care, a patient is considered a periodontitis case if:

– interdental clinical attachment loss (CAL) was detected in 2 or more adjacent teeth;
– buccal or oral CAL ≥ 3 mm (caused by non-periodontitis causes) with pocketing ≥3 mm was detected in 2 or more adjacent teeth.

Inclusion and exclusion criteria

The patients included in this study were adults with periodontitis who required non-surgical treatment, patients with 10 or more teeth and with the full capacity to provide consent.

Pregnant and breastfeeding women, patients with localized aggressive periodontitis, patients who received periodontal treatment in the last 6 months, and patients with other severe concomitant diseases were excluded from the study.

Patient information

After screening 50 patients, only 40 met the inclusion criteria. The included individuals were treated at the Department of Periodontology of Hospital Universitari Dexeus in Barcelona, Spain, from 2018 to 2021. They were diagnosed with periodontitis and required non-surgical

periodontal treatment. All patients provided written informed consent to participate in this study and for their data to be used for the purposes of research. The study was approved by the Ethics Committee of Hospital Universitari Dexeus – Grupo Quironsalud, Barcelona, Spain (No. 2018/ODI-2018-01).

During the initial evaluation, the demographic, periodontal and clinical characteristics of the patients were recorded. Collected demographic data included sex, age, height, weight, and body mass index (BMI). According to the BMI values, the standard weight status categories are as follows: underweight (<18.5 kg/m^2), healthy weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obesity (≥30 kg/m^2).[19]

A periodontogram, including the data on periodontal pocketing, CAL, bleeding on probing, tooth mobility, furcation lesions, number of teeth present, and plaque index, was created for each patient. All individuals completed a dental habits questionnaire, which included questions about the frequency and type of toothbrush used, interdental hygiene practices (dental floss, interproximal brushes, or none), whether there is bleeding on brushing, and the regularity of dental appointments.

Collected clinical data included blood pressure measurements (SDP, DBP and heart rate). As recommended by the ESC/ESH guidelines, these measurements were taken by a trained operator using a validated electronic upper-arm cuff device (boso Medicus Family 4; Bosch+Sohn GmbH & Co. KG, Jungingen, Germany), with the cuff appropriately sized for the patient's arm. Blood pressure measurements were taken in both arms after 5 min of rest. If there was a consistent difference between the obtained values, the arm with the higher blood pressure value was used. Three measurements were taken at 2-min intervals, and the average of the last 2 measurements was used. The patient was seated with their back supported, feet flat on the floor, and the arm bare and resting at heart level. The patient was instructed not to talk during and between the measurements as well as not to smoke, drink coffee or exercise for 30 min prior to the visit.[3] In order to perform the study, blood pressure measurements were taken during the first 2 visits. The presented results are the average of the first- and second-visit blood pressure measurements. Additionally, cardiovascular risk factors, such as diagnosed hypertension (or antihypertensive medication prescribed), diabetes mellitus, dyslipidemia, systemic diseases, and family history were recorded. Toxic habits, including smoking (the number of cigarettes per day or years after quitting), alcohol consumption (the number of drinks per day) or the consumption of sugar drinks (the number of drinks per day), as well as healthy habits such as physical activity and healthy diet were noted.

The data was recorded manually and converted to an electronic data sheet. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement was followed throughout the entire process.[20]

Data analysis

In this descriptive study, data analysis was performed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). Blood pressure level was described as a quantitative variable and expressed in mmHg. The mean and standard deviation (*M* ±*SD*) were calculated and followed a normal distribution.

The remaining important variables were categorical. Gender was dichotomous (male and female). Age was considered a quantitative variable, but it was divided into 3 groups (patients under 35 years, patients between 36 and 59 years, and patients aged 60 years or older). Smoking habit was also a dichotomous variable (smoker or non-smoker). Former smokers were included in the non-smoker group. Body mass index was calculated as a quantitative variable, but it was divided into 2 groups: values between 18.5 and 25 kg/m^2 were classified as healthy weight, and values above 25 kg/m^2 were categorized as overweight and obese.

Results

Forty Caucasian patients were included in the study: 19 men (47.5%) and 21 women (52.5%). Their mean age was 47.7 years (47.88 for men and 47.7 for women) (Table 1).

Of these 40 patients, 6 had hypertension (15%). Two individuals were taking medication but were included in this group due to high blood pressure measurements. They were referred to a specialist for a check-up. The other 4 patients went to a doctor after our appointment to confirm the diagnosis. Most of the patients (27; 67.5%) were classified as having high-normal blood pressure, and 7 individuals (17.5%) had blood pressure measurements classified as normal. The hypertensive group had a mean age of 54.3 years; 3 were men (50%) and 3 were women (50%). The high-normal blood pressure group had a mean age of 45.9 years; 15 were men (55.5%) and 12 were women (44.5%). The normotensive group had a mean age of 48.7 years; 1 was a man (14.3%) and 6 were women (85.7%). The mean blood pressure measurements for the hypertensive, high-normal blood pressure and normotensive patients were: SBP: 140.58 mmHg ±10.74 mmHg and DBP: 91.16 ±6.7 mmHg; SBP: 126.9 ±6.5 mmHg and DBP: 85.83 ±6 mmHg; and SBP: 111.42 ±7.28 mmHg and DBP: 73.14 ±3.93 mmHg, respectively (Fig. 1).

Three patients presented with other cardiovascular risk factors: 1 had diabetes (in the high-normal blood pressure group) and 2 had hypercholesterolemia (1 in the normotensive group and 1 in the hypertensive group). In addition, 18 patients (45%) had a BMI above 25 kg/m^2, which is considered overweight or obese. Four of these patients were hypertensive (22.2% of the total in this subgroup), 11 were in the high-normal blood pressure group (61.1%) and 3 were in the normotensive group (16.7%).

Table 1. Prevalence of hypertension, high-normal blood pressure and normal blood pressure in patients with periodontitis

Variable		Total population (%) $N = 40$	Hypertension (15%) $n = 6$	High-normal blood pressure (67.5%) $n = 27$	Normal blood pressure (17.5%) $n = 7$
Gender *n* (%)	male	19 (47.5)	3 (50)	15 (55.56)	1 (14.29)
	female	21 (52.5)	3 (50)	12 (44.44)	6 (85.71)
Age [years] *n* (%)	<35	6 (15)	0 (0)	5 (18.6)	1 (14.28)
	35–59	29 (72.5)	4 (66.7)	20 (74)	5 (71.43)
	≥60	5 (15.5)	2 (33.3)	2 (7.4)	1 (14.28)
Blood pressure levels [mmHg] *M* ±*SD*	SBP	126.25 ±11.07	140.58 ±10.74	126.9 ±6.5	111.42 ±7.28
	DBP	84.41 ±7.96	91.16 ±6.7	85.83 ±6	73.14 ±3.93
Smoking *n* (%)	yes	20 (50)	2 (33.3)	16 (59.26)	2 (28.57)
	no	20 (50)	4 (66.7)	11 (40.74)	5 (71.43)
BMI *n* (%)	18.5–25 (healthy weight)	22 (55)	2 (33.3)	16 (59.26)	4 (57.14)
	>25 (overweight or obesity)	18 (45)	4 (66.7)	11 (40.74)	3 (42.86)

M – mean; *SD* – standard deviation; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure.

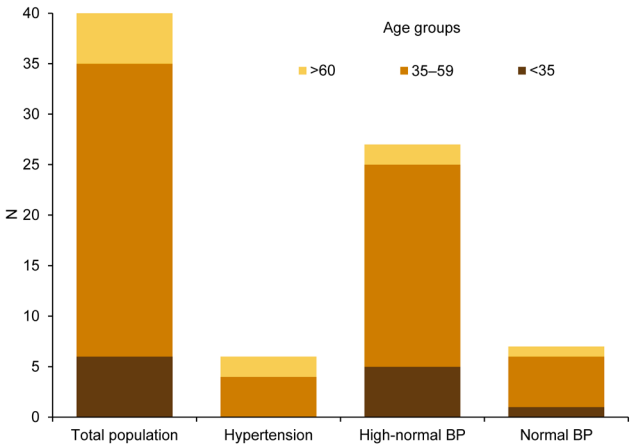

Fig. 1. Distribution of the patients' age by blood pressure (BP) values

Fifty percent (n = 20) of the patients were smokers; among the non-smoking individuals, 9 were ex-smokers. Of the smoking patients, 13 (65%) smoked more than 10 cigarettes per day. Regarding the blood pressure groups, 2 of the smokers were in the hypertensive group (33.3% of the hypertensive patients), 16 of the smokers were in the high-normal blood pressure group (59.2% of the high-normal blood pressure patients), and 2 of the smokers were in the normotensive group (28.6% of the normotensive patients).

Regarding alcohol consumption, 3 patients reported consuming more than 2 alcoholic drinks per day (7.5%), 32 reported occasional alcohol consumption (e.g., weekends and holidays) (80%) and 5 patients indicated that they did not consume alcohol (12.5%). Among the 3 patients who reported no alcohol consumption, 1 was in the hypertensive group, another was in the normotensive group, and the 3rd was in the high-normal blood pressure group.

Evaluating physical activity (more than 3 workouts per week), 9 patients (22.5%) declared that they had regular physical activity, 19 patients (47.5%) reported occasional physical activity, and 12 (30%) declared no physical activity. Of these 12 sedentary patients, 25% were in the hypertensive group (n = 3), 58.3% were in the high-normal blood pressure group (n = 7) and 16.7% were in the normotensive group (n = 2).

The majority of patients (92.5%; n = 37) declared that they consumed a healthy diet based on the Mediterranean diet, with no consumption of carbonated and sugar-sweetened beverages or junk food.

Regarding oral hygiene habits, 50% (n = 20) of the patients used an electric toothbrush, 35% (n = 14) used a manual toothbrush, and 15% (n = 6) used both types of toothbrush. The average time spent brushing their teeth was 2.125 min per day. Only 37.5% (n = 15) of the patients performed interproximal hygiene with either dental floss or interproximal toothbrushes, with an average of 1.4 uses per day. There were no differences between the groups.

Discussion

Recent studies have shown that the prevalence of high-normal blood pressure in the general population is 30–50%.[21–24] Patients with periodontal disease often present with hypertension, which may include individuals in the early stages, such as those with high-normal blood pressure.

In our study, only patients with periodontitis were included, and 67.5% had high-normal blood pressure. While the relationship between periodontal disease and arterial hypertension is well documented, there is limited research on prehypertensive states. Our findings may suggest that patients with periodontal disease are more likely to have high-normal blood pressure than patients with healthy periodontal tissues.

High-normal blood pressure is associated with a 3 times greater likelihood of developing hypertension.[4] Moreover, a strong association has been found between this status, coronary artery disease and cardiovascular mortality.[21,24] High-normal blood pressure is also associated with a worse cardiovascular risk profile and a high prevalence of metabolic disorders.[25] Thus, it is essential to implement early detection, prevention and treatment strategies, not only for hypertensive patients, but also for those in prehypertensive states.[23,24]

Some hypertension risk factors are gender (men are more likely to have hypertension than women), age (older people have a higher risk of hypertension), heart rate (>80 beats/min), smoking habits, diabetes, hypercholesterolemia, overweight or obesity, early onset of menopause, and family history of cardiovascular diseases or hypertension.[23]

The majority of patients with high-normal blood pressure (69%) do not qualify for drug therapy and are advised to make lifestyle changes.[26] These changes include salt reduction, healthy food and drink consumption, moderation of alcohol consumption, weight reduction, smoking cessation, regular physical activity, and stress reduction. The findings of this study suggest that the oral and periodontal status of prehypertensive patients should be assessed and periodontal treatment performed if necessary. Moreover, dentists may play an important role in the management and follow-up of high-normal blood pressure patients. During the course of the study, 4 patients had repeatedly high blood pressure measurements and were unaware of their hypertension status. These patients were referred to general practitioners for a check-up, and in all 4 cases, hypertension was diagnosed and treated pharmacologically.

This observational pilot study has some limitations, the most important being the small sample size. The low number of hypertensive and normotensive patients may have been a source of potential bias. In addition, there was no control group of healthy periodontal patients. It is also important to note the possible overestimation of blood pressure levels due to white-coat hypertension. To confirm the diagnoses, it would be necessary to obtain 24-hour blood pressure measurements of the patients.

Our results emphasize the importance of identifying the underdiagnosed patients who do not meet strict criteria for hypertension. These patients have more cardiovascular risk factors and can benefit from early preventive measures.

Further research with a larger population and prospective clinical trials is necessary to confirm the findings of this study. Additional investigation is needed to establish whether periodontal treatment could help control blood pressure levels in patients with prehypertension.

Conclusions

This descriptive and observational study suggests that there is a high prevalence of high-normal blood pressure among patients with periodontal disease. Additionally, we highlighted the role of dentists in the process of prevention, diagnosis, control, and treatment of this state prior to the development of established arterial hypertension in order to improve patients' health and reduce their cardiovascular risk.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Hospital Universitari Dexeus – Grupo Quironsalud, Barcelona, Spain (No. 2018/ODI-2018-01). All participants provided written informed consent.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Neus Lanau https://orcid.org/0000-0003-1624-0693
Javier Mareque-Bueno https://orcid.org/0000-0003-4440-4114
Michel Zabalza https://orcid.org/0000-0001-5573-4982

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Original paper

Mechanical performance of a conventional resin composite and its bulk-fill restorative counterpart after long-term accelerated aging

Fereshteh Naser Alavi[1,B–D], Mehrsima Ghavami-Lahiji[1,A,D–F], Parham Habibi[2,B,C]

[1] Department of Restorative Dentistry, Dental Sciences Research Center, School of Dentistry, Guilan University of Medical Sciences, Rasht, Iran
[2] Dental Sciences Research Center, School of Dentistry, Guilan University of Medical Sciences, Rasht, Iran

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Address for correspondence
Mehrsima Ghavami-Lahiji
E-mail: dr.m.ghavami@gmail.com

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Abstract

Background. The long-term mechanical properties and stability of various resin composites in areas under stress are questionable.

Objectives. The aim of the study was to determine the effects of long-term hydrothermal aging on the mechanical properties of a microhybrid conventional resin composite and its bulk-fill counterpart.

Material and methods. We used a conventional and high-viscosity restorative bulk-fill resin-based composites (RBCs) from one company. Bar-shaped specimens of each type of resin composite were fabricated using steel molds and divided into 2 groups. The specimens were stored at 37°C for 24 h, and half of the specimens in each group were subjected to the 3-point bending flexural test and microhardness measurement. The remaining specimens were aged for 10,000 thermal cycles between 5°C and 55°C, and then subjected to flexural testing and microhardness measurement. A Vickers microhardness tester was used to estimate the surface microhardness of the specimens. Data was analyzed using an independent *t* test and the Mann–Whitney *U* test. The statistical significance level was set at $p \leq 0.05$. Scanning electron microscopy (SEM) was used to investigate the surface of each material.

Results. The bulk-fill RBC showed similar flexural strength and modulus to its conventional counterpart before aging. The flexural strength of both resin composites was significantly decreased after thermocycling ($p < 0.001$). Hydrothermal aging had no significant effects on the flexural modulus ($p = 0.84$). There was a significant decrease in the surface microhardness of the bulk-fill RBC. Scanning electron microscopy photomicrographs showed several pits as a result of the exfoliation of the filler particles on the surface of the bulk-fill RBC after aging.

Conclusions. The flexural strength of both resin composites decreased significantly after aging. The flexural properties, surface changes and microhardness of the bulk-fill type were additionally affected by the aging process.

Keywords: aging, hardness, flexural strength, elastic modulus, composite resin

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Introduction

Scientific advances in dental materials have led to the expanded use of resin-based composites (RBCs) for reconstructing large posterior stress-bearing areas previously reconstructed with amalgam.[1,2] However, due to the depth-of-cure problems and the possibility of incorporating voids or contaminations between the increments, restoring deep cavities using 2-mm thick resin composite increments is extremely complicated. Some manufacturers have launched new forms of resin composites, called bulk-fill materials, which are curable up to a 4–5-mm increment thickness, thus speeding up the dental treatment process.[3,4]

Bulk-fill materials offer a promising solution to the challenges of technique-sensitive and time-consuming restorative treatments by employing the incremental technique, which uses low-shrinkage RBCs that allow clinicians to apply layers up to 4–5 mm of thickness. Placing resin composites in bulk results in more compact fills and prevents void contamination between composite layers.[5]

The increased depth of cure can be controlled by increasing the translucency of the material. Due to a linear correspondence between translucency and the amount of filler particles, reducing the filler content is an easy way to increase the depth of cure. The difference in refractive indices between the filler particles and the resin matrix, which governs how light is scattered within a material, also affects the translucency of dental materials. Translucency of experimental dental materials improved when the components of an RBC had similar refractive indices, as observed for bisphenol A-glycidyl methacrylate (BisGMA) and silica filler particles. Manufacturers have tried a number of strategies to increase the depth of cure of bulk-fill resin composites. Reducing filler content and increasing filler particle size are methods for reducing scatter at the resin–filler interface and improving light penetration.[6,7]

Some bulk-fill RBCs are called nano or nanohybrid RBCs because they contain a certain amount of low-size fillers. Because their diameter is smaller than the wavelength of visible light, nanoparticles are unable to scatter or absorb it. As a result, they play a key role in light curing, increasing translucency and aesthetics.[8] For example, Tetric EvoCeram Bulk Fill (Ivoclar Vivadent, Schaan, Liechtenstein), in addition to having a conventional photoinitiator system also includes an initiator booster (Ivocerin®) that can polymerize the material in-depth.[9] There are a few important details about the chemical composition of some bulk-fill RBCs.

Resin-based composites are subject to temperature variations caused by nutrition in the oral cavity as well as complicated mastication forces, including a significant degree of flexural stress.[10] A polymer-based material, such as composite resin, can endure deterioration when used in the mouth but its characteristics such as hardness, flexural strength and elastic modulus may suffer.[11,12] Although various laboratory tests for dental composite resins are available, mimicking clinical performance is difficult. They can, however, help us understand how changes in the composition or processes affect the material's qualities. Flexural strength testing simulates clinical conditions in which materials must endure flexing, particularly in the posterior area. High flexural strength is required for the materials that may fail under large masticatory forces, albeit this has not been clinically proven.[13]

Thermal stresses are easily formed in resin composites due to various coefficients of thermal expansion and the heterogenous composition of their components, and they can be intensified by thermal cycling in the oral environment. The thermocycling test has gained widespread acceptance as a method to mimic the degradation of the material's mechanical properties and the quality of the bond strength.[14] According to Gale and Darvell, 10,000 hydrothermal cycles with water temperatures ranging from 5°C to 55°C are recommended to demonstrate the durability of the restorations and resemble 1 year of in vivo functioning.[15]

Changes in the formulation of bulk-fill material in terms of fillers, novel resins, stress modulators, and initiator systems have an impact on the long-term mechanical properties and stability of areas under stress. The mechanical stability of RBC fillings restored with bulk-fill material in stress-bearing areas has been the subject of some debate, presuming that the bulk-fill RBCs are adequately cured and mechanical properties within the incremental thickness remain constant.[9,16] Clinical studies on the bulk-fill class of RBCs are limited and have short follow-up periods.[17] However, bulk-fill RBCs seem to be a promising alternative for posterior restorations due to their ease of use and faster restoration time.[18]

Some studies have explored the behavior of bulk-fill RBCs in terms of degree of conversion (DC), depth of cure and the appropriate light-curing unit.[19,20] To the best of our knowledge, there is little information in the literature on the physical and mechanical behavior of aged bulk-fill RBCs, such as flexural properties and microhardness, in the long-term aging process. Therefore, the purpose of the current study was to compare the mechanical performance of a bulk-fill and a conventional RBC before and after hydrothermal aging using 10,000 thermal cycles.

Material and methods

A conventional RBC and a restorative bulk-fill RBC (Master-Fil™ BULKFILL) from the same company (Dentonics, Monroe, USA) were selected for this study. Eighteen bar-shaped specimens of each composite resin were fabricated using a steel mold and divided into 2 groups (n = 9 for each group).

The International Organization for Standardization (ISO) recommends the use of 25 mm × 2 mm × 2 mm specimens for flexural testing.[21] Producing samples of this size without any defects and flaws is very challenging and requires several overlapping irradiations due to the smaller size of the curing tips used in the light-curing units compared to the length of the specimen. Additionally, even though these ISO samples consume large amounts of material, they have no clinical relevance because the cervico-incisal length and mesiodistal width of teeth usually do not exceed 13 mm and 11 mm, respectively.[2]

The mini-flexural test was chosen for this investigation because of its clinical relevance, higher efficiency and a significant correlation with the ISO flexural test.[2,9] The material for RBCs was compressed between 2 glass plates with intervening mylar sheets, separated by a steel mold measuring 16 mm × 2 mm × 2 mm, to produce the specimens.[9] The specimens were irradiated on the top surface, as indicated by ISO 4049 standards.[21]

The assembly was clamped using a small screw clamp capable of exerting pressure. The middle thirds of the specimens were cured first with a 1200 mW/cm^2 multiwave led light-curing unit (X-cure; Guilin Woodpecker Medical Instrument Co., Ltd., Guilin, China) for 20 s, and then the remaining thirds, which overlapped the middle thirds, were treated. Test specimens were separated from their molds, and the flash was removed using 600, 800 and 1000 grit silicon carbide (SiC) papers and then stored in distilled water for 24 h. The specimens of each type were randomly divided into 2 groups. Half of the specimens of each type were subjected to the 3-point bending flexural test using a universal testing machine (STM-20; SANTAM Engineering and Design Co., Tehran, Iran). The remaining specimens were placed in a basket that alternated between 5°C and 55°C water baths with a dwell time of 20 s and a rest time of 20 s for 10,000 thermal cycles (Delta Tpo2; Nemo, Mashhad, Iran).

For flexural testing, the specimens were loaded until fracture using a universal testing machine (STM-20; SANTAM Engineering Design Co.) with a load cell of 6 kg (Bongshin Loadcell Co., Ltd., Seongnam, South Korea) at a crosshead speed of 0.5 mm/min and a 12-mm distance between the supports. The maximum load applied to the specimen was recorded, and the flexural strength [MPa] was calculated using the following formula (Equation 1):

$$\text{Flexural strength} = \frac{3FL}{2bh^2} \quad (1)$$

where:
F – maximum load on the specimen [N];
L – distance between the supports [mm] (12 mm);
b – specimen's width [mm]; and
h – specimen's height [mm].

The flexural modulus [MPa] was determined using the following formula (Equation 2):

$$\text{Flexural modulus} = \frac{FL^3}{4dbh^3} \quad (2)$$

where:
d – sample's deflection corresponding to F.

After flexural testing, fragments larger than 8 mm were used to determine the micromechanical properties of the specimens in each group.[9] Measurements were taken at the top of bar-shaped specimens, approx. 4 mm away from the breaking edge, with 3 measurements per sample. The surface microhardness of the specimens was measured using a microhardness tester (Bareiss Prüfgerätebau GmbH, Oberdischingen, Germany) under a load of 300 g for 15 s for 9 specimens. The average value of the 3 indentations for each specimen was taken as the Vickers hardness number (VHN).[12] An independent t test and the Mann–Whitney U test were used to analyze the data. The statistical significance level was set at $p \leq 0.05$.

A scanning electron microscope (SEM) (VEGA3; TESCAN, Brno, Czech Republic) was used to investigate the surface of each material. Two specimens from each group were randomly chosen for this evaluation, sputter-coated and observed with the use of SEM.[22] The SEM photomicrographs were taken at ×2,000 magnification.

Results

The flexural strength and the flexural modulus of conventional and bulk-fill RBCs are shown in Table 1. The bulk-fill RBC showed similar flexural strengths and moduli to its conventional counterpart before aging. No statistically significant differences were found between conventional and bulk-fill RBCs in terms of flexural strength and modulus at the beginning of the experiment ($p = 0.34$ and $p = 0.28$, respectively). The flexural strength of both RBCs was significantly decreased after thermocycling ($p < 0.001$). The lowest flexural strength (52.62 MPa) was observed for the bulk-fill RBC after aging. The flexural modulus was decreased after thermocycling, but the difference was not statistically significant ($p = 0.84$). Both materials met the ISO requirement for the average flexural strength (80 MPa) for sculptable RBCs before aging. However, after aging, a statistically significant decrease was observed in the flexural strength, which was more pronounced in the bulk-fill composite.

Before aging, the microhardness values of the bulk-fill RBC were similar to those of the conventional RBC ($p = 0.848$). However, after aging, the bulk-fill RBC showed significantly lower microhardness values when compared to the baseline. There was no statistically significant difference in the conventional RBC microhardness values before and after aging (Table 2).

Table 1. Flexural strength and flexural modulus of the tested materials

RBC type	Flexural strength [MPa]			Flexural modulus [GPa]		
	before aging	after aging	*p*-value	before aging	after aging	*p*-value
Conventional	129.50 ±21.32[a]	73.59 ±14.39[b]	<0.001*	3.59 ±0.86[a]	3.03 ±0.79[a]	0.17[#]
Bulk-fill	120.05 ±19.35[a]	52.62 ±8.19[c]	<0.001*	4.09 ±0.99[a]	3.12 ±0.91[a]	0.136*
p-value	0.34[#]	<0.001*	–	0.28[#]	0.84[#]	–

RBC – resin-based composite; * Mann–Whitney *U* test; [#] independent *t* test. Values marked with different superscript letters in each test are significantly different ($p \leq 0.05$).

Table 2. Microhardness of the tested materials

RBC type	Microhardness [kgf/mm^2]		
	before aging	after aging	*p*-value
Conventional	84.05 ±14.88[a]	86.43 ±7.16[a]	0.616[#]
Bulk-fill	83.03 ±10.45[a]	75.88 ±4.88[b]	0.048[#]
p-value	0.848[#]	<0.001*	–

* Mann–Whitney *U* test; [#] independent *t* test. Values marked with different superscript letters in each test are significantly different ($p \leq 0.05$).

Scanning electron microscopy images of the 2 types of resin composites are shown in Fig. 1 and Fig. 2. Irregularly shaped filler particles were found in both resin composites. In the bulk-fill type, clusters of fillers with approximate nano dimensions can be seen. The mechanical properties of the composite resins deteriorate during the aging process, as evidenced by surface changes, resin–matrix degradation and filler debonding, all of which can be seen in the SEM images. As the composite resin aged, several pits appeared as a result of exfoliation of filler particles. These findings may explain the reported behavioral variations before and after the aging process.

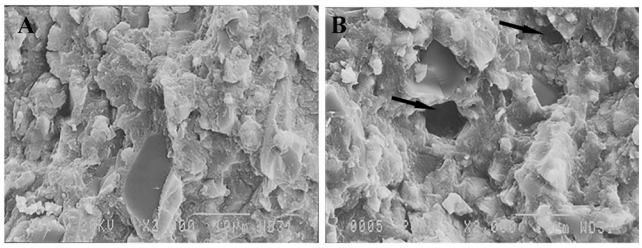

Fig. 1. Scanning electron microscopy photomicrographs of a conventional Master-Fil™ resin composite before (A) and after (B) hydrothermal aging
Pit defects are marked with an arrow (×2,000 magnification).

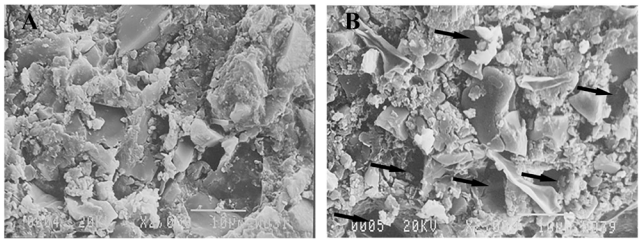

Fig. 2. Scanning electron microscopy photomicrographs of a Master-Fil™ bulk-fill resin composite before (A) and after (B) hydrothermal aging
Pit defects are marked with an arrow (×2,000 magnification).

Discussion

Few studies have compared the mechanical properties of bulk-fill RBCs with other types of RBCs after a short period of aging. Eweis et al. compared the flexural strength and the flexural modulus of high-viscosity and flowable bulk-fill RBCs to conventional RBCs obtained from the same company after 7 days of aging in different solutions.[2] However, longer exposure to the medium is necessary to mimic the wet environment of the oral cavity and evaluate the clinical performance of the material.

In the study conducted by Benalcázar Jalkh et al.,[14] the mechanical properties of different materials, including bulk-fill composites, were examined after 500 thermal cycles, but the authors used the biaxial flexural test that differs from the flexural test recommended by ISO 4049. In the studies by Haugen et al.,[23] Ilie et al.[9] and Rizzante et al.[24] on bulk-fill materials, the aging period did not exceed 24 h. Therefore, our objective was to evaluate long-term artificial aging-induced alterations up to 10,000 hydrothermal cycles in a bulk-fill composite resin and its conventional counterpart. We examined the mechanical properties of a microhybrid RBC from Dentonics and its bulk-fill counterpart.

Composite resins with a lower modulus may not provide an adequate occlusal stress buffer, so they should be capped with conventional materials. Composites with increased stiffness are necessary in high-stress situations to prevent the deformation of the restoration, which may lead to fractures. For stress-bearing situations, various physical properties of the composites, such as strength, fracture and abrasion resistance, must be addressed in addition to the modulus. Therefore, a high-modulus material is preferred when using bulk-fill (full-body) RBCs to reconstruct large posterior stress-bearing areas.[2,23]

In the present study, the bulk-fill RBC exhibited similar flexural strength to its conventional counterpart at the beginning of the experiment. The flexural strength of the bulk-fill RBC was significantly reduced after thermocycling compared to the conventional composite. Studies have reported that bulk-fill RBCs contain polymerization modulators and novel monomers in the resin matrix, which are intended to reduce the polymerization stress of these materials.[9] These changes may weaken the polymer network and decrease the resistance of these materials to moisture.[25] A further reduction of the flexural

strength values observed in the present study after aging may be due to differences in the type, composition and properties of the resin matrix in the bulk-fill RBCs.

Conversely, in the study by Benalcázar Jalkh et al.,[14] the decrease in the flexural strength of composite resins after thermocycling was lower than in our study. The disparity in the aforementioned study's results could be attributed to the variety of the materials tested and the study method used (a biaxial flexural strength test). In addition, the authors conducted only 500 thermal cycles to age the RBCs in their investigation.

The ISO 4049 recommends a minimum flexural strength of 80 MPa for restorative materials affecting outer occlusal surfaces.[21] The results of this investigation for both materials exceeded this value at baseline; however, after 10,000 hydrothermal cycles, the flexural strength values were less than 80 MPa, not meeting the ISO 4049 requirements for use as occlusal fillings. Although using bulk-fill composites for large posterior stress-bearing restorations makes the treatment easier and faster, our findings demonstrated that the flexural strength of the bulk-fill composite was further reduced after aging compared to the conventional RBC.

At the beginning of the present study, the flexural modulus of the bulk-fill composite was higher than that of the conventional type, but this difference was not statistically significant. This may be due to the fact that the bulk-fill composite was comparable in filler content to its conventional counterpart. El-Safty et al. discovered a strong relationship between modulus and filler loading.[26]

Although ISO 4049 does not specify the exact values for the flexural modulus of resin composite materials, according to earlier studies, the flexural modulus should be similar to that of dentin, which necessitates a highly filled composite resin. A lower flexural modulus is preferable to reduce the negative effects of polymerization stress.[24,27]

The mechanical properties of composite resins depend on their composition. Resin-based composites with higher amounts of inorganic fillers have a better flexural modulus.[9,28] However, there is an exception to this rule. For example, Tetric EvoCeram Bulk Fill, despite its high filler content, shows moderate flexular modulus values due to the presence of pre-polymerized fillers in its composition, which are included in the total amount of filler.[9,23] Therefore, the content of inorganic filler, which actually increases the modulus of elasticity, is lower in this material compared to its corresponding high-viscosity counterpart.[9] Based on the information provided on the Dentonics website (https://www.dentonics.com/composites-and-restoratives?page=2), there is no evidence of incorporating pre-polymerized fillers in the bulk-fill RBCs employed in this investigation. Furthermore, while barium glass filler particles, BisGMA and methacrylate monomers are mentioned, no information is given on the filler loading percentage.

Radiopaque fillers such as barium, strontium and zirconium are commonly employed in the composition of RBCs. These types of fillers, particularly barium glass, can cause a loss in flexural properties due to their weak hydrolytic stability.[22,29]

In a study conducted by Rizzante et al.,[24] bulk-fill RBCs presented a wide variety of elastic modulus values, but they were generally comparable to RBCs with a regular viscosity. Furthermore, when compared to conventional composites, bulk-fill RBCs generated the same amount of shrinkage stress, especially when larger increments were employed. However, Janda et al.[30] found no statistically significant differences in the modulus after thermocycling that would be consistent with the results of our study.

The microhardness of resin composites is not only determined by the organic matrix and the inorganic fillers but also depends on the density and structure of the polymer as well as the DC after polymerization. The higher the number of filler particles, the higher the surface hardness of the material. Hardness is also used as an indirect method of measuring the DC; the higher the conversion rate, the higher the hardness value.[31,32]

The initial results of microhardness in the conventional and bulk-fill RBCs in this study were similar, which may have been due to the loading of similar filler content in these 2 types of composites. The results of the studies by Ilie et al.[9] and Puspitasari et al.[31] demonstrated that some bulk-fill RBCs show less hardness than conventional RBCs with the same filler content. This difference is related to the presence of pre-polymerized organic fillers in addition to inorganic fillers in these materials. According to the information on the Dentonics website, pre-polymerized fillers were not present in the composite resins used in this study. In addition, filler loading is not mentioned.

The results of the studies by Tuncer et al.[33] and Ghavami-Lahiji et al.[12] showed that 10,000 cycles of thermocycling significantly reduced the hardness of the composites, which could be due to the absorption of water into the composite resins. Prolonged thermal cycling can cause water absorption and matrix swelling. Water can act as a plasticizer, weakening the polymer network and impairing the matrix–filler interface. This is due to the breakdown of chemical bonds at the silane–filler interface caused by water. Filler–matrix debonding, resin softening and even hydrolytic degradation of fillers could all be caused by the water absorbed by the polymer network,[12,22] which could impact the mechanical properties of the resin composite.

Our findings revealed a significant reduction in the microhardness of the bulk-fill RBC after thermocycling, which was not observed in the conventional RBC from the same company. The presence of a different polymer network in the bulk-fill material may be the cause of this issue. Studies have shown that polymer chains with a high crosslinking density absorb less water because of reduced

free space, resulting in greater thermal stability.[34,35] The greater microhardness of the conventional RBC compared to the bulk-fill material after thermocycling may be attributed to a denser resin network in the conventional composite or a higher DC. Furthermore, microhardness values of the bulk-fill composite decreased after aging, which was consistent with the flexural strength of this material.

Scanning electron microscopy photomicrograph demonstrated several pits due to filler particle exfoliation on the surfaces of the RBCs after aging. The number of pit defects and the deterioration of the resin network were large enough to affect their characteristics, and these changes were more pronounced in the bulk-fill RBC (Fig. 2).

The findings of this study show that the mechanical properties of composites deteriorate over time, especially in bulk-fill materials. According to Leprince et al., the swelling behavior of some bulk-fill RBCs can be problematic, and a capping substance is required not only for aesthetic reasons but also to decrease the destructive properties of the material.[27]

Limitations

It is important to note that the study investigated only one conventional and one bulk-fill RBC from one manufacturer. Furthermore, the success of composite resin restorations is complex and dependent on a number of factors. Material, clinical, patient, and professional factors can all affect the longevity of dental restorations. Despite the deteriorative alterations seen in the current investigation, such factors should be considered for RBCs, especially in the high-viscosity bulk-fill class exposed to aging in the posterior region. Further research into the functioning of RBCs in clinically relevant situations is required.

Conclusions

The bulk-fill RBC showed similar flexural strength, modulus and surface microhardness to its conventional counterpart before aging.

Hydrothermal aging decreased the flexural strength of both composite resins, and no statistically significant difference was found in the flexural modulus.

Aging had a negative effect on the surface microhardness of the bulk-fill RBC but had no significant effect on the surface microhardness of the conventional RBC.

The flexural strength, surface microhardness and surface changes of the bulk-fill type were further influenced by aging.

Ethics approval and consent to participate

Not applicable.

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

ORCID iDs

Fereshteh Naser Alavi https://orcid.org/0000-0002-4879-5434
Mehrsima Ghavami-Lahiji https://orcid.org/0000-0002-2640-2281
Parham Habibi https://orcid.org/0000-0001-8329-868X

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Original paper

Viability of bacteria associated with root caries after Nd:YAG laser application in combination with various antimicrobial agents: An in vitro study

Janina Golob Deeb[1,A–F], Nitya Reddy[1,A–C,F], Todd Kitten[2,A–D,F], Caroline Kelly Carrico[3,C,E,F], Kinga Grzech-Leśniak[1,4,A,C–F]

[1] Department of Periodontics, School of Dentistry, Virginia Commonwealth University, Richmond, USA
[2] Department of Oral and Craniofacial Molecular Biology, Philips Institute for Oral Health Research, School of Dentistry, Virginia Commonwealth University, Richmond, USA
[3] Department of Dental Public Health and Policy, School of Dentistry, Virginia Commonwealth University, Richmond, USA
[4] Laser Laboratory, Department of Oral Surgery, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Kinga Grzech-Leśniak
E-mail: kinga.grzech-lesniak@umw.edu.pl

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Abstract

Background. The neodymium-doped yttrium aluminum garnet (Nd:YAG) laser has various therapeutic applications in dentistry, including the treatment of dentin hypersensitivity and the bacterial reduction therapy in periodontology. The addition of antimicrobial agents may enhance the impact of the laser on bacterial viability.

Objectives. This in vitro study aimed to assess the effect of Nd:YAG laser application in combination with various chemical antimicrobial agents, including hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl), chlorhexidine (CHX), and sodium fluoride (NaF), on the viability of bacteria implicated in the etiology of root caries.

Material and methods. Three oral bacterial species were examined: *Streptococcus mutans* (*S. mutans*); *Streptococcus sanguinis* (*S. sanguinis*); and *Enterococcus faecalis* (*E. faecalis*). The bacteria were grown in broth at 37°C, and then treated with the chemical agents and/or irradiated with an Nd:YAG laser for 30 s. Each treatment modality was repeated 3 times: group 1 – no treatment; group 2 – 0.5% H_2O_2; group 3 – 0.5% NaOCl; group 4 – 0.12% CHX; group 5 – 2% NaF; group 6 – Nd:YAG laser irradiation; group 7 – laser and 0.5% H_2O_2; group 8 – laser and 0.5% NaOCl; group 9 – laser and 0.12% CHX; and group 10 – laser and 2% NaF. The viability of the bacteria was determined by plating them, counting viable colonies, converting the data into colony-forming units (CFUs)/mL, and transforming them into the log form. Statistical analysis was performed using the two-tailed paired *t* test.

Results. Irradiation with an Nd:YAG laser alone did not show a statistically significant effect against any of the bacterial species. The only effective antimicrobial used alone was CHX for *S. mutans*. Chlorhexidine with Nd:YAG resulted in a greater reduction in *S. mutans* and *E. faecalis* than either treatment alone. Meanwhile, H_2O_2 with Nd:YAG also showed an enhanced *S. mutans* reduction. Treatment with 0.5% NaOCl in conjunction with Nd:YAG brought the most significant reduction in viability for all bacteria in comparison with other treatment modalities.

Conclusions. The Nd:YAG laser combined with 0.5% NaOCl resulted in the most substantial reduction in bacterial survival as compared to the antimicrobials or the Nd:YAG laser used alone.

Keywords: antimicrobials, root caries, neodymium laser

Introduction

Periodontitis is a multifactorial chronic inflammatory disease in a susceptible host, initiated by bacteria and driven by the interaction between the biofilm and the host immune response, resulting in tissue destruction and the development of periodontal pockets.[1] The progression and treatment of periodontal disease cause attachment loss, gingival recession and root exposure, which, apart from being esthetically unpleasing, may lead to dentin hypersensitivity and root caries.[2] Root exposure can occur independently as a consequence of aggressive tooth brushing, and the presence of thin alveolar housing or gingival phenotype. Additionally, recession is associated with aberrant frenal attachments, mucogingival deficiencies, the orthodontic therapy, the positional characteristics of the teeth,[3] and natural aging.[4]

The roots may become susceptible to developing caries, which presents as progressive lesions on the root surfaces exposed to the oral environment due to some degree of periodontal attachment loss.[5] The demineralization of the root surface is twice as rapid as in the case of enamel.[6] The pooled prevalence of root caries is reported at 41.5%, and is growing due to the increasing human life span and dentition longevity.[7] Furthermore, isolation, access, the adhesive properties of root surfaces, and the lack of retention in preparations, associated with the root form and anatomy, present a challenge in the treatment of root caries lesions.

The microflora associated with root caries is different from that found in dentinal caries,[8] with *Streptococcus mutans* (*S. mutans*), *Streptococcus sanguinis* (*S. sanguinis*) and *Enterococcus faecalis* (*E. faecalis)* being 3 of the bacterial species implicated in root caries etiology.[8] The onset and progression of caries on the root surface occur due to the bacteria metabolizing fermentable carbohydrates into acids, which initiate the demineralization of the root surface by removing calcium (Ca) and phosphate ions from the surface apatite.[9] While this process starts in enamel at pH of 5.5, pH of only 6.4 is enough for demineralization to begin on less mineralized cementum and dentin on the surface of the exposed root. This lower degree of mineralization makes the initiation and progression of root caries considerably faster.[10]

Lasers have gained significant popularity in dentistry since the 1990s, and are used for various kinds of treatment.[11,12] The neodymium-doped yttrium aluminum garnet (Nd:YAG) laser has a wavelength of 1,064 nm and can penetrate deeper into the tissue, targeting dark pigments, such as melanin and hemoglobin, as its chromophores. The Nd:YAG laser, through exerting a photothermal effect, is capable of killing bacteria by evaporation, destruction or denaturation, which results in their devitalization or inactivation.[13,14] It can be achieved with a quartz fiber-optic tip, 200–320 μm in diameter, placed into the periodontal pocket[15] up to 5 mm[16] to target pigments, with a little effect on the non-pigmented tissues. Due to its ability to target the pigmented and inflamed gingival tissues, the Nd:YAG laser can effectively treat periodontal disease, leading to periodontal regeneration, with new cementum, periodontal ligament and alveolar bone observed during a histological analysis.[17]

Antimicrobial agents, such as chlorhexidine (CHX), have broad antimicrobial activity, as do the irrigants hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl), and are commonly used in dentistry to control supragingival plaque. Chlorhexidine is a potent allopathic reagent that has been used as a wide-spectrum antiseptic agent since 1950 to target Gram-positive and Gram-negative bacteria, fungi, and some viruses, and has the ability to inhibit the formation and development of bacterial plaque for several hours.[18,19] Hydrogen peroxide has been used in dentistry for more than 70 years[20]; it shows a wide-spectrum antimicrobial activity against bacteria, yeasts, fungi, viruses, and spores.[21] Sodium hypochlorite is also known for its antimicrobial effect, as well as fast bactericidal action and non-toxicity at a proper concentration.[22] When combined with curettage, NaOCl effectively reduces soft tissue inflammation in periodontics,[23] and using 0.1% NaOCl during periodontal surgeries could potentially improve the healing and regeneration of the connective tissue.[24] The American Dental Association (ADA) Council on Dental Therapeutics proposed using diluted NaOCl (0.1–0.5%) as an antiseptic mouth rinse for its rapid bactericidal action, relative non-toxicity, the lack of color and staining, a low cost, and having no known contraindications.[25] Applying sodium fluoride (NaF) to tooth surfaces is a well-established and commonly used method for preventing caries, as it promotes the remineralization of enamel and inhibits the production of bacterial acids.[26] Furthermore, in vitro studies have demonstrated the inhibition of demineralization by NaF combined with a carbon dioxide (CO_2) laser.[27]

Limited research has been published on the effect of chemical antimicrobial agents combined with an Nd:YAG laser on the viability of bacterial strains associated with root caries. Therefore, this in vitro study aimed to evaluate the effectiveness of H_2O_2, NaOCl, CHX, and NaF as adjuncts to Nd:YAG laser irradiation on the viability of *S. mutans*, *S. sanguinis* and *E. faecalis*, with the ultimate goal of assessing the efficacy of their application in the prevention of root caries. The proposed hypothesis is that combining a chemical agent with an Nd:YAG laser will lead to a more substantial reduction in viable *S. mutans*, *S. sanguinis* and *E. faecalis* colonies than using the chemical agent or the laser alone.

