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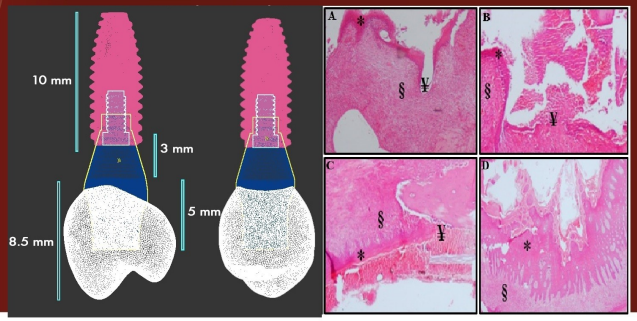


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*INDEXING*

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***ORIGINAL RESEARCH***

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**Original research**

# EVALUATION OF THE EFFECT OF SURGICALLY ASSISTED RAPID MAXILLARY EXPANSION ON TEMPOROMANDIBULAR JOINT DISC POSITION WITH MAGNETIC RESONANCE IMAGING*

## ABSTRACT

**Objective:** To evaluate, by magnetic resonance ımaging (MRI), the effects of surgically assisted rapid maxillary expansion (SARME) on the temporomandibular joint (TMJ) disc position.

**Methods:** Patients with maxillary transversial discrepeancies treated SARME analyzed prospectively. The magnetic resonance imaging assessments of the TMJ were obtained before SARME operation and after expansion process. Retention period, gender and presence of wisdom teeth were the predictor variables. Disc position index (DPI) values were calculated and analyzed as an outcome variable.

**Results:** The study included 13 subjects (4 male, 9 female) with a mean age of 19.5±2.3 years. After treatment there was excess changing position seen in three articular disc relative the condyle in three patient. Retention period, gender and presence of wisdom teeth were not significantly effected TMJ disc in terms of DPI values in mouth opened or closed position (p>0.05).

**Conclusion:** According to our study TMJ disc position was not effected significantly by SARME (p>0.05).

**Keywords:** Surgically assisted rapid maxillary expansion, Temporomandibular joint, Magnetic resonance imaging.

*This study was previously presented as an oral presentation at AÇBİD 13th International Congress with EACMFS Endorsement held in Antalya, Turkey on April 26, 2019.

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## INTRODUCTION

Transverse discrepancies are common problems in adolescents and adults with orthodontic disorders. Transverse maxillary discrepancies can be skeletal and/or dental, and clinically, they are seen as a unilateral or bilateral posterior cross-bite.[1,2] A surgically assisted rapid maxillary expansion (SARME) is the preferred treatment for transverse maxillary discrepancies in individuals with completed growth who have transverse discrepancies of more than 5 mm.[2] Whether or not a morphological or functional interaction on the temporomandibular joint (TMJ), either directly or indirectly, is caused by a surgical intervention to change the position of the jaws is a suggestive parameter.

Magnetic resonance imaging (MRI) is the gold standart to determine the chewing muscles, morphology, disc position and pathologies, bone structures, retrodiscal tissues, posterior attachment, inflammatory diseases of the joint (and the soft tissue changes caused by them), and any postoperative changes can be determined. Previous cadaver studies have indicated that MRI exhibited 95% accuracy in the evaluation of the disc location and disc form, and 93% accuracy in the evaluation of the structural changes of the bone.[3] The standard TMJ MRI protocol contains parallel, oblique, coronal, and oblique sagittal images parallel to the long axis of the condylar head. The sagittal images should be obtained with the mouth in both closed and open positions in order to determine the disc movements.

A systematic review[4] evaluating conventional rapid maxillary expansion in growing patients showed that there is no change in the position and shape of the articular disc immediately after expansion. However, there is no evidence available on the subject when using, in adults, the rapid expansion of the surgically assisted maxilla.

The aim of this study was to evaluate, by MRI, the effects of the SARME on the TMJ disc position. We hypoestesized that occlusal changes after SARME could effect the TMJ and disc. Spesifically gender, wisdom tooth presence and retention period could rate the effectivity.

## METHODS

The subjects included in this study were selected from the patients who presented to the Orthodontics Department of the Faculty of Dentistry at Suleyman Demirel University for orthodontic treatment between 2016 and 2018. Ethical approval was obtained from the local Ethics Committee (Date/Number: 04.11.2015/ 212).

The patient inclusion criteria were as follows:

- Skeletal transverse maxillary discrepancy with a unilateral or bilateral cross-bite.
- No congenital craniofacial deformities (cleft palate, syndromes, etc.).
- No surgical procedures performed in the upper or lower jaws.
- Non-growing patients with bone maturation evidence by hand and wrist radiography.
- The patient exclusion criteria were as follows:
- Lack of pre-treatment and/or post-treatment records.
- Closed-field phobia.
- Artifacts in the MRI images that prevent disc evaluations.

All of the patients had appropriate skeletal development based on the results of hand-wrist films, skeletal transverse maxillary discrepancies and surgical maxillomandibular transverse difference index values (>5mm), as measured in postero-anterior radiographs. For the maxillary expansion in each of the patients, a dental support device with a Hyrax screw was placed parallel to the midline by the orthodontist one or a few days before the operation. It was cemented with glass ionomer cement via banding to the first premolar and first molar teeth.

*Surgery and Expansion Protocol*

In order to ensure standardization, all of the operations were performed under general anesthesia according to the asepsis and antisepsis rules using the same technique by the same team in the Maxillofacial Surgery Department of Suleyman Demirel University. All of the patients underwent bilateral Le Fort I osteotomies with piezoelectric surgeries. Midpalatal suture separations were conducted in all of the patients without pterygomaxillary osteotomies. In all of

the patients, the Hyrax was activated until a 1 mm midline diastema was achieved in order to ensure symmetrical bone expansion. No complications were seen in any of the patients.

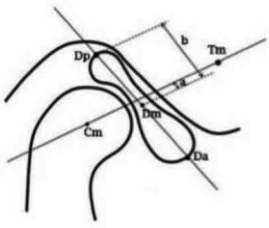
After a latency period of 5 days, the expansions were performed at a rate of 0.5 mm/day for 21 days, with no over corrections. Then, the appliance was stabilized for 4 or 5 months (retention period).

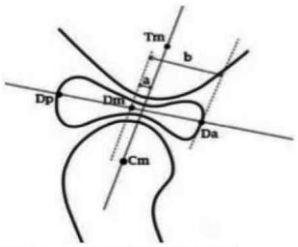
*Radiological Evaluation*

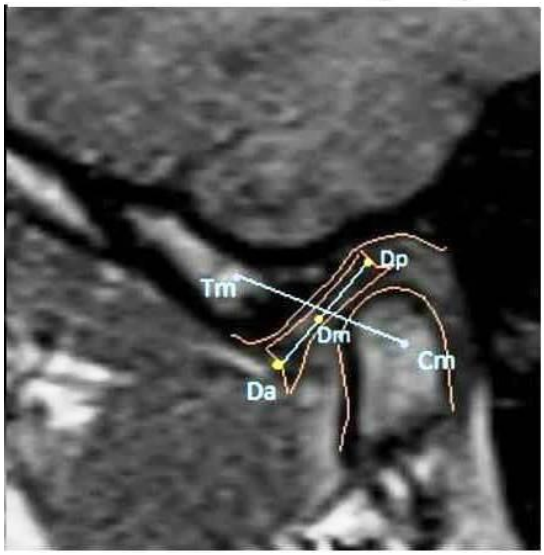
All of the MRI images were evaluated by 2 specialist radiologists and the image evaluations were based on the criteria of Tasaki and Westesson (3). In the sagittal plane, with a mouth closed position, the TMJ disc position was considered to be normal if the posterior band was located at the between 11 and 12 o'clock relative to the condyle. After that, the numerical measurements were performed using the same slice thickness that was used with the disc position index (DPI).