Material and methods

Bacterial cultures

Three oral bacterial species associated with root caries were used as representatives in the study, including *S. mutans* (UA159), *S. sanguinis* (SK36) and *E. faecalis*. The bacteria were grown individually and treated in parallel. The bacterial species were obtained from the freezer stocks kept at –80°C, with 5 µL of a single-use aliquot inoculated into 5 mL of the Brain Heart Infusion (BHI) broth (Becton Dickinson, Franklin Lanes, USA), and then incubated overnight in an aerobic environment at 37°C. A Genesys™ 150 spectrophotometer (Thermo Fisher Scientific, Emeryville, USA) measured the optical density (OD) of the cultures at 660 nm (OD_{660}), which were then normalized to an OD of 0.5. Ten 150-microliter aliquots of each bacterial strain were transferred to a 96-well plate, to non-adjacent wells to provide 10 treatment groups for each experiment. The stock solutions of the chemical antimicrobial agents (3% H_2O_2, 5.25% NaOCl, 2% CHX, and 75% NaF) were diluted in sterile distilled water to 4 times the desired final concentration. Then, 50 µL of each diluted agent was added to the 150 µL of bacteria already present in each well, resulting in the concentrations listed below for each study group.

Laser irradiation was performed on the designated study groups in a sterile biological safety cabinet, with the Nd:YAG and/or chemical agent treatment done individually to ensure that each bacterial culture received contact with the chemical agent and/or the laser for 30 s. Following Nd:YAG laser irradiation, the treated samples were diluted 50-fold into fresh BHI broth, and then further diluted before being spread onto BHI plates with the use of an Eddy Jet 2 spiral plater (Neutec Group Inc., Farmingdale, USA). The plates were incubated in anaerobic conditions at 37°C for 24–48 h. Each plate was examined, with viable colonies counted and converted into colony-forming units (CFUs)/mL, which were log-transformed for statistical analysis.

Table 1. Model results for the average colony count after treatment

Model	*F*-value (ANOVA)	*p*-value
Bacteria	12.91	**<0.0001**
Irrigant	170.92	**<0.0001**
Laser (Y/N)	134.98	**<0.0001**
Bacteria*Irrigant	7.71	**<0.0001**
Irrigant*Laser(Y/N)	32.13	**<0.0001**
Bacteria*Laser(Y/N)	0.91	0.4055
Bacteria*Irrigant*Laser (Y/N)	3.34	**0.0015**

Y – yes; N – no; values in bold indicate statistical significance ($p < 0.05$).

Laser irradiation parameters

A LightWalker Nd:YAG laser (Fotona, Ljubljana, Slovenia) with a wavelength of 1,064 nm and a 300-micrometer fiber tip was used for the irradiation of the bacterial cultures in direct contact at 150 mJ, 20 Hz and 3 W for 30 s in the micro-short pulse (MSP) mode (pulse duration of 100 µs). A disinfected aluminum foil barrier was applied to isolate the treated wells during laser irradiation and prevent the contamination of other wells by spatter. Each experiment was repeated 3 times.

Study groups

The groups were formed as follows:
– group 1: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*);
– group 2: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*) + H_2O_2 (0.5%);
– group 3: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*) + NaOCl (0.5%);
– group 4: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*) + CHX (0.12%);
– group 5: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*) + NaF (2%);
– group 6: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*) + Nd:YAG;
– group 7: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*) + Nd:YAG + H_2O_2 (0.5%);
– group 8: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*) + Nd:YAG + NaOCl (0.5%);
– group 9: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*) + Nd:YAG + CHX (0.12%); and
– group 10: bacteria (*S. mutans*, *S. sanguinis* or *E. faecalis*) + Nd:YAG + NaF (2%).

Statistical analysis

The combined effect of the irrigants and the laser on the log CFU bacterial count was assessed using the analysis of variance (ANOVA) model. Post hoc pairwise comparisons were adjusted using Tukey's adjustment. The significance level was set at 0.05.

Results

Significant differences were observed in bacterial recovery with regard to the irrigant (the chemical antimicrobial agent) used, the Nd:YAG laser and the bacterial species for all combinations. Table 1 summarizes the models, while Table 2 presents pairwise comparisons for the effect of the irrigants with and without the laser (irrigant + laser vs. irrigant alone), and Table 3 shows pairwise comparisons for the effect of the laser with and without the irrigants (laser + irrigant vs. laser alone).

Table 2. Pairwise comparisons of the effect of the irrigants with and without the laser on the average colony count [log CFU/mL])

Comparison	Bacterial species	Irrigant	Estimated average change	*SE*	Adjusted *p*-value (Tukey's test)
Nd:YAG laser vs. no laser	*E. faecalis*	CHX	–5.23	0.81	<0.0001*
		H_2O_2	–2.06	0.81	0.7396
		NaF	–0.03	0.81	>0.9990
		NaOCl	–5.74	0.81	<0.0001*
		none	0.08	0.81	>0.9990
	S. mutans	CHX	–0.26	0.81	>0.9990
		H_2O_2	–3.27	0.81	0.0240*
		NaF	0.06	0.81	>0.9990
		NaOCl	–6.63	0.81	<0.0001*
		none	–0.03	0.81	>0.9990
	S. sanguinis	CHX	–5.24	0.81	<0.0001*
		H_2O_2	–1.52	0.81	0.9885
		NaF	0.05	0.81	>0.9990
		NaOCl	–6.41	0.81	<0.0001*
		none	–0.08	0.81	>0.9990

SE – standard error; Nd:YAG – neodymium-doped yttrium aluminum garnet; *E. faecalis* – *Enterococcus faecalis*; *S. mutans* – *Streptococcus mutans*; *S. sanguinis* – *Streptococcus sanguinis*; CHX – chlorhexidine; H_2O_2 – hydrogen peroxide; NaF – sodium fluoride; NaOCl – sodium hypochlorite; CFU – colony-forming unit; * statistically significant.

Table 3. Pairwise comparisons of the effect of the laser with and without the irrigants on the average colony count [log CFU/mL]

Comparison	Bacterial species	Laser	Irrigant	Estimated average change	*SE*	Adjusted *p*-value (Tukey's test)
Irrigant vs. no irrigant	*E. faecalis*	Nd:YAG	CHX	–6.81	0.99	<0.0001*
			H_2O_2	–3.11	0.99	0.2959
			NaF	–0.06	0.99	1.0000
			NaOCl	–9.28	0.99	<0.0001*
	S. mutans	Nd:YAG	CHX	–8.89	0.99	<0.0001*
			H_2O_2	–4.24	0.99	0.0101*
			NaF	0.08	0.99	1.0000
			NaOCl	–8.89	0.99	<0.0001*
	S. sanguinis	Nd:YAG	CHX	–8.57	0.99	<0.0001*
			H_2O_2	–3.86	0.99	0.0388*
			NaF	0.14	0.99	1.0000
			NaOCl	–8.57	0.99	<0.0001*

* statistically significant.

Figure 1 shows that the Nd:YAG laser demonstrated a synergistic effect with NaOCl, reducing significantly more *E. faecalis* than NaOCl alone (–5.74 log CFU/mL; adjusted $p < 0.0001$) or the laser alone (–9.28 log CFU/mL; adjusted $p < 0.0001$), and reducing substantially more *S. mutans* than NaOCl alone (–6.63 log CFU/mL; adjusted $p < 0.0001$) or the laser alone (–8.89 log CFU/mL; adjusted $p < 0.0001$). Furthermore, combining the Nd:YAG laser with NaOCl reduced more *S. sanguinis* than NaOCl alone (–6.41 log CFU/mL; adjusted $p < 0.0001$) or the laser alone (–8.57 log CFU/mL; adjusted $p < 0.0001$).

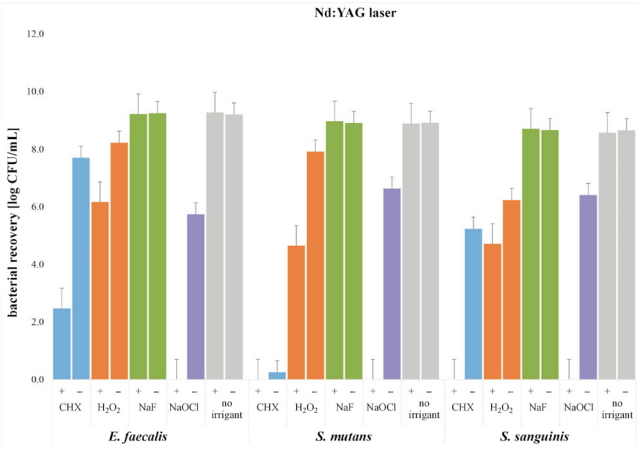

Fig. 1. Mean bacterial recovery [log CFU/mL] with a standard error (*SE*) with regard to various treatment modalities

+ Nd:YAG laser used; – no laser used.
The significance of pairwise comparisons is reported in Tables 2 and 3.

The Nd:YAG laser had a synergistic effect with H_2O_2, reducing significantly more *S. mutans* than H_2O_2 alone (–3.27 log CFU/mL; adjusted $p = 0.0240$) and the laser alone (–4.24 log CFU/mL; adjusted $p = 0.0101$). However, the combination of Nd:YAG and NaOCl was more effective than Nd:YAG and H_2O_2 for *S. mutans* (–4.6 log CFU/mL; adjusted $p = 0.0020$ (data not presented)).

The Nd:YAG laser acted synergistically with CHX, killing significantly more *S. sanguinis* than CHX alone (–5.24 log CFU/mL; adjusted $p < 0.0001$) or the laser alone (–8.57 log CFU/mL; adjusted $p < 0.0001$). The same was true for the Nd:YAG laser combined with CHX, which killed significantly more *E. faecalis* than CHX alone (–5.23 log CFU/mL; adjusted $p < 0.0001$) or the laser alone (–6.81 log CFU/mL; adjusted $p < 0.0001$). For *S. mutans*, CHX was effective on its own, with no additional benefit from the Nd:YAG laser (adjusted $p = 0.300$).

Chemical antimicrobial agents

Sodium fluoride did not reduce the viability of *S. mutans*, *S. sanguinis* or *E. faecalis* when used alone or in conjunction with the Nd:YAG laser. The only statistically significant reduction in bacterial growth by H_2O_2 was observed for *S. mutans* when it was used with the Nd:YAG laser, though it did not eliminate all *S. mutans* bacteria (–3.27 log CFU/mL; adjusted $p = 0.0240$). Chlorhexidine proved to be an effective monotherapy for *S. mutans*, as it reduced the bacterial count to undetectable levels whether or not laser irradiation was used. Additionally, CHX was more effective on *S. sanguinis* (–5.24 log CFU/mL; adjusted $p < 0.0001$) and *E. faecalis* (–5.23 log CFU/mL; adjusted $p < 0.0001$) when used alongside the Nd:YAG laser.

The most extensive effect of NaOCl occurred in combination with the Nd:YAG laser; the synergistic effect was observed for all 3 bacterial species.

Bacterial strains

Enterococcus faecalis was reduced to undetectable levels with the NaOCl and Nd:YAG laser combined treatment. When used separately, neither NaOCl nor the Nd:YAG laser was able to achieve the same level of *E. faecalis* reduction. The combination of NaOCl and the Nd:YAG laser had a synergistic antimicrobial effect and was the most effective treatment for *E. faecalis*.

For *S. mutans*, the most effective treatment was CHX as a monotherapy or in conjunction with the Nd:YAG laser, with both resulting in undetectable amounts of bacterial recovery after treatment. While the synergistic effect of NaOCl and the Nd:YAG laser was observed, *S. mutans* reduction with CHX was not enhanced by the addition of the Nd:YAG laser.

Streptococcus sanguinis achieved the highest reduction when CHX or NaOCl were used in conjunction with the Nd:YAG laser. Combining either CHX or NaOCl with the Nd:YAG laser resulted in undetectable bacterial levels, and was the most effective synergistic antimicrobial treatment for *S. sanguinis*.

This in vitro experiment evaluated only 3 bacterial species, providing a small-sample representation with regard to abundant bacteria engaged in the complex interactions present in the clinical environment.

Discussion

In this investigation, we showed the effect of the synergistic application of an Nd:YAG laser and chemical antimicrobial agents on reducing the viability of *S. mutans*, *S. sanguinis* and *E. faecalis* in vitro. Based on our data, NaOCl combined with the Nd:YAG laser resulted in the greatest growth reduction to undetectable levels for all 3 bacterial species. However, the laser as individual treatment was unable to reduce counts for any of the tested bacteria. A previous study confirmed the bactericidal and synergistic effect of the Nd:YAG laser when combined with CHX, H_2O_2 or NaOCl in reducing periodontal pathogens, specifically *Porphyromonas gingivalis* (*P. gingivalis*) and *Fusobacterium nucleatum* (*F. nucleatum*).[28] In both investigations, the laser parameters were selected to reflect the clinical settings used for periodontitis treatment, so that the protocols could be clinically translated and implemented as part of a supportive periodontal therapy (SPT) in patients with attachment loss, identified as being at high risk of caries.

Chlorhexidine is an effective antimicrobial agent capable of reducing a bacterial load by 97% when used as a preoperative rinse. However, it is not indicated for continued long-term use due to its side effects, such as altered taste and the staining of the teeth.[29,30] In the present study, CHX effectively reduced the *S. mutans* count to undetectable levels as a monotherapy, and also worked in combination with the Nd:YAG laser.

Hydrogen peroxide has been used as a mouth rinse for plaque control and the treatment of oral infections. A recent review reported that H_2O_2 had no side effects, but was not superior to CHX in antiplaque efficacy, and in reducing gingival inflammation and the oral bacteria count.[18] The concentration of H_2O_2 used in most studies is 1.5%, which is lower as compared to the present study. However, higher concentrations, such as 3%, did not cause mucosal irritation in an animal model at a maximum contact time of 7 min, and it is the concentration that is most commonly available over the counter.[19] Further examination of the hydroxyl radicals generated during the photolysis of H_2O_2 showed that they were a powerful oxidizing agent capable of inducing oxidative damage to oral bacteria.[19] The results of the present study align with these findings, as the Nd:YAG laser combined with H_2O_2 had an increased bactericidal effect on *S. mutans* as compared to either treatment alone.

While we do not fully understand the mechanisms underlying the synergistic effect found when combining laser treatment with NaOCl, there is evidence that thermal energy can potentiate the effect of NaOCl. Indeed, the intracanal heating of NaOCl in endodontic therapy has been shown to increase bacterial reduction as compared to the ultrasonic and non-heated agitation techniques.[31] Therefore, we speculate that the thermal effects from the Nd:YAG laser contributed to the enhanced bactericidal effect of NaOCl.

Sodium fluoride did not cause bacterial reduction when used alone or in combination with the Nd:YAG laser, which is in agreement with the studies reporting that various concentrations of fluorides did not significantly decrease the growth of *P. gingivalis* or *S. mutans* on titanium disks,[22] and even found a slight increase in bacterial growth with a 1% gel concentration.[23] Sodium fluoride, when applied to a tooth surface, reduces the demineralization and promotes the remineralization of enamel. Fluoride-treated teeth exhibit higher pH values, as fluoride inhibits the production of bacterial acids, which proves its antimicrobial rather than direct bactericidal effect.[26] The observations of the present study align with these reports, as we did not find any bacteria-reducing effect with NaF alone or when used alongside the Nd:YAG laser.

The prevention of root caries is important for periodontists, as 2/3 of periodontally treated patients may develop root caries during the first 4 years of periodontal maintenance. Furthermore, the incidence of new root caries persists longitudinally at 4, 8, 12, and 14 years of periodontal maintenance.[32–36] Moreover, a cross-sectional study reported a high prevalence of root caries and high caries risk rates in 20% of patients referred for periodontal treatment.[37] The current study showed that using an Nd:YAG laser with low concentrations of NaOCl may constitute an effective method that is easy to implement during SPT when treating patients at high risk of caries.

The application of the Nd:YAG laser with the settings used herein is already established for periodontitis treatment, and similar findings as in this study have been reported for the reduction of bacteria associated with periodontitis when the Nd:YAG laser was used in conjunction with chemical agents (H_2O_2 and NaOCl).[28,38–41] Such chemical agents could be applied along the gingival margin, over the exposed root surfaces before using the laser in the maintenance therapy.

Limitations

Limitations to the present study include its in vitro nature. Thus, determining the clinical significance of the observed effects of the Nd:YAG laser and chemical irrigants in the periodontal therapy remains unclear. Furthermore, evaluating only 3 bacterial species and the small sample size means the findings cannot be clinically extrapolated.

Conclusions

Treatment with an Nd:YAG laser and low concentrations of chemical antimicrobial agents provided synergistic effects, reducing the viability of bacterial species associated with root caries. In comparison with the chemical antimicrobial agents or the Nd:YAG laser used alone, the greatest reduction in bacterial viability was achieved when using the Nd:YAG laser with 0.5% NaOCl.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Janina Golob Deeb https://orcid.org/0000-0002-3189-0136
Nitya Reddy https://orcid.org/0000-0002-4206-2707
Todd Kitten https://orcid.org/0000-0001-6097-7583
Caroline Kelly Carrico https://orcid.org/0000-0001-9521-9854
Kinga Grzech-Leśniak https://orcid.org/0000-0002-5700-4577

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Original paper

Physical analysis of an acrylic resin modified by metal and ceramic nanoparticles

Luciana Machado-Santos[1,A–D,F], Kusai Baroudi[1,2,A,B,E,F], Nikolaos Silikas[3,A–D,F], João Paulo Mendes Tribst[4,C–F], Mario Alexandre Coelho Sinhoreti[5,A,B,D,F], William Cunha Brandt[6,A–D], Priscila Christiane Suzy Liporoni[1,A,B,D,F]

[1] Department of Restorative Dentistry, School of Dentistry, University of Taubaté, Brazil
[2] RAK College of Dental Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, UAE
[3] Department of Dentistry, School of Medical Sciences, University of Manchester, UK
[4] Department of Reconstructive Oral Care, Academic Centre for Dentistry Amsterdam (ACTA), Universiteit van Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, the Netherlands
[5] Department of Dental Materials, Piracicaba Dental School, State University of Campinas, Brazil
[6] Department of Dentistry, University of Santo Amaro, São Paulo, Brazil

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
João Paulo Mendes Tribst
E-mail: joao.tribst@gmail.com

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Abstract

Background. Nanoparticles (NPs) have gained significant attention in various fields due to their unique properties and potential applications. Polymethyl methacrylate (PMMA) is an acrylic resin widely used in dentistry and medicine. However, the effect of different types of NP fillers on the physical properties of PMMA-based resins has not been thoroughly explored in the literature.

Objectives. The present study aimed to evaluate the effects of 3 different types of NP fillers on the physical properties of an experimental PMMA-based resin as a function of the NP content and concentration.

Material and methods. Ten groups ($n = 10$) were designed. The specimens were composed of an acrylic resin, silicon dioxide (SiO_2), cerium dioxide (CeO_2), and titanium dioxide (TiO_2) at the following ratios (wt%): group 1 (G1) – control; group 2 (G2) – 0.5% SiO_2; group 3 (G3) – 1% SiO_2; group 4 (G4) – 3% SiO_2; group 5 (G5) – 0.5% CeO_2; group 6 (G6) – 1% CeO_2; group 7 (G7) – 3% CeO_2; group 8 (G8) – 0.5% TiO_2; group 9 (G9) – 1% TiO_2; and group 10 (G10) – 3% TiO_2. Transmission electron microscopy (TEM) was used to assess the quality of NP dispersion. Thermal stability was assessed with thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The effects of the abovementioned NPs on the properties of the resin were evaluated using the Archimedes principle for density, the Vickers hardness (VH) test and the impact strength (IS) test. Data analysis employed the one- and two-way analysis of variance (ANOVA), followed by Duncan's post hoc test at a significance level of 0.05.

Results. Transmission electron microscopy showed partial NP dispersion. All types of NPs enhanced the mechanical properties of the acrylic resin except for IS, which was similar to that of the control group. Among the types of NPs, irrespective of the weight percentage, CeO_2 showed higher thermal stability and higher IS for 0.5 wt% and 1 wt% as compared to other groups, as well as the highest values of density at 0.5 wt%, 1 wt% and 3 wt%. Titanium oxide at 1 wt% presented a higher VH as compared to other groups. The fracture pattern was the same for all groups.

Conclusions. Incorporating the tested NPs into the acrylic resin resulted in enhanced physical properties, primarily attributed to a lower NP content.

Keywords: nanoparticles, silicon dioxide, cerium dioxide, titanium dioxide, polymethyl methacrylate

Introduction

Acrylic resins have been used in dentistry and medicine since 1936, and have remained the primary material for manufacturing dental and craniofacial prostheses.[1] The material is typically fabricated with the use of polymethyl methacrylate (PMMA). The polymer has beneficial biological properties, as it is tasteless, insoluble and compatible with oral tissues. The favorable physical characteristics of PMMA include dimensional stability, resilience, resistance to compression, the ease of handling, and a low cost.[2] However, PMMA is not an ideal material in terms of mechanical properties required for long-term clinical use, such as flexural strength, hardness, density, and thermal stability.[3,4]

To improve the physical properties of different dental biomaterials, composites have been reinforced with inorganic nanofillers.[5–7] Studies have shown that decreasing the size of the reinforcement particles to a nanoscale can completely alter the way they interact with the matrix, either due to the increased surface area or the possible interactions with the matrix in the molecular sphere, changing the chemical characteristcs of the matrix.[8]

Silicate-based minerals, such as silica (silicon dioxide (SiO_2), approx. 40 nm), have been used as inorganic fillers in methacrylate-based resins, and are associated with good mechanical and polishing properties. Numerous studies have demonstrated the correlation between the quantity of silica and the modulus of elasticity, wear resistance and polymerization shrinkage.[9–11]

A previous study incorporated 3 different metal oxides in different proportions into PMMA, namely titania (titanium dioxide (TiO_2)), zirconium dioxide (ZrO_2) and aluminum oxide (Al_2O_3), and concluded that all experimental groups showed better resistance to impact, sorption and solubility as compared to the control group.[3] Cerium-based metallic material, such as ceria (cerium dioxide (CeO_2)), is transparent to visible light, can act as a catalyst, and has been linked to better compression resistance properties and thermal expansion values of ceramics.[12] Ceria has demonstrated antibacterial properties against *Pseudomonas aeruginosa* after being treated with polyacrylic acid.[13] However, there are no reports on the use of the material in the reinforcement of acrylic resins.

Titanium dioxide nanoparticles (NPs) are chemically inert, resistant to corrosion, non-toxic, and inexpensive. They have a high refractive index and antibacterial properties under a variety of spectra.[14] For these and other characteristics, TiO_2 NPs are incorporated into polymeric materials, such as PMMA.[15] In comparison with its larger particle version, TiO_2 NPs primarily offer heat stability, clearly define the color of the polymer and provide improved reinforcing properties. As such, the particles do not lose their features during the polymerization process at high temperatures, contributing to the control of polymerization shrinkage.[3] The existing studies indicate that incorporating surface-treated TiO_2 into a PMMA matrix leads to a reduced shrinkage stress of the polymeric matrix, which is due to the formation of agglomerates that serve as concentration points for these stresses.[16] According to a study by Acosta-Torres et al., with regard to the physical properties of conventional and nanopigmented PMMA, the matrices containing TiO_2 show less porosity.[17] However, certain studies have highlighted the adverse effects associated with incorporating TiO_2 and SiO_2 into polymeric materials.[18,19]

In light of the current literature,[1–19] there is a notable research gap regarding the effects of 3 distinctive NPs – SiO_2, CeO_2 and TiO_2 – as potential fillers for experimental PMMA-based resins. While the use of NPs as additives has been explored in various materials, their interactions with PMMA-based resins remain surprisingly underrepresented in the current research. Therefore, the present study aimed to bridge this gap by conducting a comprehensive analysis of the influence of these NPs on the mechanical properties of a resin across a spectrum of concentrations. Our study encompassed a thorough examination of density, Vickers hardness (VH) and impact strength (IS) to assess the mechanical behavior of the resin. Furthermore, the study evaluated the thermal stability of the NP-integrated resin via thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

The purpose of the present study was to investigate the influence of different content and concentration of SiO_2, CeO_2 and TiO_2 on the physicochemical properties of an PMMA-based resin. The present hypothesis is that the incorporation of NPs does not alter the properties of PMMA.

Material and methods

To investigate the effects of different NPs on the properties of a PMMA-based resin, 100 samples (N = 100) were prepared and divided into 10 distinct groups (n = 10 per group). A commercially available acrylic resin (Acron™ MC; GC America Inc., Alsip, USA) composed of powder and liquid was used. Three types of NPs, ranging in size from 15 nm to 50 nm, were added to the powder before the polymerization procedure by using a Hauschild SpeedMixer® DAC 150.1 (Hauschild & Co., Hamm, Germany) at 50/60 Hz and 3,500 rpm. The NPs comprised SiO_2 (15 nm), CeO_2 (25 nm) and TiO_2 (50 nm) in standardized sizes, as provided by the manufacturer (SkySpring Nanomaterials Inc., Houston, USA). The specimens in each group were composed of an acrylic resin, SiO_2, CeO_2, and TiO_2 at the following ratios (wt%): group 1 (G1) – control; group 2 (G2) – 0.5% SiO_2; group 3 (G3) – 1% SiO_2; group 4 (G4) – 3% SiO_2; group 5 (G5) – 0.5% CeO_2; group 6 (G6) – 1% CeO_2; group 7 (G7) – 3% CeO_2; group 8 (G8) – 0.5% TiO_2; group 9 (G9) – 1% TiO_2; and group 10 (G10) – 3% TiO_2.

The preliminary test using transmission electron microscopy (TEM) suggested that the NPs had better dispersion when added to the powder instead of the liquid. After microwave polymerization, the samples were cut with a vitreous fragment at 45°, and then an ultramicrotome (EM UC6; Leica Microsystems, Wetzlar, Germany) was used to obtain ultrathin (80-nanometer) sections. The slices were placed in a fine mesh (grid storage box; Agar Scientific Ltd., Stansted, UK) and observed under a TEM at 300 Kv (Tecnai G2 F30; FEI Company, Hillsboro, USA) to identify the NP content. The NPs were incorporated into the powder, and the flask was closed and placed inside the mixer at 2,000 rpm for 3 min, then rested on the bench for another 3 min. The liquid was added to the mixture and stirred at 500 rpm for 1 min to prevent the premature packing of the resin, which may occur due to the agitation and shear forces generated during this step. The liquid needs to be thoroughly and evenly dispersed within the powder to achieve a consistent resin.

The size of the samples depended on the specific characteristics of the respective tests. For instance, disk-shaped samples measuring 5 mm × 4 mm were employed in the VH test, while samples measuring 65 mm × 10 mm × 2 mm were utilized for the Charpy IS test. Similarly, the sample mass for TGA was standardized at 20 ±2 mg. By adjusting the sample sizes according to the unique demands of each test, we aimed to obtain reliable and comprehensive data that would accurately reflect the properties under investigation. However, the inclusion and polymerization processes were standardized for all samples.

Metal molds were used for the TEM and Charpy IS test samples, and composite resin molds were used for all other tested samples. The molds were invested individually with laboratory silicone (Titanium Zetalabor; Zhermack, Badia Polesine, Italy) and covered with type III dental stone (Scola-cast powder; Scola, Cheshire, UK) in a ceramic muffle oven (GC Europe, Leuven, Belgium). The experimental acrylic resin was prepared as previously described and immediately packed into the silicone index at its dough stage. The flasks were slowly and gradually pressed in a hydraulic press until establishing 1.25 kg, and then polymerized in a microwave oven (Daewoo KQG-6L6B, 800 W maximum power; Daewoo Electronics, Wokingham, UK) for 10 min: phase 1 – 3 min at 40% power; phase 2 – 4 min at 0% power; and phase 3 – 3 min at 90% power. Then, the flasks were placed on the bench until completely cooled. The samples were deflasked and their surfaces were smoothed with an electric minigrinder (Dremel® 3000; Robert Bosch Tool Corporation, Mount Prospect, USA) at 5,000–33,000 rpm. For the VH test, one side of the sample was polished with 600–1,200 grit silicon carbide sandpaper (Jiangsu Jianda Grinding Technology Co. Ltd., Wuxi, China). The samples were stored dry at 24 ±1°C for 24 ±2 h before the tests.

A sample from each group was submitted for TGA. Thermal stability was determined by means of the thermogravimetric trace record (STARe software; Mettler-Toledo, Greinfensee, Switzerland) in an atmosphere of nitrogen (N), using small sample fragments. A heating rate of 10°C/min, with a temperature ranging from 35°C to 600°C, and a sample portion weighing 20 ±2 mg were used for this test. The balance arm was held in a horizontal reference position and changes in the sample mass caused the beam to bend, which was detected by the photoelectric cells. The TGA curve was obtained by recording variations in temperature and the sample mass continuously. Differential scanning calorimetry was conducted simultaneously with the TGA test, and was used to better understand the thermal behavior and homogeneity of the experimental resin samples. The same fragment from each group (20 ±2 mg) was analyzed to compare the glass transition temperature (T_g) of the polymer (control) and of the NP-modified polymer.

One hundred disk-shaped samples, divided into 10 groups (n = 10 per group), measuring 5 mm × 4 mm and with the top surfaces polished, were evaluated for VH in a microhardness tester (FM-700; Future-Tech Corp., Kawasaki, Japan). A 100-gram load was applied to the polished surface of the sample at the speed of 0.05 mm/s for 10 s, and the values were recorded in Vickers pyramid numbers (HV). Three indentations were made in each sample. Then, the Archimedes principle was applied to investigate density changes, with the mass (m) measured on an analytical precision scale and the volume (V) determined by the principle. The density of the sample (ρ) was calculated using the following formula (Equation 1):

$$\rho = m/V \quad (1)$$

where:
ρ – density [g/cm^3];
m – mass [g]; and
V – volume [cm^3].

One hundred samples, divided into 10 groups (n = 10 per group), measuring 65 mm × 10 mm × 2 mm were subjected to the IS test in the Charpy system (Otto Wolpert-Werke, Ludwigshafen am Rhein, Germany) with an impact load of 40 kpcm. The impact load recorderd at the moment of specimen failure was changed to the IS value [kgf/cm^2] using the following formula (Equation 2):

$$IS = I/L \times H \quad (2)$$

where:
IS – impact strength [kgf/cm^2];
I – impact load (kpcm);
L – sample length in the impact region [cm]; and
H – sample height in the impact region [cm].

A fractographic analysis was conducted for the 2 sample fragments obtained in the IS test, and a macroscopic analysis was done by the visual inspection of the fractured surfaces, using a claw-mounted magnifier (Western 9051L; Western Ophthalmics Corp., São Paulo, Brazil) at ×4 magnification. The fragments of each specimen were called fragment A (FA) and fragment B (FB). During visual inspection, when FA and FB could be repositioned at the fracture line, presenting a smooth surface, the fractures were classified as brittle. Those presenting plastic deformation, or exhibiting rough and jagged surfaces were recorded as intermediate (ductile-to-brittle transition) fractures. The fractographic examination verified that all samples could have FA and FB perfectly placed together, and not even the fracture line could be seen at ×4 magnification; therefore, all samples were classified as brittle. The control and the 1 wt% experimental samples were covered with a fine layer of gold, using a sputter coater (Emitech K550X; Quorum Technologies Ltd., Ashford, UK) and observed under a scanning electron microscope (SEM) (EVO MA10; Carl Zeiss, Oberkochen, Germany) to analyze and compare the fracture morphology and microstructure.

Statistical analysis

Data analysis employed the one- and two-way analysis of variance (ANOVA), followed by Duncan's post hoc test at a significance level of 0.05.

Results

Transmission electron microscopy showed enhanced dispersion when NPs were incorporated into the powder component of the acrylic resin. However, the incorporation method did not completely prevent the formation of agglomerates (Fig. 1). Furthermore, a strong bond between the NPs and the matrix was evident in cases of effective dispersion, while regions with more pronounced agglomeration displayed voids (Fig. 1).

Table 1 presents the TGA and DSC values from the respective thermographs. The curve profiles in both analyses exhibited pattern similarity, displaying only slight temperature shifts. The control group displayed a relatively higher mass loss following the initial event, and preliminary observations suggested that 1% TiO_2 exhibited a relatively higher initial degradation temperature (T_i). Moreover, all ceria groups presented higher T_i values than the control group. The final residue percentage increased proportionally to the amount of incorporated NPs in each group, except for the control group. In the 2nd significant mass loss event, the control group

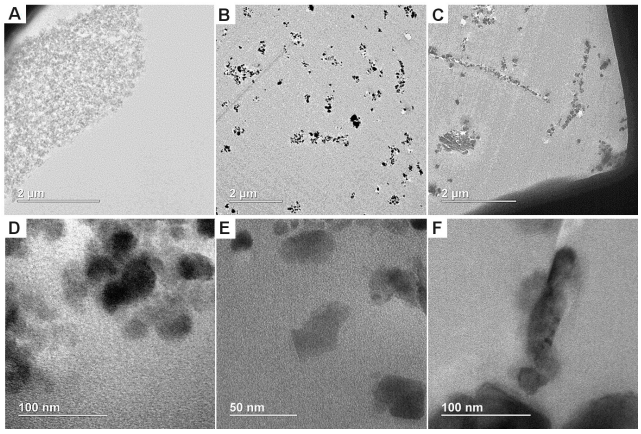

Fig. 1. Transmission electron microscopy (TEM) micrographs showing the dispersion of the nanoparticles (NPs) (3 wt%) within the polymeric matrix (A–C) and the TEM observations of the NP (3 wt%)–matrix bonded interface (D–F)

A – silicon dioxide (SiO_2); B – cerium dioxide (CeO_2); C – titanium dioxide (TiO_2); D – SiO_2; E – CeO_2; F – TiO_2.

Table 1. Resin decomposition temperatures, mass loss (ML), residue, and glass transition temperature (T_g)

Groups	Thermal degradation event						Residue at 600°C [%]	T_g [°C]
	1st			2nd				
	T_i [°C]	$T_{midpoint}$ [°C]	ML [%]	T_i [°C]	$T_{midpoint}$ [°C]	ML [%]		
G1	194.46	288.94	13.48	332.63	373.78	84.17	2.36	102.50
G2	195.73	286.40	12.36	328.29	370.95	85.56	2.08	112.50
G3	187.44	286.84	11.01	327.07	372.90	86.52	2.46	105.20
G4	174.37	291.62	11.02	329.49	370.65	84.82	4.15	108.25
G5	198.08	289.51	11.00	329.40	375.20	86.74	2.25	112.50
G6	212.73	297.34	10.10	332.35	375.37	87.03	2.87	107.00
G7	203.09	296.61	8.95	330.12	374.20	86.63	4.42	106.50
G8	198.71	289.95	11.78	328.27	372.66	85.80	2.42	110.50
G9	218.43	296.14	8.97	328.43	372.45	88.03	2.99	109.50
G10	177.47	289.74	13.37	328.61	371.52	82.07	4.56	104.50

Groups: G1 – control; G2 – 0.5% SiO_2; G3 – 1% SiO_2; G4 – 3% SiO_2; G5 – 0.5% CeO_2; G6 – 1% CeO_2; G7 – 3% CeO_2; G8 – 0.5% TiO_2; G9 – 1% TiO_2; and G10 – 3% TiO_2. T_i – initial degradation temperature; $T_{midpoint}$ – midpoint temperature (the point at which the thermal degradation reaction is approx. halfway complete).

displayed slightly higher temperatures, aligning with those observed in the ceria groups, which recorded relatively higher values as compared to the control.

The mean and standard deviation (*M* ±*SD*) values for VH, ρ and Charpy IS for all groups, including the control group, are reported in Fig. 2–4. The experimental results

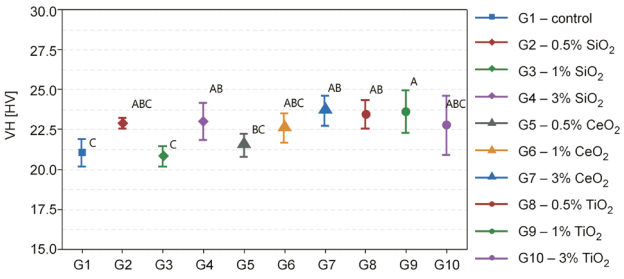

Fig. 2. Mean (*M*) and standard deviation (*SD*) values for Vickers hardness (VH)
The *SD* values were used to calculate 95% confidence intervals (*CIs*). Different letters in the graph show statistical differences between the groups ($p < 0.05$; one-way ANOVA followed by Duncan's post hoc test).

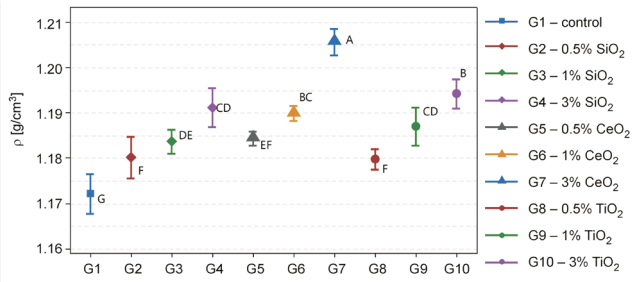

Fig. 3. Mean (*M*) and standard deviation (*SD*) values for density (ρ)
The *SD* values were used to calculate 95% *CIs*. Different letters in the graph show statistical differences between the groups ($p < 0.05$; one-way ANOVA followed by Duncan's post hoc test).

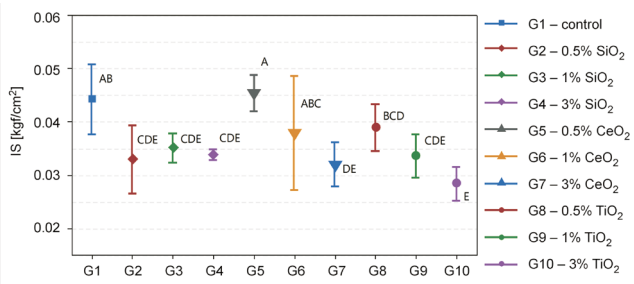

Fig. 4. Mean (*M*) and standard deviation (*SD*) values for impact strength (IS)
The *SD* values were used to calculate 95% *CIs*. Different letters in the graph show statistical differences between the groups ($p < 0.05$; one-way ANOVA followed by Duncan's post hoc test).

demonstrated significant variations in the mechanical properties among the different groups. Notably, the discernible differences in VH, ρ and IS could be attributed to variations in group composition. The one-way ANOVA revealed a statistically significant *p*-value ($p < 0.05$), indicating the substantial influence of the NP content and concentration on the aforementioned mechanical properties, underscoring the importance of group composition in shaping the material characteristics.

The VH mean values for the control and 1% SiO_2 groups were significantly lower as compared to the 3% SiO_2, 3% CeO_2, 0.5% TiO_2, and 1% TiO_2 groups (Fig. 2). Regarding density, the results show that the 3% CeO_2 group exhibited relatively higher scores, while the control group showed relatively lower values. The 0.5% SiO_2 and 0.5% TiO_2 groups had significantly lower means than the 3% TiO_2 group, though the results for the 3% SiO_2 and 1% TiO_2 groups did not differ statistically (Fig. 3). For IS, the mean values in the 3% TiO_2 group were significantly lower than those in the control, 0.5% CeO_2, 1% CeO_2, and 0.5% TiO_2 groups. The 3% CeO_2 group scores were lower than those in the 0.5% SiO_2, 1% SiO_2, 3% SiO_2, 1% CeO_2, 0.5% TiO_2, and 1% TiO_2 groups, and significantly lower than those in the control, 0.5% CeO_2 and 1% CeO_2 groups (Fig. 4).

A comprehensive multiple two-way ANOVA yielded significant outcomes, further confirming the substantial impact of the NP weight percentage and type on the observed results. The specific details and outcomes of this analysis are succinctly presented in Table 2. With regard to VH, only the 1% TiO_2 group showed higher values. There were no significant differences among the NPs for the tested weight percentages within the range of 0.5 wt% and 3 wt%. Regarding the NP type, TiO_2 showed no differences when various weight percentages were used. For density, CeO_2 showed higher values; for all NP types, the density values increased along with the weight percentage. Moreover, CeO_2 displayed higher IS than SiO_2 and TiO_2, which behaved similarly across all weight percentages. For SiO_2, the lowest IS value was obtained for 0.5 wt%, and all types of NPs showed lower values when 3 wt% was incorporated, with no difference between 0.5 wt% and 1 wt%.

The fractographic examination verified that all samples could have FA and FB perfectly placed together, and not even the fracture line could be seen at ×4 magnification. Therefore, the fracture patterns were similar (Fig. 5).

Table 2. Multiple comparison analysis of the nanoparticle (NP) weight percentage (wt%) and type

Mechanical property	NP wt%			NP type		
	0.5%	1%	3%	SiO_2	CeO_2	TiO_2
VH	Si = Ce = Ti	(Si = Ce) < Ti	Si = Ce = Ti	0.5% = 3% > 1%	0.5% = 1% = 3%	0.5% = 1% = 3%
ρ	Si = Ce = Ti	Si < Ce = Ti	Si < Ti < Ce	0.5% < (1% = 3%)	0.5% < 1% < 3%	0.5% < 1% < 3%
IS	(Si = Ti) < Ce	Si = Ce = Ti	Si = Ce = Ti	0.5% = 1% = 3%	(0.5% = 1%) > 3%	(0.5% = 1%) > 3%

VH – Vickers hardness; ρ – density; IS – impact strength; Si – silica; Ce – ceria; Ti – titania.

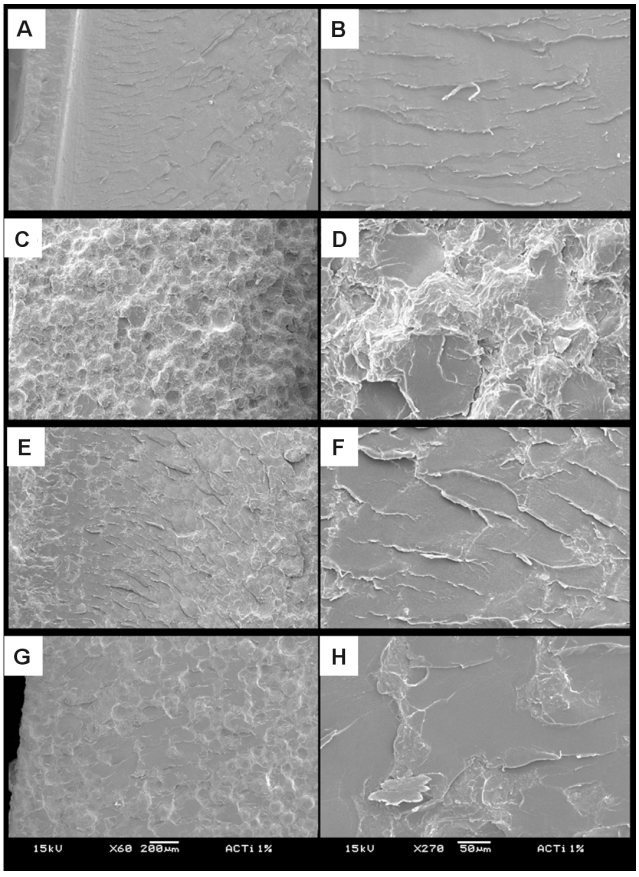

Fig. 5. Scanning electron microscopy (SEM) images of fracture topography with the incorporation of the nanoparticles (NPs) (1 wt%)

A – control (×60); B – SiO_2 (×60); C – CeO_2 (×60); D – TiO_2 (×60); E – control (×270); F – SiO_2 (×270); G – CeO_2 (×270); H – TiO_2 (×270).

Discussion

The hypothesis for this study was rejected, since the incorporation of NPs into the acrylic resin altered all the tested properties. The concept of adding reinforcement materials to enhance the mechanical properties of a dental polymer is not novel.[20] Nanoscale fillers have a significantly greater surface area per mass unit than their larger versions, which can entirely change the way they interact with the polymer matrix. The filler particles used in this study were smaller (15–50 nm) than the resin powder particles (121.2 µm); thus, it was expected that they would fill the interstices between the particles of the polymer, forming a homogeneous mixture without forcing the displacement of the polymer chain segments.[3,21]

The percentage of particles was kept low so that all of them could be involved in the matrix. Increasing the percentage to 3% was expected to improve mechanical properties, as, theoretically, there would be less space between the oxide NPs. However, it has often proved difficult to achieve a stable dispersion of NPs in polymer matrices, as NPs tend to agglomerate.[3,11,22] The agglomerates can form stress concentration sites within the polymer matrix, leading to premature failure with the application of load. Synthesizing the nanocomposite and separating the aggregated NPs were the main challenges of this study.