*DPI*

The articular disc position was assessed using a modification of the method used by Vargas Pereira.[5] The following reference points and measuring variables were used (Figure 1):



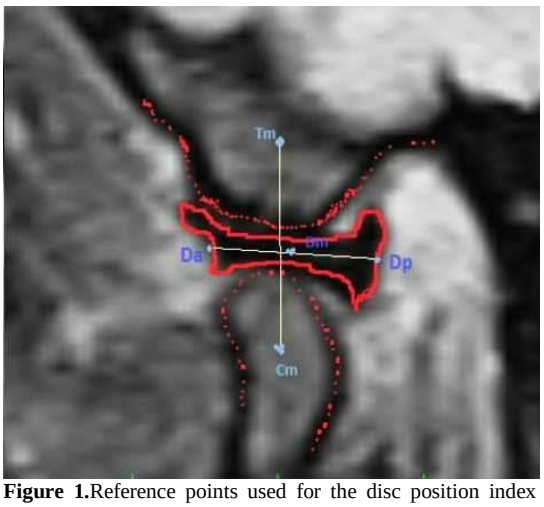




**Figure 1.**Reference points used for the disc position index in the mouth closed and mouth open positions in MRI.

Cm: Midpoint of the mandibular condylar head (as assessed by visual inspection).

Tm: Midpoint of the tuberculum articulare (as assessed by visual inspection).

Da: Anterior point of the articular disc.

Dp: Posterior point of the articular disc.

Dm: Midpoint of the articular disc (the midpoint of line Da-Dp).

a: Position of the articular disc as defined by the distance from the Dm (on line Da-Dp) to line Cm-Tm.

b: Half the length of the articular disc as defined by the distance (Da-Dp).

For the final assessment of the disc position, the disc position index was calculated as follows:(a:b) × 100.

The DPI describes the position of the articular disc in relation to the mandibular condyle and the temporal articular eminence. In a centered disc position, the midpoint of the disc (Dm) is on line Cm-Tm, and distance a is zero. In a protrusive disc position, the Dm is in front of line Cm-Tm, and distance a is positive. In a retrusive disc position, the Dm is behind line Cm-Tm, and distance a is negative.[6]

In order to standardize the DPIs of all of the TMJs in the mouth open and mouth closed positions, the measurements were performed 3 times by two radiologists, with one surgeon observing, and the means of the DPI measurements were used.

*Variables*

The predictor variable was the retention period, gender and presence of wisdom teeth. TMJ disc position change values after SARME evaluated by DPI was the outcome variable. of the study. Demographical and clinical data of the patients were obtained from the forms collected in patients files.

*Statistical Evaluation*

Data were analyzed using IBM SPSS Statistics for Windows (version 21.0; IBM Corp., Armonk, NY, USA. For the DPI, the arithmetic mean and the standard deviation were calculated. The Mann-Whitney U test was used for the comparisons between two groups, and the Kruskal-Wallis H test was used for the comparisons between four groups. The level of significance was set to $p<0.05$.

## RESULTS

13 patients included in this study (9 females and 4 males), and all of them were older than 17 years. All of the patients had bilateral cross-bites and no history of bruxism. 6 patient's wisdom third molars are absent and remaining 7 was not extracted in SARME operation. In terms of retention period 8 patient was consolidated 4 months whereas 5 was consolidated 5 months to prevent relapse.

After evaluating the MRI images, it was determined that 4 patients had anterior disc displacements before the treatment. However, after the expansion treatment, there were disc position changes in only three patients (Table 1).

**Table 1.** Preoperative and post retention measurements in the mouth open and mouth closed positions.

| Patient | Mouth closed | | | | Mouth open | | | |
|---|---|---|---|---|---|---|---|---|
| | Preoperative | | Post-retention | | Preoperative | | Post-retention | |
| | Right | Left | Right | Left | Right | Left | Right | Left |
| 1* | **+191.6** | -19.6 | **+22.5** | -19 | +6 | -13.3 | 0 | -9.1 |
| 2 | -13.9 | +0.4 | -13.9 | +0.4 | -9.3 | 0 | -15.9 | +0.6 |
| 3 | -2.5 | -2.9 | -5.9 | 0 | -6.6 | 0 | -6.2 | 0 |
| 4 | +3 | +2 | +4 | 0 | 0 | 0 | +5.1 | +4 |
| 5 | +3 | +6.3 | -7 | +5 | 0 | 0 | -6.4 | +5.2 |
| 6 | 0 | 0 | 0 | -7.9 | -7.1 | -0.1 | -5.3 | 0 |
| 7* | 0 | **-1** | +6.2 | **+56.25** | +6.9 | +4.3 | +2.5 | -2.3 |
| 8 | +156.9 | +0.4 | +156.1 | +0.4 | +156.7 | -8.9 | +149.2 | -9.3 |
| 9* | +120 | **+6.5** | +123.6 | **+116.6** | 128.8 | -6.7 | +128.8 | -1.8 |
| 10 | 0 | +0.2 | 0 | +0.2 | 0 | +5.8 | -7.5 | +9.4 |
| 11 | +9 | +4.1 | +7.1 | +1 | -6.6 | -0.1 | 0 | +1.7 |
| 12 | -4,7 | +100 | -11,3 | +100 | +0,1 | 0 | 0 | +6,6 |
| 13 | -3.7 | -5.4 | +4.2 | +3.5 | 0 | 0 | +2.3 | +1.7 |

***** Disc position was changed in three patients and values was shown as bold.

There were no significant differences between the gender, retention periods and wisdom tooth extractions in terms of value changes between the right and left TMJ DPI values in the mouth open and mouth closed positions ($p>0.05$) (Table 2-4).

**Table 2.** Left and right differences according to gender (Sd:Standart deviation).

| Gender | | Group | | Kruskal-Wallis H test | | |
|---|---|---|---|---|---|---|
| | | n | Mean±Sd | Average | H | P |
| Right % difference | Mouth closed-female | 9 | 22±55.3 | 13.61 | 0.264 | 0.967 |
| | Mouth closed-male | 4 | 3.1±2.3 | 11.75 | | |
| | Mouth open-female | 9 | 4.2±3.2 | 14.06 | | |
| | Mouth open – male | 4 | 4.1±2.6 | 13.75 | | |
| Left % difference | Mouth closed-female | 9 | 14.31±36.09 | 11.5 | 1.4 | 0.704 |
| | Mouth closed-male | 4 | 16.31±2.73 | 16.75 | | |
| | Mouth open-female | 9 | 3.03±2.39 | 14.11 | | |
| | Mouth open- male | 4 | 3.1±2.85 | 13.38 | | |

**Table 3.** Left and right differences according to the retention period (Sd:Standart deviation).

| Retention period distribution | | Group | | Kruskal-Wallis H test | | |
|---|---|---|---|---|---|---|
| | | n | Mean±Sd | Average | H | p |
| Right % difference | Mouth closed-4 months | 8 | 23±59.1 | 10.69 | 3.7 | 0.291 |
| | Mouth closed-5 months | 5 | 5.2±2.4 | 16.8 | | |
| | Mouth open-4 months | 8 | 5.2±2.7 | 16.13 | | |
| | Mouth open-5 months | 5 | 2.7±2.8 | 10.5 | | |
| Left % difference | Mouth closed-4 months | 8 | 1.84±2.67 | 9.69 | 5.6 | 0.131 |
| | Mouth closed-5 months | 5 | 35.87±47.58 | 18.6 | | |
| | Mouth open-4 months | 8 | 2.26±2.18 | 11.94 | | |
| | Mouth open-5 months | 5 | 4.32±2.45 | 17 | | |

**Table 4.** Left and right differences according to the wisdom tooth status (Sd:Standart deviation).

| Wisdom tooth | | Group | | Kruskal-Wallis H test | | |
|---|---|---|---|---|---|---|
| | | n | Mean±Sd | Average | H | p |
| Right % difference | Mouth closed-no tooth | 6 | 30.3±68.1 | 12.17 | 3.3 | 0.339 |
| | Mouth closed-wisdom tooth | 7 | 4.1±3.0 | 13.79 | | |
| | Mouth open-no tooth | 6 | 6±2.1 | 18 | | |
| | Mouth open-wisdom tooth | 7 | 2.7±2.7 | 10.5 | | |
| Left % difference | Mouth closed-no tooth | 6 | 2.15±3.04 | 10.42 | 1.5 | 0.668 |
| | Mouth closed-wisdom tooth | 7 | 25.88±42.43 | 15.43 | | |
| | Mouth open-no tooth | 6 | 2.55±2.10 | 13.17 | | |
| | Mouth open-wisdom tooth | 7 | 3.49±2.75 | 14.5 | | |

## DISCUSSION

Although skeletal maturation is completed earlier in female patients, skeletal development continues chronologically in male patients for a while longer. Therefore, rapid maxillary expansion treatments in male patients can be continued at a later age when compared to female patients.[2] In the literature, the incidence of TMJ disorders is reported to be higher in females[7] In our study most of the patients were female however be based on gender distribution TMJ disc position was not changed statistically significant in terms of DPI values ($p>0.05$).

Many researchers have reported that their patients had TMJ disorder signs and symptoms of various degrees before undergoing orthognathic surgery. Laskin *et al.*[8] reported that 14% of the patients undergoing orthognathic surgery had symptoms and signs of TMJ disorders. Kerstens *et al* [9] reported that of 480 patients with dentofacial deformities, 16% had TMJ disorder symptoms before surgery. However, Link and Nickerson found the incidence of TMJ internal irregularities to be very high (97%) in their orthognathic surgery population.[10] In our study, it was determined that 4 of the 13 patients already had anterior disc displacement in their TMJs before treatment. However, based on the number of patients in our study, in order to determine the TMJ internal irregularity rate in patients with transverse maxillary discrepancies, we suggest that a multi-sample study be performed to reveal the statistical findings.

Studies investigating the effects of orthognathic surgery on the TMJ disc have been performed mainly on sagittal split ramus osteotomy (SSRO), vertical ramus osteotomy, and double jaw osteotomy procedures that were combined with Le Fort I osteotomies. In their study of patients undergoing intraoral vertical ramus osteotomy (IVRO) or SSRO procedures with or without the combined use of a Le Fort I procedure, Ueki *et al.* reported that the mandibular condyles were displaced in the anterio-inferior direction after the osteotomy in the patients undergoing IVROs. In the MRI examinations of the TMJ discs, they found that the discs with normal positions were not affected, but the discs with anterior displacement had significantly improved. In the patients with mandibular prognathism who underwent SSROs, no significant changes were observed in the TMJ disc positions according to the MRI findings 6 months after the orthognathic surgery, but the TMJ symptoms had improved.[11-13] In addition to the changes in the three-dimensional positions of

the jaws in space, we can see that the bad interdigitation became good interdigitations after the orthognathic surgery. Consequently, this condition led to an improvement in the TMJ symptoms due to occlusal healing, and thus, a favorable condition in terms of intra-TMJ irregularities. However, interdigitation is present after a SARME is impaired, and this leads to interference, which may affect the TMJ during functional movements, such as the occlusal morphology, chewing, and biting. However, when we considered our findings, we observed that the SARME had no significant effects on the TMJ disc position within a certain period of time.

The clockwise rotation of the mandible is a major effect of SARME on the mandible, although there is no consensus about the amount or stability of this change.[14,15-18] Altug-Atac *et al.*[14] and Gunbay *et al.*[16] reported a clockwise rotation of the mandible after SARME, while Iodice *et al.*[17] and Parhiz *et al.*[19] reported no significant change in the rotational movement of the mandible. These changes are presented with the downward rotation of the menton point. In fact, there are differences in the time intervals for the evaluations of the mandibular rotations in these studies. However, according to the results, if the mandibular rotation is based on the tendency to go back to the preoperative position 6 months after the SARME, the motion is a temporary movement.[18] The methodological differences between these studies and evaluations at the different time points confirm the differences in their mandibular rotation findings.

In our study, according to the retention period (4 or 5 months) there were no significant differences in terms of the DPI values in mouth opened or closed position ($p>0.05$). These periods correspond to the time during which the mandible moves to its original position. Therefore, taking this parameter into consideration, in another study, the mandibular rotation can be confirmed using cephalometric films, and the MRI images can be taken at an earlier time following the expansion; then, the disc position evaluations can be performed.

Some of the factors that may affect the mandibular position, functional forces, and occlusal relationship are the shape, extent, and extent of enlargement of the maxillary segments. Kılıç *et al.*[20] stated that the tipping of the alveolar segments was higher and the decrease in the palate depth was greater in the group without pterygoid separation. Ferraro *et al.*[21] examined the effects of pterygoid separation in a study using cone beam computed tomography, and they reported that the same expansion amount was obtained with or without pterygoid separation. The expansion was greater in the molar region in both groups, but the dentoalveolar tipping amount was greater if no pterygoid separation was performed. In our study, a non invasive technique without pterygoid separation was used. Therefore, there was a more rotational opening and occlusal template change. It is also possible to say the opposite. The effects of a pterygoid separation on the occlusal morphology or the movement of the mandible, indirectly, on the TMJ components can be considered in a different study.

In the literature, TMJ disease has been shown as a complication caused by the surgical extraction of the wisdom teeth, and there are different studies on this subject. In 2002, Huang *et al.*[7] examined the risk factors causing painful TMJ disorders, and they collected these factors under subgroups, such as bruxism, facial trauma, third molar extraction, orthodontic treatments, frequent dental treatments, female gender, depression, and psychogenic disorders. When opening the mouth, the ligaments forming the TMJ are stretched considerably. Traumatic factors, such as intubation, wisdom tooth extraction, and long-lasting dental treatments can also cause the ligaments to be affected, with the disc motion becoming irregular and joint dysfunction starting. In our study, we divided the patients into two groups, those who underwent wisdom tooth extraction surgery before the SARME and those who did not. Thus, in this procedure, which can be considered to be a macro-trauma in terms of the TMJ, we tried to see the effects of the TMJ disc on the MRI findings after the upper jaw expansion. However, according to the wisdom tooth presence in SARME no significant

difference was found in terms of DPI values ($p>0.05$).