To disperse the NPs in the hydrophobic polymer matrix, the modification of the NP surfaces was required; it involved silanization and dual asymmetric centrifugal mixing processing.[23] Transmission electron microscopy was employed to characterize the uniformity, or lack thereof, of the NP dispersion in the resin matrix. Inorganic nanofillers were uniformly dispersed, more or less, when incorporated into the powder rather than the liquid. However, agglomeration could not be avoided, and the uniform dispersion throughout the polymer matrix was questionable (Fig. 1). Nonetheless, this could be explained by the suspected imbalance between depletion effects and osmotic pressures in PMMA, adversely impacting the fracture behavior.

Thermogravimetric analysis is a thermal analysis technique in which the mass variation of the sample (loss or gain) is determined as a function of temperature and/or time while the sample is subjected to a controlled temperature program.[24] Such changes are evidenced by a decrease or increase in mass registered by the sensor. Our TGA analysis presented 2 distinct events. The 1st event, at 175–330°C, could be related to the decomposition of secondary groups, such as the free CH_3 groups attached to the main polymer chain, generating volatiles. The 2nd and main mass loss event, at 328–455°C, can be attributed to the degradation of the main PMMA chain. As can be seen in Table 1, increases in the NP concentration and the NP content did not affect the initial degradation temperature (T_i). The glass transition temperature is the temperature at which the movement of the polymer chain segments starts, and at which the material changes from a 'glassy' state (frozen) to a 'rubber' state (more flexible), which can be observed in the DSC curve. Since PMMA is an amorphous polymer, it showed a T_g of approx. 105°C, as expected, which could be observed in the endothermic curve obtained in the heating run. Furthermore, we showed that the NPs may have interfered with the flowing process, which allows molecular chains to slide past each other, decreasing stiffness, and hence increased T_g.[25] The incorporation of NPs showed satisfactory thermal stability for use in dental PMMA, since all types, depending on the weight percentage, exhibited degradation at a temperature comparable to that for the control group and a T_g higher than in the control group.

The current study found that the incorporation of NPs increased microhardness, irrespective of the weight percentage, except for 1% SiO_2. The different densities of the NPs interfere directly with the light weight of the acrylic resin, which can be desirable. In this study, the incorporation of NPs, regardless of their type and weight percentage, significantly increased the density of the resin. A low density can be partly associated with the formation of pores or microgaps, resulting from processing, a problem that affects acrylic resins for prostheses in general.

Such high-porosity-level sites facilitate fluid transport into and out of the polymer, serving as places for the sequestration of molecules.[26]

The NPs used in this study are insoluble in water and were meant to reduce the overall volume of the susceptible polymer matrix. Therefore, the higher density could be explained by a 'packing' effect, and not necessarily a lower porosity. In practice, impact fracture damage occurs when the patient accidentally drops the prosthesis. Studies have provided different proposals to minimize the susceptibility of the material to this flaw, such as increasing the thickness of the prosthesis in regions of increased susceptibility and modifying the matrix to improve the mechanical properties by adding polyfunctional agent crosslinks,[27] and the incorporation of a rubber phase,[28] fibers[29] and metal oxides.[3] Impact strength can be defined as the energy required to fracture a material under the impact force. In this study, IS was not affected by the NPs as much as the other properties. Most groups presented lower IS values than the control, which could be related to an increase in density, thus reducing stiffness.

Regarding IS, the groups that received SiO_2 showed very similar mechanical behavior, which might have been caused by the clustering of the NPs heterogeneously inside the PMMA matrix, resulting in weaker spots to propagate fractures. In this case, the incorporation of NPs into the polymer with a mixer seems to be insufficient to ensure a fully uniform dispersion of NPs. Further studies evaluating different methods should be developed to incorporate NPs into PMMA for dental and medical applications.

No cracks or missing material were observed under macroscopic evaluation. The A and B fragments of all samples could be placed together perfectly, suggesting that the NPs limited the crack propagation and kept the samples from breaking into several pieces. Some macrophase separations could be observed in the silica and titania groups under SEM, while the ceria morphology was similar to that of the control group. The SEM images showed a honeycomb-like configuration for silica due to intense porosity, and compact and wave-like surfaces for the control or in the presence of ceria. The titania groups had both of these characteristics. Nanoparticles play a relevant role in polymerization configuration, and thus, in the fracture propagation patterns. A previous study used SEM to observe agglomeration in PMMA specimens containing 0.91% silica, and stated that the particles appeared to be embedded in and semi-bonded to the PMMA matrix, indicating a relatively strong NP–matrix interaction.[30] The present study corroborates this interaction pattern, though it was more visible in the ceria groups.

Balos et al. added nanosilica to the liquid component of a commercial PMMA-based resin, and found that it increased the microhardness and fracture toughness values for the lowest content.[30] Cerium oxide has been used in dental materials to stabilize the polycrystalline tetragonal structure of zirconia.[31] To our knowledge, there are no reports of its use for acrylic resin reinforcement. Interestingly, in this study, CeO_2 NPs showed better overall behavior. In comparison with other groups containing NPs, the CeO_2 groups had a significantly higher degradation temperature, a higher mean IS, and similar VH and density values. Titanium oxide NPs are chemically inert, have a high refractive index and antibacterial properties under a variety of spectra, and are corrosion-resistant, non-toxic and inexpensive.[14] These NPs have been previously incorporated into polymeric materials, such as PMMA,[15] and were reported to provide better reinforcing properties than their micrometric version.[16] The impact of CeO_2 and TiO_2 on the resin color was readily apparent, potentially offering valuable insights. However, this effect should be given significant consideration in the evaluation and application of these NPs, warranting further in-depth investigation.

Limitations

Some limitations of the current study are acknowledged. Firstly, the dispersion method should be refined, perhaps with the use of ultrasonication. Also, surface roughness analysis could be very useful for corroborating the hypothesis of increased wear resistance in two- and three-body wear, as well as when considering biofilm formation and biological aspects.[32–34]

Conclusions

Incorporating all 3 types of NPs into the PMMA matrix significantly enhanced thermal stability, surface hardness and density. Moreover, the impact resistance in the tested NP groups remained comparable to that of the control group, indicating that modifying the microwave-cured acrylic resin with specific amounts of NPs, particularly CeO_2, could serve as a viable solution to address the physicochemical limitations of the material and to prevent unwanted clinical failure.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Luciana Machado-Santos https://orcid.org/0000-0001-5656-8760
Kusai Baroudi https://orcid.org/0000-0002-1009-0869
Nikolaos Silikas https://orcid.org/0000-0003-4576-4584
João Paulo Mendes Tribst https://orcid.org/0000-0002-5412-3546
Mario Alexandre Coelho Sinhoreti https://orcid.org/0000-0002-1932-2902
William Cunha Brandt https://orcid.org/0000-0002-6297-6258
Priscila Christiane Suzy Liporoni https://orcid.org/0000-0001-6787-1167

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Original paper

Effectiveness of methods for removing the *Candida albicans* biofilm from the dental acrylic surface

Zuzanna Tokarz[1,B–D], Pawel Krzysciak[1,A,C,D,F], Aneta Wieczorek[2,A,E,F]

[1] Department of Infection Control and Mycology, Chair of Microbiology, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[2] Department of Dental Prosthetics and Orthodontics, Institute of Dentistry, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Aneta Wieczorek
E-mail: aneta.wieczorek@uj.edu.pl

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Abstract

Background. Approximately half of the adult population in Europe have used some form of dental prosthesis. Much effort has been put into developing denture cleaning methods and the most recommended are brushing the prosthesis after meals and cleaning it with special liquids (sometimes prepared just before the procedure). However, these simple techniques are often omitted or insufficient due to, i.e., age-related mental or motor disabilities.

Objectives. The aim of the study was to compare a range of techniques that can be performed at home and do not require patient dexterity in order to find the most efficient method of reducing the viability of the *C. albicans* biofilm and removing it from acrylic surfaces.

Material and methods. The 20 mm × 25 mm × 1 mm unpolished acrylic plates were inoculated with *C. albicans* and incubated for 72 h. Plates with formed biofilms were divided into 6 equal groups: a control group and 5 groups for different cleaning procedures: a dental cleaner with liquid, a dental cleaner with phosphate-buffered saline (PBS), air drying, antiseptic liquid, and an ultrasonic cleaner. Biofilm viability was assessed by plating serial dilutions and counting the colonies of *C. albicans* on the Sabouraud dextrose agar (SDA) medium.

Results. The study found that both MultiClean fluid and Sonic-3 ultrasonic cleaner were effective against *Candida* cells. MultiClean fluid showed the strongest biocidal properties, both when used with the Sonic Denture Cleaner and independently.

Conclusions. Cleaning acrylic surfaces with a dental cleaner followed by antiseptic liquid is more effective than using these methods separately.

Keywords: *Candida albicans*, polymethyl methacrylate, denture cleaners

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Introduction

Approximately half of the adult population in Europe have used some form of dental prosthesis. Removable dental prostheses are used by 13–29% of adults. Among them, 3–13% of edentulous subjects wear complete dentures in both jaws.[1] In Poland, more than 66% of people aged >60 years wear dental prostheses, and the use of dentures increases with age.[2] Due to the material characteristics of dentures[3] and the favorable environmental conditions that they create for the growth of microorganisms, denture wearers often suffer from denture stomatitis.

Denture stomatitis is an inflammatory process that occurs due to the use of removable prosthetic restorations. Poor oral and denture hygiene is the cause of polymicrobial biofilm formation on the surfaces of prostheses. *Streptococcus* species and *Staphylococcus aureus* were the most common microorganisms isolated from the dental prosthesis. Prolonged usage of the prosthesis resulted in the growth of other bacteria, including *Escherichia coli*, coryneform bacteria and *Micrococcus*.[4] The dental prosthesis offers a reservoir for microorganisms associated with bacterial endocarditis, aspiration pneumonia, gastrointestinal infections, and chronic obstructive pulmonary disease. Gram-positive cocci such as *Streptococcus mutans* show synergistic interactions within biofilm formations in inter-kingdom biofilms.[5] These are often complicated by fungal infection.[6,7] The adhesion of *Candida* strains to the mucosa and dentures as well as the formation of biofilms are responsible for the development of *Candida* stomatitis.[8,9]

Candida albicans is the most common commensal of the digestive and reproductive tract mucosa and, under some conditions, becomes the most common fungal pathogen. It is present in the oral microbiome of 30–75% of the world population.[6,10] The immune system activity modulates its colonization of numerous niches in the human body. When immune reactions are suppressed to some extent, for example, as a result of corticosteroids, diabetes or elimination of the natural human microbiota, these yeasts can cause local infections of the mucous membranes and, in predisposed individuals, can lead to life-threatening invasive infections.[11] One of the key mechanisms of the pathogenicity of *C. albicans* is its ability to form biofilms on the surfaces of host tissues and inanimate surfaces (catheters, prostheses, etc.).[12,13] These structures are difficult to clean and often act as reservoirs for infection. Therefore, proper hygiene of dental prostheses, including effective removal of biofilms, is crucial to prevent denture stomatitis.

The biofilm of *C. albicans* is difficult to remove from acrylic resin surfaces. The most common physical method of controlling plaque development is brushing with a denture brush, which can remove 90–96% of yeast cells from the acrylic surface.[14,15] However, the effectiveness of brushing depends on the skill of the person performing the task,[14] and can be reduced due to diseases and disorders common to older people. Alternative methods, including dedicated mechanical and chemical cleaners, may solve the problem of denture hygiene. Older people who develop dementia are at an increased risk of developing oral health problems due to diminished self-care and motor skills. Daily removal of denture plaque is essential in maintaining good oral health, especially in older age.[16]

Denture stomatitis can be prevented by controlling the formation of *C. albicans* biofilms on the acrylic.[8,9,17,18] This approach is critical in preventing the overgrowth of mycobiota and the development of oral mucosal infection. Significant effort has been put into developing effective cleaning methods. The most recommended is brushing with a denture brush after meals and cleaning with dedicated liquids (sometimes prepared just before the procedure). However, these simple measures are often neglected or insufficient due to age-related mental and motor disabilities.

A few independent studies on this topic have tested commercially available denture cleaning devices, but the results were contradictory. This study compared a range of methods that can be used at home and do not require patient dexterity, such as cleaning with sonification and denture liquid, to find the most efficient method of limiting the viability of *C. albicans* biofilms and removing them from acrylic surfaces for patients with limited motor skills. The study employed an in vitro single-species biofilm model.

Material and methods

Preparation of acrylic plates

A total of 180 acrylic plates made of routine denture material, Vertex Rapid Simplified (Vertex Dental, Soesterberg, The Netherlands), were prepared using the method described by Johnson et al.[19] First, molds were made for the casting of stone acrylic tiles (type III). Standard flasks were used.

While still warm, the stone was coated with sodium alginate solution and left to dry. Following the manufacturer's instructions, the acrylic resin was prepared by mixing 2.3 g of acrylic powder with 1 mL of water. The liquid was measured and poured into a clean and dry mixing vessel. The powder was added slowly, ensuring that each particle was wetted by the monomer.

After the powder was mixed with liquid, the mixture was stirred and left to settle in a closed container for 15 min. After this time, the acrylic mass had the consistency of dough; it was placed in the mold and pressed firmly until the halves of the mold joined. The flask was

placed in a spring clamp and tightened. Then, it was placed in a water curing bath at room temperature that was subsequently raised to 100°C. The boiling time was 2 h. Afterwards, the flask was removed from the curing bath, and the clamps were released to open the flask and remove the plates.

According to denture manufacturing procedures, it is recommended that denture surfaces in close proximity to mucosa are not polished. During the polishing treatment, acrylic material is lost, which may result in a poorer fit of the finished denture. The unpolished acrylic plates used in this study imitated the real conditions.

Strain and inoculum preparation

The effectiveness of the cleaning method was evaluated by using a *C. albicans* biofilm model. We adapted the technique described by Krom and Willems.[20] The biofilm-forming strain, *C. albicans* LIG.1.2. F.A. was isolated from the human oral cavity.[21] A loop of overnight yeasts cultured on Sabouraud dextrose agar (SDA; BioMaxima S.A., Lublin, Poland), was used to prepare the standardized suspension (10^7 CFU/mL). Subsequently, the yeast suspension was diluted to 1:25 in Roswell Park Memorial Institute (RPMI) 1640 medium (cat. No. R6504; Sigma-Aldrich), supplemented with 18 g of glucose and buffered with 3-(N-morpholino)propanesulfonic acid (MOPS; Fluorochem UK, Hadfield, UK) for further use.

Biofilm formation

The 180 unpolished acrylic plates measuring 20 mm × 25 mm × 1 mm were used in this experiment. Before use, all plates were sterilized with ethylene oxide. Next, they were placed in duplicate into Petri dishes (Φ 5 cm) and sunk in 10 mL of the previously inoculated RPMI 1640 medium. The incubation was carried out at 35 ±2°C for 4 h to ensure that the cells could adhere. The medium was then changed, and further incubation was carried out at 35 ±2°C for 72 h, with the medium changed daily.

Evaluation of antibiofilm properties

Acrylic plates with a formed biofilm were divided into 6 groups of 30 pieces each: one positive control group (1) and 5 groups for different cleaning procedures: (2) Sonic Denture Cleaner, consisting of a vibration-generating part and a removable denture container (referred to as DC or dental cleaner; Roko Dental Systems, Częstochowa, Poland), used simultaneously with MultiClean antiseptic fluid (30–40% of potassium peroxymonosulfate and <5% of potassium persulfate, referred to as liquid and prepared according to the manufacturer's instruction: adding 5 g of MultiClean powder to 200 mL of water, stirring and leaving for 3 min before use; Rokodent); (3) dental cleaner with phosphate buffered saline (PBS); (4) air drying; (5) MultiClean antiseptic fluid; and (6) ultrasonic cleaner (Sonic-3; Polsonic Palczyński Sp. J., Warsaw, Poland) corresponding to the test procedure. Cleaning the samples with an ultrasonic cleaner was used as a negative control because, according to the literature,[15] no growth was expected when using this method. Similar to the positive control (no intervention), the negative control provides a benchmark for the results obtained with other experimental methods.

All cleaning procedures were carried out by the same person on a single day to ensure maximum repeatability.

The plates were washed with PBS and air-dried for 15 min. The following test procedures were then carried out in the plate groups: (2) 2 cycles of 7 min in DC using 200 mL of antiseptic liquid; (3) 2 cycles of 7 min at 25°C using DC with PBS; (4) air drying for 24 h; (5) immersion in 200 mL of MultiClean antiseptic fluid for 20 min at 25°C, following the manufacturer's recommended procedure; (6) ultrasound cleaning in 2 L of distilled water for 7 min using a Sonic-3 ultrasonic cleaner at 25°C with a 40 kHz frequency/160 W. The positive control group (1) was processed for further actions.

After the cleaning procedures, the plates were washed again with PBS and divided into 2 groups of 90 pieces each. In the first group of plates, 2 biofilm cell samples of 1 cm^2 each were collected with a swab from the same surface. The swab was vortexed for 20 s with 1 mL of saline, and a series of 10-fold dilutions was prepared. The dilutions were inoculated on SDA and incubated at 25 ±2°C for 24 h, after which the yeast colonies were counted.

The second half of the plates, from which the biofilm was not collected, were stained with 0.1% crystal violet solution for 30 min. The stained biofilm was washed with cold water, air-dried for 24 h, and 2 biofilm cell samples of 1 cm^2 each were collected from the plates with a scalpel. Each sample was then placed in a well of a 96-well microtiter plate and discolored with ethyl alcohol for 2 h. The alcohol with the dissolved stain was transferred to fresh microtiter plates. The amount of the biofilm was estimated by measuring the absorbance of the stained alcohol at a wavelength of 560 nm.

Statistical analysis

Statistical calculations were conducted using the R 4.1.1 statistical package.[22] The Kruskal–Wallis χ^2 test for non-parametric variables was used to test the differences between the groups. Pairwise comparisons were evaluated by using the Tukey and Kramer (Nemenyi) test for post hoc analysis with a Tukey lambda distribution approximation for independent samples. A p-value <0.05 was considered significant.

Results

Evaluation of *C. albicans* viability

The first step of this research involved determining the effect of cleaning procedures on biofilm viability by comparing the recovery of colony forming units in samples treated with the tested methods to positive and negative control groups (samples untreated and cleaned with an ultrasonic cleaner, respectively). This allowed us to assess the cell-damaging or cell-killing potential of the cleaning methods.

Of all the methods tested, air drying (Tukey and Kramer (Nemenyi) test, p = 0.41) and DC with PBS (p = 0.99) did not result in a significant reduction in recovered colonies of *C. albicans* compared to the control samples. MultiClean fluid and Sonic-3 ultrasonic cleaner (p = 0.00) showed effective activity against *Candida* cells. MultiClean fluid showed the strongest biocidal properties when used with the Sonic Denture Cleaner (p = 0.00) and independently (p = 0.00). Detailed data is presented in Fig. 1 and Table 1.

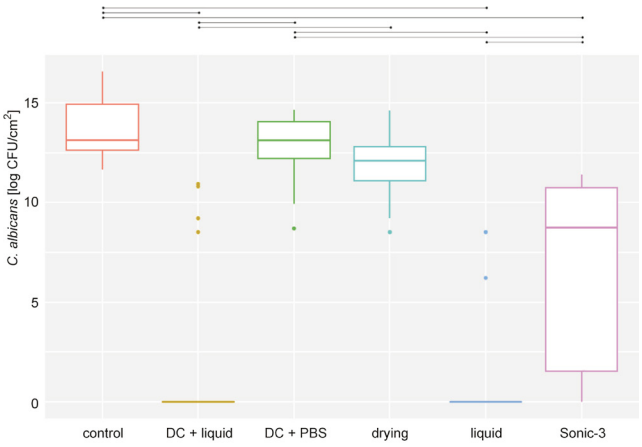

Fig. 1. Impact of different cleaning procedures on the recovery of *Candida albicans* cells

The Kruskal–Wallis test revealed significant differences between the cleaning methods (χ^2 = 82.302, df = 5, p = 2.768e-16). The line represents the median, the box shows the interquartile range, the whiskers stand for the minimum and maximum ranges, and the colored circles represent the outliers. The horizontal lines indicate statistically significant differences between variables.
Cleaning methods: control – untreated samples; DC + liquid – Sonic Denture Cleaner and MultiClean fluid; DC + PBS – Sonic Denture Cleaner and phosphate-buffered saline; drying – air-dried samples; liquid – MultiClean fluid; Sonic-3 – Sonic-3 ultrasonic cleaner.

Assessment of biofilm biomass: Crystal violet staining

Crystal violet staining shows the effectiveness of the cleaning method on the biofilm removal without assessing its viability. The Tukey and Kramer (Nemenyi) test showed that the ultrasonic cleaner (p = 0.00) and the Sonic Denture Cleaner with PBS (p = 0.00) almost completely removed the biofilm from the surface of the acrylic plates. Air drying (p = 1.00), MultiClean fluid (p = 0.21), and the Sonic Denture Cleaner with MultiClean fluid (p = 1.00) were insufficient to remove *C. albicans* cells from the acrylic plates compared to the control group. Detailed data is presented in Fig. 2.

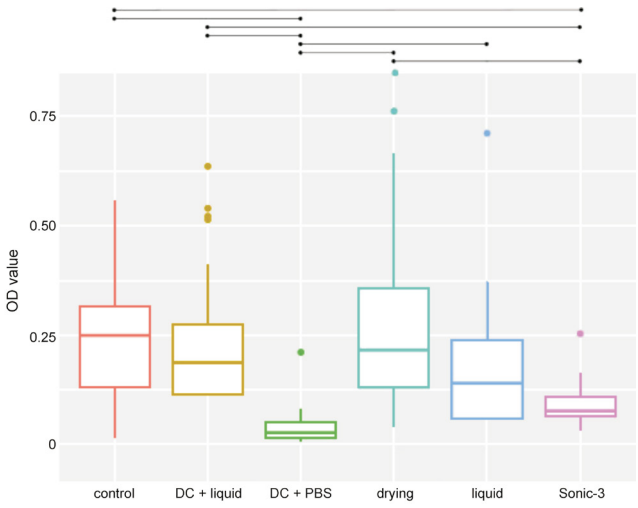

Fig. 2. Effectiveness of different cleaning procedures on biofilm removal as measured by crystal violet staining

The Kruskal–Wallis test revealed significant differences between the cleaning methods (χ^2 = 65.473, df = 5, p = 0.000); OD – optical density.

Table 1. *Candida albicans* count in the evaluated study groups

Group	Variable [CFU/cm²]					
	mean	median	min	max	Q1	Q3
Control	2,319,588	505,000	116,000	15,945,000	260,000	3,082,500
DC + liquid	6,694	0	0	56,000	0	2,500
DC + PBS	764,222	510,000	6,000	2,295,000	209,750	1,477,500
Drying	308,778	179,750	5,000	2,240,000	62,250	337,500
Liquid	583	0	0	5,000	0	0
Sonic-3	24,278	6,250	0	90,000	0	58,750

Cleaning methods: control – untreated samples; DC + liquid – Sonic Denture Cleaner and MultiClean fluid; DC + PBS – Sonic Denture Cleaner and phosphate-buffered saline; drying – air-dried samples; liquid – MultiClean fluid; Sonic-3 – Sonic-3 ultrasonic cleaner.

Discussion

The surfaces of removable dentures used by patients are often covered with plaque, which can promote infections in the denture-bearing area. In the case of partial dentures, inflammation can be observed on the gingival surfaces that are in contact with the denture plate. Plaque can be removed from dentures by using several different methods, such as brushing with a denture brush or soaking the plaque in chemical solutions (e.g., Corega tabs), as well as using devices such as microwave ovens or ultrasonic apparatus.[23] If patient dexterity has decreased with age, effective cleaning of the dentures becomes more difficult, which can significantly affect the health of the oral tissues.

This study aimed to compare denture cleaning methods that do not require dexterity and are available for home use.

Some patients believe that drying the prosthesis overnight dehydrates and kills microbes present on the denture. It can hardly be called a hygienic procedure, but it does not require skilled hand movements or the help of third parties in its application. The results of this study showed that this method was not effective in killing *C. albicans* biofilm cells. Drying the dentures does not involve any actions that would remove the biofilm from its surface or even lead to its fixation (Fig. 3). This method should never be recommended for patients with limited dexterity.

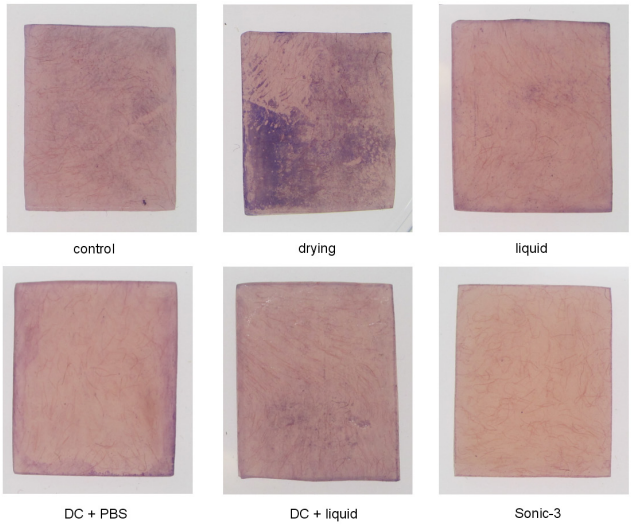

Fig. 3. Acrylic plates with *Candida* biofilm stained with crystal violet

The second method often used by patients is placing the dentures in the denture cleaning fluid. This method does not require a lot of dexterity but may require a little help in preparing the liquid, especially for people with diseases that affect cognitive abilities. The results show a significant antifungal activity of the liquid used in this study, but, like drying, it does not remove the biofilm from the surface of the prosthesis.

Other studies have shown that peroxide-based cleansers are the most effective cleaning method.[12,14,15,24] However, de Andrade et al.[25] and Nishi et al.[26] did not observe any antifungal activity resulting from the use of denture cleaning tablets alone, which is in contradiction with the results of the present study. The explanation for this situation may be the use of the single-species rather than the multi-species biofilm model in the aforementioned studies, which included the *Streptococcus* species. The *Streptococcus–Candida* biofilm tends to be more difficult to break due to synergistic actions between these microorganisms.[5] Peroxide-based cleansing solutions were found to be highly effective in killing *C. albicans* cells, but they tended to fix the biofilm structure,[25] which could then act as a base and a medium for microorganisms to rebuild the biofilm. These results are consistent with the outcomes of our study.

Sonic cleaning methods involve the mechanical removal of the biofilm from the surface coupled with biocidal activity by using sonic or ultrasonic vibrations to disintegrate the biofilm and release cells; however, the use of only ultrasonic waves with specific parameters can damage yeast cells.[27]

The tools for sonic cleaning of dentures used in this study included a professional ultrasonic cleaner and devices that generate vibrations to mimic ultrasound (Sonic Denture Cleaner with MultiClean fluid or with PBS). The Sonic Denture Cleaner, due to its size and ease of use, was considered an alternative method of denture cleaning for individuals with limited dexterity. Vibrations generated by this device effectively removed the biofilm from the surface of the plates, but no reduction in the cell viability was observed. The results obtained with the Sonic Denture Cleaner and PBS regarding the biofilm removal were comparable to those of the ultrasonic cleaner. Although the technical specifications were not provided by the manufacturer, due to its structure, method of operation and power source, it can be concluded that the Sonic Denture Cleaner generates a mechanical wave at a much lower frequency and less power than an ultrasonic cleaner, which resulted in a much lower efficiency of the Sonic Denture Cleaner in damaging the structure of the biofilm. However, the combination of the Sonic Denture Cleaner with MultiClean fluid had the strongest anticandidal properties.

Ultrasonic devices without peroxide-based cleaning agents were not effective in reducing the number of *C. albicans* cells in complete dentures.[25,26] However, according to Ghazal et al.,[15] the use of ultrasonic cleaners resulted in a significant reduction in the number of *C. albicans* cells, and their findings are relatively consistent with our results. Despite the efficiency with which ultrasonic cleaning disintegrates biofilms, its main disadvantage is the lack of access to these cleaners. Ultrasonic devices capable of generating frequencies that reduce the viability and adhesion of biofilms are used primarily by professionals.[25]

Pellizarro et al.[28] suggested that the best results could be obtained by combining chemical and mechanical methods. They reported that sodium carbonate peroxide solutions can remove *C. albicans* from denture bases; however, soaking dentures in antiseptic liquid should be combined with brushing to control fungal growth more effectively. A similar effect can be achieved by cleaning the denture with the Sonic Denture Cleaner, followed by soaking in a disinfectant. Combining chemical disinfectants with dental cleaners might help avoid the difficulties associated with decreased mobility. However, simultaneous use of peroxide-based cleaners and ultrasonic or sonic dental cleaners may not produce the desired results due to the biofilm-fixing properties of the antiseptic fluid.

The main limitation of this study is the use of an in vitro model to assess the removal of *C. albicans* biofilms from acrylic surfaces. The results may not be applicable to the cleaning of multi-species biofilms from more complex denture surfaces. Most studies[12,15,26,29] evaluated the effectiveness of denture plaque cleaning methods on removable dentures that have been used for some time. This research model has obvious advantages in that it can confirm the observed ex vivo efficacy. On the other hand, we assessed the ability of different cleaning methods to kill and remove *C. albicans* from a porous material such as prosthetic acrylic. This study is also easily reproducible and adaptable to other biofilm models.

Our approach does not take into account the changes that occur in the physical properties of acrylic during denture use. The introduction of a multi-species biofilm model and coating of the acrylic with saliva would certainly help to obtain a better picture of the effectiveness of these methods.

Conclusions

The Sonic Denture Cleaner is a reasonably efficient tool for removing *C. albicans* biofilms from acrylic surfaces. The combination of cleaning with a dental cleaner and subsequently soaking the acrylic tiles in antiseptic liquid gave better results than using either method separately. Further studies evaluating the effectiveness of these tools for denture cleaning and taking into account multi-species complex biofilm models as well as other clinical conditions associated with denture wearers are required.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Zuzanna Tokarz https://orcid.org/0000-0002-2958-4412
Pawel Krzysciak https://orcid.org/0000-0002-2554-9409
Aneta Wieczorek https://orcid.org/0000-0003-2506-7394

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Review

Effect of mechanical forces on the behavior of osteoblasts: a systematic review of in vitro studies

Parisa Motie[1,A–F], Sadra Mohaghegh[1,A–F], Farnaz Kouhestani[2,A–F], Saeed Reza Motamedian[3,A–F]

[1] Student Research Committee, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Periodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[3] Department of Orthodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Saeed Reza Motamedian
E-mail: drmotamedian@gmail.com

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Abstract

Mechanical loading can play a critical role in bone modeling/remodeling through osteoblasts, with several factors being involved in this process.

The present study aims to systematically review the effect of mechanical stimulation on human osteoblast cell lineage combined with other variables.

The PubMed and Scopus databases were electronically searched for studies analyzing the effect of compression and tension on human osteoblasts at different differentiation stages. Studies that used carcinogenic osteoblasts were excluded. In addition, studies that did not analyze the osteogenic differentiation or proliferation of cells were excluded. The risk of bias of the studies was evaluated using the modified CONSORT (Consolidated Standards of Reporting Trials) checklist. a total of 20 studies were included. The cells were subjected to tension and compression in 5 and 15 studies, respectively. The application of uniaxial and cyclic strain increased the proliferation of osteoblasts. The same increased pattern could be observed for the osteogenesis of the cells. The impact of the tensile force on the expression of the osteoclastic markers differed based on the loading characteristics. On the other side, the impact of compression on the proliferation of osteoblasts varied according to the magnitude and duration of the force. Besides, different patterns of alternations were observed among the osteogenic markers in response to compression. Meanwhile, compression increased the expression of the osteoclastic markers. It has been shown that the response of the markers related to bone formation or resorption can be altered based on the differentiation stage of the cells, the cell culture system, and the magnitude and duration of the force.

Keywords: proliferation, differentiation, compression, osteoblast, tension

Cite as
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Introduction

Mechanical forces of physiological magnitudes applied to the bone tissue of the human body lead to the preservation and strengthening of the body's bone mass. Weightlessness caused by space flight decreases bone mineralization, while moderate mechanical loading, such as regular exercise, can improve bone tissue mineralization and density.[1,2] The force produced during occlusion is naturally transmitted to the alveolar bone through the periodontal ligament fibers. It was reported that masticatory hypofunction, followed by a chronic soft diet intake, reduces bone mass.[3] In distraction osteogenesis, mechanical strain induces bone formation by osteoblasts.[4] The clinical success of orthopedic and dental implant osseointegration relies on appropriate mechanical loading factors.[5]

Mechanical loading can cause biological changes in various human cells. It stimulates the osteogenic differentiation in human amniotic epithelial cells and human dental pulp stem cells.[6,7] Compressive forces induce bone resorption and remodeling in human periodontal ligament cells.[8,9] External cyclic forces enhance osteogenic differentiation of mesenchymal stem cells (MSC) of the axial skeleton through Notch signaling induction.[10] Extracellular matrix production and tenogenic differentiation of human adipose-derived stem cells are enhanced by receiving an appropriate mechanical loading regimen.[11]

The osteoblast lineage includes bone-forming and bone-remodeling cells in the human body[12] that play critical physiologic and therapeutic roles by responding to various types of stimuli caused by mechanical forces. These cells are mechanical sensors that can transduce mechanical stimuli into biochemical signals (cell-to-cell communication or the production of paracrine factors).[13] Therefore, mechanical forces can affect the bone modeling/remodeling process. a wide range of in vitro experiments have investigated gene expression and proliferation changes of osteoblasts under different methods of loading application.[14] Mechanical strain can affect bone formation using bone matrix deformation and, therefore, the fluid shift within the osteocyte's canaliculi.[15]

In orthodontic treatments, mechanical forces are used to move the tooth bodily or change its inclination. During typical orthodontic tooth movement, a compression side and a tension side are created by the applied force.[13] The effect of these two forces on osteoblasts has been investigated in several studies in vivo and in vitro. It has been shown that there are various factors affecting bone regeneration in the presence or absence of loading application, such as strain parameters (frequency, cycle number, and stimulation duration)[4] and scaffolds and medium growth factors.[16] Nevertheless, this systematic review aimed to summarize and compare the osteoblast's behavior in response to the application of tension or compression in vitro and examine the factors that can influence these responses.

Methods

Eligibility criteria

Type of participants and interventions

Studies that analyzed the impact of mechanical forces on each type of the human cell osteoblast lineage were included. Articles analyzing the behavior of carcinogenic cells were excluded. Studies that merely assessed the effect of medium mechanical features on the behavior of cells were excluded. Additionally, those that stimulated the cell through non-mechanical forces or forces other than compression or tension were excluded.

Type of outcome measurement

Studies that analyzed the effects of mechanical stimulation on the human osteoblast cell's behavior (proliferation and differentiation) were included. Studies that merely assessed factors other than those mentioned were excluded.

Type of studies

All in vitro studies stimulated human cells of the osteoblast lineage at different stages of differentiation through mechanical force (tension and compression) were included. All animal studies, abstracts, letters, and reviews were excluded.

Information source and search strategy

PubMed and Scopus electronic databases were searched based on the combination of relevant keywords (Table 1). In addition, the reference lists of indicated articles were manually searched to find possible related studies.

Study selection and data extraction

Two reviewers performed study selection and data extraction independently. Any disagreement was discussed and resolved by a third independent expert. After remov-

Table 1. Key Words

Mechanical Force (free text)	Behavior (Mesh Term)	Behavior (Free Text)	Cells (Mesh Term)	Cells (free text)
Tens*	"Cell Prolifera-tion"[Mesh]	Prolif*	"Osteoblasts"[Mesh]	Osteoblast*
Compress*	"Cell Differentia-tion"[Mesh]	Diff*	"Osteoclasts"[Mesh]	Osteoclast*

ing duplicated studies using the EndNote reference manager (EndNote X9.1), the initial screening of titles and abstracts was done according to the mentioned eligibility criteria. Full texts of potentially eligible studies were reviewed in the next step. The study was designed according to The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (Fig. 1).[17]

Data items

The study's methods and results were reviewed for data extraction. Extracted data items are as follows: 1) Cell lineage, 2) Type of mechanical stimulation, 3) Mechanical device and loading characteristics, 4) Cell culturing medium, and 5) Cell response to mechanical stimulation (proliferation and differentiation).

Critical appraisal

Assessing the quality of studies was done based on the modified CONSORT checklist,[18] including the following 14 items (Table 2): structured summary (yes/no), scientific background and explanation of rationale (yes/no), specific objectives and/or hypotheses (yes/no), explained interventions insufficient details (yes/no), defined outcome measurements methods (yes/no), sample size determina-

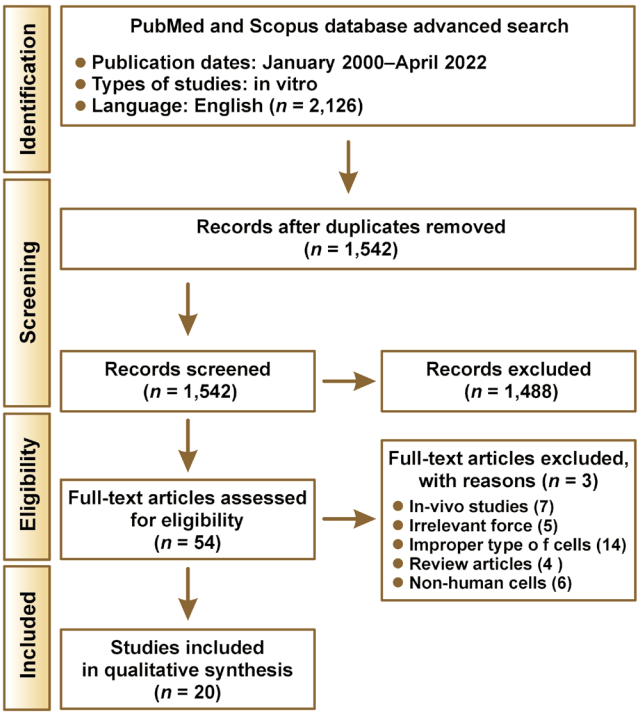

Fig. 1. Flow diagram

Table 2. Modified CONSORT checklist for in vitro studies

Auther/year	Abstract	Introduction		Method								Result	Discussion	Other	
	1	2a	2b	3	4	5	6	7	8	9	10	11	12	13	14
Bhatt et al (2007) (7)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	Y
Brezulier et al (2020) (46)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Grimm et al (2015) (20)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Ignatius et al (2005) (35)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Jansen et al (2004) (30)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y
Jansen et al (2006) (31)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y
Jansen et al (2010) (71)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Kaspar et al (2000) (43)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Kaspar et al (2002) (40)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Kokkinos et al (2009) (8)	Y	Y	Y	Y	Y	N	N	N	N	N	N	Y	Y	N	Y
Kreja et al (2008) (36)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Kusumi et al (2005) (41)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
Rath et al (2012) (39)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y
Sanchez et al (2012) (45)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y
Tripuwabhrut et al (2012) (47)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y
Tripuwabhrut et al (2013) (23)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y
Weyts et al (2003) (34)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	Y
Wozniak et al (2000) (42)	Y	Y	Y	Y	Y	N	N	N	N	N	N	Y	N	Y	Y
Zhang et al (2018) (38)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y
Zhu et al (2008) (33)	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	Y	Y

1:structured summary, 2a: background, 2b: objective, 3: intervention, 4: outcome, 5: Sample size, 6:Randomized Sequence generation, 7: allocation concealment, 8: implementation, 9: blinding, 10: statistical method, 11: outcomes and estimation, 12: limitations, 13: funding, 14: protocol, Y:yes, N:no

tion (yes/no), randomizing sequence generation (yes/no), allocation concealment (yes/no), implementation, usage of proper statistical method (yes/no), expressing the results for each group and the estimated size of the effect and its precision (yes/no), addressing trial limitations (yes/no), addressing sources of potential bias (yes/no), imprecision, and, if relevant, the multiplicity of analyses (yes/no), identifying the sources of funding (yes/no), and availability of the full study protocol (yes/no).

Results

Among 1542 studies**,** 1488 studies were excluded based on their titles and abstracts (Fig. 1). The full text of the 54 remaining studies was analyzed, and 34 studies were excluded due to the following reasons: 7 studies performed the analysis in in-vivo conditions, five studies applied irrelevant forces such as microgravity and shear stress, 14 studies used improper lineages such as osteosarcoma cells, and four articles used animal osteoblasts. Finally, 20 studies were included for qualitative data synthesis. Among the included studies, tension and compression were applied in 15 and 5 studies, respectively (Table 3).

Cell lineage

Human fetal (SV-HFO)[19–24] and adult osteoblast from the tibia, femur,[25,26] calvaria,[27] or from subchondral bone pieces[28]; primary human osteoblasts from Cambrex Bio Science[4]; osteoblast-like cells from tibia/femur/calvaria/ilia[29]; human bone marrow-derived osteoblasts (HBMDOs) from the femoral diaphysis[5]; and Clonetics normal human osteoblasts (NHOst),[30] preosteoblasts, and osteoprogenitors[31] were used to evaluate the effect of tension. For compression assessment, the included studies used alveolar bone osteoblasts,[14,32] human fetal osteoblasts,[13] human tibia osteoblasts,[33] and commercial human osteoblast cell lines.[12]

Force type

The effect of tension on the biological activities of the primary human osteoblast lineage at different stages of differentiation was evaluated in fifteen studies, and five articles analyzed the effect of compression.

Force device

In the included studies, the desired tension was applied by an FX-4000T Flex-cell BioFlex Tension Plus Unit,[4,19–23,27,31] a 4-point bending device,[5,26,29] a six-station stimulation apparatus,[24,25] or other stretching systems.[28,30] To apply the compressive force, two studies added lead weights to glass wells,[14,32] one study used a Flex-cell compression system,[33] one study used plastic tube caps,[13] and one used a centrifuge system.[12]

Medium

Assessing the tension effect, an osteogenic growth medium was used for cell culture in twelve studies. In one of them, 1-alpha,25-dihydroxycholecalciferol, vitamin K1, and ascorbic acid were added to cultures that were tested for osteocalcin (OCN) synthesis.[34] One study supplemented the osteogenic medium with osteogenic protein-1 (OP-1)[26] and another added hams F12.[24] Three studies used standard mediums[5,25,28] supplemented with vitamin D3[25] or collagenase II.[28] Among the compression-related studies, cells were cultured in a standard medium in two studies[32,33] and an osteogenic medium in one study.[12] One study added hams F12 to the medium[13] and one study analyzed the behavior of osteoblasts in both standard and lysates cultures.[14] In addition, one study assessed the effect of clodronate on the behavior of osteoblasts.[12]

Cell response

Tension

Proliferation

The proliferative response of the cells depends on loading characteristics such as frequency and cycle number[29] and the differentiation stage of osteoblasts is another factor affecting the cells' proliferation and apoptosis after the force is applied.[23] It was shown that mechanical loading could positively affect the proliferation of osteoblastic precursor cells in a (COL 1) matrix[24] and promote the viability of the cells, decreasing the expression of two apoptosis 'executioner' caspases, and increasing proliferation in a time-dependent manner.[27] Eight hours of 9% uniaxial strain increased the osteoblasts' proliferation rate by up to three times, more than 3% and 6%.[4] Also, the application of cyclic stretch (two days for 30 minutes per day with a frequency of 1 Hz and a strain magnitude of 1000 μstrain) increased the proliferation rate by about 10–48%.[26] In contrast, it has been reported that the immediate effect of stretch on DNA synthesis (0/5 hours) is not significant. Also, the cells which received the most intense stretching exhibited the lowest proliferation.[5]

Osteogenesis

Mechanical strain can stimulate bone formation using different signals and pathways. Wozniak et al. claimed that avb3-integrin activation is one of the mechanisms. It was shown that strain causes the redistribution of avb3-integrin on the cell's surface.[31] Extracellular signal-regulated kinase (ERK1/2) signaling is also affected by mechanical loading. The effect of force on ERK1/2 is different based on the osteoblasts' strain characteristics and differentiation stage.[19] Wnt/b-catenin signaling has an inhibitory role in osteoblastic differentiation independent of the

Table 3. Comparison of studies

Study	Cell Type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation Tests	Prolifera-tion Tests	Result
Tripuwabhrut et al. (2012) (47)	HOB from alveolar bone cultured on plates	Compression (continues)	1.0, 2.0, 3.0, and 4.0 g/ cm2 for 1, 3, 24, 48, and 72 h by adding lead weights into glass wells	Standard	- RT-PCR - human cytokine group I 2plex express assay kit	- MTT	- Proliferation: 1h had no ss effect, 3, 4 g/cm2 for 3–48 h de-creased - IL6 and CXCL8 MRNA increased in force depend-ent manner after 24h (peaked at 4g) - IL6 and CXCL8 protein reduced in force dependent man-ner after 24h (lowest at 4g)
Brezulier1 et al (2020) (46)	HFO cultured on 2D and 3D model	Compression (continues)	1, 4 g/cm2 using plas-tic tube cap	DMEM + hams F12	- RT-PCR - ELIZA	- Glucose consump-tion - Propidium iodide and Hoechst staining	- Proliferation (2D): 24h: no ss difference; 72h: control = 1g/ cm2 > 4g/cm2 - Proliferation (3D): 24h: con-trol=1g/cm<4g/cm; 72h: no ss difference - ALP (2D, 3D): no ss differ-ence - COL1 (2D, 3D): no ss difference - OCN: 2D: no ss diff; 3D: 4g/ cm2 > control - OPN (3D): no ss diff - RUNX2 (2D, 3D): 4g/cm2 > control - IL-6,8, OPG (2D,3D): in-creased from 24 to 72, - OPG: no ss difference between 2D and 3D
Grimm et al (2015) (20)	HOB	Compression (continues)	34.9 g/cm2 by centri-fuge	Osteogenic - Osteogenic + clodronate	- PCR - ELI-SA - IS	- MTT	- Proliferation: No ss differ-ence - RANKL: In-creased - OPG: Decreased - Clodronate re-duced the effect of force - OPG protein: in-creased, clodro-nate + compres-sion decreased
Sanchez et al (2012) (45)	HOB from tibia cul-tured on plate	Compression (cyclic)	1 MPa, 1 Hz for 4h by Flexercell Compres-sion Plus system	DMEM + 5% FBS + penicil-lin + streptomycin + glutamine	RT-PCR - ELI-SA - IS	N/A	- IL-6, IL-8, CC-2, RANKL, FGF-2, MMP-3, MMP-9, MMP-13: Increased - OPG, Col-1, MMP-2: No ss diff
Tripuwabhrut (2013) (23)	HOB from alveolar bone cultured on plate	Compression (continues)	2 and 4.0 g/cm2 for 1d by adding lead weight to glass	Standard - Standard + lysate	- RT-PCR - ALP activity - IS - ELISA	NM	- OCN and OPN: No ss diff - Col 1: Increased - Runx2, OPG: Decreased - RANKL: In-creased at 4g/ cm2 - ALP: Increased - ALP (lysate me-dium): De-creased - PGE-2: Increased
Bhatt et al. (2007) (7)	HOB cultured on plas-tic dishes	Stretch (cy-clic uniaxial)	3%, 6%, 9% 1 Hz for 8 h by Flexcell strain apparatus	Osteogenic	- RT-PCR - IS	H thymi-dine	- Proliferation: Increased peaked at 9% - Col 1: Increased peaked at 9% - BMP-2: Increased at 9% - OPN, ON: Increased peaked at 3% - OCN: Increased

Study	Cell Type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation Tests	Prolifera-tion Tests	Result
Kaspar et al. (2000) (43)	HOB from tibia and femur cultured on sili-con dishes	Sinusoidal strain (cyclic)	1000 µstrain 1 Hz over two days for 30 min per day by 4-point bending device	Osteogenic (For OC synthesis eval-uation: + 1alpha,25-di hydroxy chole calciferol + vita-min K1+ ascorbic acid)	- ALP activity - ELISA	Coulter Counter	- Proliferation: Increased - pre COL: In-creased - ALP: Decreased - OCN: Decreased
Kaspar et al. (2002) (40)	HOB from tibia, femur, calvaria and iliac cul-tured on silicon dishes	Sinusoidal strain (cyclic)	-A: 1000µstrain 1Hz 4, 60, 1800 and 3600 cycles -B: 1000µstrain 0.1, 1, 10, 30Hz 1800 cycles -C: 1000 µstrain 0.1, 1, 10, 30Hz 30, 300, 3000 and 9000 cycles 5 min All by 4-point bending device	Osteogenic + penicillin	N/A	Coulter Counter	- Prolifera-tion: -A: peaked at 1800 cycles 3600 cycles: Decreased -B: no ss difference between differ-ent frequencies > control -C: 1Hz and 300 cycle= high-est 30Hz and 9000 cycles= unstimulated group Other frequencies and cycle numbers= Increased
Zhang et al (2019) (38)	HOB from calvaria cultured on collagen coated Bioflex plate	Tension (cy-clic, equibiaxi-al)	2% 0.2 HZ 5 s, every 60 s for 6, 12, and 24 h by a Flexer-cell FX-4000 Strain Unit	Osteogenic + antibiotic	- RT-PCR	-MTT	- Proliferation: Increased after 24 h (higher than 6,12h) 6h, 12h vs control : No ss diff - CALCR: decreased at 6h and increased at 12,24h - CTSK, COL10A1, CHRD: in-creased in a time dependent man-ner - COL10A1, CHRD: de-creased at 24h
Kreja et al. (2008) (36)	HOB from the tibia or femur cultured on sili-cone dishes	Strain	-A: continuous 1% for 30 min, 6 h, 24 h, 72 h cell harvest-ing immediately after stimulation -B: continuous 1% for 30 min cell harvesting at different time points (0 min, 30 min, 1 h, 3 h, and 5 h) -C: continuous 1% for 6 h cell harvesting at 3 h and 18 h -D: Intermittent 1% on 3 consecutive days (3×30 min, 3×3 h, and 3×6 h) cell harvesting immediate-ly after stimulation -E: 8% continuous (30 min) or intermit-tent on 3 consecutive days (3×3 h) cell harvesting immediate-ly after stimulation	DMEM + FCS + vitamin D	- RT-PCR	N/A	- A, B, C, E: - RANKL, OPG, M-CSF, OCIL: No ss diff - D, E: - RANKL: Increased by intermittent stim-ulation 3×3 h and 3×6 h at a magni-tude of 1% and 3×3 at a magni-tude of 8% strain

Study	Cell Type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation Tests	Prolifera-tion Tests	Result
Rath et al (2012) (39)	HOB from subchondral bone pieces cultured on BioFlex® culture plates coated with col-lagen type I	tensile strain (continuous)	5% for 4 and 24 hours	TCM+ FCS+ anti-biotic/antimycotic solution	- RT-PCR	N/A	-4h: -COLA1, COX2: In-creased - BMP2, BMP7, OCN, OPN: No ss diff -24h: -COLA1, COX2, BMP2, BMP7, OCN, OPN: No ss diff
Kusumi et al (2005) (41)	NHOBs	Tensile strain (cyclic and continuous)	Cyclic: 2%, 7%, 14% 0.2, 0.25, 0.3 Hz 10, 20, 30, or 45min, and once a day for 4h for 1, 2, or 3 successive days Continuous: 7% for 3 days	OGM	-enzyme-linked immunosorbent assay -RT -PCR -Western immunoblotting analyses	N/A	- Cyclic: -OPG: In-creased - sRANKL, RANKL: De-creased - Continuous: -OPG, sRANKL, RANKL: No ss diff
Ignatius et al (2005) (35)	SV-HFO	tension	1% 1 HZ 1800 cycles 30min every day Cell harvesting: immediately after loading on days 3, 7, 10, 14, 17, 21 six-station stimula-tion apparatus	Osteogenic+ Hams F12	-RT-PCR	- Coulter Counter	- Proliferation: Increased -COL I: constant from day7 to 21, increased at 3, 7 - ALP: in-creased from day1 to 21 in both groups (control and stimulated), Day 7,17>control -OPN: : increased from day1 to 21 in both groups Day 3, 14>control -OCN: : increased from day1 to 21 in both groups Day 3, 21>control -cbfa1: increased at day 3,7
Weyts et al (2003) (34)	SV-HFO cultured on collagen coated plates	Stretch (bi-axial)	0.4, 0.9,2.5% 0.5 HZ For 72h in the presence or absence of osteogenic factors	aMEM without phenol red + FCS + glycerophos-phate+ dexametha-zone	-PICP RIA -DNA quantities -ALP activity -calcium detection kit	- Sysmex cell coun-ter	- Prolifera-tion: -day 7: decreased at all magnitudes in both standard and inducing medium -day14: increased at all magnitudes in inducing me-dium and no diff in standard me-dium -day21: No ss diff - DNA levels: increased from 7 to 21d, at 21d ss higher in induc-ing medium -pro COLI: de-creased from 7d to 21d, no ss diff between medi-ums - ALP: peaked at 14d, ss higher in induc-ing medium at 14,21d -calcium accu-mulation: in-creased from 7d to 21d in induc-ing medium, ss higher in induc-ing medium at 21d
Jansen et al. (2004) (30)	SV-HFO cultured on collagen coated Bioflex plates	Stretch (bi-axial)	0.4% for 5, 15, 60 min on day 7, 14, or 21 by Flexercell strain apparatus	Osteogenic	-Western blot - ALP activity	N/A	- ERK1/2 phos-phorylation: -duration: rapid increase with a max between 5-15 min 60 min: decrease toward baseline -day: strongest at day 14,21 -Day 21: differentiated cells>>non-differentiating -After day 21: in the presence of osteogenic fac-tors>>in the ab-sence of osteo-genic factors

Study	Cell Type	Mechanical force	Device, duration, frequency of the force	Medium	Differentiation Tests	Prolifera-tion Tests	Result
Jansen et al (2006) (72)	SV-HFO cultured on collagen coated Bioflex plates	Stretch (bi-axial, cyclic)	0.5 Hz for 15 min on days 7, 14, 21 by Flexercell strain appa-ratus	aMEM without phenol red + HEPES+ charcoal treat-ed fetal calf se-rum + CaCl2 + streptomycin + penicillin + dexa-methasone + b-glycerophosphate	-ALP activity -DNA level -Calci-um Deposition -Alizarin Red S -Western blot		- DNA levels: increase in mineralizing cultures > non-mineralizing cultures -ALP: - miner-alizing cultures: increase, peaked at day 14. - nonmineralizing cultures: no change -Calcium Deposi-tion, Alizarin Red S: Day 7: no mineraliza-tion, day 14: onset of mineral-ization, day 21: full mineraliza-tion -MMP-1, MMP-3: in-crease -gene expres-sion: most= MMP-1, -2, -14, TIMP-2. Least= MMP-8
Jansen et al (2010) (71)	SV-HFO cultured on collagen coated Bioflex plates	Stretch (bi-axial, cyclic)	-short term: single bout cy-clic 0.4% 0.5 Hz for 15 min on days 5,14 -long term: repetitive bouts of 15 min for five times per day, day 5-21 by Flexercell strain apparatus	aMEM without phenol red+ HEPES+ charcoal treat-ed fetal calf se-rum+ CaCl2+ streptomycin+ penicillin+ dexa-methasone+ b-glycerophosphate	-ALP activity -DNA level -Calci-um Deposition -Alizarin Red S -Western blot	N/A	- DNA levels: No ss diff -ALP: peak at day14 ,stretched: lower at first, higher during the min-eralization phase -Alizarin Red S: peak at day14 ,stretched: lower at first, higher during the mineralization phase
Zhu et al (2008) (33)	SV-HFO cultured on collagen coated Bioflex plates	tension	0.8%, 1.6%, 2.4%, 3.2% 1 HZ for 48h by Flexercell strain apparatus	Osteogenic + G418	-RT-PCR -ALP activity	N/A	- COLI: en-hanced by the increasing strain gradually - ALP: increased at 0.8% and 1.6%, no change at higher magni-tudes. - OCN: increased at higher magni-tudes of strain (2.4% and 3.2%).no change at 0.8% and 1.6%. elongation had no effects - Cbfa1/Runx2 mRNA: in-creased only at the highest mag-nitude of strain
Kokkinos et al (2009) (8)	HBMDO from the femoral diaphysis cul-tured on Ti-6Al-4V	Homogeneous strain	500, 1000 µstrain 0.5, 1 Hz for 0.5 h, 1.5 h, 3 h, 6 h by four point bending device	standard	-RT-PCR	- DNA synthesis	- DNA synthesis: - 0.5 h: no effect - 1000 µstrain, 1 Hz: lowest stimulato-ry result - Cbfa1 mRNA: peak =0.5 Hz, 500 µstrain, 3 h
Wozniak et al (2000) (42)	preosteoblasts, oste-oprogeni-tors, osteoblasts from human bone marrow cultured on colla-gen/ vitronectin-coated supports	Strain (cyclic)	70,000 µstrain 0.05 Hz For 48h at 3 cycles/minute (10s on/10 s off)	Osteogenic + OP1	-IHC -flow cytometric analysis -Western analysis -immuno-blotting of cell lysates -Alizarin red-S	N/A	-avb3: - syn-thesis: no diff - redistribution: increased the number and size of the plaquelike sites of avb3 expression -OCN: : in-creased -OPN: strain stimulate secretion of the 168-kDa mole-cule such that it does not accumu-late in the cell -Alizarin Red S: increased the intensity of min-eralized nod-ules

HOB: human osteoblasts, HFO: human fetal osteoblasts, HBMDO: human bone marrow derived osteoblasts, NHOBs: normal human osteoblasts, OGM: osteogenic growth medium, TCM: tissue culture medium, Ss: statistically significant

ERK pathway. Mechanical loading affects this signaling in a time-dependent manner (initial increase followed by a long period of inhibition after stretch).[21] There are some ways that osteoblastic differentiation can be monitored, such as the detection of DNA levels (cell density), matrix production (procollagen secretion), maturation (alkaline phosphatase (ALP) activity), and mineralization (calcium levels) for instance.[23] Mechanically strained HBMDOs and SV-HFO/SV40 produced higher intensities of mineralized nodules.[20,21,23,31] ALP activity was increased in stretched HFOs,[20–24] but higher strain magnitudes did not affect it.[22] Mechanically stimulated osteoblasts showed lower levels of it.[26] Biomechanical loading increases the COL 1 expression in human osteoblasts and HFOs in general,[4,22,26] but the day of culture[24] and duration of loading application[28] can change its expression results. HFOs,[24] preosteoblasts, and osteoprogenitors[31] showed higher OCN and osteopontin (OPN) expression and secretion levels after mechanical stimulation. It was reported that the mRNA expression of OCN and OPN in osteoblasts and HBMDOs increased, but the peak depended on the strain magnitude.[4,5,22] Kasper et al. concluded that strain reduces OCN expression in osteoblasts.[26] In contrast, Rath et al. reported that it does not affect the expression of OCN and OPN in the same cells.[28]

Cyclic uniaxial stretch increased bone morphogenetic protein-2 (BMP-2) expression in primary human osteoblasts,[4] but continuous tensile strain made no significant change in the expression of BMP-2 and BMP-7.[28] Core binding factor-α1 (Cbfa1) is the osteoblast-specific transcription factor through which mechanical loading can affect osteoblast differentiation.[21] Cbfa1 expression in HBMDOs peaked at 500 με and decreased at higher magnitudes of mechanical loading (1000 με),[21] while HFOs exhibited increased expression of it only at the highest magnitude (3.2%).[22]

Osteoclastogenesis

Changes in RANKL/RANK/OPG mRNA and protein synthesis are influenced by loading characteristics.[25] Differences in the results of changes due to cyclic and continuous force confirm this statement.[30] Among the various matrix metalloproteinases (MMPs), MMP-1, and MMP-3 significantly increased under the influence of the applied force.[20]

Compression

Proliferation

Among three studies[12,13,32] that assessed osteoblast proliferation, one study mentioned the magnitude and duration-dependent manner of compression.[32] In addition, one study[13] mentioned that osteoblasts had different reactions to compression in 2D and 3D conditions, and only one study[12] mentioned that compression has no significant effect on the proliferation of osteoblasts.

Osteogenesis

To assess the osteoblastic differentiation/activity, the expression, release, or production of the following factors was analyzed: ALP, COL 1, OCN, OPN, and osteoprotegerin (OPG). It has been mentioned that the alternation in the expression of osteogenic factors differs in 2D and 3D conditions except in the case of runt-related transcription factor-2 (RUNX2), which increased in both conditions.[13] However, the decrease in the expression of RUNX2 can also be seen.[14] It has been mentioned that the expression of OPG decreased following compression.[12,33] One study[14] mentioned that the expression of ALP and COL 1 increased, while one study showed lower rates of COL 1 following compression, and another study[33] mentioned no significant difference in the expression of COL 1. In addition, no significant difference in the expression of OCN and OPN was seen.[14]

Osteoclastogenesis

The rate of interleukins (IL-6, IL-8), MMP, RANKL, and cytocolagenase 2 were analyzed to assess osteoclastogenesis. Four studies[12,32,33] mentioned that compression increased the rate of osteoclastogenic factors. However, one study[33] mentioned no statistically significant difference in the expression of MMP-2.

Discussion

Osteoblasts face different types of mechanical forces in the human body. The success of treatment procedures such as distraction osteogenesis and orthodontic treatments is related to the reaction of osteoblasts to mechanical forces, specifically tension and compression. Several studies have been done using in-vitro conditions to analyze the behavior of osteoblasts and monitor the expression of specific factors that play a crucial role in bone formation and absorption. The results can be beneficial in anticipating the reaction of osteoblasts to the mechanical forces in the human body.

Cell lineage

Primary osteoblasts have different sensitivities to mechanical strain compared to SV40-immortalized osteoblasts.[20] It can be hypothesized that due to the rigidity of the extracellular matrix following mineralization, the effect of mechanical forces on osteoblasts will be reduced.[35] In fact, the differentiation stage affects the mechanosensitivity of osteoblasts. For instance, the expression of MMP-3 increased 25-fold after applying tensile force

on the fifth day of cultivation.[20] However, it increased by lower amounts (5 folds) when the force was applied on the seventh day of cultivation when the medium was more mineralized.[20] In addition, the tensile force did not affect MMP-3 expression when the force was applied on the 21st day of cultivation.[20]

Force device

The included studies applied tension through different devices. The protocol of mechanical force has a more significant effect on the accuracy of results compared to the specific device that was used. In terms of tensile force, it is essential to make sure that mechanical force is distributed evenly between cells. It is mentioned that this goal can be reached by placing cells in the center of the bio-flex plate accurately.

Force characteristics

Despite the controversies about the exact amount of physiological mechanical strain in the human body, it is claimed that its normal range is between 50–1500 μs. Below this range is defined as the disuse mode and causes bone weakness. Bone remodeling is started at 1000–1500 μs and can be continued up to 3000 μs, which is the start of micro-damage manifestations.[36] a high number of included studies applied supraphysiological forces, which may jeopardize the reliability of the results. This may have resulted from not seeing significant differences at lower magnitudes.

Some studies mentioned that prolonging the duration of mechanical force leads to desensitization of cells and reduces the positive/negative effect of mechanical forces on osteoblasts.[14,37] The duration of force application among studies that applied continuous tension did not exceed several hours.[28,35,38] Only Kreja et al.[39] applied continuous force for 72 hours. However, they did not mention a significant effect of continuous force, while gene expression altered following intermittent force application in the mentioned study.

Cell culture system

The effect of mechanical stimulation can be altered based on the cell culture system.[24] To assess the molecular mechanisms concerning the effect of mechanical forces on gene expression and how mechanical forces convert to molecular signals, it is recommended to use 3D conditions rather than conventional 2D environments. In addition, the 3D condition will provide an environment that more resembles in-vivo conditions. It must be considered that before the cultivation of cells under 3D conditions, such as what has been done in Brezulier et al.[13] and Ignatius et al.,[24] it is essential to assess the viability of 3D conditions through cell viability assays such as MTT or BrdU assays prior to the commencement of the experiment. However, among all included studies, only two studies analyzed the behavior of osteoblasts in 3D conditions.[13,24] In addition, 2D conditions have some artifacts compared to the in-vivo conditions. For instance, Bhatt et al.[40] mentioned that in in-vivo conditions, tensile stretch with a frequency of 1 cycle/12 hours is used. However, this frequency had no significant effect on the behavior of osteoblasts using in-vitro conditions.[40]

The composition of the medium can affect the response of stem cells to mechanical forces. For instance, Kreja et al.[39] aimed to analyze the osteoclastogenic response of osteoblasts to different magnitudes and frequencies of strain. Considering the positive effect of vitamin D3 on the RANKL expression, it has been added to the medium to induce osteoclastogenesis.[39] In addition, FCS, which was added to the medium in this study,[39] contains osteoclastogenic cytokines, proteins, and growth factors.

Proliferation and viability

The proliferative response of osteoblasts is one of the primary changes that occur after force application.[29] Factors such as culture conditions, mechanical stimulation parameters, and duration of loading application can strongly affect the results,[5,23,29,34,37,41] but no correlation was found between cellular responsiveness and donor variability of bone cell origin.[29] an appropriate number of cycles and strain frequency intensifies proliferation and cell viability[23,27,29,34] while compressive force generally inhibits cell proliferation[37] except in cases in which proliferation had already been down-regulated because of other reasons (by clodronate for example).[12] In comparison with 2D cultures, seeding the cells in 3D cultures resulted in better proliferative responses and adaptation to compressive forces because of better nutrient access or low cell concentration density.[24,41]

Differentiation

To assess osteogenesis/osteoclastogenesis, the expression and protein production of osteogenic factors such as ALP, OCN, OPN, RUNX2, and COL 1 can be analyzed. Considering the fact that these genes consist of long-term and short-term markers, elongation of cultivation times may provide more accurate results. Therefore, a portion of included studies[12,41] extended the duration of the experiment by several weeks to obtain more reliable results, while some performed analyses several hours following the stimulation.[37] This variety can be justified by considering that the duration of the analysis would be set based on the type of markers that were analyzed.

Cells obtained from different patients may exhibit different behaviors.[26,39] For instance, Kaspar et al.[26] mentioned that even in the control group of their study, which was not subject to mechanical forces, osteoblasts showed different

behaviors. Included studies that derived osteoblasts from human bone did not discuss the common source of osteoblasts in the control or experimental groups.

Alkaline phosphatase

ALP is an early osteogenic differentiation marker. Studies showed that mechanical forces can increase the proliferation in the high ALP activity stage of osteoblasts. For instance, Weyst et al.[42] mentioned that the ALP activity of HFO will peak 14 days following the mechanical force. In this stage, mechanical strain increases proliferation. However, in the earlier or later phases, stretch decreased or did not alter proliferation, respectively.

Different types of osteoblasts may show contradicting behavior to compression. For instance, the amount of 4 gr/cm^2 compression has no effect on the ALP expression of HFO while increasing the expression in HOB. In the case of tensile forces, different magnitudes and frequencies of stimulation increased ALP activity in HOB and HFO.[20,21,35,42] Moreover, among all included studies, the rate of ALP activity peaked after 14 days of force application, which is considered the initiation of mineralization.

PGE2 can induce both osteoblastic and osteoclastic procedures. It has been hypothesized that PGE2 may increase the production of ALP and COL 1.[14] The same increasing pattern following the expression of ALP can confirm the increasing effect of PGE2 on ALP and COL 1.[14]

Collagen type I

It makes up 90% of organic materials in the bone matrix[20] and is considered an early osteoblast differentiation marker. It is apparent that proliferation and the expression of COL 1 downregulate before the upregulation of osteogenic genes. However, it has been shown that COL 1 enhances osteogenesis.[43,44] Cells that were cultured on collagen matrixes had higher osteogenic gene expression and expressed the osteogenic factors earlier compared to those cultured on plastic dishes.[45] In addition, collagen causes uniform mineralization compared to focal mineralization on plastic dishes.[45]

It has been assumed that mechanical stimulation will alter the expression of COL 1 directly. However, Sanchez et al.[46] mentioned that the production of MMP-3 caused by mechanical stimulation affects the rate of COL 1 expression. They mentioned that 4 hours following the compression, there were no changes in the rate of COL 1. However, MMP-3 increased at this time point. They hypothesized that the rate of COL 1 will increase in the next phase of differentiation.

Osteocalcin

It is the most plentiful non-collagenous protein expressed only by fully differentiated osteoblasts and is critical for bone metabolism.[47] The effect of mechanical forces on OCN expression depends on the force's type, magnitude, frequency, and duration. For instance, it has been mentioned that compression has no significant effect on OCN expression. In contrast, tension alters its expression in different patterns. Among all included studies, the expression of OCN decreased when 0.1% strain was applied.[26,38] However, Ignatius et al.[24] mentioned higher expression of OCN following 0.1% strain. Different types of osteoblastic cells were used in the mentioned studies, which may be the reason for conflicting results.

The amount of 0.05%, 2.4%, 3%, 3.2%, and 7% strain increased OCN expression in osteoblasts with no conflicts between studies. Since OCN expression will be recognized in the late stages of the differentiation, performing the analyses at least 48 hours after the stimulation, which was most common among the included studies, may give more accurate results. For instance, Rath et al.[28] analyzed the expression after 4 hours and 24 hours of mechanical force and did not mention any significant differences. It does not mean that OCN cannot be recognized at the earlier time points. For instance, Bhatt et al.[40] analyzed the expression 12 hours after the stimulation and showed higher rates of OCN following the mechanical force.

Cbfa1 is the key to converting mechanical stimulation to osteogenic differentiation. The expression of this marker can be increased following mechanical tension.[22,38] It can increase MSCs differentiation to osteoblasts and regulate osteoclastic function by binding to osteoblastic acting elements (OSEs), which are located in the promoter region of the osteoblast's specific genes such as OCN, OPN, BSP, and COL I.[48]

Osteopontin

This factor is a late osteoblastic differentiation marker. a 4 gr/cm^2 compression will decrease the expression of OPN in HFO[13] while having no significant effect on HOB.[14] The expression of OPN increased following the tensile force in all of the studies, independent of the magnitude, duration, and frequency of the cyclic tensile force.[24,40,49] However, same as OCN, since it is a late-stage factor, a minimum time is needed to recognize significant differences in the expression of this gene. Rath et al.[28] was the only study that mentioned no significant difference in the rate of OPN. This can be because Rath et al.[28] was the only study that applied continuous tensile force while others applied cyclic forces.

Runt-related transcription factor 2

Runx2 is a transcription factor playing a crucial role in MSC functionality in forming osteoblasts, osteoclasts, osteocytes, and bone lining cells. Its positive and negative regulation can impact the bone formation process.[50]

Among the included studies, ALP and COL 1 factors did not increase or decrease in the same manner as RUNX2, which suggests that they are produced in a RUNX2-independent manner.[13,14] However, it has been mentioned that OCN may be expressed through a Runx2-dependent pathway.[14] HOB and HFO showed contradicting results about the effect of compression on RUNX2 expression. In fact, a 4 gr/cm^2 compressive force increases the expression of RUNX2 in HFO[13] while decreasing the expression in HOB.[14] On the contrary, with compression, high magnitudes of tensile force can increase the expression of RUNX2.[22]

Osteoclastogenesis

Receptor activator of nuclear factor kappa-B ligand

This factor has a significant effect on orthodontic tooth movement by increasing the bone resorption rate. Besides osteoblasts, this factor can be found in odontoclasts, osteocytes, and fibroblasts during tooth movement. The expression of RANKL will increase following compression in both HOB and HFO, independent of the force's magnitude. In addition, continuous tensile forces have no significant effect on the expression of RANKL, while cyclic tensile force decreases RANKL expression[30,39]. It has been proven that IL-8 can increase the expression of RANKL, which can be confirmed by considering that among included studies, RANKL and IL-8 alter in the same pattern in response to mechanical forces.[46]

Interleukin 6 and interleukin 8

These two markers, which can act in a paracrine and autocrine manner, are considered osteoclastogenic factors. These two markers can be found in the gingival fluid during orthodontic tooth movement. Tripuwabhurt et al.[32] mentioned that although compression increased the expression of IL-6, IL-8, and C-X-C motif chemokine ligand 8 (CXCL8), it reduced the rate of IL-6 and CXCL8 protein levels.[32] This controversy can be justified by considering the negative feedback control in post-transcriptional procedures.[32] The expression of IL-6 and IL-8 increased following the compression in both HOB and HFO, independent of the magnitude and type of compression.[13,32,33]

Osteoprotegerin

This factor neutralizes the enhancing effect of RANKL on osteoclastogenesis.[13] Grimm et al.[12] mentioned that compression decreased the expression of OPG. However, the protein levels of OPG increased. This contradiction can be justified by considering the physiological sequences that lead to protein production.[12] Compression increased the expression of OPG in HFO,[13] while lower rates of this marker were seen following the compressive force in HOB.[14,33] In addition, the continuous tensile force had no significant effect on the expression of OPG.[30,39] However, the rate of OPG increased following the cyclic tensile force.[30] It must be mentioned that the effect of tension on HFO was not evaluated.

Limitations

More studies are needed to assess the independent effect of magnitude, duration, and frequency of force on the behavior of osteoblasts.

A portion of the studies did not mention the differentiation stage of osteoblasts at the time of analyses.

Studies with the same osteoblast source for control and experimental groups are needed.

More studies that analyze the effect of mechanical forces in 3D conditions are needed.

More studies that apply mechanical forces in the magnitudes close to in-vivo conditions are needed.

Conclusions

This study aimed to analyze the in-vitro studies that applied tension or compression forces (two significant forces in dentofacial deformity treatments) to osteoblasts from different aspects. It has been shown that the response of markers that are related to bone formation or absorption can be altered based on the differentiation stage of the cells, the cell culture system, and the magnitude and duration of the force. Our results can be useful to compare different in-vitro conditions to physiological conditions to specify what best resembles in-vitro conditions of the human body environment during treatments such as orthodontic tooth movement and distraction osteogenesis.

Ethics approval and consent to participate

Not applicable.

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

ORCID iDs

Parisa Motie https://orcid.org/0000-0001-5598-3575
Sadra Mohaghegh https://orcid.org/0000-0002-4494-5685
Farnaz Kouhestani https://orcid.org/0000-0002-9341-1209
Saeed Reza Motamedian https://orcid.org/0000-0001-7337-2145

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Review

Dental implants for patients with oral mucosal diseases: A narrative review and clinical guidance

Ahmad Mubarak Al Ramil[1,B–D], Shatha Lamfoon[1,B–D], Hani Mawardi[2,A,D–F]

[1] Ministry of Health, Riyadh, Saudi Arabia
[2] Department of Oral Diagnostic Sciences, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Ahmad Mubarak Al Ramil
E-mail: ahmadmr84@gmail.com

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Abstract

Oral mucosal diseases are a group of conditions that affect the oral mucosa with variable severity and include recurrent aphthous stomatitis (RAS), oral lichen planus (OLP), pemphigus vulgaris (PV), mucous membrane pemphigoid (MMP), and systemic lupus erythematosus (SLE). These may manifest clinically as painful oral ulcerations, reticulations and/or erosions, with differences between each. Management protocols often include initial topical and/or systemic corticosteroid (CS) therapy to control the patient's acute symptoms, followed by CS-sparing agents for long-term maintenance therapy. Patients with oral mucosal diseases often require dental implants to replace missing teeth. However, data on potential complications and success rates for these cases is still lacking. Considering the steady increase in the incidence of immune-related systemic conditions in the general population globally, dentists are expected to have the needed knowledge and ability to safely place dental implants in this group of patients. Therefore, this review aims to discuss the underlying pathogeneses of common oral mucosal diseases, clinical presentations, best practice approaches, and recommendations for the placement of dental implants in patients with similar conditions.

Keywords: dental implants, oral lichen planus, recurrent aphthous stomatitis, systemic lupus erythematous, vesiculobullous diseases

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Background

For the past several decades, replacing missing teeth with dental implants has become the first choice for patients in need of better predictability, longevity and esthetics.[1] This preference can be attributed to factors such as the ability to preserve neighboring teeth, improved quality of life and the high survival rate of placed implants.[1] When compared to other available treatments, dental implants continue to be the closest option to mimicking natural teeth and restoring lost function.

Although the reported success rate of dental implants is approx. 95–98% in healthy subjects, the outcome data in patients with oral mucosal diseases is still lacking.[2–4] Oral mucosal diseases include a wide range of conditions that have a significant impact on the patient's quality of life, ranging from mild to more severe, such as recurrent aphthous stomatitis (RAS), oral lichen planus (OLP) and pemphigus vulgaris (PV).[2] Management protocols for these conditions often involve initial topical and/or systemic corticosteroid (CS) therapy to control the patient's acute symptoms, followed by CS-sparing agents for long-term maintenance therapy.[5] In some clinical scenarios, combinations of more than one agent with long-term follow-up are indicated for an optimal outcome.

Considering the steady increase in the incidence of immune-related systemic conditions in the general population globally, dentists are expected to have the needed knowledge and ability to safely place dental implants in this group of patients.[3] Therefore, this review aims to discuss the underlying pathogeneses of common oral mucosal diseases, clinical presentations, best practice approaches, and recommendations for the placement of dental implants in patients with these conditions.

Recurrent aphthous stomatitis

Overview

Recurrent aphthous stomatitis is a common chronic inflammatory disease characterized by recurrent, painful ulcerations of the oral mucosa.[6] The estimated prevalence of RAS ranges from 5% to 25% in the general population and can reach up o 60% in selected groups with higher stress levels, such as students or professionals in demanding jobs.[6,7] Recurrent aphthous stomatitis is most commonly diagnosed in young patients, with a peak onset between 10 and 29 years of age and a female predominance.[6] The etiology of RAS is still unknown but it is reported to be hereditary with multiple implicating factors such as stress levels, oroantral microbiota, and deficiencies in iron and vitamin B complex.[8] Clinically, RAS manifests as a painful ulcer or multiple ulcers, covered by a grey-yellowish pseudomembrane, and surrounded by an erythematous halo on the non-keratinized oral mucosa (Fig. 1).[9] There are several types of RAS, including minor, major, herpetiform, or complex type. The type of RAS determines the selection of the most suitable treatment approach (Table 1).

Management

In order to diagnose RAS, other potential causes (e.g., iron, vitamin B_{12} or folate deficiencies and trauma) and underlying medical conditions such as Crohn's disease, ulcerative colitis, Behçet's disease, and systemic lupus erythematosus (SLE), which may present clinically with

Fig. 1. Patient with severe and minor recurrent aphthous stomatitis (RAS) involving the maxillary and mandibular labial and buccal mucosa

Table 1. Clinical types of recurrent aphthous stomatitis (RAS)[8,51]

Characteristic	Type of RAS			
	minor	major	herpetiform	complex
Prevalence	80–90%	10%	1–10%	1–5%
Size	<1 cm	>1 cm	multiple 2–3-mm aphthae that coalesce into a large one	3 mm or more, oral or genital aphthae
Shape	oval	ragged oval, crateriform	oval	combination of all 3 forms (oval, ragged oval and crateriform)
Location	non-keratinized mucosa	non-keratinized mucosa	any intra-oral site	depend on the RAS form present
Healing	4–14 days	up to 6 weeks	10–14 days	

aphthous-like oral ulcerations, should be excluded.[6,9] In addition to a clinical examination, laboratory investigation (e.g., colonoscopy for possible Crohn's disease) and medical consultations may be needed to rule out secondary RAS.

As of today, there is no cure for RAS. However, it is a self-limiting condition that takes from 4 to 14 days to heal completely. In some situations, longer periods of healing are observed, particularly in cases of major RAS, resulting in scarring at the ulcer site.[8] Several options are available to manage the patient's symptoms, including topical administration of lidocaine (i.e., gel, spray, or suspension) for pain control and cyanoacrylate-based sealing agents such as isoamyl-2-cyanoacrylate to create a protective layer over the ulcer and improve function.[10] Mild to moderate symptoms may respond to topical CS therapy in the form of gel or ointments (i.e., fluocinonide or clobetasol propionate), or elixir (i.e., dexamethasone or compounded clobetasol) for cases with multiple ulcers (Table 2). Intralesional injections of triamcinolone acetonide provide a useful option for painful, larger ulcers to expedite the healing process. Cases with more acute and severe symptoms may require a short course of systemic CS therapy.[6] Long-term prophylaxis therapy with colchicine, pentoxifylline or dapsone can be considered for patients with few or no ulcer-free days to keep the disease in a remission state.[11]

RAS and dental implants

Literature on the impact of RAS on dental implant success is lacking. However, several factors should be considered in such cases. Placement of dental implants is often associated with increased anxiety and stress levels, which could trigger a RAS episode in the first days post-surgery.[12] As sites with active oral ulcerations are more likely to have friable and less resilient soft tissues, it is advised to initiate RAS treatment before attempting to place a dental implant, with the aim of keeping the disease in a remission state. Careful tissue management during the surgery is also recommended to prevent dehiscence and delayed wound healing at ulcer sites.

Oral lichen planus

Overview

Oral lichen planus is a common, chronic T-cell-mediated mucocutaneous disease that affects the oral cavity, skin and/or genitalia.[9,13,14] The estimated prevalence of OLP ranges from 0.22% to 5% worldwide. The condition is more prevalent in females and the age of onset ranges between 40 and 80 years.[13,15,16] The pathogenesis of OLP has been linked to the apoptosis of epithelial basal cells induced by $CD8^+$ cytotoxic T-cells as a result of endogenous or exogenous

Table 2. Common therapies used for the management of oral mucosal diseases

Treatment		Dose/concentration	Formulation	Frequency	Indication
Topical CS therapy	fluocinonide	0.05%	gel	2–3 times daily	localized oral ulcers
	clobetasol propionate	0.05%	gel		
	dexamethasone	0.5%	elixir		multiple oral sites involved
	compounded clobetasol	0.05%	elixir		
	prednisolone	15 mg/5 mL	syrup		
Systemic CS therapy	prednisone prednisolone	0.5–1 mg/kg/day	tablets	once daily	moderate to severe OLP, VBDs, SLE
CS-sparing agents/ immunosuppressive agents	hydroxychloroquine	200 mg	tablets	twice daily	maintenance therapy for OLP
	azathioprine	1 mg/kg/day	tablets	once daily	maintenance therapy for OLP, VBDs
	mycophenolate mofetil	500 mg	tablets	1–4 times daily	maintenance therapy for OLP, VBDs

CS – corticosteroid; OLP – oral lichen planus; SLE – systemic lupus erythematosus; VBDs – vesiculobullous diseases.

antigens.[17] Common triggers include local and systemic inducers of cell-mediated hypersensitivity (such as a local reaction to dental restorative material or flavoring agents), stress, and microorganisms such as hepatitis C virus, which remain controversial among OLP experts.[16] Clinically, OLP may present as a multifocal disease, often with symmetrical distribution on the buccal mucosa, tongue, lips, gingiva, palate, and, rarely, the floor of the mouth.[13] Several forms of OLP have been described in the literature, including reticular, erythematous, erosive, papular, plaque, and bullous types (Fig. 2).[14] The plaque form, in particular, may mimic other oral pathological conditions, including leukoplakia, which must be excluded by the treating dentist and biopsied if needed.[18] Desquamative gingivitis is often an early sign of OLP in active disease and presents as desquamation (peeling) of the gingival margin with erythema and bleeding on brushing.[13] Patients with oral lichen planus may also experience discomfort, pain and sensitivity with acidic and spicy foods or drinks and/or a burning sensation in the oral cavity of varying severity. In addition to the oral cavity, OLP patients may report other body sites affected by the same process, such as skin, scalp and genitalia, presenting with purplish, scaly and itchy plaques.[19] Diagnosis of OLP is usually given based on a combination of the patient's reported history and clinical manifestations; however, a biopsy may be indicated for less classical presentations, suspicious for pre-malignant or malignant lesions.[19]

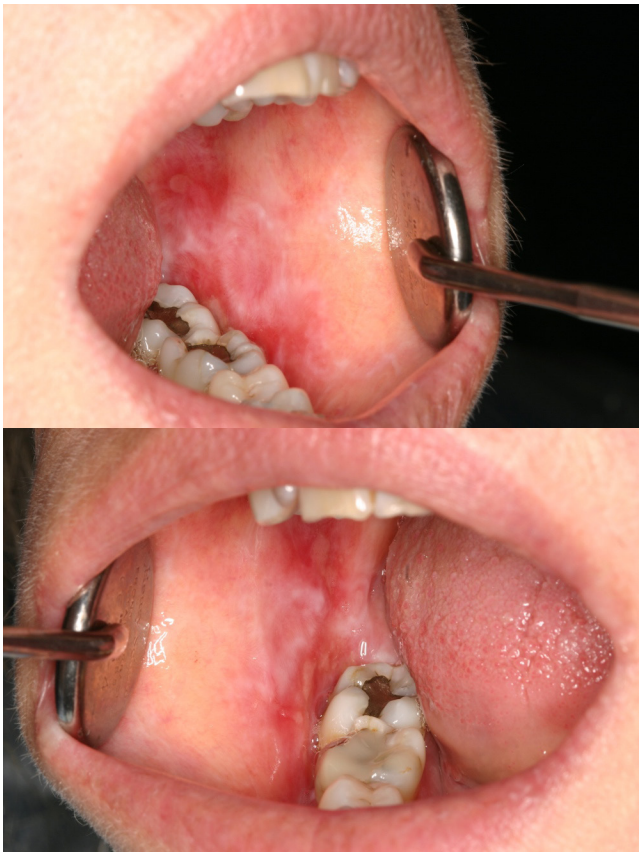

Fig. 2. Patient with reticular, erythematous and ulcerative forms of oral lichen planus (OLP) affecting buccal mucosa on both sides

Management

Oral lichen planus is a chronic disease with no approved cure to date. Patients with drug-induced lichenoid reactions may benefit from switching to an alternative agent after consultation with their physician. Otherwise, the management of OLP is typically tailored to address the symptoms reported by the patient. Education regarding the overall disease and its prognosis should be offered to asymptomatic patients. Patients with more symptomatic diseases need CS therapy as the first line of treatment in a topical formulation, which requires multiple daily applications, such as clobetasol or fluocinonide. In clinical situations where multiple oral sites are involved, topical dexamethasone elixir can be prescribed. Similar to RAS, intralesional triamcinolone injections expedite the healing process of single and large painful ulcerations. Systemic CS therapy is a viable and effective option with a rapid onset for achieving the remission in moderate to severe disease. Prednisone or prednisolone can be administered at a dose of 0.5–1 mg/kg/day. In most cases, OLP tends to have a favorable prognosis with less impact on the person's quality of life. However, a small number of cases could progress to more severe forms or continue to be symptomatic for a longer period of time, requiring maintenance therapy.[20] For these cases, steroid-sparing agents such as hydroxychloroquine, azathioprine and mycophenolate mofetil are often considered.[19,21] However, patients with more frequent episodes of exacerbated symptoms during the year may be treated with topical agents, with or without short-term systemic CS therapy.

OLP and dental implants

As of today, there are no guidelines or recommendations for the management of OLP patients with dental implants. In general, OLP patients can undergo dental procedures with a low risk of complications.[22] Yet, data on dental implant placement in OLP patients and potential complications has been scarce. A systematic review reported that patients with controlled OLP had a mean dental implant survival rate of 98.3%, with a mean follow-up of 44.6 months.[2] However, a prospective study involving 55 dental implants placed in patients with active OLP showed that 42 (76%) dental implants failed and were successfully replaced after controlling disease activity with systemic CS therapy.[23] Moreover, the marginal bone loss of implants placed in active OLP patients was compared to that in controlled OLP patients on systemic CS therapy and healthy individuals. During a 4-year follow-up, more bone loss was observed in the active OLP group (2.53 ±0.44 mm) than in the controlled OLP group and healthy individuals (0.75 ±0.56 mm and 0.79 ±0.73 mm, respectively).[24] However, a retrospective study that included 66 short implants placed in patients with controlled OLP showed the reported success rate of 98.5%, with marginal bone loss of 0.96 mm mesially

and 0.99 mm distally within a mean follow-up period of 68 months.[25] No significant differences were observed between the reticular and erosive forms of OLP, and the study did not include a control group.[25] In a prospective study comparing 18 patients with controlled erosive OLP to 18 healthy subjects who received dental implants, the implant survival rate was 100% in both groups; however, OLP patients with desquamative gingivitis had higher rates of peri-implant mucositis and peri-implantitis.[26] Studies indicate that OLP patients in remission are likely to have more predictable outcomes with dental implant treatment than those with uncontrolled disease.