## CONCLUSIONS

Be based on the small sample size used in our study, SARME was not effected the TMJ disc position. Gender, retention period, and wisdom tooth presence are not effected the TMJ disc position in terms of DPI's after SARME. There were no complaints of bruxism based on the clinical histories of the patients. However, no objective diagnosis of bruxism was obtained. For this reason, large sample studies are needed to compare the disc change incidences according to the morphological differences in the MRI images. The samples should also be grouped according to the different surgical techniques.

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**Original research**

# EFFECT OF TOPICAL HUMIC ACID ON EXCISIONAL PALATAL WOUND HEALING: A HISTOPATHOLOGICAL AND HISTOMORPHOMETRIC STUDY IN RATS

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## ABSTRACT

**Objective:** The purpose of the present study was to examine the effects of topical humic acid application on healing in oral mucosa wound in rats.

**Material and Methods:** A total of 12-week-old 72 Wistar male rats weighing 280-300 gr were used in the study. The rats were randomly grouped in 4 groups as the Control Group (K) to which no applications were made, Chlorhexidine (0.12%) Group (CHX), 80 mg/kg Humic Acid Group (HA80), and 150 mg/kg Humic Acid Group (HA150). Mucosal defects of 5-mm-diameter were induced with punch in the palatal areas of the rats. These groups were further divided into 3 sub-groups to be sacrificed on days 7, 14 and 21. Epithelization, ulceration, polymorphic nuclear leukocytes (PNL), mononuclear cells (MNL), fibroblast and vascularization were examined in histopathologic evaluations. In addition, photos of the tissue samples were taken and transferred to the computer medium for histo-morphometric examinations.

**Results:** As a result of the statistical analyses, no significant differences were detected among the groups in terms of epithelization degree, PNL and MNL cell infiltration on days 7, 14 and 21. The ulcerated areas were low in HA150 Group compared to the other groups, and there was a significant difference in this respect ($p<0.05$). Vascularization degrees were evaluated, the K Group and HA150 Group showed better results on day 7 ($p<0.05$). On days 14 and 21, no significant differences were detected among the groups ($p>0.05$). Wound area measurement scores were lower in HA150 Group compared to the other groups, and this result showed that the healing in HA150 Group was better ($p<0.05$).

**Conclusions:** As a result of the present study, it was found that humic acid increased wound healing in oral cavity.

**Keywords:** Wound, palatal mucosa, chlorhexidine, humic acid.

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## INTRODUCTION

Wound healing is characterized by coming together of epithelial, endothelial, inflammatory cells, platelets and fibroblasts, and performing their normal functions in a certain order. Wounds in the mouth that occur due to physical, chemical, surgical or microscopic reasons can negatively affect the vital functions, work and social life, nutrition, and breathing of people. For this reason, it is desired that such wounds heal as soon as possible.[1] Previous researchers have worked on materials and techniques that would accelerate the healing process for many years, and examined the effects of different wound healing agents on different types of wounds.[2-4] In addition, many agents have been used to decrease post-operative complications and to accelerate oral wound healing. Topical antimicrobials are recommended because of these effects.[5-8]

Humic substances, which constitute one of the largest carbon reserves in the nature, consist of many different sources like lignite, peat, live plants, algae, etc. In medicine, humic substances were used for different purposes against different diseases 3.000 years ago.[9] The idea of using humic substances in pharmaceutical industry emerged because of their antiviral activities, and anti-inflammatory and proinflammatory characteristics, effects on blood coagulation and fibrinolysis, estrogenic activity, antibacterial, antiallergic, and antiulcerogenic properties.[9] İn the structure of humic acids; there are molecules of various components such as amino acids, lignins, pectins or carbohydrates.[10,11] The main elements that make up the structure of humic acid are carbon, hydrogen, oxygen, nitrogen and sulfur (C, H, O, N and S).[12]

Previous studies showed that humic acid does not have any toxicity.[13-18] In an experimental bone fracture study, it was determined that osteoid formation and mineralization were accelerated with humic acid application during the first week after fracture, while osteoid formation and mineralization were significantly reduced when humic acid treatment was delayed to the second week.[19] Another study on bone fractures in children found that humic acid has a positive effect on bone regeneration.[20] In a study by Derre *et al.*[21], experimentally induced herpes infection in the mouse ear was treated by applying a topical humic acid-derived substance, and it was concluded that the humic acid-derived agent significantly reduced or completely suppressed the infection. Vucskits *et al.*[22] In their study in rats, they stated that the humic acid diet both increases the immune response and prolongs the immune response time. Ji *et al.*[23] conducted a study on rats, and showed that humic acid accelerates the healing of wounds on the surface of the skin. Calisir *et al.*[24] reported that humic acid was effective in closing wounds on the palatal mucosa in rats. Although these results suggest that humic acids may be useful in wound healing in palatal mucosa, there is currently not enough evidence showing the effects of humic acid on wound healing in oral cavity. The purpose of this study was to evaluate the effects of humic acid on the healing of excisional wounds in the palatal mucosa of rats.

## MATERIALS AND METHODS

Approval was obtained from Sivas Cumhuriyet University, Animal Experiments Ethics Board on 01.02.2018 and with number 136 for the present study. As subjects, 12-week-old 72 Wistar male rats with an average weight of 280-300 g were used. The rats in each group were fed in separate cages under the same conditions. All rats were fed with standard feed and water, observing 12-hour-night/day cycle at 21±1ºC temperature and 40-60% humidity. The rats were kept in metal cages for 10 days to adjust to new living conditions before the study commenced. The experimental stages of the present study were conducted at Sivas Cumhuriyet University, Medical Faculty Animal Laboratory.

### *Surgical Method*

The rats were anesthetized by injecting 30 mg/kg Ketamine-HCL (Ketalar, Eczacibasi, Turkey) and 5 mg/kg Xylazine HCL (Rompun, Bayer, Germany) before creating the wounds in veterinary control. Five-mm-diameter mucosal defects were created in the rats in the palatal area. A round and stainless steel punch was used in this process. After hemorrhage control, the wounds were left to

secondary recovery. The first day when the wounds were created was recorded as day 0.

***Preparation and Application of Humic Acid***

Considering the weight of the rats, appropriate concentrations of humic acid was prepared for the rats in each group. The amount of humic acid (0.5 cc) was applied topically with a blunt-tip injector.

***Creation of Groups***

The rats were randomly selected and divided into 4 main groups as the Control Group (K) (n=18), Chlorhexidine Gluconate Group (0.12%) (CHX) (n=18), 80 mg/kg Humic Acid Group (HA80) (n=18), and 150 mg/kg Humic Acid Group (HA150) (n=18). After creating wounds in the palatal areas of the rats, the rats were divided further into 3 sub-groups to be sacrificed on days 7, 14 and 21.

***Histological evaluation***

Full-layer samples were taken from the wounds created in the palatal areas in all groups on days 7, 14 and 21 including 1x1 cm of intact tissue. These tissue samples underwent routine tissue follow-up procedures in the Department of Pathology of Sivas Cumhuriyet University, Faculty of Medicine. The samples taken from the rats with biopsies were fixed for 48 hours in 10% formalin solution for light microscope examination, and were blocked by using routine paraffin blocking method. The slides were prepared by taking three 3-4-μm sections from the blocks with microtome (Leica D80 LX, Germany). The slides were then evaluated in the light microscope with Hematoxylin Eosin Staining to observe inflammatory changes and to show morphology in healed wounds.

Histopathological examination was performed by the same pathologist who was blinded about the groups. In histopathological evaluation, Epithelization and Ulceration were evaluated as Yes (1) and No (0), and Polymorphic Nuclear Leukocytes (PNL), Mononuclear Cells (MNL), Fibroblast and Vascularization degrees were rated as No (0), Low (1), Moderate (2), or Severe (3).

***Photographical evaluation***

The course of healing in all the defects on days 7, 14 and 21 was photographed (Canon EOS 1000D, Tokyo/Japan) after the tissues taken from the palatal areas of the rats were fixed under x8 magnification on stereomicroscope (Carl Zeiss Stemi DV4, Germany). After the photographs were taken, the images were transferred to the computer medium. The measurements of the wound surface areas were performed by using the ImageJ and NIH Image Software, National Institutes of Health, Bethesda, Md. program.

***Statistical Method***

The data of the study were uploaded to the SPSS (Ver 22.0) program; and since the parametric test assumptions were met in the evaluation of the data, the Kolmogorov - Simirnov Test was used. Then, variance analysis was made. When measurements obtained from more than two independent groups were compared, and when the difference between the groups was found to be significant as a result of the analyses, the Tukey Test was used to find the group(s) that differed. When the scores at different times were compared, the Kruskall-Wallis test was used. The Man-Whitney U test was employed when the difference was found to be significant as a result of the analyses to find the group that differed. The Chi-Square test was used in the evaluation of the qualitative data obtained with numbers, and the error level was taken as 0.05.

**RESULTS**

No significant differences were found in terms of epithelization level, PNL and MNL cell infiltration and fibroblast values among the groups on days 7, 14 and 21. In terms of ulcerated areas, the difference between HA150 Group and other groups on days 7 and 14 was statistically significant ($p<0.05$). Ulceration was lower in HA150 Group than in any other group. On day 21, the difference was statistically significant between HA150 Group and K and CHX Group, and between HA80 Group and K and CHX Group ($p<0.05$); however, there was no statistically significant difference between HA80 Group and HA150 Group. When vascularization values were evaluated, it was found that the vascularization values were higher at statistically significant level on day 7 in Group K and HA15 Group, compared

to the CHX and HA80 Group ($p<0.05$). On days 14 and 21, the degree of vascularization was at a similar level in all groups (Table 1).

**Table 1:** Statistical analysis results of histopathologic variables

| | | K Group (n=6) | CHX Group (n=6) | HA80 Group (n=6) | HA150 Group (n=6) | p |
|---|---|---|---|---|---|---|
| Day 7 | Epithelization | 0.66±.51 | 1.00±.00 | 1.00±.00 | 1.00±.00 | 0.223 |
| | Ulceration | 1.00±.00 | 0.50±.54[a] | 0.83±.40 | 0.16±.40[b] | 0.016* |
| | MNL | 1.50±.54 | 2.00±.89 | 2,66±.81 | 2.33±.88 | 0.088 |
| | PNL | 1.50±.54 | 1.33±1.03 | 2.66±.81 | 2.16±.98 | 0.066 |
| | Fibroblast | 3.00±.00 | 2.00±.89 | 2.00±.75 | 2.16±.98 | 0.537 |
| | Vascularization | 2.00±.00 | 1.33.75 | 1.33±.75 | 2.00±.00 | 0.227 |
| Day 14 | Epithelization | 0.66±.51 | 1.00±.00 | 1.00±.00 | 1.00±.00 | 0.223 |
| | Ulceration | 0.83±.40 | 0.83±0.40 | 0.66±.57 | 0.00±.00[c] | 0.014* |
| | MNL | 1.50±.54 | 1.66±.81 | 1.50±.54 | 2.33±.81 | 0.211 |
| | PNL | 2.00±.63 | 1.33±.51 | 1.50±.54 | 2.33±.81 | 0.121 |
| | Fibroblast | 1.54±.54 | 2.00±.63 | 2.00±.63 | 2.16±.75 | 0.321 |
| | Vascularization | 1.83±.40 | 1.50±.54 | 1.66±.51 | 1.83±.75 | 0,695 |
| Day 21 | Epithelization | 0.83±.40 | 1.00±.00 | 1.00±.00 | 1.00±.00 | 0.406 |
| | Ulceration | 0.67±.57 | 0.50±.54 | 0.16±.40[d] | 0.00±.00[e] | 0.002* |
| | MNL | 1.50±.54 | 1.00±.00 | 1.00±.00 | 1.16±.40 | 0.075 |
| | PNL | 1.16±.51 | 1.00±.00 | 1.00±.00 | 1.00±.63 | 0.052 |
| | Fibroblast | 2.00±.89 | 1.66±.51 | 2.00±.00 | 2.00±.89 | 0.771 |
| | Vascularization | 1.16±.40 | 1.33±.75 | 1.66±.51 | 1.66±51 | 0.227 |

**p<0.05; C: Control; CHX: Chlorhexidine; HA80: Humic Acid 80 mg/kg; HA150: Humic Acid 150 mg/kg; MNL: Mononuclear cell; PNL: Polymorph nuclear leukocyte*

*a= The difference between CHX, K and HA80 was statistically significant*

*b= The difference between HA150 and other groups was statistically significant*

*c= The difference between HA150 and other groups was statistically significant*

*d= The difference between HA80 Group, CHX and K Group was statistically significant*

*e= The difference between HA150 Group, CHX and K Group was statistically significant*

When the wound surface area measurements between the groups on days 7 and 14 were evaluated, it was determined that none of the groups was superior in terms of healing. However, on day 21, HA150 Group was found to be more effective in decreasing the wound surface area than other groups (Table 2).

**Table 2**: Statistical analysis results of the intergroup wound area surface measurements

| | Groups | n | Average Area | p |
|---|---|---|---|---|
| **Day 7** | K | 6 | 13.14±2.64 | 0.432 |
| | CHX | 6 | 15.00±6.13 | |
| | HA80 | 6 | 17.13±3.16 | |
| | HA150 | 6 | 14.56±7.21 | |
| **Day 14** | K | 6 | 12.10±5.10 | 0.358 |
| | CHX | 6 | 10.80±4.93 | |
| | HA80 | 6 | 8.53±3.55 | |
| | HA150 | 6 | 8.88±5.62 | |
| **Day 21** | K | 6 | 7.33±3.05 | 0.035* |
| | CHX | 6 | 5.88±1.96 | |
| | HA80 | 6 | 6.86±3.03 | |
| | HA150 | 6 | $2.70^{a}$±2.72 | |

*p<0.05; C: Control; CHX: Chlorhexidine ; HA80: Humic Acid 80 mg/kg; HA150: Humic Acid 150 mg/kg
a=The difference between HA150 Group and K, CHX and HA80 Group was statistically significant

When the wound surface area measurements within the groups were compared, it was days 7, 14 and 21 in the K group. In the CHX group, the healing on day 21was better than the healing on day 7, and this difference was statistically significant (p<0.05). According to the area measurement results in the HA80 group, the healing on day 14 was better than the healing on day 7 and the healing on day 21 was better than the healing on day 7. In the HA150 group, the healing on the 21st day was higher to the healing on the 7th day, while the difference between the healing on the 7th day and the 14th day and the healing in the 14th and 21st day were not statistically significant (Table 3).