Vesiculobullous diseases

Overview

Pemphigus vulgaris

Pemphigus vulgaris is a rare mucocutaneous vesiculobullous disease (VBD) and the most common subtype of the pemphigus group. It is an autoimmune condition characterized by autoantibodies targeting desmoglein 1 and/or 3, which results in loss of adhesion between keratinocytes and the suprabasal layer with blister formation.[27] The estimated global prevalence of PV is approx. 0.1–0.5% and tends to be more common in certain ethnic groups, such as Ashkenazi Jews, Japanese and populations of Mediterranean descent.[28] It primarily affects adults with a female predilection, and the age of onset ranges from 50 to 60 years. In addition to the skin, PV can also affect the pharynx and the oral cavity. The condition is usually chronic and frequently causes blisters, erosions and ulcers at the affected sites (Fig. 3).[29] The oral cavity is often the first site to be affected, causing a series of examinations. These include a biopsy for hematoxylin and eosin (H&E) and direct immunofluorescence (DIF) examination in addition to serum testing using indirect immunofluorescence (IDIF) to make an accurate diagnosis followed by disease management.

Mucous membrane pemphigoid

Mucous membrane pemphigoid (MMP) is a VBD that predominantly affects body mucosal surfaces, resulting in the formation of subepithelial blisters.[27] It typically affects the oral mucosa, conjunctiva, anogenital tissues, and upper aerodigestive tract with occasional skin involvement. The pathogenesis of MMP is driven by autoantibodies targeting various autoantigens in the basement membrane zone (BMZ), including collagen XVII (COL17, also known as BP180), BP230, laminin 332, integrin α6/β4, and collagen VII (COL7), which results in the separation between the epithelium and connective tissue.[5] Mucous membrane pemphigoid has an incidence rate of approx. 5–7.5 cases per 10,000 adults, with a female predilection

Fig. 3. Patient with pemphigus vulgaris (PV) on the anterior maxillary alveolar ridge presenting as erythema with ulceration

and an age range of 50–80 years.[30] Clinical manifestations of MMP include painful blisters and erosions on mucosal surfaces and skin, often healing with scar formation (Fig. 4). Similar to PV, obtaining a biopsy for H&E and DIF examination is the gold standard for diagnosing MMP and facilitating treatment.

Management

Most VBD cases can achieve disease remission with proper treatment, based on affected sites. Longstanding, untreated disease may result in life-threatening events such as dehydration, infections and sepsis.[31] The treatment options available for both PV and MMP start with topical and systemic CS therapy to achieve disease remission. Systemic immunosuppressive treatments are recommended for moderate to severe cases with multi-system involvement to control disease activity. For long-term maintenance purposes, patients are commonly treated with CS-sparing agents to reduce the total dose and duration of CS therapy while still controlling the patient's symptoms. Several agents with evidence-based benefits are available and include mycophenolate mofetil, rituximab and intravenous immunoglobulins (IVIG).[30]

Fig. 4. Oral erythema with large ulceration in the soft palate in a patient with mucous membrane pemphigoid (MMP)

Vesiculobullous disease and dental implants

Similar to other previously discussed mucosal diseases, few studies have evaluated the success of dental implants in patients with VBD. In active VBD, the presence of autoantibodies against hemidesmosomes, as part of the disease pathogenesis, may jeopardize the formation of the junctional epithelium between the gingival epithelium and the implant surface.[32] This can result in plaque and bacterial accumulation, increasing the risk of peri-implant mucositis and implant failure. A single case of an edentulous female patient diagnosed with PV on low-dose systemic CS therapy who received 2 dental implants to support the denture was reported. The patient was followed up every 6 months for 32 months and demonstrated 100% implant survival rate after at least 24 months.[29]

Systemic lupus erythematosus

Overview

Systemic lupus erythematosus is a chronic, autoimmune multi-system disease that affects the joints, tendons, kidneys, skin, blood vessels, and other organs such as the heart, lungs and brain, with a broad spectrum of clinical manifestations.[30] The oral mucosa can be affected in 40% of SLE patients, with symptoms including desquamative gingivitis, erosions, xerostomia, and/or temporomandibular joint symptoms such as edema and pain (Fig. 5). The incidence rate of SLE is estimated to range between 0.3 and 23 cases per 100,000 individuals per year, with the disease most commonly affecting those aged from 16 to 55 years.[33] Systemic lupus erythematosus is 10 times more likely to affect women than men.[30] The pathogenesis of SLE has been linked to B- and T-cell dysfunction with the production of antinuclear antibodies (ANA).

Diagnosis of SLE is often based on clinical characteristics and detecting serological abnormalities, including elevated ANA, anti-Ro antibodies (Abs), anti-La Abs, anti-Smith Abs, anti-U1-ribonucleoprotein Abs, anti-rheumatoid factor Abs, anti-dsDNA Abs, anti-cardiolipin Abs, lupus anticoagulant, and beta2-glycoprotein-I. In addition, the serum of SLE patients may show low C3 and C4, which helps reach the proper diagnosis.[30] Other imaging modalities are indicated on a case-by-case basis to assess organ involvement.

Fig. 5. Patient with systemic lupus erythematosus (SLE) presenting with islands of reticulation and erythema in the mid and right hard palate

Management

Similar to most autoimmune conditions, the main goal in managing SLE is to reach disease remission or low disease activity with no flares. In general, the treatment of SLE is customized based on the severity and number of organs involved. Patients with acute symptoms may require a short course of systemic CS therapy to achieve rapid disease remission.[34] Hydroxychloroquine is commonly prescribed to stabilize SLE activity and reduce flares and other constitutional symptoms. In some cases, long-term use of low-dose systemic CS therapy is indicated. Several biological agents with promising efficacy are now available for the treatment of SLE, including rituximab and belimumab.[35] In the oral cavity, ancillary topical CS therapy is an effective tool to treat persistent or non-responsive lesions.

SLE and dental implants

As of today, only a single case of implant placement in a patient with SLE treated with hydroxychloroquine 200 mg/day and low-dose CS (4 mg/day) therapy has been reported in the literature. In this case, the placed implant survived for 2 years without complications.[36] Other studies examined periodontal health in SLE patients. A cross-sectional study found no differences in periodontal parameters between SLE patients and systemically healthy individuals.[37] In addition, no significant correlation was found between SLE biomarkers and periodontal parameters assessing disease activity and prognosis.

General considerations

Currently, there are no established guidelines for the management of patients with oral mucosal diseases who are receiving dental implants (Table 3). Hence, most of the needed clinical decisions made in dental practice today are based on expert opinions. Therefore, to minimize potential dental and medical complications, several factors should be considered for this group of patients when planning dental implant therapy. In general, asymptomatic patients with disease remission can be treated as healthy subjects without special precautions. For all other patients, comprehensive assessment and evaluation of related risk factors, in addition to medical consultations and careful handling of oral tissues, are crucial for successful treatment.

Table 3. Current literature on dental implant placement in patients with oral mucosal diseases

Oral mucosal disease	Study	Study type	Patients, *n*	Implants placed, *n*	Mean age [years]	Mean follow-up [months]	Implant survival rate (%)
OLP	Aboushelib and Elsafi 2017[23]	prospective	23 OLP	55 (uncontrolled disease)/42 (after controlling the disease activity with systemic CS therapy)	56.7	36	13 (uncontrolled disease)/100 (after controlling the disease activity with systemic CS therapy)
	Khamis et al. 2019[24]	prospective	42 OLP/59 controls	59	56.7	48	100
	Anitua et al. 2018[25]	retrospective	23 OLP	66	58	68	98.5
	Hernández et al. 2012[26]	prospective	18 OLP/18 controls	56 OLP/62 controls	52.2	52.3	100
PV	Altin et al. 2013[29]	case report	1	2	70	32	implant survived with mean peri-implant bone resorption of 0.9 mm at follow-up
SLE	Ergun et al. 2010[36]	case report	1	6	49	24	success at 2 years of follow-up

PV – pemphigus vulgaris.

Long-term use of several medications for oral mucosal diseases should be considered, as it may have a direct or indirect effect on the osseointegration and success of dental implants. For instance, systemic CS therapy is used to treat VBD and SLE.[11] There is no consensus on the direct effect of CS therapy on the osseointegration of dental implants.[38] However, some studies have discussed the negative effect of CS therapy on osteoblasts via induction of cellular apoptosis and reducing the number of pre-osteoblasts while promoting the differentiation of bone marrow stromal cells to adipocytic lineage cells.[38] In addition, CS has the potential to extend the life span of osteoclasts and promote bone resorption. On the contrary, a systematic review found no association between lower implant survival and systemic CS therapy.[39]

Medication considerations and other risk factors should be discussed with the patient before treatment. In addition, potential complications must be explained in detail. Once all questions have been addressed, obtaining the patient's consent to the procedure is the last step before initiating treatment and should be documented in medical records. In the next section, we will discuss specific considerations for pre- and post-dental implant surgery as well as the long-term maintenance phase and follow-up (Table 4).

Before dental implant surgery

Several points have to be considered for patients with oral mucosal diseases before dental implant surgery. First, a complete medical history, including current medications and allergy history should be obtained in detail during the consultation visit. It is recommended to obtain oral mucosal disease history and status and to discuss the proposed dental plan with the oral medicine specialist/oral pathologist of the patient. Consultation with the

Table 4. General considerations regarding patients with oral mucosal diseases receiving dental implants

Treatment phase	Considerations
Before implant placement	– if the patient is asymptomatic and in a remission stage, no specific intervention is needed; – if oral lesions and/or ulcerations are present, initiate indicated treatment to achieve disease remission; – consult with the treating physician for updated treatment regimen and considerations; – maintain good oral hygiene to avoid the risk of infection or exacerbation of oral mucosal disease; – implement a stress-reduction protocol; – ensure gentle handling of soft tissue during dental surgery; – initiate antibiotic therapy (e.g., amoxicillin, clindamycin) the day before or on the day of surgery
Postoperative phase	– prescribe systemic antibiotics, as indicated, or continue previously initiated antibiotic therapy; – antimicrobial, alcohol-free mouthwash (chlorhexidine 0.12% solution) twice daily until wound healing is complete; alternatively – Betadine 1%; – effective pain control measures; – continue to maintain good oral hygiene; – close and frequent follow-up; – instruct the patient to avoid irritating food items, specifically for OLP and VBD patients
Long-term maintenance	– regular follow-up and maintenance visits; – close monitoring of OLP patients for early detection of malignant transformation process

treating physician is also recommended for a complete understanding of the patient's medical status and active medications, as well as any necessary recommendations and precautions. It is also recommended that all patients receive dental prophylaxis for complete removal of accumulated local deposits (i.e., plaque and calculus) as these can act as a trigger for disease activity in VBD.

In general, dental implants should be placed when the mucosal disease is in partial or complete remission (i.e., minimal or no mucosal ulceration, absence of signs of inflammation). This approach applies to all conditions included in this report that have been demonstrated to improve dental implant outcomes.[23] On a case-by-case basis, antibiotic coverage may be considered for patients on long-term (>6 weeks) systemic CS therapy or immunosuppressive therapy who are prone to infection.[40] Eligible patients usually begin antibiotic therapy (e.g., amoxicillin, clindamycin) the day before or on the day of surgery, continuing for 5–7 days during the initial healing phase.

Adrenal suppression is another potential complication in patients on the long-term systemic CS therapy due to the increased stress and pain levels associated with dental procedures.[41] Even with the reduced risk of adrenal crisis in dental clinics, the indication for perioperative CS therapy as a prophylaxis measure remains controversial.[41] Few studies have suggested an increased risk of adrenal insufficiency for patients receiving more than 7.5 mg prednisolone/day or equivalent for more than 30 days, or more than 20 mg prednisolone/day for more than 2 weeks.[42] Other studies recommended CS supplementation regimens based on the surgery type and anticipated stress.[41] For instance, minor surgeries require 25–75 mg of hydrocortisone, while major surgeries given on the same day would require 100–150 mg of hydrocortisone. However, the World Workshop on Oral Medicine VI indicated insufficient evidence to support the use of supplemental perioperative CS therapy for most dental procedures performed under local anesthesia, except for the most stressful procedures, procedures performed under general anesthesia, patients with poor health, or patients on medications that metabolize cortisol or inhibit its synthesis.[43] Consultation with the treating physician on a case-by-case basis before the day of the surgery is recommended.

There is insufficient data on the impact of chronic hydroxychloroquine use on the success of dental implants. Hydroxychloroquine is an antimalarial and immunomodulatory agent used in SLE and OLP. A meta-analysis reported no significant delay in healing time after implant placement in patients on long-term hydroxychloroquine for specific medical conditions. The same applies to other CS-sparing agents, including mycophenolate mofetil, azathioprine, pentoxifylline, and dapsone. Therefore, no specific precautions are indicated for patients treated with one or more of these drugs.[44]

In addition to current medications, certain conditions may require further consideration. For instance, increased stress levels during implant placement may be a potential trigger for RAS. Therefore, the implementation of stress-reduction protocols, including early morning appointments and pain-free procedures, could be of potential benefit. Other measures include pre-emptive analgesia and implant placement under nitrous oxide or oral sedation before the day of the surgery using short-acting benzodiazepines (e.g., 0.5–1 mg diazepam or 0.125–0.25 mg triazolam given 1 h before the procedure). These may benefit high-risk patients without medical contraindications.

Post-operative phase

After the placement of dental implants, patients are given specific post-operative instructions to reduce the risk of complications and facilitate healing. In the context of oral mucosal diseases, additional instructions are required. For instance, patients with OLP and VBD should be advised to avoid potentially irritating food items (i.e., citrusy and spicy agents) during the initial healing phase. In addition to wound care and oral hygiene instructions, proper pain control is crucial to reduce post-surgical stress that may trigger an acute episode of RAS or reactivation of the dormant herpes simplex virus. The treating dentist should pay special attention to potential drug interactions of the prescribed pain medications with the patient's current medications. For example, hydroxychloroquine is a known substrate for cytochrome P450 2D6 (CYP2D6), which is a liver enzyme involved in the body metabolism.[45] Therefore, hydroxychloroquine has the potential to undermine the effectiveness of codeine and tramadol, which use the CYP2D6 pathway for activation of the prodrug, as well as increase serum levels of metoprolol in patients with heart disease.[46] In addition, non-steroidal anti-inflammatory drugs (NSAIDs) combined with prednisolone may increase the risk of gastrointestinal tract (GI) bleeding and ulceration.[47] Moreover, ibuprofen, together with pentoxifylline, may also increase the risk of GI bleeding. Treatment with both ibuprofen and tacrolimus requires monitoring of renal functions in patients with compromised kidneys. Post-surgical prescription of amoxicillin also carries a risk for drug interactions as it has the potential to reduce the serum levels of mycophenolate mofetil and increase methotrexate levels in the blood.

Antimicrobial therapy is critical to the success of dental implants,[48] including systemic antibiotics prescribed for 5–7 days during the initial healing phase. In addition, less irritating antimicrobial mouthwash should be prescribed, such as alcohol-free chlorhexidine (0.12%). Alternatively, Betadine 1% has similar antimicrobial effects to chlorhexidine. Patients should be instructed to identify unusual symptoms following implant placement and to report them immediately in case an intervention or treatment is needed. The symptoms may include a sudden increase in their pain level, acute or persistent edema, progressive gingival inflammation, and/or bleeding and implant mobility.

After surgical placement of dental implants, prosthetic treatment should be considered carefully. Prosthetic components that maintain minimal contact with the surrounding mucosal surfaces through proper pontic design should be used. In addition, careful handling of soft tissues with minimal trauma and the use of tissue-compatible materials are recommended.[25] The excessive use of potentially irritating materials (e.g., retraction cords and acrylic materials for temporary restoration) may exacerbate the patient's symptoms or trigger an inflammatory gingival response.[49] Furthermore, the procedure time for the surgical and prosthetic parts should be kept to a minimum to reduce the risk of edema and tissue irritation.

Long-term maintenance and follow-up

Similar to healthy subjects, patients with oral mucosal diseases who receive dental implants require regular follow-up to ensure treatment success and early detection of complications. In addition, meticulous oral hygiene regimens should be reinforced on a regular basis and may have to be conducted through scheduled office visits. Patients with OLP, in particular, should be followed up every 6–12 months for early detection and management of suspicious lesions due to the risk of malignant transformation.[20] Based on a recent report published by the World Health Organization (WHO), OLP has been classified as a pre-malignant disorder with a malignant transformation rate of 0.1–0.5%, which remains controversial.[50] However, patients should be educated about this risk and the process of self-examination to facilitate the surveillance process.

Conclusions

Oral mucosal diseases are common conditions with variable severity in the general population. Currently, there are no contraindications for the placement of dental implants in patients with oral mucosal diseases. However, there are no established guidelines on best practices and protocols to increase the success rate of dental implants in such cases with minimal complications. The current review provides dentists with insight into special considerations for placing dental implants in patients with oral mucosal diseases.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

ORCID iDs

Ahmad Mubarak Al Ramil https://orcid.org/0000-0003-3426-9406
Shatha Lamfoon https://orcid.org/0000-0001-6103-3170
Hani Mawardi https://orcid.org/0000-0002-4768-1523

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Review

Impact of nutrition on the condition of the oral mucosa and periodontium: A narrative review

Aleksandra Strączek[1,A–D], Justyna Szałkowska[1,A–D], Paulina Sutkowska[1,A–D], Alicja Srebrna[1,A–D], Natalia Puzio[1,A–D], Aleksandra Piasecka[1,A–D], Natalia Piskorz[1,A–D], Jan Błaszczyk[1,A–D], Karolina Thum-Tyzo[2,A,E,F]

[1] Student Research Group, Department of Oral Medicine, Medical University of Lublin, Poland
[2] Department of Oral Medicine, Medical University of Lublin, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Aleksandra Strączek
E-mail: aleksandra.straczek98@gmail.com

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Abstract

Diet and eating habits significantly affect health and quality of life. Various diets and food eliminations can lead to nutritional deficiencies and malnutrition. This article discusses the relationship between nutrition, nutritional deficiencies, and the condition of the periodontium and oral mucosa. An analysis of PubMed materials was conducted to assess the impact of nutrition on the condition of the oral mucosa and periodontium. We also considered dietary habits such as vegetarianism, the ketogenic diet, the Paleo diet, the Mediterranean diet, the Western diet, and intermittent fasting. Vitamin deficiencies, both water-soluble and fat-soluble, as well as macro- and microelements, can manifest in the oral cavity, among others, as gingivitis and bleeding, recurrent aphthous stomatitis, enamel hypomineralization, cheilitis, angular cheilitis, halitosis, glossitis, lingual papillae atrophy, and stomatitis. Malnutrition does not cause periodontal disease, but it increases the risk of its occurrence and accelerates disease progression. Inadequate nutrition, combined with other predisposing factors, may contribute to an increased risk of oral cancer and the development of leukoplakia.

Keywords: diet, oral health, nutrition, oral mucosa, periodontal health

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Introduction

Diet and eating habits significantly affect health and quality of life. Various diets and food eliminations can cause nutritional deficiencies and malnutrition. There are many conditions that influence the health of the oral cavity such as the diseases of the teeth's hard tissues, periodontium and mucosa.[1] This article will discuss the relationship between nutrition, nutritional deficiencies, and the condition of the periodontium and oral mucosa. It will take into consideration eating patterns such as vegetarianism, the ketogenic diet, the Paleo diet, the Mediterranean diet, the Western diet, and intermittent fasting. This will enable the reader to better understand the topic of diet and nutrition in the context of oral health.

Malnutrition can significantly affect oral health, and poor oral health, in turn, can result in malnutrition. A diet low in nutrients can lead to the progression of oral disease through altered tissue homeostasis, reduced resistance to microbial biofilm, and a decrease in tissue healing.[2]

The first signs of deficiency of some micronutrients, for example, the B vitamins, are seen in the mouth and include glossitis, cheilitis and angular stomatitis.[1] Vitamins and minerals play an important role in the rapid cell turnover of the oral mucosa. Due to its unique anatomical environment, the oral cavity may manifest early signs of nutritional disorders as well as other systemic diseases.[3]

There is a strong link between some systemic diseases and periodontitis; diet and nutrition can have an impact on a patient's condition.[4] The primary causative agent of periodontal disease is the mixed bacterial colonization in the oral tissue.[5] However, micronutrient and macronutrient deficiencies in the diet modify the course of periodontitis. Higher levels of vitamins A, B and C, calcium, zinc, and polyphenols have been shown to prevent periodontal disease.[6] Furthermore, periodontal disease has been associated with non-alcoholic fatty liver disease (NAFLD) induced by a Western diet.[2] Thus, following different diets and the associated deficiencies of individual nutrients will have an impact on the progression of periodontal disease.

Scientists have noted a significant influence of popular eating patterns on the condition of the oral mucosa. For example, the biggest concerns regarding the vegetarian diet are the iron deficiency of animal origin and the inhibitors found in some plants that can be ingested abundantly. Some oral changes observed in vegetarians may indicate iron deficiency anemia and vitamin B12 deficiency.[1] However, another study suggests that the antioxidants found in fruits and vegetables can have a positive impact on periodontal health because they protect the oral tissues from oxidative damage and modify the inflammation present in periodontal diseases such as periodontitis.[7] Other eating patterns, which are further described in this review, may also have a significant impact on the condition of the oral mucosa. Technological and cultural advances are changing people's lifestyles and eating habits, which can lead to the development of many diseases. Our study provides a comprehensive review of the latest research. This article is noteworthy and important from a public health perspective.

Objectives

This study aimed to illustrate the influence of many aspects of nutrition on the condition of the oral mucosa and periodontium.

The article lists considerations concerning general malnutrition as well as nutritional deficiencies of individual substances. Additionally, it outlines the beneficial effects of different substances on the oral mucosa. Current popular nutritional patterns have been analyzed for their good and bad effects on oral health.

Material and methods

The conducted review focused on the search for material on the impact of broadly understood nutrition on the condition of the oral cavity.

The authors conducted a comprehensive review of the most current research in this field, identified relevant sources and gathered the available knowledge in one place. The inclusion criteria covered the broadly understood diet, eating habits, and nutritional deficiencies and their implications for oral health. The preliminary search allowed for the selection of a set of keywords that seemed to be the most relevant and consistent with the topic of the review: diet, oral health, nutrition, oral mucosa, and periodontal health. English-language articles available in the PubMed database and published in the last 20 years were selected. The PubMed database was considered the most appropriate database due to the possibility of searching many scientific databases. The exclusion criteria were articles that did not take into account the correlation between dietary patterns, nutritional deficiencies and oral health. The studies meeting the inclusion criteria were analyzed in detail, and the main issues and conclusions were presented.

Influence of diet and nutritional deficiencies on the condition of the oral mucosa and periodontium

Oral and systemic homeostasis is closely related to diet, which means nutrition has both local and systemic effects on the human body. Local impact is understood as the influence of food on the tissues of the oral cavity, taking into account their composition and consistency as well as

frequency of eating.[8] A balanced diet affects the growth and development of the body tissues. Proper nutrition is vital for human growth and development. Nutritional imbalances during the period of active growth may cause severe developmental defects.[9] The bones and soft tissues of the mouth respond quickly to nutritional deficiencies because they are constantly renewed, unlike the teeth, which are primarily affected by nutrition during their formation.[8]

Symptoms of nutritional deficiencies may affect some or all tissues within the oral cavity, including the teeth, periodontium, salivary glands, mucous membranes, and the skin around the mouth.[3]

On the other hand, an excess of certain nutrients, such as carbohydrates or food products with a low pH level, can lead to the appearance of tooth cavities or non-carious tooth defects.[10] Food residues settle on the surface of the teeth, forming plaque. Plaque consists of bacteria that convert carbohydrates supplied by food into acids. A decrease in pH level below 5.5 results in demineralization of enamel hydroxyapatite crystals and proteolytic breakdown of the structure of tooth hard tissues. Therefore, the more sugar we consume, the more substrate we provide to the bacteria present in the unremoved plaque.[11] Studies showed that fermentable carbohydrates, apart from free sugars, have an impact on dental caries, but the consumption of starchy staple foods and fresh fruits is associated with lower levels of dental caries.[12] According to the American Heart Association, the American Academy of Pediatrics and the World Health Organization, free sugar intake should be limited to less than 10% of the total energy intake for adults and children.[13] Another issue that affects the formation of carious cavities is the frequency of sugar consumption and its consistency. Frequent consumption of sticky products that are difficult to rinse out when swallowing saliva or drinking fluids as well as products containing carbohydrates provides the greatest risk for caries.[10,14] Thus, it is very important to eat a certain number of meals per day and at regular intervals. Snacks and products rich in carbohydrates should be minimized in the diet.[15] Moreover, the susceptibility to caries in the presence of carbohydrates may be influenced by genetics and micronutrients, such as vitamin D.[16] Non-carious defects are of a non-bacterial origin, and their effects are irreversible.[12] Their presence can be influenced by the composition of saliva, general diseases, mechanical stress, and nutrition.[17] Regular consumption of acidic foods and beverages, occupational acid exposures, and drugs or diseases that affect saliva flow rate all contribute to an increased risk of erosive dental hard tissue defects.[18] Each type of acid has erosive potential. Solutions that are undersaturated with respect to enamel tissues will dissolve it. For instance, beverages containing calcium-chelating acids, such as citrate, may cause erosion at higher pH levels.[19] Erosion is commonly associated with citrus fruits, juices, pickled cucumbers, vinegar, and energy drinks.[20,21] In the Western diet, soda, wine and citrus-based drinks are often consumed because they are refreshing. In the European culture, the consumption of cheese and wine is a wise solution as cheese buffers the acidity of the wine and provides substrates for remineralization. The erosive potential of a diet depends on the frequency of acidic food consumption, acid strength and the buffering capacity of saliva.

The consistency and hardness of consumed products may lead to the formation of non-carious cavities. The mechanical loss of mineralized tooth tissues can lead to abrasion or attrition. Attrition is associated with occlusal surfaces, while abrasion is associated with buccal or lingual surfaces. Enamel can be worn away as a result of a hard diet. Plant products are rich in phytoliths, which are abrasive[20]; chewing abrasive substances such as tobacco can cause visible abrasion on the occlusal surfaces. It can also be caused by eating vegetables that have not been properly washed and still contain traces of soil. Abrasive changes also occur as a result of shelling sunflower seeds.[21]

The symptoms of nutritional deficiencies are often primarily visible in the oral cavity due to the rapid exchange of mucosal cells and the presence of a bacterial biofilm. A healthy epithelium undergoes cellular renewal every 3–7 days and acts as an effective barrier to toxins. Nutrient deficiency can lead to tissue breakdown, resulting in more frequent infections and the development of oral lesions.[7] For example, vitamin B deficiencies manifest as the inflammation of the tongue, lips and corners of the mouth, and anemia manifests as generalized pallor, atrophic glossitis, angular cheilitis, and recurrent aphthous stomatitis (RAS).[7,22]

Periodontal diseases are pathological conditions of the tissues that support the teeth. If left untreated, they can result in tooth loss, which will adversely affect chewing, food intake and nutritional status. Malnutrition does not cause periodontal disease, but it increases the risk of its occurrence and contributes to faster disease progression. The condition of the periodontium is influenced not only by malnutrition of the body but also by the quantitative and qualitative composition of the diet.[23]

Oxidative stress resulting from the advantage of oxidants over antioxidants is partly involved in the pathogenesis of periodontal diseases. A component of the inflammatory process is the production of reactive oxygen species (ROS) by immune cells stimulated by insufficiently buffered pathogens, causing an oxidative imbalance. Reactive oxygen species contribute to the damage of periodontal cells and tissues at the molecular level, with a particular affinity for lipids.[7,22,24] They damage lipids by initiating a chain of lipid peroxidation.

A diet high in refined carbohydrates and saturated fats is pro-inflammatory, while a diet rich in polyunsaturated fats (e.g., fish oils) and antioxidants present in fruits, vegetables and nuts (e.g., cashews) is anti-inflammatory.

Consuming a pro-inflammatory diet can cause oxidative stress after a meal, which can lead to inflammation. This condition is known as meal-induced inflammation; it is also associated with a postprandial increase in glucose.[7] A 2020 study performed in 240 individuals found a correlation between poor diets and deepening of periodontal pockets in middle-aged adults. Healthy diets included white meat, fish, fruits, vegetables, cereals rich in fiber, such as rye, oats and barley, and those with a higher ratio of unsaturated fatty acids to saturated fatty acids. Negative components included sugar, salt, red meat, and alcohol.[25,26] Research has shown that even the consumption of highly glycemic foods may increase gingivitis and periodontitis.[27] This phenomenon can be counteracted by adding fiber to the diet, which reduces the postprandial glucose spike.[7]

Woelber et al. demonstrated the effect of an anti-inflammatory diet on periodontal disease. The diet was based on eliminating processed carbohydrates (including sugar, honey, white flour, and white rice), reducing starch consumption, supplementing omega-3 fatty acids, and limiting the amount of trans and omega-6 fatty acids. The consumption of industrial animal proteins (dairy products and meat) was also reduced; plant proteins and small amounts of organic meat were favored. A daily supply of vitamin C, vitamin D, antioxidants, fiber, and nitrate-containing plants was provided. The study showed that the anti-inflammatory diet significantly reduced gingivitis to a clinically relevant extent.[28,29]

Antioxidants can help reduce the severity of the disease by scavenging free oxygen radicals and can counteract periodontitis associated with the presence of ROS. The antioxidant micronutrients include vitamins A, C and E, glutathione, melatonin, and lycopene.[7,24]

Melatonin is secreted by various organs in the human body, and is derived from plants and grains. It is not classified as a major nutrient but has been suggested to be more antioxidant than vitamin E. Studies have reported that the topical application of melatonin may complement periodontal treatment. The anti-ROS effect of melatonin reduces periodontitis and bone loss in diabetic animal models and, therefore, has the potential for the treatment of diabetic periodontitis.[24] These reports require further investigation.

Lycopene is a red pigment present in tomatoes, carrots and watermelons. It has antioxidant properties. Some studies suggest that lycopene supplementation may improve periodontal health; however, its mechanism of action on periodontal tissues has not been established and warrants further research.[24]

Malnutrition and poor oral hygiene are important predisposing factors for periodontal disease, including necrotizing gingivitis.[9] Dental plaque is considered the main etiological factor in the development and progression of periodontal diseases, and the increase in its volume is associated with high sucrose consumption. However, the maximum reduction of sugar in the diet does not limit the progression of gingivitis, which proves that periodontal diseases are caused by multiple factors.[23]

Susceptibility to periodontal disease increases as a result of vitamin C, folate and zinc deficiencies, as these nutrients increase the permeability of the gingival fissure barrier. Calcium, phosphates, vitamins A and E, and beta-carotene maintain the integrity of the gums and the proper functioning of the immune system.[8] Therefore, supplying the abovementioned ingredients in the diet has an impact on periodontal health. Protein–energy malnutrition (PEM) in early childhood is associated with the deterioration of the periodontal condition in adolescents; it affects the developing immune system, which reduces the individual's ability to respond to periodontal pathogens.[7,23]

Studies have shown that a diet characterized by the consumption of highly processed foods with low micronutrient value promotes gingivitis and periodontitis. On the other hand, a plant-based diet rich in low-glycemic complex carbohydrates (fruits, vegetables and legumes), omega-3 fatty acids, micronutrients, plant nitrates, and fiber not only is conducive to overall health but also positively affects the health of the periodontium and gums. The inflammatory potential of dietary proteins depends on their origin. Animal proteins are associated with increased levels of insulin-resistant growth factor 1, which plays an important role in carcinogenesis. Plant-based proteins, on the other hand, appear to reduce the risk of cardiovascular disease, type 2 diabetes and kidney disease.[27] This, in turn, may have implications for periodontal health. However, more research is needed to thoroughly investigate this issue.

Certain dietary probiotics are believed to contribute to the maintenance of periodontal health. There are several mechanisms that may explain the effects of probiotics: production of lactic acid that inhibits the proliferation of periodontal bacteria by penetrating the bacterial membrane and acidifying the cytoplasm; production of hydrogen peroxide that inhibits the growth of pathogenic bacteria; modification of proteins at the point of attachment; and the production of vitamins and other substances.[6] Prebiotics, which mostly include undigested fibers produced from complex carbohydrates, have a number of benefits for the body, including the reduction of inflammation and the modulation of appetite. There is no evidence of an effect of prebiotics on periodontal disease. However, 2 prebiotics may be useful in promoting oral health-related bacteria, namely methyl-beta-d-galactoside and N-acetyl-d-mannosamine.

In addition to the described systemic effects of diet on periodontal health, there are several dietary antimicrobials that may cause local effects. Examples of these compounds include unsweetened green tea, cocoa, coffee, wine, ginger, garlic, curry, coriander, cinnamon, and oregano. Studies have shown both negative and positive ef-

fects of some of these foods on periodontal disease.[27] The study by Liu et al. found that 7 bacterial taxa, particularly *Streptococcus* sp., *Ruminococcaceae* sp., *Haemophilus* sp., *Veillonella* spp., *Actinomyces odontolyticus*, and *Gemella haemolysans*, underwent significant changes after oolong tea consumption. These studies suggest that a long-term consumption of oolong tea may have an impact on the oral flora and a negative impact on the condition of the periodontium.[30]

A healthier lifestyle, a proper body mass index (BMI), good oral hygiene, and regular visits to the dentist, as well as a higher consumption of antioxidant micronutrients, contribute to a good condition of the periodontium.[7]

Deficiencies in certain micronutrients can cause oral mucosal diseases. Their identification through early oral symptoms can prevent the development of serious and irreversible systemic and neurological damage. Deficiencies in B vitamins, iron and folate are associated with RAS, glossitis, cheilitis, and angular cheilitis. Recurrent aphthous stomatitis is characterized by recurrent single or multiple painful ulcers confined to the oral mucosa (Table 1) (Fig. 1).[9,23] Glossitis caused by a deficiency of B vitamins may manifest as changes in the color, size and sensitivity of the tongue, as well as changes in the papillae, such as enlargement, flattening and eventual loss if left untreated. The tongue may appear pale or erythematous, ranging from shades of red to purple. Vesicles and ulcers may be visible, first at the apex, then covering the rest of the tongue. Patients may have problems with sensation of taste, burning and pain. Angular cheilitis is primarily associated with deficiencies in riboflavin, niacin, pyridoxine, folic acid, cobalamin, protein, and iron. In the course of the disease, erythema, maceration, fissures, and furrows arranged radially around the corners of the mouth can be observed. Secondary bacterial and yeast infections are also common.[3] All of the abovementioned oral mucosal diseases may cause difficulties in eating, which can aggravate the underlying malnutrition.

Fig. 1. Erosion of the buccal mucosa within the oral cavity

In the literature, one may encounter conflicting data on the relationship between retinol and beta-carotene and the incidence of oral lichen planus. Researchers have shown lower levels of vitamins E, C, D, and B12, zinc, calcium, and folic acid in patients with oral lichen planus. The presented data suggests that an unhealthy diet may increase the risk of oral lichen planus.[31] Oral lichen planus manifests in different forms affecting the gingival mucosa. Oral lichen planus may present as a desquamative gingivitis or a vulvovaginal gingival lichen planus due to genital involvement.[32] These forms are associated with periodontal problems, while the effects of malnutrition and poor oral hygiene on periodontal status are well documented. Supplementation with folic acid is recommended for patients with folic acid deficiency and lesions in the oral cavity. Attention should also be paid to the relationship between vitamin A, C, B12, and folic acid deficiencies with the occurrence of leukoplakia. Available data confirms that a diet rich in fruits and vegetables, especially tomatoes and tomato products, significantly reduces the risk of leukoplakia.[9]

Table 1. Effects of nutrient deficiency on the oral mucosa

Nutrient	Effects of deficiency on the oral mucosa
Vitamin A	gingivitis, gingival hypoplasia, proliferation of crevicular epithelium, alveolar bone resorption[36,37]
Vitamin D	painful erosions, ulcerations and aphthous ulcers in the oral cavity, damage to the secretory salivary glands that leads to xerostomia,[8] periodontitis[37,38]
Vitamin E	none
Vitamin K	submucosal and gingival bleeding, both spontaneously and after trauma, ecchymoses of the buccal mucosa and palate[3]
Vitamin C	gingival edema, friable and erythematous interdental papillae, poorly formed, soft, and often loose teeth, tiny hemorrhages on tips of the interdental papillae, aphthous-like lesions,[3] xerostomia[37]
Vitamin B complex	lower resistance to bacterial insults, RAS, enamel hypomineralization, cheilosis, cheilitis, halitosis, gingivitis, glossitis, atrophy of the lingual papillae, stomatitis, rashes around the nose, dysphagia, pallor, stomatodynia, erosions in the mouth[36,37] angular cheilitis, recurrent ulcerations, oral candidiasis[1,47]
Calcium	increased risk of periodontal diseases and tooth loss, increased severity of periodontitis[3,36,40]
Iron	RAS, atrophic glossitis, angular cheilitis, pallor of mucous membranes,[1,9,23,47] higher gingival index, BOP, probing pocket depth, higher percentage of sites with a CAL ≥ 6 mm[43]

RAS – recurrent aphthous stomatitis; BOP – bleeding on probing; CAL – clinical attachment loss.

Protein–energy malnutrition causes significant changes in the ecology of oral microbes, resulting in the predominance of anaerobic pathogens, an increased tendency of bacteria to bind to the cells of the oral mucosa, weakened responses of acute phase proteins, cytokine system dysfunction, and atrophy of the salivary glands. Therefore, malnutrition may reduce the body's ability to defend against oral infections and, consequently, lead to the development of life-threatening diseases.[7,33] Protein–energy malnutrition is associated with the exfoliation of the mucosa. The gums are often affected by scurvy with hemorrhagic gingivitis, manifested by red swelling of the interdental papillae. Bleeding gums are also a common symptom of vitamin K deficiency.[3]

Oral cancer is caused by a complex interaction between genetic factors and environmental exposures. Poor diet combined with other predisposing factors may contribute to an increased risk of oral cancer. Certain foods, such as areca nuts and complex products containing them (betel and gutka), are significantly associated with the development of oral cancer. Frying or baking protein-containing foods can generate heterocyclic amines, which are carcinogenic. The consumption of charcoal-based products is a significant risk factor. In addition, foods high in fat (pasta and cheese) and dishes cooked at high temperatures or in a microwave oven are significantly correlated with an increased risk of oral cancer. In addition, the temperature of the food consumed is also important. The Diet, Nutrition and the Prevention of Chronic Diseases report[22] concluded that there is convincing evidence linking hot food and drink consumption with the risk of oral cancer. It should also be mentioned that poor diets combined with alcohol consumption and smoking significantly increase the risk of oral cancer.

Some products have been reported to protect against oral cancer. These include green vegetables, carrots and cruciferous vegetables. Daily consumption of fruits and vegetables can reduce the risk of oral cancer by 50%. Importantly, the greatest benefits of these foods were observed when eaten raw. A special role should be assigned to citrus fruits. According to some studies, fruits are more beneficial than vegetables. Inadequate consumption of these products, poor eating habits and an unhealthy lifestyle can cause oxidative damage that induces the process of carcinogenesis by the DNA strand damage mechanism. For prevention, a daily diet should include antioxidants such as vitamins A, C, E, beta-carotene, lycopene, and selenium. In addition, the proper supply of dietary fiber plays an important role in reducing the risk of oral cancer by protecting the body from oxidative stress.[8,9,22] It is also worth to consider the treatment of oral cancer with the natural product – propolis. Propolis consists of flavonoids, phenolic acids, vitamins, and minerals. It is used in many dental specialties due to its antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, and anticancer properties. What is more, 100% of its natural polymers, such as cellulose, proteins and nucleic acids, are produced by living organisms and can be used in the treatment of periodontitis, oral mucosal diseases and oral cancer therapy, among others.[34,35]

Relationship of vitamin deficiencies and micro- and macroelements with diseases of the mucosa and periodontium

Vitamin A

Vitamin A plays an important role in the maintenance of the epithelium; its deficiency may cause gingivitis, gingival hypoplasia, proliferation of the crevicular epithelium, and alveolar bone resorption (Table 1).[36,37]

Vitamin D

Preclinical and clinical studies confirmed that vitamin D, through its metabolic pathways, may participate in the pathogenesis of periodontitis by influencing the mineral density of the teeth, and is inversely correlated with the severity of periodontal disease. Its main function is to regulate the calcium and phosphorus balance, which promotes the growth of bony tissue.[38] Vitamin D has visible tuning, anti-inflammatory and mineralizing effects on the periodontium, and can reduce the number of viable *Porphyromonas gingivalis* through active autophagy.[37,39] Vitamin D may also affect the periodontal immune response by reducing the expression of interleukin (IL)-8 and IL-6.[37] There is a scientifically confirmed correlation between vitamin D levels and RAS, Behçet's disease, PFAPA (periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis), Sjögren's syndrome, periodontitis, and oral squamous cell carcinoma (OSCC).[38] Recurrent aphthous stomatitis is characterized by the presence of painful erosions and ulcers in the oral cavity (Table 1). Their size varies from 1 cm to a few centimeters in diameter. There are 3 categories of RAS, depending on their size. Vitamin D has an important role in modifying the course of the disease. Behçet's disease is an immune-mediated condition whose symptoms include oral and genital ulcerations, arthritis, uveitis, retinal vasculitis, neurological disturbances, sporadic deep vein thromboses, erythema nodosum, and gastrointestinal inflammation. Patients with Behçet's disease have lower vitamin D levels than healthy individuals.[38] PFAPA is characterized by the presence of aphthous ulcers in the oral cavity, episodes of fever, pharyngitis, and cervical lymphadenopathy (Table 1). Vitamin D is an important modifier of PFAPA. Studies have shown that the supplementation of vitamin D during the winter season reduced the number and duration of fever episodes in patients with PFAPA.[38] Sjögren's syndrome is an autoimmune condition that can be modified by vitamin D. It is character-

ized by damage to the secretory salivary glands, leading to xerostomia, tear secretion dysfunction, conjunctivitis, and keratitis (Table 1).[38] Clinical studies have shown a significant relationship between the endocrine activity of vitamin D and periodontitis, confirming the role of vitamin D as a modifier of the immune response in the periodontium (Table 1).[37,38] Patients with generalized aggressive periodontitis, an early form of the disease with marked familial aggregation, showed significantly elevated plasma levels of vitamin D binding protein, which suggest an association between D binding protein and specific genotypes of generalized aggressive periodontitis.[37] The experiments have shown the ability of 1,25(OH)-D_2 (active vitamin D metabolite) to inhibit the monocyte production of pro-inflammatory cytokines IL-1B and tumor necrosis factor alpha (TNF-α), both of which play significant roles in the pathogenesis of periodontitis by impairing wound healing and inducing bone resorption. Studies showed a correlation between vitamin D deficiency and the risk of oral candidiasis in human immunodeficiency virus (HIV)-positive patients.[38] This is due to the role of vitamin D in the human immune response.[37–39] Additionally, adequate vitamin D levels are reported as an anticancerogenic factor. Studies showed that low levels of vitamin D serum may increase the incidence of cancer and mortality in men, specifically alimentary system cancers, such as OSCC.[38]

Vitamin E

Vitamin E is a membrane antioxidant. It is essential for the heart's health and cell wall protection. Vitamin E is reported to maintain the gingival health and the integrity of the immune system.[8] It has no oral manifestations of deficiency.[3]

Vitamin K

The typical oral manifestations of vitamin K deficiency are submucosal and gingival bleeding, both spontaneously and after trauma, and ecchymoses of the buccal mucosa and palate (Table 1).[3]

Vitamin C

The relationship between vitamin C and periodontal disease may be due to vitamin C's role in maintaining and repairing healthy connective tissue along with its antioxidant properties. Vitamin C deficiency-related scurvy, manifested in the oral cavity by gingival edema, friable and erythematous interdental papillae, and poorly formed, soft and often loose teeth (Table 1), is strongly associated with periodontitis.[36] Interestingly, these findings are not present in edentulous patients. Periodontal disease is more common in individuals with a low dietary intake of vitamin C. Scorbutic gingivitis, manifested by tiny hemorrhages at the tips of the interdental papillae, may be accompanied by aphthous-like lesions (Table 1).[3] In bone, due to the inability of osteoblasts to form osteoid, there is an increase in oxidative stress and susceptibility to infections caused by the alteration of the oral-periodontal ecosystem. In addition to periodontal changes, vitamin C deficiency can also cause xerostomia (Table 1).[37]

Vitamin B complex

Vitamin B complex deficiency is associated with lower resistance to bacterial insults, RAS, enamel hypomineralization, cheilosis, cheilitis, halitosis, gingivitis, glossitis, atrophy of the lingual papillae, stomatitis, rashes around the nose, dysphagia, and pallor of mucous membranes (Table 1).[36,37] Current research indicates that low levels of folic acid are associated with the periodontal tissue's reduced ability to act against bacterial irritants. Vitamin B12 deficiency can be manifested by stomatodynia, glossitis and erosions in the mouth (Table 1).[36]

Macro- and microelements

Macro- and microelements, such as fluorine, aluminum, molybdenum, cobalt, sodium, potassium, phosphorus, and chlorine significantly affect the incidence of dental caries and have an influence on bone structure, but their impact on the oral mucosa will not be discussed in the following sections.[40–42]

Calcium

Calcium deficiency is associated with an increased risk of periodontal diseases and tooth loss (Table 1). Calcium absorption is dependent on adequate vitamin D levels, and patients with low calcium levels may have the same oral signs of enamel pitting and hypoplasia as those with vitamin D deficiency.[36] There is an inverse relationship between calcium intake and the severity of periodontitis. Unfortunately, increasing calcium intake does not lead to an increase in salivary calcium levels, which are beneficial in preventing dental caries and periodontitis.[40]

Iron

Iron deficiency anemia is the most prevalent nutritional deficiency in the world. It is associated with an increase in oxidative stress resulting from hypoxia in the body's tissues. Studies show that patients with chronic periodontal disease and accompanying iron deficiency anemia exhibited higher levels of gingival index, bleeding on probing (BOP), probing pocket depth, and a higher percentage of sites with a clinical attachment loss (CAL) ≥6 mm than patients with chronic periodontal disease without iron deficiency anemia.[43]

A summary of the effects of nutrient deficiencies on the oral mucosa and periodontal diseases is presented in Table 1.

Nutrition and systemic diseases involving the oral cavity

Oral health is closely related to diet, including nutritional influences on craniofacial development, oral infectious diseases and oral cancer. However, the most significant effect of nutrition on the teeth is caused by its local action, resulting in the development of dental caries and enamel erosion. The problem of dental erosion is increasing and is associated with dietary acids, a major source of which is soft drinks.[33] Dental erosion, tooth hypersensitivity, dental caries, and oral mucosa alterations can be the oral manifestations of eating disorders, such as anorexia nervosa and bulimia nervosa.

The associations between oral health conditions, nutritional status, dietary practices, and general health status are complex and involve many interrelated factors.[23] Dental diseases have a considerable impact on self-esteem and the quality of patients' lives.[33] High-protein and high-carbohydrate diets have a big influence on oral halitosis. Oral halitosis is a common condition that causes persistent bad breath and affects approx. 25–30% of the global population.[44] Lichen planus is a chronic inflammatory skin disease involving the mucous membranes of the oral cavity. A study conducted by Di Stasio et al. showed that patients suffering from oral lichen planus presented with symptoms of depression, anxiety and distress.[45] The relationship between mental disorders and eating habits is well known. Diet, the consumption of high-calorie and processed foods, and changes in people's lifestyles are considered risk factors for the development of many diseases, including psychiatric disorders. Compounds such as vitamin D, vitamin B complex (including folic acid), zinc, and magnesium, have a positive effect on mental health.[46]

The influence of popular eating patterns on the condition of the mucosa

Ketogenic diet

The ketogenic diet involves a high intake of fat, a moderate intake of protein, and a low intake of carbohydrates. The maximum carbohydrate intake is 50 g per day, which puts the body in a state of physiological ketosis.[7,47,48] The ingested fats break down into ketones, which are used as an energy source. By burning ketones, ketone bodies are formed, which are excreted through exhalation and urine. This leads to xerostomia and a characteristic breath with a fruity or acetone odor. The breakdown of proteins, which produces ammonia, also leads to bad breath.[47,49] Large amounts of consumed carbohydrates contribute not only to the formation of dental caries but also to periodontitis due to the stimulation of the accumulated dental plaque. The use of the ketogenic diet reduces the severity of these conditions. A significant reduction in BOP was observed in the individuals who adhered to the ketogenic diet, even in the absence of oral hygiene for 4 weeks.[49] The implementation of a ketogenic diet largely eliminates the formation of carious lesions, which is particularly important in patients receiving radiotherapy or immunotherapy, as they often suffer from xerostomia. The ketogenic diet causes a decrease in the BMI index, which is important because being overweight promotes the development of periodontal diseases.[22,47–49]

Paleo diet

The Paleo diet involves eating substantial amounts of nutrient-rich foods such as vegetables, fruits, lean meats, and seafood, and eliminating inflammatory foods such as grains, dairy, refined sugars, refined oils, and processed foods.[4,22,47] Due to the small amount of carbohydrates consumed, the severity of the appearance of carious lesions is reduced. Fruits and vegetables are the main sources of carbohydrates in this diet.[6,23] Eating starchy vegetables that are rich in vitamin A helps to keep the lining of the mouth healthy, and the vitamin C present in fruits and vegetables helps heal wounds and prevent infections.[39] Folic acid, which supports the growth and repair of cells, is also present in fruits and vegetables. Since this diet is rich in fruits and vegetables, it contains a lot of antioxidants that reduce the risk of mouth and throat cancer.[23,47]

Vegetarian diet

A diet that excludes meat and meat products and is mainly based on foods of plant origin is called a vegetarian diet. There are several types of vegetarian diets: vegan, raw vegan, pesco-vegetarian, ovo-vegetarian, flexitarian, lacto-vegetarian, and lacto-ovo-vegetarian diets. A vegetarian diet can contribute to a lack of various nutrients, such as protein, which can negatively affect the health of the body and mouth. Therefore, a vegetarian diet should be rich in whole grains, seeds, nuts, and soy, which are high in protein.[1,47,50,51]

According to the Academy of General Dentistry, patients on a vegetarian diet may suffer from vitamin and element deficiencies. These deficiencies manifest in the oral cavity in the form of glossitis, angular cheilitis, taste alterations, and glossodynia. Vitamin B12 deficiency manifests itself as glossitis, angular cheilitis, recurrent ulcerations, and oral candidiasis. Iron deficiency can cause atrophic glossitis, pallor of mucous membranes, and angular cheilitis (Table 1). The effects of a zinc deficiency in the oral cavity include an increased number of flattened filamentous cells and warts, ulcers, and xerostomia.[1,47]

Studies indicate that the saliva of individuals following the vegetarian diet is less able to form a barrier to free radicals and pollutants than that of non-vegetarians. A plant-based diet is rich in antioxidants and has a positive effect on the condition of the periodontium. It protects the teeth from oxidative damage and alters the inflammatory response in periodontitis.[47] Studies show that vegetarians have good overall oral health.[50]

Intermittent fasting

Intermittent fasting involves eating only during a certain period of the day. It consists of abstaining from eating for a certain number of hours. For example, fasting at 16:8 means a 16-hour fasting period and an 8-hour eating period during which food can be consumed. There are no restrictions regarding food choices and carbohydrate intake.[47]

Fasting increases gluconeogenesis and levels of hormones such as human growth hormone and ghrelin. Limiting the eating period to 8 h a day contributes to the shortening of the bowel, in which the saliva pH drops to an acidic level. The reduction in chewing time may result in decreased salivation, a greater risk of xerostomia and caries, and gingivitis. Intermittent fasting inhibits inflammation and reduces the production of free radicals, which is beneficial in combating periodontitis.[52] Thus, patient education should include hygiene instructions and xerostomia prevention.[47]

Western diet

Diet, physical inactivity, sedentary lifestyle, and obesity (a consequence of an unhealthy lifestyle) are, after smoking, the main risk factors for the onset of cancer. It is estimated that changes in alimentary habits can decrease the likelihood of cancer onset by 30–50%.[48,53] Generally, oral cancer is a growing global problem, and it is not unified.[54] The incidence of head and neck cancers – oral cavity tumors, pharyngeal cancers – registered a global incidence of 5.2% in 2018. The 5-year survival rate is 40–50%.[55] Alcohol consumption and papillomavirus infections also play a crucial role in carcinogenesis. Epidemiological studies underline a protective effect of a high intake of fruits and vegetables, especially in the case of oral cavity cancer.[56] The prevalence of the fast food culture in developed countries and its effects on the Western diet, which is low in fiber and rich in sugar and processed foods, are tightly linked to a loss of microbial diversity and dysbiosis. The Western diet is also closely associated with a high risk of obesity and cardiovascular disease.[48]

Mediterranean diet

The traditional Mediterranean diet is characterized by a high intake of vegetables, legumes, fresh fruits, non-refined cereals, nuts, and olive oil, obtained through mechanical pressing and having an acidity rate lower than 0.8%. It involves a moderate consumption of fish and dairy, a low consumption of red meat, and a moderate use of ethanol (mainly red wine), consumed during the main meals. The Mediterranean diet is considered an effective and manageable method to fight cancer occurrence owing to its protective effects in reducing oxidative and inflammatory processes in cells and avoiding DNA damage, cell proliferation, inflammations, angiogenesis, and metastasis.[57] Regular consumption of olive oil may have a protective effect against periodontitis, although the temporal association needs to be further examined.[58] Many studies have demonstrated a strong and antagonist relationship between some chronic diseases and a high adherence to the Mediterranean diet.[57]

Conclusions

Nutrition has a significant impact on the human health and well-being, including oral health. Nutritional deficiencies may affect not only the teeth but also the periodontium and oral mucosa. It is essential for dentists and dental hygienists to have knowledge of symptoms associated with a particular nutrient deficiency as they are often the first to diagnose them. We hope that this article will help dental professionals detect nutritional deficiencies earlier and more efficiently. This may lead to prompt and effective treatment as well as may help in preventing long-term deficiencies and their complications. This article is a source of knowledge not only for medical professionals but also for non-medical people. It is very important for individuals to have practical knowledge about their health and the importance of the chosen diet. The included information can help medical professionals take care of their own and their patients' well-being as well as look more consciously at their own diet and notice possible dietary mistakes that can result in unpleasant consequences, for example, those that appear in the oral cavity. The described effects of deficiencies on the oral cavity may be the first sign of nutritional deficiencies that require immediate treatment.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

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ORCID iDs

Aleksandra Strączek https://orcid.org/0000-0003-3305-5545
Justyna Szałkowska https://orcid.org/0000-0001-9210-6934
Paulina Sutkowska https://orcid.org/0000-0002-2127-1809
Alicja Srebrna https://orcid.org/0000-0001-5953-9164
Natalia Puzio https://orcid.org/0000-0001-6420-1459
Aleksandra Piasecka https://orcid.org/0000-0003-3552-6726
Natalia Piskorz https://orcid.org/0000-0002-1267-6599
Jan Błaszczyk https://orcid.org/0000-0001-6325-4321
Karolina Thum-Tyzo https://orcid.org/0000-0001-7468-7384

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Review

A critical review of dental biomaterials with an emphasis on biocompatibility

Marta Joanna Woźniak-Budych[1,A–F], Maciej Staszak[2,A–F], Katarzyna Staszak[2,A–F]

[1] NanoBioMedical Centre, Adam Mickiewicz University in Poznan, Poland
[2] Institute of Chemical Technology and Engineering, Faculty of Chemical Technology, Poznan University of Technology, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Marta Joanna Woźniak-Budych
E-mail: marta.budych@amu.edu.pl

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Abstract

This paper presents the major achievements in the field of biomaterials in restorative dentistry and tissue regeneration reported over the past 3 years. The review aims to summarize the knowledge on important biomaterials and the emerging modification strategies to improve their biointegration, biological activity, mechanical properties, and resistance to the harsh oral environment. We also discuss the main opportunities and challenges associated with the use of biomaterials in dentistry.

Much contemporary research focuses on the interactions between biomaterials and the surrounding tissues in the oral environment regarding adhesion, associated stresses and strains, and the durability of dental restoration materials. Dental biomaterials should support cell adhesion and activity, leading to dental tissue regeneration, and are also expected to effectively prevent bacterial infections and inhibit material corrosion in saliva. The degradation, dissolution or corrosion of restorative materials due to exposure to body fluids can alter the structure and mechanical properties of the material, causing various adverse effects.

Another aspect addressed in recent literature is the improvement of the mechanical properties and esthetics of restorative materials. The surfaces of biomaterials are usually modified with polymers or nanomaterials to reduce friction while maintaining biocompatibility.

Although all modern biomaterials are promising, there is an urgent need for more in vivo and clinical studies to investigate their biological advantages and disadvantages in detail. The computational techniques used to assess the properties of modern dental materials, particularly the mechanical ones, could assist in the development of the materials. Such an approach can help bring new biomaterials to the market by reducing complicated, tedious and expensive experimentation.

Keywords: biomaterials, orthodontics, restorative dentistry, prosthodontics, periodontology

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Introduction

Only a few commercially available dental biomaterials have passed all biocompatibility screening assays, thus confirming their low toxicity and patient health protection.[1,2] Most dental materials have been approved based on the grandfathering process, which allows for sale without prior toxicity tests if a biomaterial is characterized by a similar chemical composition, sterilization procedure, production procedure, and the same dose of active substances as compared to the marketed products.[3] Since May 2021, the European Union (EU) have been implementing a new regulation for marketing and using medical devices,[4] Regulation (EU) 2017/745 or the Medical Device Regulation (MDR), which replaces the former Medical Device Directive (MDD) 93/42/EEC.[5] The new regulation will substantially impact those involved in manufacturing, distributing and using medical devices, as it requires the entire lifecycle of a medical device to be represented by a comprehensive set of dental product data, including clinical outcomes.[4–6]

Biocompatibility is vital for all treatment strategies and refers to the ability of the material to function in the oral environment without causing local or systemic harm, essentially meaning the interaction between the material and the host must be harmonious. Generally, adverse reactions in patients can be classified as local or systemic. The first category concerns a direct interaction with the oral mucosa and pulp, which may cause mucosal irritation or oral lichenoid reactions, while systemic adverse reactions (mainly hypersensitivity and anaphylactic shock) relate to the overreaction of the immune system.[7] Interestingly, after many years of exposure to dental materials, patients and dental staff experience various adverse reactions, from cracking or flaking skin, swelling and irritation to peripheral neuropathy.[8] Hence, an objective assessment of dental material biocompatibility is crucial for ensuring patient safety and successful treatment. Additionally, it is equally important to emphasize that proactive diagnostics and material selection can substantially decrease the likelihood of complications and the need for follow-up treatment.

The most commonly used dental biomaterials are restorative materials applied to fill tooth cavities or treat dental caries, and materials for hard and soft tissue repair. The former group includes resin composites, titanium (Ti) and zirconium (Zr) alloys, polymers, ceramics, casting investments, and impression materials. The latter category comprises biomaterials that may serve as scaffolds for cell adhesion and proliferation, as well as carriers in drug delivery systems to treat fractures, and for temporomandibular joint reconstruction, dentin/enamel reconstruction, periodontal ligament (PDL) replacement, and the pre-osseointegration of dental implants.[9,10] Examples of the applications of dental materials are presented in Fig. 1.

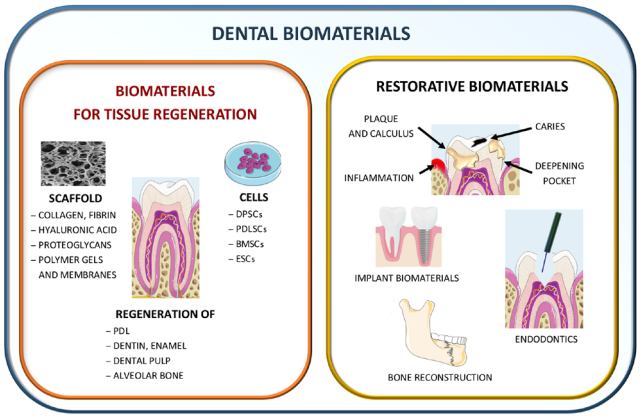

Fig. 1. Various applications of biomaterials in dentistry

PDL – periodontal ligament; DPSCs – dental pulp stem cells; PDLSCs – periodontal ligament stem cells; BMSCs – bone marrow stem cells; ESCs – embryonic stem cells.

Despite the wide availability of dental biomaterials, no material has ideal properties. The selection of biocompatible materials depends on several factors, such as their mechanical and biological characteristics, stability in the oral environment (including corrosion in saliva), functionality, the final cost, and esthetics. Furthermore, biomaterials are expected to be multifunctional, biologically active, and offer combined features, as in the case of composite dental fillings that exhibit antibacterial and remineralization effects.[11,12] Dental biomaterials have evolved extensively over the last 10 years, with their functionality being progressively improved. However, there is a vast space for further investigations on the longevity of biomaterials, their wear behavior in the changing environment of the oral cavity, their bioactive potential, and the enhancement of their biological, optical and mechanical properties.

The repair and regeneration of injured hard and soft tissues remains challenging. There have been many attempts to favor the regrowth process of different soft tissues with advanced dental materials. However, these approaches could not re-establish the complex structure–function interactions with other tissues, and additional studies are required.[13–15] In the case of hard tissues, the primary problem is polymerization shrinkage at the composite–tooth interface, which usually leads to micro-leakage and secondary caries.[16–20] Thus, improving the prolonged release of active components from various dental restorative materials, taking into account their wear in the oral environment, remains a significant challenge in dentistry.[21–23]

This review presents the major achievements in the field of biomaterials reported over the past 3 years (Fig. 2), and aims to summarize the emerging modification strategies to improve the biointegration, biological activity and mechanical properties of the materials, as well as their resistance to the oral environment. We also discuss the main opportunities and challenges associated with the use of biomaterials in dentistry.

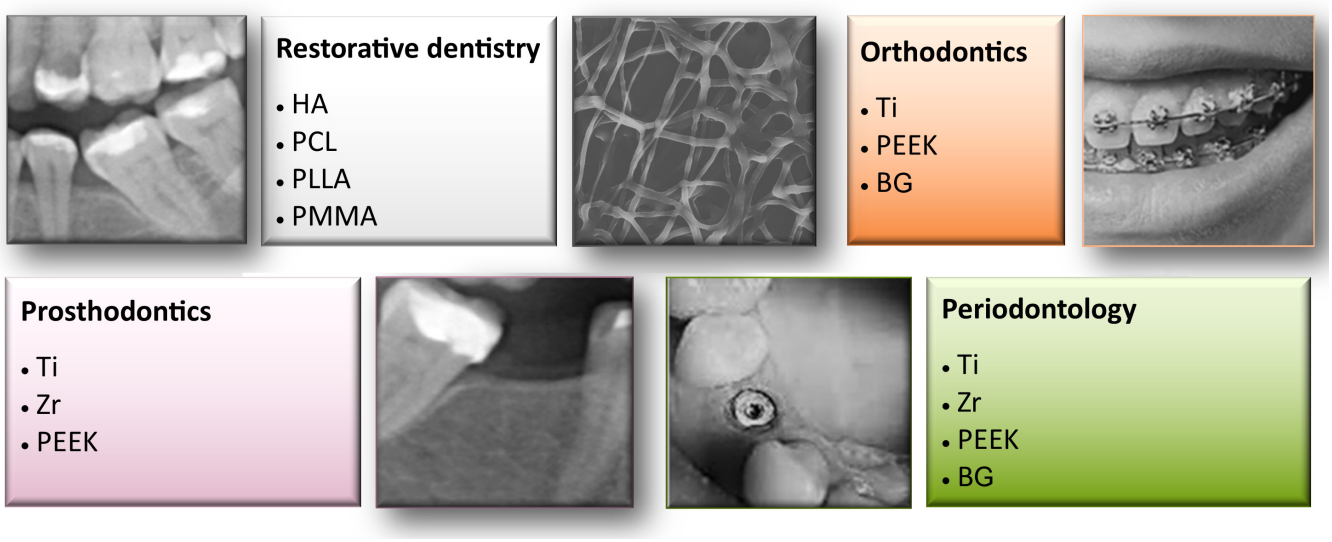

Fig. 2. Various materials used in dentistry

HA – hydroxyapatite; PCL – poly(e-caprolactone); PLLA – polylactic(L-lactic) acid; PMMA – polymethyl methacrylate; Ti – titanium; PEEK – polyetheretherketone; BG – bioactive glass; Zr – zirconium.

Examples of the most important reports on biomaterials published over the past 3 years are presented in Table 1 and discussed in the relevant sections.

Methods

The PubMed, Scopus and Google Scholar search engines were used in the bibliometric analysis, using a combination of the following keywords: 'dental material' with 'biocompatibility', 'tissue regeneration', 'restorative dentistry', 'orthodontics', prosthodontics, 'Ti', 'polyetheretherketone' (PEEK), 'bioactive glass' (BG), 'resin', 'ceramic', 'implant', 'polymethyl methacrylate' (PMMA), 'in vivo', 'in vitro', 'clinical studies', 'simulation', and 'deep learning'.

The exclusion criteria were manuscripts written in a language other than English, those not available, and articles published before 2020 (with a few exceptions of articles essential to the development of the techniques described). At the same time, to ensure originality and accuracy, the search focused on research papers and excluded review articles. As a result, articles with titles containing words such as 'review', 'meta-analysis' or 'overview' were not included, with few exceptions. Additionally, we added a description of the method used for conducting the systematic literature review in the supplementary materials (available on request from the corresponding author).

Biomaterials for soft and hard tissue regeneration

Over the past 3 years, there has been a significant increase in research related to dental biomaterials for tissue regeneration and reconstruction as compared to other restorative materials.[24] Dental tissue engineering allows the replacement of missing teeth with bioengineered products and the regeneration of damaged dental tissues with the use of dental stem cells.[25] Appropriate stem cells are seeded on the surfaces of biomaterials (bioscaffold surfaces), which stimulate them to create a biocomplex. Various mesenchymal stem cells are found in the tooth, such as dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), dental follicle stem cells (DFSCs), and stem cells from the dental apical papilla or the exfoliated deciduous teeth. However, the first 2 cell lines are recognized as the most powerful in dental tissue engineering.[25–27] Biomaterials for scaffolds are designed to facilitate cell proliferation, differentiation, adhesion, and migration. Additionally, scaffolds loaded with appropriate growth factors or biomolecules can mimic tissue-specific micro-environments, and regulate the cell–matrix interactions, angiogenesis and the formation of the extracellular matrix (ECM).[28]

Various polymers (natural and synthetic) are suitable bioscaffolds for the proliferation and differentiation of dental stem cells.[29] Natural scaffolds are usually made of polysaccharides, such as alginate, hyaluronic acid and its derivatives, or chitosan, and are endowed with various proteins, including collagen, fibrin and silk. Natural scaffolds exhibit excellent biocompatibility and degrade faster than synthetic polymers, without releasing toxic components. However, their mechanical properties are much worse as compared to synthetic bioscaffolds.[30] On the other hand, synthetic bioscaffolds, such as poly(e-caprolactone) (PCL), polylactic acid (PLA) and polyethylene glycol (PEG), display better physicochemical properties, but degrade much slower and have lower biocompatibility. Synthetic polymers are often enriched with various bioactive compounds, such as nano-hydroxyapatite (nHA), nanofluoroapatite or BG, to reduce their cytotoxicity or add new features.[31–36]

Table 1. Biomaterials in dentistry reported during the last 3 years

Area	Biomaterials	Functionality/results	Research advancement	Reference number
Biomaterials for soft and hard tissue regeneration				
Resin composites	Bis-GMA/TEGDMA resin composites modified with Zn/Sr-doped HA	– restorative materials for hard tissue treatment; – improvement of antibacterial activity and mineralization capacity without the loss of biocompatibility	in vitro studies: cytotoxicity (L929 fibroblasts); antibacterial test (*S. aureus*)	53
	silica aerogel/Bis-GMA/TEGDMA resin composites (size of silica aerogel particles: 15 mm and 50 mm)	– restorative materials for hard tissue treatment; – improved mechanical and antimicrobial properties, higher hardness and lower water absorption than in the case of pure composites	in vitro antibacterial studies (*S. aureus*)	54
	CP/Bis-GMA/TEGDMA resin composites	– restorative materials for hard tissue treatment; – remineralization of caries-affected dentin and the prevention of caries lesions under orthodontic brackets, controlled release of ions after 2–3 months	in vitro/ex vivo remineralization studies with human teeth and artificial saliva (review article)	55
	SiO_2- and Al_2O_3-based UDMA resin composites	– restorative materials for hard tissue treatment; – increased surface roughness and porosity; – only Al_2O_3-enriched resins showed sufficient physicomechanical properties to be used as resin fillings	lack of biological studies	57
GIC	encapsulated commercial GIC: Fuji II LC, Fuji IX, Ketac™ Fil Plus, and EQUIA Forte™ HT Fil	– restorative materials for hard tissue treatment; – encapsulation eliminated the manual mixing problem and improved the surface hardness of GIC in the post-aging studies	in vitro aging studies investigating mineral deposition	171
	RMGIC modified with ZnO NPs (20–40 nm, 2 wt%)	– restorative materials for hard tissue treatment; – improvement of antibacterial activity without sacrificing fluoride release properties and flexural strength	in vitro antibacterial studies (*S. mutans*)	60
	commercial GIC (Fuji II LC) modified with a propolis extract	– restorative materials for hard tissue treatment; – lack of antibacterial properties after the addition of the propolis extract, the deterioration of flexural and shear strength	in vitro antibacterial studies (*S. mutans*)	61
	GIC (Fuji II) modified with 3 active components: nHA (4 wt%), Zn L-carnosine (6 wt%) and ciprofloxacin (1.5 wt%)	– restorative materials for hard tissue treatment; – improved thermal, mechanical and biological properties of biomaterials; – combination of GIC and bioactive components can help treat caries in non-compliant patients, including children	in vitro studies: cytotoxicity (human gingival fibroblasts BSCL138, human keratinocytes BSCL143L); antibacterial test (*S. mutans*)	62
Bioscaffolds	chitosan/gelatin/HA scaffolds	– restorative materials for tissue engineering; – excellent biomimetic microenvironment for cells, supporting their odontogenic differentiation and biomineralization in vitro	in vitro studies (DPSCs)	38
	ECM from DPSCs	– restorative materials for tissue engineering; – improved osteogenic differentiation/mineralization of gingival fibroblasts	in vitro/ex vivo tests (DPSCs were isolated from the teeth or gingival tissues of healthy adult patients)	39
	laminin-DPEM	– restorative materials for tissue engineering; – the introduction of laminins to DPEM enhances the adhesion of DPSCs and promotes their odontogenic differentiation	in vitro/ex vivo tests (the dentin matrix was extracted from the premolars of beagles)	40
	decellularized bioscaffolds isolated from rat DP tissue	– restorative materials for tissue engineering; – ability to support dental stem cell repopulation	in vitro/ex vivo studies (DP was isolated from rat jaws, and the decellularized scaffolds were recellularized with human DPSCs in vitro)	42
	L-arginine-containing mesoporous SiO_2 NPs	– restorative materials for tissue engineering; – controlled release of L-arginine inhibits bacterial growth and neutralizes the acidic environment responsible for the development of secondary caries	in vitro antibacterial studies (*S. mutans*, *L. casei*)	64
nHA	commercial toothpaste containing F and HA (F1400, F500, Biorepair®)	– restorative materials for tissue engineering; – the toothpaste reaches the enamel matrix and the apatitic mineral phase by penetrating the enamel layer, thereby improving remineralization	in vitro/ex vivo studies (8 primary teeth extracted for orthodontic reasons)	66
	HA-modified commercial dental flosses (Oral-B®)	– restorative materials for tissue engineering; – efficient drug delivery system for the prevention and treatment of early caries; – ability to remove the bacterial biofilm from interproximal areas	in vitro studies (*S. salivarius* K12)	67

Area	Biomaterials	Functionality/results	Research advancement	Reference number
Biomaterials in restorative dentistry				
Ti and Ti alloys	Ni-Ti orthodontic wires	– biomaterials in orthodontics; – Ni-Ti orthodontic wires are susceptible to corrosion in the oral cavity, especially at friction points	in vitro studies on the corrosion of orthodontic wires in the oral cavity	78
	Ti implants	– biomaterials in orthodontics; – mechanical treatment of Ti implants may increase the dissolution of Ti in peri-implant tissues, potentially contributing to the development of peri-implantitis	ex vivo studies (a model of organic polymicrobial peri-implant biofilms)	68
	Ti implants	– biomaterials in orthodontics; – Ti was detectable in PICF and GCF, even in healthy subjects	clinical study evaluating the fissure fluid from both implants and the adjacent teeth for the possibility of Ti contamination (77 patients with 117 implants)	69
	CM and a synthetic BS combined with TM	– biomaterials in orthodontics; – application of CM and a synthetic BS combined with TM did not facilitate lateral bone augmentation; – in vivo results were inconclusive	in vivo studies investigating the lateral bone augmentation of chronic peri-implant defects (6 canine mandibles)	70
	Ti and Zr implants coated with β-TCP	– Ti and Zr implants coated with β-TCP can generate periodontal tissue and form biohybrid implants	in vivo studies (the implants were implanted in rabbits after the extraction of lower right central incisors, and examined at 45 and 90 days)	128
	Ti and Zr disks	– biomaterials in orthodontics; – air-polishing, the ultrasonic scaler and the Er:YAG laser significantly reduced the amount of biofilm on Ti and Zr surfaces	in vitro studies examining the decontamination methods to remove the bacterial biofilm from biomaterial surfaces	130
	Ag/HA NPs and ZnO NP coatings on orthodontic Ti mini-screws	– biomaterials in orthodontics; – orthodontic Ti mini-screws coated with ZnO NPs revealed the highest antimicrobial activity against bacteria and fungi, and showed better cytocompatibility with oral epithelium cells, bone cells and fibroblasts as compared to the Ag/HA NP coating	in vitro antibacterial tests (*E. aeruginosa*, *S. aureus*, *S. mutans*, *E. faecalis*, *E. coli*, *C. albicans*) and cytotoxicity tests (fibroblasts, osteocytes, osteoblasts, and oral epithelial cells)	81
	complete-arch implant-supported fixed maxillary prostheses made of Ti or monolithic Zr	– biomaterials in prosthodontics; – Zr prostheses showed less plaque accumulation and less inflammation of the adjacent soft tissues as compared to Ti prostheses	clinical study comparing plaque accumulation and soft tissue inflammation	77
PEEK	PEEK FDP	– biomaterials in prosthodontics; – 95% of patients maintained their PEEK FDP without fracture, while 5% reported decementation; – 10% of PEEK FDP showed marginal discoloration	clinical study examining the efficacy of PEEK FDP over 1 year; 20 patients received three-unit posterior PEEK FDP	98
	PEEK and sandblasted PEEK disks	– biomaterials in orthodontics; – sandblasted PEEK was more susceptible to bacterial adhesion; – bacterial adhesion to PEEK is lowest on materials with a rough surface	in vitro tests (*S. sanguinis*, *S. oralis*, *E. faecalis*, and *S. gordonii*)	85
	PEEK and Ti healing abutments	– biomaterials in orthodontics; – PEEK healing abutments induced a stronger inflammatory response in the tissues as compared to Ti healing abutments	clinical study examining and comparing the response of soft tissues to biomaterials (22 implants with PEEK or Ti healing abutments were placed in 11 patients for 3 months)	87
	PEEK and Ti scaffolds	– biomaterials in orthodontics; – PEEK scaffolds showed lower biomechanical strength than Ti scaffolds; – both scaffolds provide excellent osteogenic space retention as compared to traditional GBR surgery	in vivo studies investigating alveolar bone augmentation (3 beagles, 18 bone defects were created in the mandibles of 3 dogs (6 per dog))	88
	orthognathic surgery combined with simultaneous PEEK bone augmentation	– biomaterials in orthodontics; – orthognathic surgery with PEEK bone augmentation significantly improved facial symmetry, even in patients with combined soft and hard tissue hypoplasia	clinical study comparing the facial symmetry results after digitally planned surgery and bone augmentation with PEEK (30 patients, including those with and without laterognathia, patients with and without syndesmosis, and patients with and without PEEK bone augmentation)	103

Area	Biomaterials	Functionality/results	Research advancement	Reference number
Biomaterials in restorative dentistry				
BG	GIC, GIC with CP, Ag NP-modified GIC, and 45S5 Bioglass® paste	– biomaterials in orthodontics; – GIC with CP exhibited the strongest bond	ex vivo study examining the effects of bonding agents on the luting strength of orthodontic brackets (48 healthy human premolars)	108
	BioMin® F and NovaMin®	– biomaterials in orthodontics; – orthodontically induced WSLs can be diminished through BioMin F remineralization therapy	clinical study comparing the treatment of orthodontically induced WSLs with 2 types of BG (60 patients aged 14–26 years, with a total of 60 WSLs, the formulations were tested for 5 weeks)	111
Resins	epoxy resin-coated Ni-Ti orthodontic wires	– biomaterials in orthodontics; – epoxy resin coating ensures increased corrosion resistance and reduced leakage of Ni into saliva	corrosion studies using artificial saliva	84
	ZnO NP-PMMA nanocomposite (a denture base material)	– biomaterials in prosthodontics; – nanocomposite was prone to color changes after exposure to various solutions; – modification of PMMA with ZnO NPs is esthetically acceptable at a concentration of 2.5% or 5%	lack of biological studies; the degradation test was performed for 6 months to examine stability and color changes after exposure to water, coffee, red wine, black tea, and a denture cleaning solution	113
	PMMA modified with TiO_2	– biomaterials in prosthodontics – adding TiO_2 NPs ensured a significant reduction in bacterial adhesion to the biomaterial surfaces	in vitro antibacterial studies (*S. mutans*)	115
	graphene-PMMA composites	– biomaterials in prosthodontics; – significant improvement of the mechanical properties of composites with a low content of graphene (0.1027%)	lack of biological studies	117
	CAD/CAM PMMA resins	– biomaterials in prosthodontics; – improved mechanical and biological features, i.e., a higher modulus of elasticity, lower cytotoxicity and slight changes in cell morphology	in vitro studies (keratinocytes): cytotoxicity test, morphological changes of the cells after exposure to the tested material	118
	PEKK-based double-crown dental prostheses (modified with Au and Co-Cr)	– biomaterials in prosthodontics; – PEKK/PEKK and Au/PEKK primary/secondary crown combinations exhibited superior surface wear than the control group	lack of biological studies; stabilization studies for the double-crown system in a wet environment simulating 13 years of use to investigate the retentive forces of several material combinations between primary and secondary crowns	96
Ceramics	multi-layered Zr ceramics (cantilever IRFDP)	– biomaterials in prosthodontics; – acceptable fracture resistance	in vitro studies (natural teeth were used as abutments to simulate clinical conditions and create the ideal matching of the adhesive interface)	121
	metal-ceramic and ceramic FDP	– biomaterials in prosthodontics; – the highest incidence of chipping occurred in Zr FDP with a complete veneer, followed by metal-ceramic FDP with a high noble metal framework	cohort study evaluating the chipping and failure rates of FDP supported by metal-ceramic and ceramic implants and combined tooth–implant-supported FDP (434 FDP placed in 324 patients, an observation period of 0.5–12.6 years)	124
	ceramic brackets (Forestadent, G&H Orthodontics, GC Orthodontics, DynaFlex, and American Orthodontics)	– biomaterials in orthodontics; – all ceramic brackets were prone to discoloration – coffee had the greatest effect on color stability	in vitro studies examining the color stability of ceramic brackets in various fluids (100 ceramic brackets dipped in coffee, Coca-Cola®, Cedevita®, and artificial saliva)	82

GIC – glass ionomer cement; nHA – nano-hydroxyapatite; Ti – titanium; PEEK – polyetheretherketone; BG – bioactive glass; Bis-GMA – bisphenol A-glycidyl methacrylate; TEGDMA – triethylene glycol dimethacrylate; Zn – zinc; Sr – strontium; HA – hydroxyapatite; *S. aureus* – *Staphylococcus aureus*; CP – calcium phosphate; SiO_2 – silicone dioxide; Al_2O_3 – aluminum oxide; UDMA – urethane dimethacrylate; RMGIC – resin-modified glass ionomer cement; ZnO – zinc oxide; NP – nanoparticle; *S. mutans* – *Streptococcus mutans*; Zn – zinc; DPSCs – dental pulp stem cells; ECM – extracellular matrix; laminin-DPEM – laminin-modified native dental pulp extracellular matrix; DP – dental pulp; *L. casei* – *Lactobacillus casei*; F – fluorine; *S. salivarius* – *Streptococcus salivarius*; Ni – nickel; PICF – peri-implant cavity fluid; GCF – gingival crevicular fluid; CM – collagen membrane; BS – bone substitute; TM – Ti mesh; Zr – zirconium; β-TCP – beta-tricalcium phosphate; Er:YAG – erbium-doped yttrium-aluminum garnet; Ag – silver; *E. aeruginosa* – *Enterobacter aeruginosa*; *E. faecalis* – *Enterococcus faecalis*; *E. coli* – *Escherichia coli*; *C. albicans* – *Candida albicans*; FDP – fixed dental prosthesis; *S. sanguinis* – *Streptococcus sanguinis*; *S. oralis* – *Streptococcus oralis*; *S. gordonii* – *Streptococcus gordonii*; GBR – guided bone regeneration; WSL – white spot lesion; PMMA – polymethyl methacrylate; TiO_2 – titanium dioxide; CAD/CAM – computer-aided design/computer-aided manufacturing; PEKK – polyetherketoneketone; Au – gold; Co – cobalt; Cr – chromium; IRFDP – inlay-retained fixed partial prosthesis.

Another promising group of biomaterials that have shown advantages as dental scaffolds are hydrogels (HGs). They are soft, three-dimensional (3D) networks made of hydrophilic polymers, which can be natural, synthetic, or a combination of both. Hydrogels provide a tissue-like micro-environment and, due to their elasticity and flexibility, can mimic the native ECM. Moreover, various nanomaterials and bioactive compounds are incorporated into the polymer matrix of HGs to increase the efficiency of the scaffold.[37] The potential of chitosan/gelatin/hydroxyapatite (HA) scaffolds to enhance the viability and proliferation of DPSCs was examined by Vagropoulou et al., who found that these hybrid scaffolds provided a biomimetic micro-environment for cells, supporting their odontogenic differentiation and biomineralization in vitro.[38]

Bioscaffolds for tooth engineering can be prepared from the native ECM, which is unique for each cell type, provides tissue architecture and delivers specific growth factors. For instance, Nowwarote et al. reported that ECM from DPSCs exhibited improved osteogenic differentiation/mineralization of gingival fibroblasts.[39] In turn, Fu et al. proposed an excellent dental pulp (DP) tissue regeneration strategy via the laminin-modified native DP ECM (laminin-DPEM).[40] Based on in vitro tests, the authors showed that introducing laminins to DPEM enhanced the adhesion of DPSCs and promoted their odontogenic differentiation.[40]

Wang et al. fabricated a novel, biologically active scaffold for tooth engineering based on dentin, which was freeze-dried to maintain its mechanical and biological properties.[41] Dental pulp stem cells cultured on the freeze-dried dentin showed improved attachment, growth, viability, and collagen secretion.[41] The ability to support dental stem cell repopulation on the decellularized bioscaffold was evaluated by Matoug-Elwerfelli et al.[42] The bioscaffold isolated from rat DP tissue demonstrated positive expression of odontoblastic markers and growth factors.[42]

Metallic biomaterials are of great interest in dentistry due to their excellent mechanical features and biocompatibility.[43] They are applied as dentures, plates, joints, screws, and implants to restore the functions of missing tissues. Cobalt-chromium (Co-Cr), stainless steel, gold (Au), Zr, and Ti alloys are predominant among all metallic biomaterials. However, Ti- and Zr-based alloys exhibit better osseointegration than other metals and alloys. Moreover, Ti alloys possess superb mechanical properties. Thus, Ti and its alloys are still the primary materials used for dental implants.[43] However, Ti-based dental implants need to be improved, as they cause allergic reactions and the discoloration of the mucosa. Another significant drawback of Ti dental implants is corrosion, which results in the release of Ti particles into the tissues close to the implants and local lymph nodes. Conditions in the oral environment, including the application of fluoride-enriched toothpastes or mouthwashes, or high glucose levels, may enhance this unfavorable process. Zirconium-based dental materials are suitable for biomedical applications due to their low porosity, high density, high flexural strength, and resistance to fracture and corrosion. Zirconium restorative materials have other advantages, such as degradation and aging in water or water vapor.[44] Generally, both Zr and Ti implants reveal similar osseointegration. The modification of the surfaces of metal-ceramic implants improves their mechanical properties, fracture toughness and wear resistance, and inhibits bacterial adhesion. The most common modification methods are sandblasting, laser ablation, polishing, acid etching, ultraviolet (UV) treatment, or the addition of nanocomposites (e.g., nHA). Preclinical studies indicate that the surface modification of metallic biomaterials brings an enormous benefit in terms of improved osseointegration.[45]

Biomaterials can be used as carriers in drug delivery systems to facilitate implantation or treat various infections and oral diseases, and may come in multiple forms, such as nanosystems/nanoparticles (NPs), nanofibers, thin films, HGs, or scaffolds. They can be made of natural or synthetic polymers, organic or inorganic NPs, ceramic materials, or metal compounds. Such carriers protect the active components of the drugs against degradation or deactivation in the oral environment, and allow their controlled release and targeting pathogenic bacteria.[46,47] For instance, a chitosan-agarose HG was applied for the biomimetic remineralization of a native enamel surface, and a significant increase in the microhardness of the enamel-like layer was observed after a 7-day remineralization process in artificial saliva.[45] In turn, a chitosan-based HG enriched with an amelogenin-derived peptide (QP5) was suitable to inhibit the growth of cariogenic bacteria and promote the remineralization of initial caries lesions.[45]

In the last few years, regenerative therapies based on guided bone regeneration (GBR) and guided tissue regeneration (GTR) have attracted a great deal of attention.[48] Guided bone regeneration focuses on the regeneration of the alveolar bone in edentulous regions, while GTR repairs periodontal tissues.[49,50] Both GBR and GRT use a porous membrane to physically prevent unwanted cells from entering the lesion area. Such membranes should have excellent biocompatibility, and ensure the spatial and biomechanical stability of the lesion site. Based on their composition and bioactivity, polymeric membranes for GBR and GTR can be classified into absorbable, non-resorbable and inorganic-based materials.[48] The first group primarily comprises natural polymers, including collagen-, gelatin- and chitosan-based materials. The non-resorbable membranes are typically made of synthetic polymers, such as expanded or dense polytetrafluoroethylene (PTFE), while the inorganic-based membranes include calcium sulfate ($CaSO_4$) and HA compounds.[49] Guided tissue regeneration also requires the presence of osteogenic cells, as well as osteoconductive and osteoinductive materials, since biomaterials act as cell carriers

that activate the cellular processes required for tissue regeneration. The delivery of stem cells through biomaterials appears to help regenerate and restructure the oral cavity. However, more research is needed to assess their long-term efficacy.[49]

Biomaterials in restorative dentistry

Restorative biomaterials constitute the largest group among all materials in dentistry. They include those used to treat caries (resin composites, glass ionomer cement (GIC) and polymers), materials for tissue regeneration and reconstruction (bioscaffolds, drug delivery systems and implants), and multifunctional toothpastes, mousses or flosses for tooth remineralization (Fig. 3). These biomaterials have been the focus of research in recent years with the aim to improve their functionality and biocompatibility.