**Table 3:** Statistical analysis results of the intragroup wound area surface measurements

| Groups | | Average area | p |
|---|---|---|---|
| K (n=6) | 7. Day | 13.14±2.64 | 0.058 |
| | 14. Day | 12.10±5.10 | |
| | 21. Day | 7.33±3.05 | |
| CHX (n=6) | 7. Day | $15.00^{a}$±6.13 | 0.046* |
| | 14. Day | 10.80±4.93 | |
| | 21. Day | $5.88^{a}$±1.96 | |
| HA80 (n=6) | 7. Day | $17.13^{bc}$±3.16 | 0.001* |
| | 14. Day | $8.53^{b}$±3.55 | |
| | 21. Day | $6.86^{c}$±3.03 | |
| HA150 (n=6) | 7. Day | $14.56^{d}$±7.21 | 0.005* |
| | 14. Day | 8.88±5.62 | |
| | 21. Day | $2.70^{d}$±2.72 | |

*p<0.05; C: Control; CHX: Chlorhexidine ; HA80: Humic Acid 80 mg/kg; HA150: Humic Acid 150 mg/kg
a=The difference between days 7 and 21 in area measurements in CHX Group was statistically significant
b= The difference between days 7 and 14 in area measurements in HA80 Group was statistically significant
c= The difference between days 7 and 21 in area measurements in HA80 Group was statistically significant
d= The difference between days 7 and 21 in area measurements in HA150 Group was statistically significant

## DISCUSSION

The present study is the first study in which the effects of two different humic acid concentrations was evaluated on wound healing. In the present study, the groups to which 80 mg/kg humic acid, 150 mg/kg humic acid, chlorhexidine gluconate, and no agent application were compared in the defects created on palatal mucosa of rats. Humic substances, which are mostly found in lignite, peat, soil and water, have antiviral, antibacterial,

antitoxic, antiulcerogenic, antiarthritic, antiallergic, immunomodulator, and anti-inflammatory characteristics.[25] Humic acids are the most commonly found forms of organic carbon in the nature, and have strong anti-inflammatory effects, which they show by inhibiting the release of IL-1β and TNF-α activated by leukocytes.[13,26] It was shown that humic acid decreases lipopolysaccharide-mediated adhesion molecules cultured from human umbilical vein endothelial cells (ICAM-1, VCAM -1 and E-selectin) at significant levels.[27] This may be one of the ways in which the possible effects of humic acid in the inflammatory process is explained.

When intragroup wound surface area scores were compared, it was determined that the healing on days 7, 14 and 21 was similar in Group K. The healing on day 21 was better compared to the healing on day 7 in CHX Group, and the difference was statistically significant ($p<0.05$). According to the field measurement results in HA80 Group, the healing on day 14 was better than that on day 7, and the healing on day 21 was better than that on day 7. In HA150 Group, the healing on day 21 was better than that on day 7; however, the differences between healing scores on day 7 and 14, and the differences between the healing scores on day 14 and 21 were not found to be statistically significant.

The still epithelial cells become cells that migrate to the wound area with the effect of growth factors secreted from platelets and macrophages. The formation of epithelium begins with the migration of epidermal cells in the wound edge and skin supplements.[26] Mariano *et al.*[28] reported that the application of 0.2% chlorhexidine on the palatal mucosa defects induced in rats shortened healing time and facilitated wound epithelization. Teixeira *et al.*[29] created wounds on rats' tongues, and showed that the application of 0.12% chlorhexidine was highly effective on ulcerations as of the first day. Abshenas *et al.*[30] and Brzozowski *et al.*[31] reported that the application of humic acid accelerated wound healing at significant levels in rats in which they created gastric ulcers.

The regeneration and repair of the epithelium is an important part in wound healing. The major function of epithelium is to create a barrier between the body surface and the environment. After an injury, the connection to the adjacent cells on the edge of the wound is disrupted.

In the present study, when epithelization values on days 7, 14 and 21 were evaluated, there was no statistically significant difference between the groups. However, in the HA150 Group, epithelization was found to be better than in the other groups. The lowest level was in Group K (Figure 1,2 and 3).

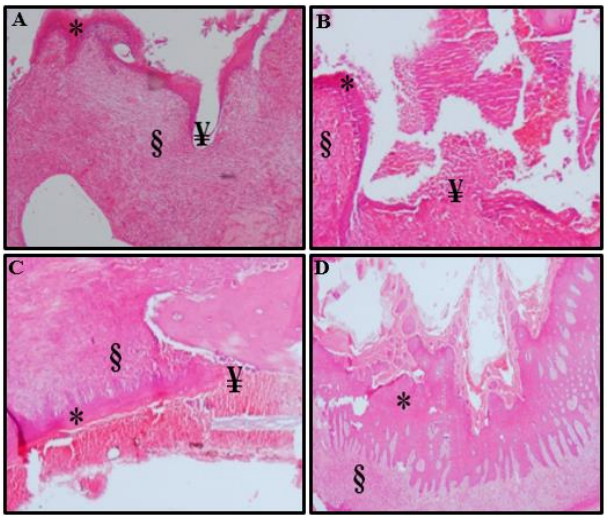


**Figure 1:** Day 7, the surface epithelium (*), ulceration (¥) and inflammatory granulation tissue (§) of K (A), CHX (B), HA80 (C) and HA150 (D) Groups (Hematoxylin-Eosin X40)

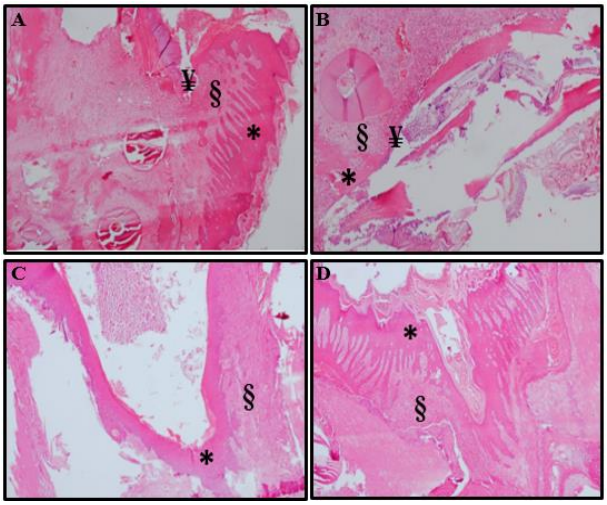


**Figure 2:** Day 14, the surface epithelium (*), ulceration (¥) and inflammatory granulation tissue (§) of K (A), CHX (B), HA80 (C) and HA150 (D) Groups (Hematoxylin-Eosin X40).