Dental resin composites are the dominant materials for filling tooth cavities and restoring the biting surface of damaged teeth. They have been used for direct restoration since 1998 to replace amalgam fillings, which are toxic due to the mercury (Hg) content, and are usually made of a dental resin reinforced with a powdered glass filler. The color of the resin can be adapted to the surrounding teeth, with the fillings often blue light-cured to build the final restoration.[51,52]

The primary issues associated with the application of resin composites are polymerization shrinkage, discoloration and cytotoxicity, though composite materials with antibacterial properties and mineralization capacity may help overcome polymerization shrinkage problems. Inhibiting bacterial growth prevents secondary caries, while the deposition of minerals can facilitate bonding

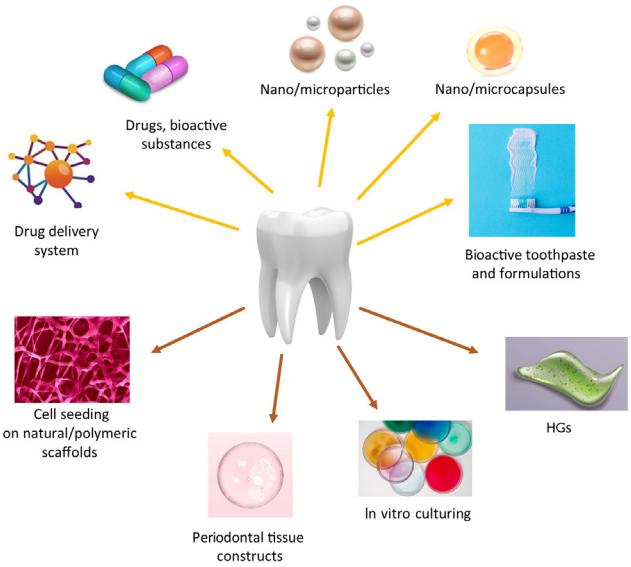

Fig. 3. Various biomaterials in periodontology
HG – hydrogel.

to the tooth structure. For instance, Li et al. designed advanced resin composites consisting of the monomer bisphenol A-glycidyl methacrylate (Bis-GMA) and the diluent triethylene glycol dimethacrylate (TEGDMA), with improved antibacterial and mineralization properties by incorporating zinc (Zn)/strontium (Sr)-doped-HA.[53] Furthermore, the Zn/HA and Sr/HA modification did not affect resin biocompatibility in vitro when using fibroblasts, which is crucial for preventing micro-leakage and secondary caries.[53] Cheng et al. achieved the mechanical and antimicrobial improvement of Bis-GMA and TEGDMA-based resins by adding silica aerogel (15 mm and 50 mm).[54] The silica aerogel/resin composite showed significantly lower water absorption and higher hardness than a pure resin matrix.[54] Bis-GMA and TEGDMA resins can also be modified with multifunctional fillers, such as calcium (Ca) orthophosphates.[55] Calcium phosphates (CPs) are the main constituents of bones and teeth, and significantly improve the remineralization of enamel and dentin. In composite materials, Ca phosphates contribute to the remineralization of caries-affected dentin and preclude caries lesions under orthodontic brackets. Additionally, Bis-GMA and TEGDMA resins are excellent for short-term treatment due to the release of ions, which subsides after 2–3 months.[55]

Composite materials based on the monomer urethane dimethacrylate (UDMA) have also been investigated as dental fillings. UDMA resins exhibit lower polymerization shrinkage than Bis-GMA/TEGDMA resins, and due to the lack of hydroxyl groups, they are less prone to water sorption. Thus, UDMA-based composites are used alternatively to prepare dental restorative materials.[56] Khan et al. designed silicone dioxide (SiO_2) and aluminum oxide (Al_2O_3)-based UDMA resin composites enriched with a spherical micro-filler (30 mm).[57] These composites were characterized by increased surface roughness and porosity. However, only the resins enriched with Al_2O_3 showed sufficient physiomechanical properties to be used as a resin filling.[57]

Another versatile material with a broad spectrum of uses in restorative dentistry is GIC, a powder-liquid formulation that usually contains a fluoroaluminosilicate glass filler, which releases fluoride over time, and a polyacrylic acid solution. The most important modification of GIC involves the incorporation of components that exhibit regenerative or self-curing potential, such as nano-sized fillers, natural resins (e.g., propolis or chlorohexidine (CHX)), nano-sized bioceramics, HA, nanofluoroapatite, various metal NPs (e.g., Zn, silver (Ag) or Ti), and natural antibiotics.[58,59]

Malekhoseini et al. examined the effects of incorporating zinc oxide (ZnO) NPs, sized 20–40 nm, on the antibacterial and mechanical properties of resin-modified glass ionomer cement (RMGIC).[60] Based on in vitro studies, the authors concluded that the modification of the RMGIC via ZnO NPs (2 wt%) improved the release of fluoride ions

and its antibacterial activity against *Streptococcus mutans* (*S. mutans*), while it did not affect its flexural strength or modulus of elasticity.[60] Similar in vitro tests were performed by Panahandeh et al.[61] The commercial Fuji II LC RMGIC powder was modified with a propolis extract. Adding propolis did not enhance the antibacterial properties of the RMGIC; instead, it deteriorated its flexural and shear strength. The lack of the antibacterial activity of the propolis-modified RMGIC can be attributed to the high viscosity of the Fuji IX cement, which may disturb the release of the antibacterial agent.[61] Pagano et al. also carefully studied the improvement of mechanical and biological properties of GIC as a result of incorporating various additives.[62] They designed advanced GIC (based on Fuji II LC), modified with 3 active components: HA (4 wt%) to enhance biocompatibility and mechanical properties; a mucosal defense agent – Zn L-carnosine (6 wt.%) to protect and repair mucous membranes; and an antibacterial agent – ciprofloxacin (1.5 wt%) to achieve bactericidal features. The combination of RMGIC and bioactive components helped treat caries in non-compliant patients.[62]

The most common polymeric materials used in dentistry are based on polycarbonate (PC), PCL, PMMA, polylactic(L-lactic) acid (PLLA), and hexamethyldisilazane (HMDC). These materials can be used as polymer coatings to improve biocompatibility, prevent the formation of the bacterial biofilm, inhibit the corrosion of restorative materials in saliva, and reduce the friction force on orthodontic elements, such as metal wires. For instance, nanofibres made of PCL or poly(p-dioxanone) (PDO), loaded with various antibiotics, such as ciprofloxacin, doxycycline or metronidazole, can be applied as efficient drug delivery systems to treat bacterial infections of oral mucosa.[63] López-Ruiz et al. designed a dental drug delivery system based on L-arginine-containing mesoporous SiO_2 NPs to prevent secondary caries.[64] The controlled release of L-arginine effectively inhibited the growth of *S. mutans* and *Lactobacillus casei* (*L. casei*), and neutralized the acidic environment responsible for the development of secondary caries.[64]

Nano- and microparticles can act as a delivery system for fluoride ions, which prevent the growth of caries-related bacteria and further acidification of saliva. In addition, fluoride ions in saliva reduce the demineralization of enamel. For example, in vitro studies showed that sodium fluoride (NaF)-loaded ethylcellulose and gelatin microparticles provided the sustained release of fluoride ions over 8 h.[65] These microparticles released fluoride ions in acidic conditions, accelerating the remineralization of hard tissues.[65]

Biomaterials have been introduced to daily-use oral care products, such as toothpastes, mousses, mouthwashes, and dental flosses, to restore enamel and dentine tissue. Fluoride- and HA-containing toothpastes or mousses are among the most popular methods of restoring the tooth structure by promoting tooth remineralization. The ability to remineralize dental surfaces has been widely examined and reported for deciduous and permanent teeth. Bossù et al. tested the effects of 3 commercial toothpastes containing fluorine (F) and HA on tooth remineralization.[66] Based on ex vivo studies (8 primary teeth extracted for orthodontic reasons), the authors concluded that all types of toothpaste reached the enamel matrix and the apatitic mineral phase by penetrating the enamel layer. The remineralization ability was associated with the chemical composition of the toothpastes and the tailored synthetic graininess of active components in the biofluid media.[66]

Various active compounds, such as fluoride, HA, CHX, and povidone-iodine, can be incorporated into dental flosses to serve as an efficient drug delivery system for the prevention and treatment of early caries.[67] Improving dental floss is essential as it helps remove the bacterial biofilm from interproximal areas particularly prone to early caries.

Developing novel advanced restorative materials provides new methods for effectively treating oral diseases and for dental tissue regeneration. Materials are modified with bioactive substances, drugs or functional additives to improve their final properties. However, different shortcomings of dental restorative biomaterials are still raising doubts, leaving room for continuous enhancement.

Titanium and titanium alloys

Titanium is widely used in dental implants because of its biocompatibility, chemically inert character, resistance to corrosion, superior stability, and various physical and mechanical characteristics. Since its introduction to dentistry, it has been the gold standard for dental implant connectors and abutments. No cases of Ti-based allergic reactions have been reported in the dental literature. Indeed, one of the main factors that make Ti suitable for use in dental and medical implants is its ability to form a strong bond with the living bone and soft tissue, a process known as osseointegration, which is essential for the long-term success of the implant; Ti is particularly well-suited for this purpose. When Ti is implanted into the body, it stimulates the growth of new bone tissue, which helps anchor the implant in place and promotes healing. However, the growing demand for esthetics has led to the advancement of alternative materials that resemble the teeth, such as ceramic and Zr-based implants. Although Ti-based materials are widely used, much research still focuses on determining their long-term use and modification.

The effects of cleaning the surfaces of Ti implants were studied by Kotsakis et al., who found that the mechanical treatment of Ti implants may increase the dissolution of Ti in peri-implant tissues, potentially contributing to the development of peri-implantitis.[68] Their results were supported by an ex vivo model of organic polymicrobial

peri-implant biofilms, which was more representative of clinical conditions than other single- or three-species biofilm models.[68] The findings were also biologically relevant, as the tests regarded the crucial cell types involved in the homeostasis of peri-implant tissues.[69] Furthermore, the study showed that Ti was detectable in peri-implant crevicular fluid (PICF) and gingival crevicular fluid (GCF), even in healthy subjects. Also, the concentrations of inflammatory mediators were increased in peri-implant disease and significantly associated with Ti concentrations, even when adjusted for the peri-implant health. The authors suggest that the effects of Ti on peri-implant and periodontal tissues require further research.[69]

An in vivo study investigated the effects of a collagen membrane (CM) and a synthetic bone substitute (BS) combined with a Ti mesh (TM) on lateral bone augmentation in a chronic peri-implant defect model in 6 canine mandibles.[70] The study found that using CM and/or BS did not appear to brought an additional benefit to lateral bone augmentation in peri-implant defects with TM. However, the study had a small sample size and the results were inconclusive.[70]

Using Ti-platelet-rich fibrin (T-PRF) as a donor site membrane during free gingival graft (FGG) surgery was recommended by Koca-Ünsal et al.[71] The research confirmed that T-PRF increased vascularity at the donor site, which might improve soft tissue healing. The study also found a positive correlation between the tissue thickness at the right and left donor sites, but no significant differences between the two. Furthermore, the study indicated that using ultrasound (US) to assess the tissue thickness and vascular density at the donor site during FGG surgery could contribute to the clinical success and reduce the risk of complications; it was the first study to evaluate the effectiveness of T-PRF using US imaging.[71]

A retrospective case series study on the use of TM in the jaw for the development of the implant site was presented by Levine et al.[72] A total of 58 mesh procedures were performed in 48 patients, using various bone grafts and biologics. The average initial ridge width was 2.0 ±1.0 mm, and the horizontal increase after TM procedures was 4.7 ±1.6 mm. Titanium mesh exposure occurred in 22% of cases, with middle-aged and older patients more likely to have mesh exposure than younger patients. All implants were placed successfully, but 56% required additional contour augmentation after placement in the appropriate prosthetic position. The study indicates that TM procedures result in significant bone regeneration in narrow alveolar ridges, but the rate of TM exposure associated with age, as well as the frequency of additional contour grafting, should be discussed with patients.[72]

Majewski assessed the application of a customized TM for GBR in atrophied alveolar ridges to achieve the optimal crest volume for implant placement.[73] Six patients who had been evaluated for at least 3 years were included in the study. A custom-made TM was used to protect the contour of the augmentation site and the stability of the xenograft particles. After 6 months, the mesh was removed and the implants were placed in the planned positions. The average volume increase of the augmented sites amounted to 5.2 mm horizontally and 2.75 mm vertically. Minor soft tissue perforation occurred in 50% of cases, but it did not affect the implant placement procedure. No implant failure was observed during the follow-up period. The study concluded that a customized TM was a predictable technique for bone regeneration in advanced 3D defects.[73]

The properties of Ti implants favor their use in prosthodontics. Recent studies have mainly focused on evaluating the mechanical properties and possible cytotoxicity of Ti, though it is essential to consider the properties of dental alloys and carefully choose materials to ensure their effectiveness and safety in clinical use. Romero-Resendiz et al. investigated the feasibility of using Ti-indium (In) alloys for dental prostheses through powder metallurgy methods.[74] They found that In acted as a grain refiner, reducing the grain size of the alloys and improving their mechanical behavior. The total porosity of the alloys decreased with an increasing In content, but the grain size and the In content had a more significant impact on the mechanical properties of the alloys than porosity. The alloys also showed non-classic mechanical performance due to the heterogeneous distribution of In, which was predominantly found on the grain boundaries and in porous regions. The release of Ti^{3+} and In^{3+} ions was below toxic concentrations, and the corrosion behavior was affected by the formation of a protective oxide layer, leading to low corrosion rates. Among the 3 prepared alloys, the Ti-10In alloy showed the most promising properties for use as a dental implant due to its smaller grain size, higher open porosity percentage, lower ion release, and lower corrosion rate.[74]

Atanasova et al. investigated the relationship between the strength of the bond of dental porcelain to selective laser-melted (SLM) Ti and the temperature of Ti preoxidation.[75] They found that the oxide scale covering Ti surfaces thickened significantly at temperatures of 750°C and above, but the bond strength was negatively correlated with the pre-oxidation temperature. The results suggest that the pre-oxidation of SLM Ti frameworks prior to the application of porcelain is not necessary, and room-temperature passivation in the air after the surface airborne-particle abrasion of SLM Ti results in a Ti–ceramic bond that is above the minimum recommended values for metal-ceramic systems. These findings indicate that SLM Ti can be clinically applied in porcelain-fused-to-metal (PFM) prostheses.[75]

Recent studies have shown that Ti cannot always compete with other available biomaterials. Abou-Ayash et al. investigated the trueness and marginal fit of computer-aided design/computer-aided manufacturing (CAD-CAM) complete-arch implant-supported screw-retained

fixed prostheses made of PEEK, polyetherketoneketone (PEKK) and Ti.[76] They found that the material type significantly affected the trueness of the frameworks, with PEEK having the lowest deviations, followed by PEKK and Ti. For marginal gaps, only the location of the abutments had a significant effect, with gaps in abutment 4 being significantly larger within the PEKK group than in abutments 2 and 3. Overall, the trueness of the frameworks differed according to the material type, but the marginal fit of the frameworks was similar and was smaller than 90 μm on average.[76]

In a clinical study, Curiel-Aguilera et al. compared complete-arch implant-supported fixed maxillary prostheses made of Ti or monolithic Zr in terms of plaque accumulation and soft tissue inflammation.[77] They found that Zr prostheses had slightly lower plaque levels than Ti prostheses, and the levels significantly decreased over time in the Zr group while remaining constant in the Ti group. The study also found that Ti prostheses had significantly higher plaque levels than Zr prostheses across all time points, and that patients with Zr prostheses responded well to plaque control measures, while those with Ti prostheses had more difficulty with controlling plaque. Overall, the results suggest that Zr prostheses may present with less plaque accumulation and less inflammation of the adjacent soft tissues than Ti prostheses.[77]

Titanium alloys have become firmly established in orthodontics due to their durability. Nevertheless, their durability is still under investigation, especially in the case of alloys containing other metals. A study by Chahine et al. aimed to evaluate the corrosion behavior of nickel (Ni)-Ti orthodontic wires in the oral cavity, using scanning electron microscopy (SEM), electrochemical analysis, and transmission electron microscopy (TEM) mapping.[78] The SEM and electrochemical analysis results showed that Ni-Ti orthodontic arches were able to initiate the corrosion process in the oral cavity, especially at the site of friction in contact with the braces. The TEM mapping revealed the presence of double or multiple layers of Ti, Ni and Al oxides on the surface of the wires. The study suggests that Ni-Ti orthodontic wires may be susceptible to corrosion in the oral cavity, especially at friction points.[78]

Nur'aini et al. aimed to compare the permanent deformation properties of 3 different Ni-Ti orthodontic wire products – IMD Orthoshape™, American Orthodontics Ni-Ti heat-activated wire and Ormco Ni-Ti thermal wire.[79] A total of 27 samples were used, divided into 3 groups, each containing 9 samples of a different wire product. The wires were placed into the brace slots on the test prototype and subjected to force loading for 28 days. Then, the permanent deformation of the wires was measured using a digital caliper, and compared to the deflection of the new or control wires. The results showed a significant difference in the permanent strain values between the 3 thermal Ni-Ti wire products, with the smallest being reported for Ormco, followed by American Orthodontics and IMD Orthoshape. The study concluded that using 0.014-inch-diameter thermal Ni-Ti wire with the least permanent strain was suitable in the early stages of orthodontic treatment to allow maximum and effective tooth movement.[79]

The issue of Ti alloy biocompatibility is not without significance, as only suitably modified surfaces can meet the specific requirements of, among other things, the environment of the application. The goal of the work by Im et al. was to improve the biocompatibility and retention of orthodontic mini-screws by imparting bioactive properties to their surfaces.[80] The proposed method consisted of performing anodization, periodic pre-calcification and heat treatment on the Ti-6Al-4V ELI alloy mini-screws. The anodization process consisted of applying 20 V to a glycerol solution containing 20 wt% water and 1.4 wt% hydrofluoric acid for 60 min, resulting in the formation of a layer of titanium dioxide (TiO_2) nanotubes on the surfaces of the mini-screws. Cyclic decalcification and heat treatment generated fine-grained Ca phosphate deposits on the surfaces of the mini-screws. The samples were then immersed in simulated body fluid (SBF) to confirm the presence of densely structured protrusions and elevated concentrations of Ca and phosphorus (P), which bind and concentrate endogenous bone morphogenetic protein (BMP). They also measured the time of the removal of the mini-screws after they were fixed in the tibias of rabbits for 4 weeks. The results showed that the mini-screws had a significantly longer removal time than the untreated ones, and could be used as orthodontic mini-screws.[80]

The aim of an in vitro study conducted by Fathy Abo-Elmahasen et al. was to evaluate the microbial activity of Ag/HA NPs and ZnO NPs on orthodontic Ti mini-screws in terms of inhibiting microbial growth.[81] The study also tested the in vitro cytotoxicity and cytocompatibility of the synthesized nano-coatings, as well as the animal models of 4 cell types (fibroblasts, osteocytes, osteoblasts, and oral epithelial cells). The results showed that the mini-screws coated with ZnO NPs had the highest antimicrobial activity against a range of bacteria and fungi, and ZnO NPs also showed better cytocompatibility with oral epithelium cells, bone cells and fibroblasts as compared to Ag/HA NPs. The authors concluded that the proposed nano-coating was a promising strategy to overcome the development of the inflammatory zone around fixed mini-screws.[81]

As mentioned earlier, in research on the alloys used in orthodontics, the esthetic aspect is taken into account. Šimunović et al. conducted an in vitro study to assess the color stability of esthetic ceramic brackets and adhesive samples after immersion in commonly consumed beverages.[82] There were a total of 100 ceramic brackets sourced from 5 different producers (Forestadent, G&H Orthodontics, GC Orthodontics, DynaFlex, and American Orthodontics) and a total of 120 adhesive samples (3M Transbond™ XT, and American Orthodontics

BracePaste® color change adhesive and BracePaste® adhesive) dipped in 4 different solutions – coffee, Coca-Cola®, Cedevita®, or artificial saliva (control group). The color readings were assessed at various intervals. The results showed that all brackets showed significant discoloration, with coffee having the greatest effect on color stability. The BracePaste color change adhesive showed the greatest discoloration, while Transbond XT the least.[82]

To improve the corrosion and esthetic properties of Ni-Ti orthodontic wires, biocompatible Al-SiO_2 coatings are proposed. Such materials are characterized by better physicochemical properties and far greater esthetics, and do not cause cytotoxicity.[83] As an alternative to uncoated Ni-Ti wires, esthetically pleasing epoxy-coated or thermally rhodium (Rh)-plated wires are also available to improve the esthetics. Furthermore, in vitro and corrosion studies using artificial saliva have shown that epoxy resin coatings increase corrosion resistance and release less Ni into saliva.[84]

Polyetheretherketone

Polyetheretherketone is a type of thermoplastic polymer of various applications, including medical and dental implants. The material is known for its high strength and stiffness, and its resistance to heat, chemicals and wear, which helps prolong the life of medical devices that are made of this polymer. Due to its durability, it can handle high stresses and deformations, and that is why it is suitable for manufacturing medical implants. Moreover, PEEK is often used as an alternative to metals in medical implants because of its biocompatibility. It does not damage or irritate the living tissues, which makes it a safe and effective material for medical and dental applications. Polyetheretherketone is commonly used in orthopedic and dental rehabilitative treatment because of its beneficial biomechanical characteristics. Nevertheless, the material is still susceptible to bacterial attachment and biofilm development.

Several surface properties are associated with biofilm development on the PEEK surfaces, one of which is roughness, with *Streptococcus sanguinis* (*S. sanguinis*) shown to build up a biofilm on rough PEEK surfaces over 72 h, and comparable findings reported for *Streptococcus oralis* (*S. oralis*), *Enterococcus faecalis* (*E. faecalis*) and *Streptococcus gordonii* (*S. gordonii*). The research by Barkarmo et al. also implies that bacteria are more likely to adhere to the PEEK surfaces if they are sandblasted.[85] The plasma spraying of the PEEK surfaces with Ag NPs effectively blocked the growth of Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Streptococcus aureus* (*S. aureus*). However, PEEK materials that have been modified with antibiotic coatings and peptide-functionalized NPs exhibit minimal adherence and growth of *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus epidermidis* (*S. epidermidis*) as compared to traditional PEEK products. These findings suggest that bacterial adhesion to PEEK is lowest on materials with a rough surface.

Two strategies for modifying the surface of PEEK to make it resistant to bacterial adhesion and biofilm formation were discussed in the work by Gao et al.[86] The 1st strategy consisted in coating the PEEK surface with antibiotics or other antimicrobial agents, such as natural extracts, antimicrobial peptides or metal oxides. The 2nd strategy involved modifying the surface morphology of PEEK to create microstructures that can kill bacteria or prevent them from adhering to the surface. The review discussed several specific methods for modifying PEEK with these strategies and presented research results indicating their effectiveness in inhibiting bacterial growth. Furthermore, the study referred to several antimicrobial agents that can be used to coat the surface of PEEK to make it resistant to bacterial adhesion and biofilm formation. These include antibiotics, such as gentamicin sulfate, minocycline, vancomycin, and tobramycin, as well as natural extracts, antimicrobial peptides, and metal oxides, such as ZnO and silicon nitride (Si_3N_4). The review also discussed the use of selenium (Se) and fluoride as antimicrobial agents for coating PEEK.[86]

Milinkovic et al. compared the response of peri-implant soft tissues to polyethylene and Ti healing abutments, using histological and immunohistochemical analyses.[87] They found that PEEK healing abutments induced a more intense inflammatory response in the tissues than Ti healing abutments, as demonstrated by the activation of histocytes and plasma cells. On the other hand, Ti healing abutments induced an inflammatory response of lower intensity, which was mainly mediated by B cells. These results suggest that PEEK healing abutments may be associated with a more intense inflammatory response in the soft tissues around the implant than Ti healing abutments.[87]

In a study by Li et al., patient-specific PEEK scaffolds proved to be a promising option for individualized alveolar bone augmentation, demonstrating similar space-holding and osteogenic properties to their Ti counterparts.[88] Such scaffolds can achieve excellent osteogenic space retention, making them a potential alternative to traditional GBR surgery. Although PEEK scaffolds showed lower biomechanical strength than Ti scaffolds, they can be used for customized alveolar bone augmentation and may be a better option than traditional collagen periosteal membranes.[88]

Improving the seal between soft tissues and PEEK implants by mimicking the chemistry of the tooth surface poses a significant problem described in the literature, is very important in dental practice and presents a challenge in material engineering. An interesting example in this respect comes from Saad et al., who modified the PEEK surface with collagen I and compared it with a Ti alloy (Ti-6Al-4V), the most widely used biomaterial

for percutaneous procedures.[89] The results showed that collagen-modified PEEK and Ti surfaces were characterized by an increased adsorption of the key proteins of the basement membrane and improved epithelial cell viability as compared to unmodified PEEK and Ti. These findings suggest that the proposed modification technique can potentially improve the PEEK–epithelial tissue sealing and expand the use of PEEK as a biomaterial for percutaneous implants. Moreover, the use of collagen-modified PEEK in percutaneous implants offers several potential advantages over unmodified PEEK and Ti. The surface of collagen-modified PEEK more effectively promotes the adsorption of essential basement membrane proteins, which are necessary for establishing a strong seal between the implant and the surrounding soft tissues, and can reduce the risk of infection and improve long-term implant stability. Furthermore, the collagen-modified PEEK surface promotes better cell viability and growth as compared to unmodified PEEK and Ti, suggesting better integration with the surrounding tissues. These benefits make collagen-modified PEEK a promising biomaterial for percutaneous implants.[89]

Another possibility for modifying scaffolds to enhance their therapeutic effect is worth mentioning at this point. Due to its high affinity, collagen can be combined with several bioactive agents to improve its effect, with the collagen scaffolds incorporating recombinant human-cartilage oligomeric matrix protein–angiopoietin 1 (rhCOMP-Ang1) and coumaric acid shown to facilitate bone formation.[90] Indeed, in vitro and in vivo models of critically sized mandible defects have shown that this material enhances the proliferation, mineralization and migration of cultured human periodontal ligament fibroblasts (hPLFs) through the activation of the angiopoietin 1 (Ang1)/TEK (Tie2) signaling axis.[90] As discussed earlier, Ti and Zr with collagen scaffolds have already been proposed, and their further modification with bioactive agents should contribute to their development into even more functional materials.

Another important aspect to consider is material durability. The influence of 3 denture cleaners upon immersion in a chemical solution that was applied to PEEK and other denture base materials was studied and compared with respect to long-term water sorption and solubility.[91] The study concluded that denture cleaners can affect the water sorption and solubility of PEEK and other denture materials over time. Specifically, the PEEK group showed a significant difference in the average water sorption values among all cleaning agent groups and a substantial difference in average solubility with regard to the distilled water and sodium hypochlorite (NaClO) groups. These results suggest that the type of denture cleaner used may affect the long-term performance of PEEK and other denture base materials.[91]

Evaluating the effects of loading and grafting on osseointegration and soft tissue healing in the implant area with immediately placed, self-loaded, progressive implants at the tissue level in a minipig model was the subject of a study by Parvini et al.[92] The researchers put 56 TLX implants (commercial Straumann®TLX RT, Roxolid®, SLActiv®, with durable PEEK matrices) immediately after the bilateral extraction of mandibular first and second premolars in 14 minipigs. The implant sites were assigned to 4 groups, including unloaded with simultaneous grafting with the use of bovine bone mineral, unloaded without grafting, loaded with simultaneous grafting, and loaded without grafting. The researchers found at weeks 4 and 12 that implant loading and grafting had no significant effect on osseointegration and soft tissue healing in the TLX implant area. However, they noted that the values tended to vary between the buccal and lingual aspects of the implants. These results suggest that TLX implants may be a promising option for dental implants, especially when immediate loading and grafting are not possible or necessary.[92]

Polyetheretherketone is being increasingly used to manufacture removable and fixed prostheses, such as dental crowns, bridges and denture clamps in removable dental prostheses, which is due to its low potential for causing allergies, low water solubility, superior biocompatibility, high thermal and chemical resistance, moderate biofilm formation, and excellent mechanical properties.[93] The material, with its good physical and mechanical properties, including high melting temperature and compressive strength as compared to other polymers, as well as good biocompatibility, is a potential alternative to Ti for long-term dental applications.[94] Research related to the use of PEEK in dental prostheses focuses on the problem of its chemical stability, which can hinder adhesive bonding due to the inert surface of the material, and on comparison with other materials.

Gentz et al. compared the retentive forces of removable partial denture clasps made of Co-Cr and 2 thermoplastic polymers (PEEK and PEKK), using the CAD/CAM processes.[95] The clasps were tested with a chewing simulator for 15,000 cycles to simulate 10 years of use, and the results showed that Co-Cr clasps had significantly higher retentive forces than thermoplastic polymer clasps. The researchers also examined the specimens under an SEM and observed that Co-Cr clasps showed wear on the glaze layer of the ceramic crowns, whereas the thermoplastic polymer group did not. Such wear may explain the reduction in retentive forces observed with Co-Cr over insertion and removal cycles as compared to the consistency noted with thermoplastic polymers. Nonetheless, PEEK and PEKK clasps had similar retentive forces, yet lower than those of Co-Cr clasps. All 3 groups of clasps demonstrated an initial increase in retentive forces, followed by a gradual decrease, still all maintained similar or higher retentive forces as compared to the baseline measurement. The study suggests that thermoplastic polymer clasps are appropriate for

clinical use due to their resistance to fatigue and the possibility to fabricate them through CAD/CAM.[95]

Igarashi et al. described the results of an in vitro study conducted to assess the use of PEKK as an alternative material for double-crown dental prostheses.[96] The study involved testing the retentive forces of primary crowns made of various materials, including PEKK, and secondary crowns made of PEKK, and comparing them with the gold standard (primary crowns made of Au and secondary crowns made of galvano-gold (GA)) after 20,000 connections and disconnection cycles. The study used a linear torsion all-electric dynamic test instrument to place the secondary crown on the primary crown with a force of 50 N·cm for 1 s, and then remove it with a frequency of 1 Hz. The results showed significant differences between the various test materials regarding their retentive forces, demonstrating that PEKK was a suitable alternative material for double-crown prostheses. Furthermore, the authors concluded that the PEKK/PEKK and Au/PEKK primary/secondary crown combinations were better in terms of retention forces than the Au/GA combination, and the combination of Co-Cr/PEKK showed stable retention throughout the testing period and superior retention strength after a total of 20,000 insertion/removal cycles. Since PEKK/PEKK and Au/PEKK showed superior surface wear than the control group, it can be concluded that PEKK offers good clinical prospects.[96]

Wang et al. discussed the use of PEEK in fixed dental prostheses (FDP), such as crowns, partial dentures and post-and-core.[97] They noted that PEEK showed excellent mechanical properties, including good stress distribution, and was biocompatible, non-mutagenic, non-cytotoxic, and non-allergenic. However, the chemical stability of PEEK can be considered an advantage or a disadvantage, as it minimizes intraoral corrosion, but makes adhesive bonding difficult due to the inert surface of the material. The authors described various strategies that had been explored to improve the adhesive properties of PEEK, including acid etching, plasma treatment, airborne-particle abrasion, laser treatment, and adhesive systems. They concluded that while PEEK was a promising alternative to conventional materials for FDP, it had some disadvantages, such as the lack of esthetics, and its inert and hydrophobic surface could make bonding with composite resins and abutment teeth difficult. They suggested that further research was needed to overcome these challenges and facilitate the wider adoption of PEEK in fixed prosthodontics.[97]

Raj et al. conducted a clinical study to evaluate the efficacy of PEEK FDP over 1 year.[98] The study included 20 patients who received three-unit posterior PEEK FDP. Clinical examinations and patient recalls were conducted at intervals of 0, 3, 6, 9, and 12 months to evaluate the longevity of restorations, using the modified Ryge criteria and the System for California Oral Health Reporting (SCOHR). Radiographic assessments were also performed after 12 months. The study results showed that 95% of patients maintained their PEEK FDP without fracture during the study period, while 5% reported the decementation of their FDP. Furthermore, 10% of the PEEK FDP showed marginal discoloration, but no significant changes in marginal adaptation, oral hygiene or periodontal health were observed over the study period. The authors concluded that PEEK FDP had satisfactory clinical efficacy and acceptable clinical outcomes during the 12-month observation period, but further research with a larger sample size was needed to build evidence on the use of PEEK for FDP in the long term.[98]

Soldatovic et al. determined the fracture load of implant-supported four-unit cantilever FDP with frameworks made of 2 differently filled PEEK compounds and veneered using 3 different techniques.[99] The study included 120 duplicate four-unit FDP frameworks, which were milled from PEEK with 20% TiO_2 filler or pressed with 30% TiO_2 filler, and veneered using digital veneers, conventional composite resin veneers or pre-fabricated veneers. Fixed dental prostheses were adhesively bonded to Ti abutments, and the fracture load was measured before and after artificial aging in a mastication simulator. The results showed that the filler content and the veneering technique significantly affected the fracture load, with the 30% filler content and pre-fabricated veneers providing the highest resistance to fracture. Aging did not affect the fracture load. The authors concluded that the filler content of PEEK compounds and the veneering technique influenced the fracture load of implant-supported four-unit PEEK FDP, and selecting the appropriate veneering method could improve the long-term success of bilayered structures. They also noted that all of the FDP in the study had a higher fracture load than the maximum occlusal forces in the posterior region, and that mastication simulation did not impact the fracture resistance of the examined PEEK FDP.[99]

A very interesting summary of works on the use of PEEK for dental prostheses was presented by Khurshid et al., who assessed if PEEK had superior mechanical and esthetic properties as compared to other materials used in the construction of dental prostheses.[100] The review included 12 articles, with 2 case studies, 3 observational studies, and 1 randomized controlled trial (RCT). The quality of the research was evaluated using various tools, and the overall quality of most studies was classified as low or medium. The review found that the evidence on the long-term viability of PEEK-based dental prostheses was insufficient, and most of it came from case reports and non-randomized observational studies. The authors concluded that future studies should focus on large-scale multicenter trials comparing the survival rate of PEEK-based prostheses with other materials, and that implant-supported PEEK prostheses should be further studied as a potential replacement for conventional materials and designs.[100]

The effects of amine group modification on the osseointegration behavior of carbon fiber-reinforced polyethylene (CPEEK) in rabbits was the purpose of a study by Wang et al.[101] Two groups of implants were used – 30%-CPEEK and A-30%-CPEEK (amine modification), with pure Ti as a control. Bone-forming capacity and osseointegration were assessed in vivo by microcomputed tomography analysis, SEM observations and histological evaluation. The results showed that all parameters differed significantly between the 2 groups, with those in the A-30%-CPEEK group being equal to or better than pure Ti. The study also found that modifying the amine group could positively affect bone regeneration and suggested that A-30%-CPEEK could be a promising non-metallic implant material. In conclusion, this study showed that the amine group modification of PEEK improved osseointegration in rabbits. Moreover, A-30%-CPEEK implants showed higher levels of new bone formation and better bone integration than unmodified implants with 30%-CPEEK.[101]

Boonpok et al. studied the effect of hydrothermal treatment on the bioactivity of the HA-titanium nitride (TiN) coating on PEEK by immersion in SBF.[102] The coating was produced by pulsed direct current magnetron sputtering. Its dissolution in SBF was studied at different time points for up to 56 days. The results showed that the dissolution of the coating and the precipitation of the Ca phosphate complex from SBF occurred continuously throughout the immersion period, causing physical and chemical changes in the coating. However, after 56 days, the coating remained on the PEEK surfaces and had a 1.16 Ca/P ratio. These results suggest that the hydrothermal treatment of the HA-TiN coating improves its bioactivity, and may have potential applications in orthopedics and dentistry.[102]

Kerkfeld et al. compared the facial symmetry results after simultaneously performed, digitally planned orthognathic surgery based on patient-specific implants (PSI) and bone augmentation with PEEK in patients with craniofacial defects.[103] The research involved 5 groups of patients, including those with and without laterognathia, patients with and without syndesmosis, and patients with and without PEEK bone augmentation. The digital process workflow involved cone-beam computed tomography (CBCT) and virtual surgery planning for all patients to produce patient-specific cutting guides and osteosynthesis plates. In addition, the deformed skulls were superimposed on a non-deformed skull and/or the healthy side was mapped to produce PEEK PSI for augmentation. The results of both surgical approaches were evaluated using conventional posterior-anterior radiographs, and en-face images taken before and 9 months after surgery. The findings showed that simultaneous orthognathic surgery with PEEK bone augmentation significantly improved facial symmetry when compared to conventional orthognathic surgery (6.5%P (3.2–9.8%P)). Indeed, PSI-based orthognathic surgery improved horizontal bone alignment in all patients, while simultaneous PEEK bone augmentation improved facial symmetry, even in patients with combined soft and hard tissue hypoplasia. A digital workflow, including virtual surgical planning, led to improved balance in all patients.[103]

Bioactive glass

Bioactive glass is a type of biocompatible glass of various applications, made from a combination of silicon (Si), Ca, sodium (Na), and P, and designed to fuse with the living tissues in the body. When implanted, BG stimulates the growth of new bone tissue, which can help repair or regenerate damaged or lost bone, making it valuable in dentistry, orthopedics and other medical fields. In addition to its ability to stimulate bone growth, BG has several other properties that make it a useful biomaterial. Indeed, it is biocompatible, resistant to infections, and can help prevent bacteria from growing on its surface. Moreover, BG is strong, durable, and can withstand the stresses and strains of daily use. Among the main advantages of BG is its ability to fuse with the living tissues; once implanted, it forms a chemical bond with the surrounding bone and soft tissues, which favors implant anchorage and promotes healing. This bonding process, known as osseointegration, is essential for the long-term success of medical implants, and is one of the key factors that make BG such a valuable biomaterial.

Ongphichetmetha et al. compared the effectiveness of a paste containing 5% calcium sodium phosphosilicate (CSPS) and 8% arginine in alleviating dentin hypersensitivity (DH) in patients receiving non-surgical periodontal therapy.[104] This double-blind RCT involved 45 volunteers who were treated with one of 3 dental formulations immediately after non-surgical treatment and continued brushing twice daily for 8 weeks. The results showed that the CSPS preparation immediately reduced DH, which declined by week 8. The study concluded that the CSPS paste and arginine were beneficial in terms of reducing discomfort in patients with periodontitis immediately and during the first 2 weeks after non-surgical periodontal treatment.[104]

Williams discussed the evidence on bioactivity mechanisms in some biomaterials, including BG, especially in case the material was modified to promote such activity.[105] The article covered a basic understanding of bioactivity phenomena and their relations with biocompatibility mechanisms in biomaterials. The author analyzed the performance of bioactive materials in various areas, including bone induction, cell adhesion, immunomodulation, thrombogenicity, and antimicrobial behavior. The effectiveness of bioactive materials was shown to be based on solid scientific evidence in a variety of applications, but their successful clinical translation remains problematic. Indeed, it is suggested

that a focus on the 'bioactivity zone' at the interface between the material and the host tissue may provide a better understanding of the mechanisms of bioactivity, and help improve the design and performance of biomaterials. Finally, the article discussed the challenges and barriers to the successful clinical translation of bioactive materials.[105]

Some reviews in the field indicate that the subject of applying BG in periodontology is well-researched. Cannio et al. reviewed the literature on the potential bioactivity and biocompatibility of some BG types, such as 45S5 Bioglass®, BonAlive® and 19-93B3, in the biomedical field.[106] The paper discussed various forms in which these materials can be obtained and their potential applications, including dentistry and reconstructive surgery. The article also highlighted the need for further research and clinical trials to fully understand the performance of these materials and optimize their design for future applications. In their systematic review, Behzadi et al. evaluated the potential effectiveness of BG and HA in dentinal tubule occlusion, which may be useful in treating DH.[107] The review included 35 in vitro studies, which revealed a low risk of bias and demonstrated the effectiveness of BG and HA in dentinal tubule occlusion. The review suggests that desensitizers containing BG and HA can be used to treat DH, but more long-term clinical studies are needed to make definitive recommendations.[107]

In orthodontics, BG is used as a bonding and cleaning agent. For example, Chopra et al. evaluated the effects of different bonding agents on the luting strength of orthodontic brackets.[108] The study involved 48 healthy human premolar teeth that were randomly divided into 4 groups, including a control group that used GIC, a group that used GIC with CPs, a group that used GIC with Ag NPs, and a group that used the 45S5 Bioglass paste. The teeth were bonded with metal locks, and a universal testing machine (UTM) was used to remove the locks and measure the strength of the luting cement. The thickness of the remaining cement was also measured using computed tomography (CT) and SEM. The results showed that the CP GIC group had the highest bond strength, followed by the luting GIC, Ag NP GIC and 45S5 Bioglass groups. The luting GIC group had the greatest thickness of the remaining cement, followed by the Ag NP GIC, CP GIC and 45S5 Bioglass groups. The study found that CPs in GIC provided the strongest bond and left the least cement on the tooth surface as compared to the other luting agents tested.[108]

Moslemi et al. proposed using the AutoCAD® and SPSS software for advanced statistical analysis of the data collected during orthodontic tests.[109] The research evaluated the effects of toothpastes with BG on the remineralization of orthodontically induced white spot lesions (WSLs). Orthodontic brackets were bonded to extracted premolar teeth, which were then immersed in a demineralization solution to create artificial carious lesions on enamel. The samples were divided into 2 groups, with one treated with a toothpaste containing NaF, and the other treated with a toothpaste containing BG. The samples were analyzed using a polarizing microscope, and the results showed that both toothpastes were effective in remineralizing WSLs, but the BG toothpaste was more effective than the toothpaste with NaF.[109]

Al Shehab et al. investigated the influence of 2 types of sealants, namely fluoride bioactive glass (FBAG) paste and the Alpha-Glaze™ resin, on the shear bond strength of orthodontic brackets to enamel, as well as their protective effects against a simulated cariogenic acid attack.[110] The study included 135 extracted premolar teeth, which were divided into 3 groups: FBAG; Alpha-Glaze; and control. The shear bond strength of the brackets was measured using an Instron UTM, and the protective effects of the sealants was evaluated using a toothbrushing simulator and light microscopy. The results showed that the shear bond strength values did not differ significantly between the FBAG (28.1 ±5.5 MPa), Alpha-Glaze (32.5 ±7.4 MPa) and control (30.7 ±6.5 MPa) groups. The Alpha-Glaze sealer provided a mechanical barrier on the enamel surface within seconds of polymerization, but did not considerably protect enamel from a simulated cariogenic acid attack. On the other hand, FBAG was able to protect the enamel surface around orthodontic brackets, but its relatively long application time posed some clinical difficulties. The study suggests that FBAG can be used as an orthodontic sealant that does not affect the bond strength of orthodontic brackets and causes minimal damage to enamel during the removal of brackets.[110]

Salah et al. evaluated the effectiveness of 2 types of BG (45S5) – BioMin® F and NovaMin® – in comparison with casein-phosphopeptide-amorphous calcium phosphate (CPP-ACP) in the treatment of orthodontically induced WSLs.[111] Sixty post-orthodontic WSLs were randomly assigned in a double-blind RCT with 3 parallel arms (n = 20): group I (Bio-BAG) received the BioMin F slurry and toothpaste; group II (N-BAG) received the NovaMin slurry and toothpaste; and the positive control group (CPP-ACP) received the Recaldent toothpaste. The products were applied daily in a dental office for 1 week, and then reinforced by self-application at home for 4 weeks. The results showed that at a 6-month follow-up, all 3 groups revealed a statistically significant regression of WSL as compared to baseline, and there was a highly significant percentage reduction in the lesion size in the Bio-BAG group as compared to the control group. The average lesion area decreased by 64.8%, 32.2% and 31.6% in respective groups. The study concluded that using the BioMin F toothpaste in a dental office and at home for 4 weeks resulted in a more significant esthetic improvement of post-orthodontic WSLs as compared to NovaMin and CPP-ACP.[111]

Acrylic resins

For acrylic resins, new research focuses mostly on esthetics, including color fastness, and on mechanical resistance and biocompatibility. For example, Almuraikhi studied the effects of different disinfecting solutions on the color stability of 2 types of denture substructure materials – Meliodent® and ProBase® Hot.[112] The materials were immersed in chemical disinfectants (2% alkaline glutaraldehyde, 0.5% chlorhexidine gluconate or 0.5% NaClO solutions) or distilled water (as control), and color stability was measured at different time points. The results indicated that all disinfectant solutions had some impact on the color stability of base materials, with the smallest effect observed with 0.5% chlorhexidine gluconate and the greatest effect observed with 0.5% NaClO. Color stability was the lowest when the materials were immersed in distilled water. These results suggest that professionals should consider the effects of different disinfectant solutions on the color stability of denture base materials when selecting a disinfectant for use on dentures, which is particularly important, as color stability is an important factor affecting the final denture appearance.[112]

Szerszeń et al. evaluated the color stability of a ZnO NP-PMMA nanocomposite as a denture base material.[113] The nanocomposite was exposed to various solutions (distilled water, coffee, red wine, black tea, and denture cleaning tablet) for 6 months and color changes were measured using a digital colorimeter. The results showed that all materials experienced significant color changes after exposure to various solutions, with the greatest changes observed for red wine and the least for distilled water. The researchers found that the modification of PMMA with ZnO NPs is esthetically acceptable at a concentration of 2.5% or 5% by weight, but color changes become more noticeable when the NP content is higher, and the use of the nanocomposite should be discussed with patients.[113]

In terms of biological, mechanical and structural properties, modified PMMA may represent a promising approach to improving denture performance and longevity.[114] Zore et al. studied the effect of adding TiO_2 NPs to PMMA on the adhesion of *S. mutans* to the material through comprehensive tests of mechanical strength, color fastness and biocompatibility.[115] The surface properties of modified PMMA, including roughness, the contact angle, zeta potential, and color parameters, were measured. Tensile strength was also measured. The results showed that adding TiO_2 NPs decreased the roughness and contact angle of the material, increased its zeta potential, and changed its color. Adding TiO_2 NPs also decreased the uniaxial tensile strength of the material and had a significant effect on the formation of the *S. mutans* biofilm on its surface, with concentrations of 10% and 20% reducing the bacterial adhesion by 58% and 60%, respectively. The authors concluded that PMMA modified with TiO_2 NPs could be a promising material for fabricating acrylic resin-based dental materials, but further studies are needed to test its antimicrobial properties and color matching with gingival tissues.[115]

Malisic et al. studied the effect of gamma radiation on the microbiological purity and material properties of a PMMA composite containing Al_2O_3 NPs.[116] The material was irradiated with doses of 0, 10, 20, and 25 kGy, and the microbiological purity, mechanical properties, thermal stability, microstructure, and the color changes were tested. The results showed that a dose of 25 kGy was sufficient for the complete sterilization of the material, and improved its mechanical properties and thermal stability. However, the dose also caused microstructural changes in the material, including the formation of cracks and pores and color changes. The authors concluded that gamma radiation could sterilize PMMA/Al_2O_3 NPs composites, but the optimal dose would depend on the intended application of the material.[116]

Punset et al. found that adding graphene to PMMA improved its mechanical properties, including compressive strength and the modulus of elasticity, and reduced its specific wear rate, determined by the mechanical compression test and the pin-on-disk wear test.[117] The presence of graphene in PMMA was studied using Raman spectroscopy and field emission scanning electron microscopy (FESEM). Shore hardness and Vickers microhardness were also determined. Despite promising results, further studies are needed to determine the optimal percentage range of reinforcement, the influence of graphene morphology, and the biocompatibility and fatigue of the composite.[117]

Pagano et al. compared the mechanical and biological characteristics of a PMMA disk for CAD/CAM prostheses (test samples) with traditional resin (control samples).[118] The test samples had a higher modulus of elasticity than the control samples and a different Brillouin frequency. Furthermore, SEM showed that keratinocytes in the test samples appeared flattened, with lamellipodia, while those in the control samples had cytoplasmic filaments. The test samples were also significantly less cytotoxic than the control samples. However, no significant differences in apoptosis were found between the 2 types of samples. Real-time polymerase chain reaction (PCR) showed increased *p53* expression in keratinocytes in both types of samples, but no significant variations in *p21* or *bcl2* expression. The authors concluded that the PMMA disc for CAD/CAM prostheses had improved mechanical and biological features as compared to traditional resin, including a higher modulus of elasticity, better keratinocyte morphology and lower cytotoxicity.[118]

Since it is important for the application of materials in dentistry that the material allows for simple processing to create accurate models, research is being carried out to determine the best possible fabrication technique variants. Sidhom et al. evaluated the precision of PMMA

working models and the marginal fit of PMMA provisional prostheses produced using 2 digital fabrication techniques – CAD/CAM milling and 3D printing.[119] The samples were evaluated using a coordinate-measuring machine (CMM), an SEM and a stereomicroscope. The results showed that the CAD/CAM-milled models had lower mean distances between the reference points as compared to the 3D-printed models, and the marginal fit of the CAD/CAM-milled provisional prostheses was better than that of the 3D-printed prostheses. The authors concluded that CAD/CAM milling is a more accurate technique for the fabrication of PMMA working models and provisional prostheses than 3D printing.[119]

The analysis of recent literature indicates that it is possible to use PMMA in dental applications, with particular emphasis on its mechanical and biological properties. Furthermore, modifying PMMA with several additives has been shown to alter its mechanical properties, including compressive and tensile strength and the modulus of elasticity, and to significantly affect biofilm formation on its surface.