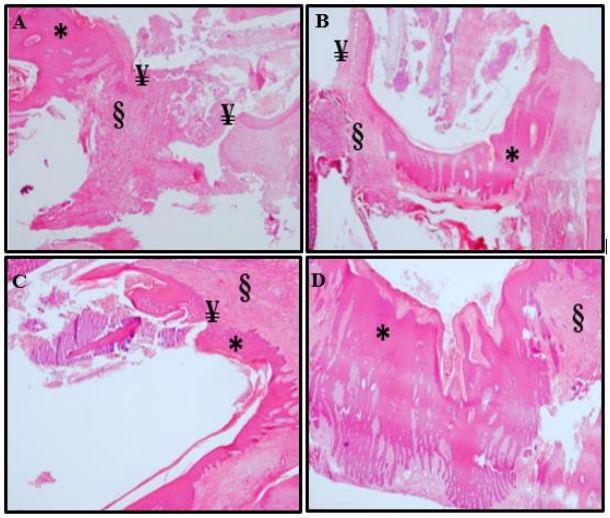


**Figure 3:** Day 21, the surface epithelium (*), ulceration (¥) and inflammatory granulation tissue (§) of K (A), CHX (B), HA80 (C) and HA150 (D) Groups (Hematoxylin-Eosin X40).

When ulceration values were evaluated, the amount of ulceration on day 7 was lower at a statistically significant level in HA150 Group than in the other groups. In addition, less ulceration was detected in CHX Group than K and HA80 Group. On day 14, a smaller amount of ulceration was detected in HA150 Group than in the other groups, and this difference was statistically significant. The ulceration amounts between K, CHX and HA80 Groups were at similar levels. When the ulceration values were evaluated on day 21, ulceration was lower in HA80 and HA150 at a statistically significant level compared to the other groups. According to the results obtained in this study, when epithelization and ulceration values were evaluated together, it was found that 150 mg/kg humic acid increased wound healing at a significant level. It was also concluded that chlorhexidine did not disrupt healing (Figure 1,2 and 3) .

Wound healing is a quite complex response process to tissue injury, and consists of interconnected and intertwined stages like inflammation, proliferation and maturation. Inflammation, which is the first step of recovery, gives the tissue a kind of resistance to microbial contamination.[32] Infection in the wound area must be prevented in order for wound healing to occur in a problem-free manner. Anti-inflammatory effect is required to shorten recovery time.[33]

In their study, Knuuttila *et al.*[34] reported that various concentrations of chlorhexidine had an anti-inflammatory effect, and reduced different types of leukocytes. Hoffman *et al.*[35] reported that 0.1% chlorhexidine yielded successful results in reducing inflammation. In a study conducted on rats by Van Rensburg *et al.*[36], they detected anti-inflammatory effects of potassium humate, and reported at the end of their study that the humic acid solution (61 mg/kg) obtained from lignite was as effective as prednisolone (steroid group), which is known to have an anti-inflammatory effect in suppressing the edema induced in the ears of the rats. Calisir *et al.*[37] conducted an experimental periodontitis study, and found that 80 mg/kg humic acid, which they applied locally, increased the inflammatory cell infiltration rate, the 80 mg/kg and 150 mg/kg of humic acid that they applied systemically reduced inflammatory cell infiltration rates. In the present study, the PNL and MNL numbers were evaluated to have an idea on the anti-inflammatory effects of humic acid. Polymorphonuclear leukocytes and mononuclear leukocytes play important roles in the defense of the live tissue against microbial contamination in inflammatory reactions. The number of these cells increases in the presence of bacterial infections, and they quickly migrate to the inflammatory zone. As a result of the findings of the present study, the difference between groups in terms of the number of PNL cells on day 7, 14 and 21 was not statistically significant; and the difference between groups in terms of the number of MNL cells on day 7, 14 and 21 was not statistically significant. However, it can be argued that humic acid has anti-inflammatory properties since it reduces inflammatory cells.

Cellular activity is dominant in the proliferation stage of wound healing.[38] In this stage, it is noted that there is pink granular tissue formation that contains inflammatory cells, fibroblasts and newly-developing blood vessels. Fibroblasts begin to synthesize new non-cellular matrix and immature Type III collagen in response to the cytokines and growth factors released from the inflammatory cells in the wound area. Also, the stimulated fibroblasts secret a

number of growth factors, which support the healing process by creating a "feedback cycle". Collagen accumulation increases the resistance of the wound to stretching in a fast way.[39]

In their cell culture study, Goldschmidt *et al.*[40] showed that chlorhexidine at a rate of 0.01% or above caused cell death in human gum fibroblasts. In another in-vitro study conducted by Alleyn[41], they reported that 0.12% chlorhexidine reduced the binding of fibroblasts on dentin. Unlike these studies, Mariano *et al.*[28] conducted a study and found a large amount of collagen fiber and a small number of fibroblasts in the group which received 2% chlorhexidine. Cheng *et al.*[42] argued that humic acid caused oxidative DNA damage, growth delay and apoptosis in human primary fibroblasts. Kreminzki *et al.*[43] conducted another study and argued that turban, which constituted the source of humic acid, had proangiogenic properties. However, there are not enough studies in the literature supporting these studies or proving the opposite.

In the present study, when the fibroblast values on days 7, 14 and 21 were evaluated, no statistically significant differences were detected among the groups. Given these results, it was concluded that the application of humic acid had no positive effects on fibroblasts. The vascularization level in Group K on day 7 was higher than the vascularization level in CHX and HA80 Group. Similarly, the vascularization level in HA150 Group was higher at a statistically significant level than the level of vascularization in CHX and HA80 Group. However, there was no difference in the vascularization levels of the groups on day 14 and 21. As a result of the findings of the present study, it is possible to speculate that humic acid has a positive effect on wound healing by increasing vascularization.

Measuring the length, width and depth of the wound is an important part of wound evaluation. Compared to defining statements like "good" or "getting better", measuring the size of the wound is an objective proof of wound healing. In the present study, after the tissues taken from the palates of the rats on days 7, 14, and 21 were fixed, the photos taken under X8 magnification in the stereomicroscope were transferred to the computer medium, and it was aimed that objective measurements were made.

Mariano *et al.*[28] conducted a study on rats, and reported that 2% chlorhexidine and metronidazole were very effective in closing wounds at the end of day 6. Hammad *et al.*[44] reported that 0.2% chlorhexidine gel showed a very good effect in reducing the wound surface on days 7 and 14 compared to the group to which they applied allantoin. Similar to our study, Calisir *et al.*[24] compared the concentrations of 0.09% saline, 0.05% chlorhexidine, and 80 mg/kg of humic acid, and they found on days 7 and 14 of the trial that the groups with chlorhexidine and humic acid were better at closing the wound surfaces than the groups that received saline. In the same study, it was also found that chlorhexidine and humic acid had similar levels of activity when compared among the groups. On day 21 of the trial, they reported that humic acid is superior in closing wound surfaces compared among the groups.

In the present study, when the wound surface areas between the groups were compared, it was determined that there were no differences between the healing of wound surfaces on day 7 and 14; however, on day 21, the improvement in the wound surface in HA150 Group was better at a statistically significant level than the improvement in the wound surfaces of the K, CHX and HA80 Groups.