Ceramic materials

Ceramic materials are now firmly established in prosthodontics, and research continues regarding their mechanical resistance and biocompatibility. The question of esthetics is also of great importance, with studies focusing on commercially available and new materials, mainly based on modifications. The aim of a study by Juntavee et al. was to evaluate the ability of different types of ceramics of varied thickness to mask the color of various substructures.[120] Ceramics used in the study were BruxZir® Anterior, Celtra™ Duo and VITA SUPRINITY®, and the substructures included natural dentine, tetracycline-stained dentine, Zr, a resin composite, and a cast metal. The researchers used a spectrophotometer to measure the color of the specimens, and determined the masking ability by comparing the color difference before and after combining the ceramics with the substructures. The results showed that the type and thickness of the ceramic and substructure significantly affected the masking ability, with tetracycline-stained dentine having the greatest impact on the appearance of the ceramic restoration. The study concluded that BruxZir Anterior, Celtra Duo and VITA SUPRINITY all had the ability to mask tetracycline-stained dentine, with BruxZir Anterior requiring a minimum thickness of 0.6 mm, VITA SUPRINITY requiring a minimum thickness of 1.2 mm and Celtra Duo requiring a minimum thickness of 1.6 mm to achieve an ideal masking capability. Monolithic Zr had a higher masking ability than a lithium silicate/phosphate glass ceramic.[120]

Al-Dwairi et al. conducted an in vitro study to examine the effect of the design of the framework on the fracture resistance and failure modes of cantilever inlay-retained fixed partial dentures (IRFPDs) made of 2 types of multi-layered monolithic Zr materials.[121] The researchers prepared 72 natural premolar teeth as abutments for IRFDPs, using 3 different designs, and then fabricated full-contoured IRFDPs from the 2 Zr materials. The samples were subjected to thermocycling and mechanical loading, and the surviving samples were then loaded until failure on UTM. The results showed that the mean failure load was not significantly different between the different designs or materials. However, during the dynamic fatigue test, the IPS e.max® ZirCAD Prime material had a significantly higher failure rate than the Zolid Gen-X material. The researchers also found that the type of attachment structure significantly affected durability. Dentures with a cantilever design, which maximizes adhesion to enamel, showed promising results, while IPS e.max ZirCAD Prime was more prone to fracture with the long palatal wing design.[121]

Daou conducted an in vitro study to compare the fit of Co-Cr alloy FDP fabricated using different techniques before and after ceramic layering.[122] The researcher prepared a Co-Cr alloy master model and manufactured 60 frameworks, using selective laser melting (SLM), soft milling and conventional casting. The replica technique was used to measure the marginal and internal discrepancies of the frameworks before and after they were layered with ceramics, and compared the results within each group. The data showed significant differences within the groups before and after ceramic layering for SLM and soft milling, but not for conventional casting. The study also found that soft milling resulted in increased gap values in the marginal and occlusal regions. However, there was no statistical difference in the marginal region between the conventional casting group and the soft milling and SLM groups. The author concluded that ceramic layering increased the discrepancy between the laser-sintered and milled frameworks, particularly in the marginal region.[122]

Another in vitro study was conducted by Spitznagel et al., who investigated the failure load and fatigue behavior of monolithic and bilayer Zr FDP supported by one-piece ceramic implants.[123] The researchers prepared 80 three-unit FDP supported by 160 Zr implants, which were divided into 4 groups: the monolithic 3Y-TZP Zr (Vita YZ® HT) group (Z-HT); the monolithic 4Y-TZP Zr (Vita YZ ST) group (Z-ST); the 3Y-TZP Zr (Vita YZ HT) group with a facial veneer (Vita VM®9) (FL); and the polymer-infiltrated ceramic network (PICN) 'tabletop' group with a 3Y-TZP framework (Vita YZ HT) (RL). Half of the samples in each group were subjected to fatigue in a mouth-motion chewing simulator with simultaneous thermocycling, and all specimens were then exposed to single-load-to-failure testing. The results showed that all samples withstood the fatigue application and that the choice of material significantly affected the failure load, with the FL group recording the highest failure load, followed by the Z-ST, Z-HT and RL groups. All FDP mate-

rial combinations survived chewing forces that exceeded physiological levels, with the bilayer FL and monolithic Z-ST groups showing the highest resilience, meaning they could serve as reliable prosthetic reconstruction concepts for three-unit FDP in ceramic implants.[123]

Due to the widespread use of implants, it is possible to carry out long-term studies that can clearly indicate the advantages and disadvantages of the materials used. Rammelsberg et al. conducted an observational cohort study to evaluate the chipping and failure rates of FDP supported by metal-ceramic and ceramic implants and combined tooth–implant-supported FDP.[124] The study included 434 FDP placed in 324 patients, with 213 implant-supported FDP, 66 implant-supported cantilever FDP, and 155 tooth–implant-supported FDP. Metal-ceramic FDP were made with a high noble metal alloy or a Co-Cr base metal alloy framework, while ceramic FDP were all Zr-based with a monolithic, completely veneered or partially veneered framework. The researchers found that during the observation period of 0.5–12.6 years, 17 FDP failed due to implant failure, tooth loss, major chipping, or the loosening of the abutment screw. The survival probability was 96% after 5 years and 91% after 10 years, and the Cox regression analysis showed that age, sex, the location of FDP, the type of FDP support, and the FDP material had no significant effect on the failure of the prostheses. However, chipping was significantly affected by the material of the framework and the type of veneer, with the highest incidence of chipping occurring in Zr FDP with a complete veneer, followed by metal-ceramic FDP with a high noble metal framework. The authors concluded that implant–implant-supported and combined tooth–implant-supported FDP have promising long-term survival rates, and that chipping seems to occur less frequently in monolithic or partially veneered FDP than in FDP with complete veneers.[124]

Balmer et al. wrote a position paper on the current level of evidence regarding Zr implants in clinical trials.[125] The authors note that Ti oral implants are considered the standard in implant dentistry, but their grey color and a high prevalence of peri-implant infections have led to controversy over whether tooth-colored, metal-free Zr ceramic implants provide sufficient potential to be considered equal in terms of treatment outcomes. The authors stated that the most available and scientifically documented Zr implant systems were one-piece implants requiring experienced surgeons and prosthodontists due to their limited flexibility in compromised angulation or vertical positioning cases. They also noted that there was evidence of a comparable outcome for one-piece Zr implants as in the case of Ti implants for the fixed replacement of 1–3 missing teeth. However, the available clinical data evaluating two-piece Zr implants with an adhesively bonded implant–abutment interface suggest an inferior outcome. The authors also mentioned that data evaluating the clinical applicability of screw-retained solutions, although revealing sufficient fracture resistance in laboratory investigations, are still missing. They concluded that more RCTs assessing patient-reported outcome measures and two-piece Zr implant systems, as well as further clinical research on the selection of materials for monolithic reconstructions are needed to allow for a comparison with Ti at the implant level.[125]

Zirconium-based ceramic implants have proven biocompatible through the tests conducted on various forms of Zr, including an yttria-stabilized ceramic, and magnesium oxide (MgO)-, Al and Ca-added ceramics. These tests demonstrated that ceramics were biocompatible materials, although Zr wear products showed some toxicity.[126] Dental implants based on Zr are also biocompatible with immune cells, including monocytes, lymphocytes and macrophages, since tests showed the absence of toxicity.[127] Also osteoblasts exhibited no toxicity during investigations. Meanwhile, in vivo tests of hard and soft tissues showed their biocompatibility with Zr-based implant materials.[127]

Several variables can affect the biocompatibility of the implant surface, such as the energy, topography and chemical composition of the surface. These factors may play an important role in the development of biofilms in implanted dental materials. Both Ti and Zr are hydrophobic materials, meaning they do not interact with water, and therefore, do not promote the growth of microorganisms. In addition, Gram-positive bacteria have a thick peptidoglycan layer and hydrophobic characteristics that allow them to be attracted to implants made of Ti or Zr. Despite this attraction, microbiological studies have not reported considerable bacterial adhesion to either material. Nonetheless, Ti and Zr differ in their electrical properties. Unlike Zr, Ti is a semiconductor due to a bioactive dioxide layer, and its electrical conductivity ultimately leads to greater plaque accumulation on Ti surfaces. Additionally, albumin present in saliva is adsorbed on Ti surfaces because of divalent Ca ions, which are attracted to the negatively charged Ti surface, and bond to Ti and bacterial surfaces.

Using stem cells for PDL tissue reconstruction in osseointegrated implants was studied by Safi et al., with Ti and Zr implants coated with beta-tricalcium phosphate (β-TCP) assessed using a long-pulse neodymium-doped yttrium-aluminum garnet (Nd:YAG) laser.[128] Isolated bone marrow mesenchymal cells and PDLSCs were tested for periosteal markers. After extraction, the coated implants were transferred to the rabbit lower right central incisors and examined after 45 and 90 days. The results showed that placing coated Ti and Zr implants without a cell sheet resulted in a well-seated implant at both healing intervals. The use of PDLSCs alone or co-cultured with bone marrow mesenchymal stem cells (BMMSCs) formed natural periodontal tissue, with no significant difference between Ti and Zr implants, resulting in a biohybrid dental implant. The study concluded that Ti and Zr

implants coated with β-TCP could generate periodontal tissue and form biohybrid implants when mesenchymal cell sheets are isolated from PDLSCs alone or co-cultured with BMMSCs.[128]

Assery et al. studied the effect of erbium-doped yttrium-aluminum garnet (Er:YAG) laser treatment on Zr and Ti disks, and differences in biofilm formation due to surface changes.[129] The disks were exposed to a laser, and the surface changes, roughness and elemental mass of the material were evaluated using SEM and atomic force microscopy (AFM). At the 2nd stage, 4 participants wore the disks in custom-made intraoral stents overnight, and the biofilm-covered disks were stained and visualized using multi-photon confocal laser scanning microscopy. The results showed that the Ti and Zr disks treated with the Er:YAG laser had visible surface changes, but showed no significant alterations in the mean surface roughness or elemental mass. Furthermore, there were no significant differences in biofilm biomass, thickness or the ratio of live to dead bacteria in the laser-treated Ti and Zr disks as compared to the non-laser-treated groups. The study concluded that the Er:YAG laser treatment of Ti and Zr implant surfaces did not significantly affect surface roughness, elemental mass or early oral biofilm formation.[129]

Investigating the efficacy of different implant decontamination methods in terms of reducing biofilm modification, as well as their potential cytotoxicity was the goal of a study by Stein et al.[130] Titanium and Zr disks were contaminated with a highly adherent biofilm consisting of 6 bacterial species. Decontamination was performed using various methods, including a Ti curette, an ultrasonic scaler, air-polishing with glycine and erythritol powder, an Er:YAG laser, 1% chlorhexidine (CHX), 10% povidone-iodine, 14% doxycycline, and a 0.95% NaOCl solution. The results showed that only air-polishing, the ultrasonic scaler and the Er:YAG laser significantly reduced the amount of biofilm on Ti and Zr surfaces. These methods also resulted in favorable cytocompatibility on both materials. In contrast, the chemicals caused potential cytotoxic effects.[130] The application of Er:YAG laser irradiation as a support for Zr materials used in dentistry, including in vitro confirmation, is also described in other works.[131–133]

The analysis of the most recent literature shows that at present, Zr implants, despite their advantages, are not a substitute for commonly used Ti implants. Comparative studies show that Ti is still superior in terms of long-term stability and satisfactory osseointegration. On the other hand, Zr implants are the preferable option when esthetics is required. According to recent reports, they are also the material of choice for protection against implants, as clinical studies indicate a better response of soft tissues as compared to Ti implants. Long-term studies are still needed in this area, particularly regarding the antibacterial effects.

Three-dimensional printing technology

From a practical point of view, researchers are increasingly focusing on questions related to the possibility of using 3D printing technology in prosthodontics, including the selection of the appropriate biomaterials. Son et al. conducted a study to evaluate the intaglio surface trueness of interim dental crowns fabricated using 3 different 3D printing and milling technologies.[134] The study used a CAD reference model as a baseline for comparison, and interim dental crowns were fabricated using the stereolithography apparatus (SLA), digital light processing (DLP) and milling machine technologies. The intaglio surface trueness of the fabricated crowns was assessed using a 3D inspection software program, and statistical analysis was conducted to compare the results. The study showed significant differences in intaglio surface trueness between the 3 different technologies, with the milling group demonstrating the highest trueness values. In addition, the milling group showed significant differences in trueness according to the location of the intaglio surface. In general, the findings suggest that 3D printing technologies may be more accurate and uniform in manufacturing interim dental crowns as compared to the milling technology.[134]

Shin et al. conducted a study to analyze the effect of the internal structure and the presence of a cross-arch plate on the accuracy of 3D-printed dental models.[135] The models were designed with a U-shaped arch or a cross-arch plate attached to the palate area, and the internal structure was divided into 5 types. The accuracy of the models was measured in terms of trueness and precision. The study showed that the presence of a cross-arch plate significantly improved the accuracy of the 3D-printed models, with lower trueness and precision values in the cross-arch plate group as compared to the U-shaped group. In addition, the internal structure of the models affected accuracy, with higher trueness values in the 1.5 mm shell group, and lower values in the roughly and fully filled models. The findings suggest that a cross-arch plate is necessary for the accurate 3D printing of dental models, and that the internal structure of the models also plays a role in determining accuracy. Overall, the results of this study highlight the importance of considering the internal structure and the presence of a cross-arch plate in the design of 3D-printed dental models.[135]

Mai et al. conducted a study to evaluate the effect of a newly developed CHX-loaded polydimethylsiloxane (PDMS)-based coating material on the surface properties and antibacterial activity of 3D-printed dental polymers.[136] The coating material was synthesized by encapsulating CHX in mesoporous silica NPs and adding them to PDMS. The coating was applied to the 3D-printed polymer specimens, using oxygen plasma and thermal treatment to form a thin film. The results of the study showed that the coating significantly reduced surface irregularity

and increased the hydrophobicity of the specimens. In addition, the coated specimens had a significantly lower count of bacterial colony-forming units (CFUs) in culture media as compared to the non-coated specimens, indicating the effective antibacterial activity of the coating. The findings suggest that coating substances may improve the surface properties and antibacterial activity of 3D-printed dental materials.[136]

Schönhoff et al. investigated the mechanical properties of polyphenylene sulfone (PPSU) and PEEK processed using fused filament fabrication (FFF) to evaluate the suitability of PPSU as an alternative material for the 3D printing of dental restorations.[137] After aging, the study tested three-point flexural strength, two-body wear, Martens hardness, and the indentation modulus. The results showed that PPSU printed with FFF had lower flexural strength than PPSU cut from pre-fabricated molded material, indicating the need to optimize the 3D printing parameters for PPSU. Polyphenylene sulfone also had lower hardness and indentation modulus values than PEEK, but comparable results for flexural strength and two-body wear. In general, the findings suggest that PPSU may be a suitable alternative to PEEK for the manufacturing of removable and fixed dental prostheses, but that the quality of the filament used in the 3D printing process is important in determining the mechanical properties of the printed material.[137]

Park et al. compared 3D-printed provisional three-unit FDP with conventionally fabricated and milled restorations in terms of flexural strength.[138] The study used 3 additive manufacturing (AM) technologies – SLA, DLP and fused deposition modeling (FDM) – as well as subtractive manufacturing (SM) and conventional methods as controls. The 3D-printed prostheses were made of a PMMA-based resin (SLA and DLP) or a PLA-based resin (FDM). The flexural strength of the prostheses was measured using UTM and analyzed statistically. The study showed that the SLA and DLP groups had significantly higher flexural strength than the conventional group, but there were no significant differences between the SLA and DLP groups or between the DLP and SM groups. The FDM group showed only dents, but no fractures. The findings suggest that 3D-printed provisional restorations made with the SLA and DLP technologies have adequate flexural strength for dental use.[138]

The discussed studies evaluated various aspects of the use of 3D printing in dentistry, including the development and application of coating materials, the accuracy and trueness of interim dental crowns, the mechanical properties of different materials, the effect of the internal structures and cross-arch plates on the accuracy of dental models, and the flexural strength of 3D-printed dental prostheses. Overall, the studies suggest that 3D printing can be useful in dentistry, but further research is needed to optimize its use in fabricating prostheses, models and restorations.[134–138]

In summary, removable and fixed removable dental prostheses are commonly used to replace missing teeth, or support and stabilize other dental restorations. Such devices are usually made of various materials, including metals, ceramics or composite resins, and can be manufactured using various techniques, such as casting, milling or AM. Research has shown that the design and material of dental prostheses can affect their performance, with particular materials and designs being more resistant to damage or chipping than others. Research is underway to evaluate the performance and clinical applicability of different dental prostheses and materials, with a focus on improving patient outcomes.

Simulations as support for material design

Structural analysis

One of the most common computational techniques used in dentistry, and especially in orthodontics, is the finite element method (FEM), a numerical technique used to solve complicated structural engineering problems. The fundamental idea of FEM is to decompose a complex geometry problem into smaller and simpler parts called 'elements', which can be easily analyzed. These elements are then combined to create a complete solution to the original problem. The FEM computations are often used in engineering fields, such as mechanical, civil and aerospace engineering, to analyze and design structures like buildings, bridges and aircraft.

The initial step in FEM calculations creates a mathematical model of the problem that includes details about the material composition, geometry and the loading conditions of the structure. The model is then divided into smaller elements that can be analyzed using mathematical equations. Next, the internal forces and stresses of each element are calculated with a combination of mathematical equations and computer algorithms. Finally, the forces and stresses of each element are combined to determine the overall behavior of the structure. Numerous software programs utilize FEM calculations for various uses. Popular examples are ANSYS, Abaqus, COMSOL Multiphysics®, SOLIDWORKS® Simulation, and Solid Edge, which are mainly used by engineers and scientists to evaluate and design complicated structures and systems. These software programs are also used in other industries, such as biotechnology, chemical engineering and energy production.

The work of Schmidt and Schrader was divided into 2 parts.[139,140] In the 1st part, the authors investigated the fracture behavior of a three-piece dental bridge restoration under different chewing speeds (1.0 mm/min and 130 mm/s), using digital image correlation (DIC). The results

showed that the material exhibited significantly different responses at different test speeds, and the forces at fracture were substantially lower at the average chewing speed (130 mm/s) than in the quasi-static test. The work underscores the importance of considering different speeds when evaluating the mechanical properties of dental materials, as well as the usefulness of the DIC method for studying the fracture performance of materials. Furthermore, these results may be important for future research on the application of FEM to predict the behavior of dental restorations under clinical conditions.[139] In the 2nd part of the study, the use of FEM for predicting the mechanical performance of a PMMA temporary dental bridge under clinically relevant loads was proposed, with the nonlinear FEM predictions validated through experiments. The analysis indicated that when a material model for PMMA in dental applications is determined, the influence of the stress-state-dependent plastic flow is negligible, and the von Mises yield criterion is sufficiently accurate. Finally, the results showed good agreement when applying the von Mises yield criterion, and the Schmachtenberg hardening law was used to account for plastic deformations. However, the study also emphasized that simplifying the assumptions of the linear elastic properties of the material should be avoided in FEM studies, as they do not consider rate dependencies or plastic flow. Therefore, accurate preliminary investigations for material characterization are necessary for precise FEM predictions.[140]

Wahju Ardani et al. conducted a study to test the feasibility of using modified PEEK with nHA as a biomaterial for orthodontic mini-implants.[141] Using numerical molecular docking simulation, the binding affinity of nHA, PEEK and a PEEK-nHA complex with 12 osseointegration-related target proteins was analyzed. The results showed that the PEEK-nHA complex had a more negative binding affinity than either PEEK or nHA alone, indicating that it has greater potential than either of these materials as a biomaterial for osseointegration and the fabrication of orthodontic mini-implants.[141]

A similar approach was represented in other works in this area. For example, Mieszala et al. determined the possibility of using PEEK in the manufacturing of braces for orthodontic treatment.[142] A variety of transpalatal arch (TPA) geometries were designed by the researchers with the use of CAD, with 4 of the designs selected and fabricated by milling PEEK. Then, the researchers conducted FEM and in vitro mechanical tests to analyze the forces acting on first upper molars. The results showed that the PEEK TPAs were capable of generating forces ranging from 1.3 to 3.1 N, and moments in the oro-vestibular direction ranging from 2.1 to 6.6 N·mm. However, some areas of the TPAs experienced von Mises stress in excess of 154–165 MPa, which could lead to the permanent deformation of the devices. Despite this, none of the TPAs showed visible deformation or cracking during in vitro tests. The researchers concluded that PEEK might be a suitable material for manufacturing orthodontic TPAs.[142]

The simulation of the tooth movement induced by orthodontic treatment can be performed with the use of FEM, which is designed to study multi-body system interactions. Orthodontic tooth movement refers to the process of moving teeth to their desired positions by using orthodontic appliances, such as braces or aligners. The process is a vital part of orthodontic treatment, and aims to correct misaligned teeth and improve the function and appearance of the patient's bite. Anh et al. simulated the mechanical behavior of a newly designed closing loop connected to the gable bends to investigate the optimal loop activation conditions to achieve the desired tooth movement during orthodontic treatment.[143] The closure loop is a commonly used system in orthodontics to close the space left after extractions, and peak bending is a type of bend in the arch that provides torque control for the anterior teeth and anchorage control for the posterior teeth. The researchers constructed a 3D model of the maxillary dentition, and used FEM to simulate the movement of both anterior and posterior teeth while varying the degree of peak bending. The results showed that using a 5° gable bend in the loop caused the lingual deviation of the crown of a central incisor and the corporeal movement of a first molar. Meanwhile, using 10° and 15° gable bends caused the corporeal movement and root movement of a central incisor and the distal deviation of a first molar. The researchers concluded that the new closing loop design, which involves reducing the thickness of the teardrop-shaped loop by 50% and using different degrees of peak bending, is effective in achieving torque control for the anterior teeth and anchorage control for the posterior teeth, and can be easily performed in a dental office.[143]

In another study on orthodontic tooth movement, researchers compared the simulated orthodontic treatment results, obtained using the Incognito™ Lite Appliance System,[144] and the actual results to assess the accuracy of the system.[145] The Incognito Lite Appliance is a lingual orthodontic system that uses CAD and CAD/CAM to create digital treatment settings. The study included 17 Angle's Class I or II malocclusion participants who received the Incognito Lite standard arch sequence. The simulation and actual results were compared using Final Surface® software and the best-fit point-to-triangle method with 1,000 reference points. The results showed that the Incognito Lite Appliance System accurately achieved the simulated tooth movement in terms of tip, torque and rotation parameters, with most discrepancies within ±3°. However, there were significant discrepancies between the simulated and actual tooth positions in the translational parameter, with a difference in position of 0.6–1.0 mm. The researchers concluded that while the Incognito Lite Appliance System was accurate in terms of tip, torque and rotational parameters, significant discrepancies in

the translational parameter might impair the treatment outcome and could be clinically significant.[145]

Assessment of mechanical properties

The field of dental implants has seen significant advancements in recent years, with the development of new materials and designs that aim to improve the osseointegration process and increase success rates. In the works summarized below, innovative approaches to dental implants, which have shown promising results in clinical trials, are presented. Work by Milone et al. compared stress distribution around the cortical bone for dental implants made of Ti and Zr, using FEM.[146] The authors found that the stress evaluated for the Zr implant was more distributed around the cortical bone, suggesting that using Zr could improve the osseointegration process and increase the lifespan of the implant.[146]

In a study by Patil et al., the use of finite element analysis (FEA) was explored to evaluate the mechanical behavior of Ti dental implants coated with graphene or graphene oxide.[147] Bones and soft tissues were modeled as homogeneous, isotropic and linearly elastic materials, and the assumption was made that the Ti implant was 100% osseointegrated. The FEA was performed using the CATIA and ANSYS Workbench software tools to analyze von Mises stress, strain and deformation at the implant and the implant–cortical bone interface. An ideal implant preload was applied to the occlusal surface of the crown, with preload values of 0.2 N·m in an anticlockwise direction and an axial load of 100 N, 150 N, 200 N, and 250 N. The results showed that the Ti implant coated with graphene oxide had better mechanical behavior than graphene, with the mean von Mises stress values of 39.64 MPa, 23.65 MPa and 37.23 MPa in pitches 1, 2 and 3, respectively (1.0 mm, 1.4 mm and 2.2 mm). The study also found that functionalizing the Ti implant helped reduce stress in the implant system. Overall, the use of FEA for solving biomechanical issues related to medical and dental devices was emphasized, and the potential for in vivo studies and practical applications was noted.[147]

Miljanovic et al. designed a mandibular implant using the Mimics® and 3-Matic software to address mandibular defects in patients suffering from trauma or disease.[148] The implant was improved by planning the positions for dental implants prior to mandibular reconstruction surgery, using the Blue Sky Plan®, v. 4, software. A surgical guide was designed in this software and 3D-printed using the SLA technology, while FEA was performed on the surgical guide with the ANSYS FEM software to evaluate its mechanical behavior during the surgery. The results showed that the surgical guide was able to withstand the forces encountered during the procedure. The proposed method significantly reduced the surgical and recovery time, increased accuracy, and provided a predictable and visualized restorative solution.[148]

Hussein evaluated the mechanical properties of a graphene-based polymer (GBP) and PEEK as materials for esthetic clasps in removable partial dentures.[149] The study included 32 latches made with the use of the CAD/CAM technology, which were subjected to retention force tests after 10,000 insertion and removal cycles and thermocycling. The deformation of the clasp arms was also measured, and the areas of stress and strain concentration were analyzed using the FEM software (ANSYS, v. 21). The results showed that PEEK had a significantly higher retention force (2.248 ±0.315 N) than GBP (2.018 ±0.298 N), with a p-value <0.001. The buckle arm strain was also significantly higher in GBP as compared to PEEK. The concentration of stress and strain were observed at the connection of the retentive arm to the minor connector and at the retentive arm clamp in both materials. Owing to the FEA of the clasp materials, it was possible to estimate maximum principal stress. The results of this study suggest that PEEK has superior mechanical properties as an esthetic bracket material as compared to GBP, and that the optimization of GBP may be necessary to improve its suitability for this application. The possibility of using calculation methods to unambiguously indicate stress distribution in a material was confirmed, which greatly facilitates the design of new materials and the assessment of their durability. Stress and strain concentration was observed in both materials, but further optimization of GBP may be necessary to improve its suitability for use as an esthetic latch material. These results may be helpful to dentists when selecting materials for esthetic clasps in removable partial dentures. Furthermore, this work highlights the potential of new materials and designs in dental implants to improve success rates and patient satisfaction.[149]

Deep learning

Deep learning is a machine learning (ML) subfield that involves the training of artificial neural networks (ANN) on large data sets to allow the network to learn and make intelligent decisions on its own. Deep learning has been successful in many applications, including image and speech recognition, natural language processing, and even games. As such, it has enabled major advances in fields such as computer vision, robotics and healthcare. Also, in the widely understood issues of dental materials, ANN have proven to be useful.[150,151]

The research of Kose et al. centered around dental biomaterials and aimed to assess the precision of ML regression models in predicting the final color of leucite-reinforced glass ceramic CAD/CAM veneer restorations.[150] The study leveraged the color space parameters within the CIELab system (the International Commission on Illumination – Commission internationale de l'éclairage), specifically L (lightness), a (green to red) and b (blue to yellow), which are fundamental for quantifying and characterizing color in this context. Leucite-reinforced glass

ceramics designed for dental use were used in different shades and thicknesses, ranging from 0.3 to 1.2 mm. Full-color measurements were taken for each sample against different background colors, such as black, white, A1, and A3. The dataset included the color difference values (CIEDE2000) and all CIELab coordinates relevant to the experimental groups. The researchers used experimental translucent resin cement and a precisely calibrated spectrophotometer for measurements. Afterward, 28 distinct regression models were applied and fine-tuned for optimal performance by considering the weights associated with the CIELab color space parameters. The findings of this study underscore the influence of various factors on the L, a and b coordinates in the CIELab color space, which collectively determine the color characteristics of dental biomaterials. It is noteworthy that the shade of the ceramic restoration was primarily affected by the type of tooth substrate, followed by the thickness of the restoration, and specific values for L, a and b. The decision tree regression model proved to be the most effective, exhibiting the lowest mean absolute error and the highest accuracy in predicting the shade of the restoration based on these CIELab coordinates. From a clinical perspective, the ML regression model presented in that study has substantial potential for dental professionals. It streamlines the forecast of the final color outcome of ceramic veneers produced using leucite-reinforced glass CAD/CAM ceramic, a material exclusively designed for dental purposes. Whether the veneers are low-translucency (LT) or high-translucency (HT), and whether they are cemented with translucent cement, this predictive tool depends on the initial color of the tooth substrate and the thickness of the ceramic material. Employing this technique enhances the predictability and precision of esthetic outcomes in dental restorations, ultimately providing advantages to both clinicians and patients in the field of dental biomaterials.[150]

With regard to Zr biomaterials, a study by Lerner et al. investigated the role of artificial intelligence (AI) in fabricating implant-supported monolithic Zr crowns (MZCs) on custom-made hybrid abutments.[151] The methodological process involved the intraoral scanning, CAD-based design, precise milling, and clinical application of hybrid abutments and provisional crowns. Artificial intelligence aided in designing the final crown with the automated margin line design. Outcome parameters covered various aspects, from the assessment of the precision of fabrication to clinical evaluations, including the marginal adaptation, interproximal contacts, occlusal harmony, chromatic integration, and survival of the MZCs. The study cohort consisted of 90 patients, predominantly female (55), with a mean age of 53.3 ±13.7 years. Over a follow-up period ranging from 6 months to 3 years, the careful evaluation of individual hybrid abutments revealed a mean deviation of 44 ±6.3 μm from the original CAD design. Notably, the MZCs achieved commendable results in terms of clinical performance, characterized by superior marginal adaptation, favorable interproximal and occlusal contacts, and harmonious chromatic integration. Crucially, the 3-year cumulative statistics indicated a 99.0% survival rate and a 91.3% success rate for the MZCs.[151]

The work by Suryawanshi and Behera focused on the evaluation of ML models used for predicting the wear of dental composite materials.[152] Dental materials degrade over time and need replacement, and resin composites are commonly used for dental restorations. In their study, the authors utilized the in vitro test results obtained from the pin-on-disk tribometer following the American Society for Testing and Materials (ASTM) standards (ASTM G99-04) to assess the performance of 3 different ML models in analyzing the wear of dental composite materials when exposed to a chewing tobacco solution. The research involved 4 distinct dental composite material samples that were immersed in a chewing tobacco solution for a specific duration, and then subjected to a wear test. Among the models under consideration there was a multi-layer perceptron (MLP), a type of ANN. The structural similarity of MLP to biological neural networks makes it versatile and applicable to a variety of tasks within the scope of ML, including regression and classification. The underlying mechanism is based on the emulation of interconnected neurons, enabling them to identify intricate patterns in data. Another important AI approach in this study is the k-nearest neighbors (KNN) algorithm. This algorithm, known for its simplicity and intuitive methodology, is useful for classifying and regressing tasks. The KNN approach is based on making predictions by using the majority class outcomes or calculating the averages from the k-nearest data points in the training dataset. The 3rd model is XGBoost – an acronym that stands for "eXtreme Gradient Boosting", an algorithm that is renowned for its effectiveness in predictive modeling situations. The XGBoost ML model stood out, as it achieved an impressive R^2 value of 0.9996, indicating a very strong predictive capability. The results suggest that XGBoost significantly outperformed the other ML approaches in predicting the wear of dental composite materials, making it a promising tool for predictive applications in the field of dentistry.[152]

Drug discovery

In the field of drug design, molecular docking is a widely used numerical approach to find the optimized protein–ligand system. The purpose of molecular docking is to find the optimal orientation and conformation of a ligand when it binds to a protein, and to predict the strength of the binding interaction. Such information can be used to assess the potential of the ligand as a drug candidate and to optimize its structure to improve its binding affinity. Molecular docking algorithms typically use a set of geometric, physical and chemical principles to predict the binding of the ligand to the protein[153] through a combination of quantum computing and molecular mechan-

ics. Quantum computing is used to predict the electronic properties of the ligand and the protein, such as electron distribution and chemical bond strength. Meanwhile, molecular mechanics calculations predict the shapes and behavior of the ligand and the protein based on their physical properties, such as size, mass and charge. These 2 types of calculations are often used together in molecular docking algorithms to provide a more accurate prediction of the ligand–protein binding. For example, PyRx is a program designed for small-molecule ligand-to-protein docking and analysis, and is commonly used in drug discovery and development to study interactions between ligands and proteins, and predict the affinity of a ligand to a target protein. In addition, PyRx can be used to optimize the positions of ligands and evaluate how ligands bind to proteins, to study the structural properties of proteins, as well as to enable the virtual screening of large libraries of compounds.

Prahasanti et al. presented an in silico study showing that HA-PMMA composites have the potential to function as dental implant biomaterials due to their advantageous mechanical, chemical and biological properties.[154] These composites not only induce osseointegration, with biocompatibility and minimal allergic reactions, but also they do not release metal ions and can be obtained from natural Indonesian resources. The 3D structure, molecular weight and identification number of the HA-PMMA complex were obtained from the PubChem database and minimized using the Open Babel software. The 3D structures of bone morphogenetic protein (BMP) 2, BMP4, BMP7, alkaline phosphatase (ALP), osteonectin, osteopontin, and osteocalcin were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) and sterilized with the use of the PyMol software to remove water molecules. Molecular docking simulations were performed using PyRx and analyzed using the Discovery Studio software to visualize the types of chemical bonds formed. The results showed that the HA-PMMA complex could increase the activity of osseointegration-related proteins such as BMP2, BMP4, BMP7, ALP, osteonectin, osteopontin, osteocalcin. The complex had the strongest binding to osteonectin and was predicted to increase the activity of ALP. Finally, the study suggested that HA-PMMA composites might be potential candidates as biomaterials for dental implants.[154]

Riaz et al. evaluated the binding affinity of 3 newly designed hydroxyquinoline compounds based on the amino acid residues at the active site of each protein and drug-like properties, including sulfanilamide, 4-amino benzoic acid and sulfanilic acid, against 5 bacterial protein targets.[155] Hydroxyquinolines have previously been shown to have various biological properties, including antibacterial activity. The researchers used molecular docking software to assess the affinity of the compounds with proteins and found that they had binding energy values ranging from –2.17 to –8.45 kcal/mol. Compounds 1 and 3 had the best binding results toward bacterial protein targets 1ZI0/3VOB and 1JIJ/4CJN, respectively, and, as the authors suggest, may serve as novel antibiotic scaffolds. The researchers conducted molecular dynamics simulations to further evaluate the potential of the compounds as bacterial inhibitors, particularly against methicillin-resistant *S. aureus*. The simulation showed that the compounds had good stability and did not form aggregates. The study identified 2 hydroxyquinoline derivatives showing a strong affinity for binding to specific proteins in certain bacterial strains. These compounds can effectively treat infections caused by these bacteria, particularly *S. aureus* and *E. coli*. The synthesis of these compounds is relatively straightforward, facilitating further research investigating their effectiveness in treating bacterial infections. It can be concluded that hydroxyquinoline compounds have the potential to develop as bacterial inhibitors and warrant further experimental studies through preclinical and clinical trials.[155]

Opportunities and challenges

A few main limitations regard all types of biomaterials in dentistry. They are associated with the biocompatibility of biomaterials, their degradation in the complex oral environment, and features that affect their cost and esthetics.

Biomaterials in dentistry are exposed to the aggressive oral environment, changes in saliva pH and flow, and various forms of gingival inflammation due to an erosive diet, rich in sugars and acids, or stress-related diseases, such as anorexia or bulimia nervosa. A highly acidic oral environment is also related to gastroesophageal reflux disease (GERD), which is becoming increasingly common in the young population due to an improper diet, severe vitamin D deficiency, obesity, and food allergies.[156] These factors contribute to the loss of the mineralized tooth structure, early caries, bacterial infections, and the loss of dental tissues.

Restorative biomaterials still suffer from signs of aging, such as marginal or surface discoloration, chipping, and changes in shape and transparency. Palacios et al. examined changes in the mechanical properties of 3 commercial resin composites immersed in artificial saliva.[157] After 30 days of incubation, the flexural strength, fracture toughness, hardness, and modulus of elasticity of the tested composites were significantly reduced.[157] Similar results were reported by Kielbassa et al., with the authors showing that high-viscosity GIC and a glass hybrid restorative system do not provide long-term protection against abrasive wear.[158]

Over the last decade, scientists have made a considerable effort to enhance the mechanical properties of biomaterials and their stability in complex media without compromising their biocompatibility. Their research

regards mainly the chemical composition of restorative materials and advanced modification techniques.[159,160] Nowadays, nanotechnology plays an essential role in developing advanced restorative biomaterials and their further improvement.[161] The application of nanomaterials in dentistry includes dental diagnostics, preventive dentistry, prosthodontics, endodontics, periodontics, implantology, and regenerative dentistry. They are used as NPs, nanocomposites, nanofibers, or implant nanocoatings. Nano-enhanced biomaterials display excellent antimicrobial activity and improved mechanical properties, especially resin composites enriched with Ag NPs, ZnO NPs or copper (Cu) NPs.[162] However, the number of in vitro and in vivo studies confirming these positive effects is limited. Additionally, only a few long-term clinical studies indicate resin composites can be used for the restoration of the posterior teeth with long-lasting durability.[163–165]

Another critical limitation of advanced dental materials is preventing the formation of salivary protein coating on the surfaces of biomaterials. Salivary proteins attach to the material surface and serve as adhesion substrates for the colonizing bacteria, reducing the bacteriostatic activity of biomaterials.[161] Improving biomaterials to achieve protein-repellent surfaces is a great challenge for material engineering, with in vitro studies indicating that the adsorption of saliva proteins on the surfaces of biomaterials is mainly determined by the surface characteristics. The lowest protein adsorption is observed for hydrophobic and negatively charged coatings. However, some studies show that the adsorption of proteins cannot be linked to the physicochemical properties of the surface. The negatively charged glycoproteins mucin-7 (MUC7) and zinc alpha 2-glycoprotein (ZAG) adsorb not only to positively charged surfaces, but also to the silica- and PEG-modified biomaterials, which are hydrophilic and exhibit negative electrokinetic potential. Scientists suggest that this effect can be explained by the unique and complex interactions between the proteins involved in salivary micelles, which are formed due to the accumulation of MUC7, lactoferrin and immunoglobulin A, and positively charged lysozyme, which mainly occurs as a single component. The proteins attach to the surface of the biomaterial through a multitude of physicochemical interactions to maintain homeostasis in the complex oral environment.[166,167]

In recent years, scientists have faced the emerging problem of monomer cytotoxicity due to incomplete composite resin polymerization reactions. The unreacted monomers remain in the polymer matrix and may be released into saliva, accumulating in the surrounding tissues. It should be pointed out that the unreacted monomers deteriorate the stability of polymer fillings, which results in their shorter clinical lifetime. Developing new resin monomer technology based on silorane and UDMA resins is an important area of dentistry research.[168,169] These new materials ensure low shrinkage stress at the tooth–biomaterial interface, and minimize contraction gaps, micro-leakage and the recurrence of caries. Although modern biomaterials are promising, more in vivo and clinical studies are urgently needed to investigate their biological advantages and drawbacks in detail (Fig. 4).

Developing modern dental materials should be supported by computational techniques to evaluate their properties, predominantly mechanical features. Such an approach can facilitate the introduction of new biomaterials to the market by reducing the number of complicated, tedious and expensive experiments. In this regard, more cooperation between dentists and engineers is needed to ensure the development of the optimal solutions (Fig. 4). Furthermore, advanced treatment methods in dentistry should include personalized strategies to design biomaterial-drug combinations specific to each individual's health state. Personalized dental biomaterials could be enhanced based on data acquisition at the individual level, including saliva composition and saliva biomarker levels.[170,171]

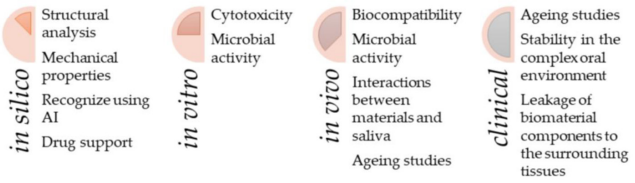

Fig. 4. Development of dental materials – necessary research steps

Conclusions

This paper summarizes the most important biomaterials and emerging modification strategies that improve their biointegration, biological activity, mechanical properties, and resistance to the harsh oral environment.

Although biomaterials are widely used, a great deal of research is still being conducted to improve their long-term use and introduce modifications to support dental tissue regeneration. Dental biomaterials are also expected to prevent bacterial infections and effectively inhibit material corrosion in saliva. However, restorative biomaterials are still susceptible to bacterial attachment and biofilm formation.

Another aspect addressed in recent literature reports is the improvement of the mechanical properties and esthetics of restorative materials. The surfaces of biomaterials are usually modified with polymers or nanomaterials to reduce friction while maintaining biocompatibility.

All modern biomaterials are promising; however, there is an urgent need for more in vivo and clinical studies to investigate their biological advantages and disadvantages in detail. The computational techniques used to assess the properties of modern dental materials, particularly the mechanical ones, could assist in the development of the materials. Such an approach can help bring new biomaterials to the market by reducing complicated, tedious and expensive experimentation.

Ethics approval and consent to participate

Not applicable.

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

ORCID iDs

Marta Joanna Woźniak-Budych https://orcid.org/0000-0002-7694-9023
Maciej Staszak https://orcid.org/0000-0002-7602-7803
Katarzyna Staszak https://orcid.org/0000-0003-1335-4791

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