When the intragroup wound surface areas were compared, no significant differences were detected in the K group among wound surface measurements on days 7, 14 and 21. As a result of wound area measurements made in the CHX Group, the wound healing on day 21 was better compared to that on day 7, and the wound healing rates on the other days were similar. As a result of wound area measurements made in HA80 Group, the wound healing on day 14 was better than that on day 7. Similarly, the wound healing on day 21 was better than that on day 7. The wound healing on day 21 was better than the wound healing on day 7 in HA150 Group. No significant differences were detected between the wound healing

measurements on day 7 and 14, and between day 14 and 21. As a result, in the present study, it was found that 150 mg/kg humic acid was very effective in closing wound surface. It was also found that chlorhexidine, which was used in the study, had no negative effects on accelerating the closure of the wound surface.

## CONCLUSIONS

Within the limitations of this experimental study, it may be argued that the humic acid, which has previously been shown to have antibacterial and anti-inflammatory characteristics, positively affects wound healing in oral cavity. Humic acid treatment has been found to be superior to chlorhexidine, which is widely used in the treatment of oral wounds.

Further studies are needed to investigate the mechanisms or pathways of the healing effect of humic acid on wounds in the mouth. In line with the results obtained here, we believe that the present study will contribute to the literature on clinical use of humic acid.

## ACKNOWLEDGEMENTS

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### *Lokal Hümik Uygulamasının Yara İyileşmesi Üzerine Etkisinin Değerlendirilmesi*

## ÖZ

***Amaç:*** *Bu çalışmanın amacı; hümik asitin topikal olarak uygulanmasının ratlarda ağız mukozasındaki yara iyileşmesine etkilerinin, histopatolojik ve histomorfometrik olarak araştırılmasıdır.* ***Gereç ve Yöntemler:*** *Denek olarak 12 haftalık, ortalama ağırlıkları 280-300 gr olan Wistar cinsi 72 adet erkek rat kullanıldı. Ratlar rastgele seçilerek hiç bir ajan uygulanmayan kontrol grubu (K), klorheksidin (%0.12) grubu (CHX), 80 mg/kg hümik asit grubu (HA80) ve 150 mg/kg hümik asit grubu (HA150) olmak üzere 4 ana gruba ayrıldı. Ratlarda palatinal bölgede, punch ile 5 mm çapında mukozal defekt oluşturuldu. Bu gruplar kendi içerisinde 7., 14. ve 21. günlerde sakrifiye edilmek üzere 3 alt gruba ayrıldı. Ratlar sakrifiye edildikten sonra histopatolojik inceleme için doku örnekleri alındı. Histopatolojik değerlendirmede; epitelizasyon, ülserasyon, polimorfo nükleer lökositler (PNL), mononükleer hücreler (MNL), fibroblast ve vaskülarizasyona bakıldı. Ayrıca doku örnekleri histomorfometrik inceleme için fotoğraflanarak bilgisayar ortamına aktarıldı.* ***Bulgular:*** *Yapılan istatistiksel analiz sonucunda tüm gruplar arasında 7.,14. ve 21. günlerde epitelizasyon derecesi, PNL ve MNL hücre infiltratı açısından anlamlı bir fark bulunmadı. HA150 grubundaki ülsere alanlar diğer gruplara oranla daha az miktardaydı ve bu fark istatistiksel olarak anlamlıydı ($p<0,05$). Vaskülarizasyon değerlerine bakıldığında 7. günde K grubu ve HA150 grubu daha iyi sonuçlar gösterdi ($p<0,05$). 14. ve 21. günlerde ise gruplar arasında anlamlı bir fark bulunmadı ($p>0,05$). Yara yüzey alan ölçümleri, HA150 grubunda diğer gruplara kıyasla daha düşük miktardaydı ve bu sonuç, HA150 grubundaki iyileşmenin daha iyi olduğunu gösterdi ($p<0,05$).* ***Sonuç:*** *Bu çalışmanın sonucunda hümik asitin, ağız boşluğundaki yara iyileşmesini arttırdığı görüldü. Ayrıca klorheksidinin yara iyileşmesini olumsuz yönde etkilemediği tespit edildi.* ***Anahtar Kelimeler:*** *Yara, palatinal mukoza, klorheksidin, hümik asit.*

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**Original research**

# EVALUATION OF BIOMECHANICAL EFFECTS OF PROSTHETIC COMPONENTS WITH DIFFERENT MATERIALS ON THE ABUTMENT SCREW

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## ABSTRACT

**Objectives:** The aim of this study was to assess the effects of different resin-based and ceramic superstructure materials and two different abutment types on the stress distribution of the abutment screw using the method of three-dimensional finite element stress analysis.

**Materials and Methods:** A three-dimensional implant, abutment (zirconia and titanium), abutment screw, crown (zirconia reinforced lithium silicate, lithium disilicate, polymer-infiltrated resin ceramic, and PEEK), and alveolar bone were designed using Rhinoceros 3D modeling software and VRMesh Studio software to form 8 simulations. On the models prepared, loading was made on the lingual tubercle of the maxillary right first premolar crown at an angle of 30° with 150 N force obliquely in the buccolingual direction. The von Mises stress values obtained from the abutment screw were compared according to the types of abutment and crown materials.

**Results:** The von Mises stress values in the abutment screw were higher in the models using a titanium abutment (on average 1336.24 MPa), and the lower stress values were obtained in the models using a zirconia abutment (on average 964.26 MPa). When the prosthetic material used was changed, the stress values on the abutment screw was similar.

**Conclusions:** Considering that the abutment screw is the weakest component of the implant-system, zirconia abutments can be used reliably in the maxillary first premolar region where aesthetic expectations are high.

**Keywords:** Dental stress analysis, dental abutments, glass ceramics

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## INTRODUCTION

The improvements in dental implant technology since the end of the last century have opened a new era in the field of prosthetic dentistry. Compared to traditional restorations, implant-supported restorations offer a wide range of options for both clinicians and patients. Thus, highly satisfactory aesthetic and functional results are achieved with them. However, implant treatments are not perfect under all conditions. In implant practice, high material cost, surgical trauma, and long duration of treatment, biological and mechanical complications associated with implant-supported restorations cause troubles.[1,2] In the implant system, complications such as peri-implantitis, loosening or fracture of the abutment screw, fracture of the abutment or prosthetic superstructure, loosening or decementation of the crown, and separation of the veneer porcelain are the most common.[2,3] Among these complications, loosening of the abutment screw is one of the main mechanical complications.[3,4] The rate of loosening of abutment screw was found to be 5.3% in the first year after loading[5] and 5.8-12.7% after 5 years of follow-up.[2,4] If abutment screw loosening is not noticed and intervened, it has a high risk of resulting in screw or implant fracture.[6]

In addition to managing the manufacturing process using the chairside/laboratory procedure, the selection of the appropriate crown material can contribute to lasting success. Resin-based or highly resistant monolithic ceramics with shock-absorbing capacity can be preferred to overcome or minimize the risk of fracture in prosthetic components. Nevertheless, despite the promising results of resin-based materials in implant-supported restorations[7,8], their mechanical strength is lower compared to ceramics.[9] Resin-based prosthetic materials provide a biomechanical advantage by compensating the lack of periodontal ligament in implant-supported restorations and minimize the risk of mechanical complications between the implant-abutment-crown complex.[9]

The abutment screw is generally known as the weakest component in the implant system, as the screw head and the surrounding area have the highest concentration of torque and stress.[10] In the clinical and laboratory studies, technical complications (loosening or fracture) related to abutment screws were the most frequently reported problems for two-piece systems.[10,11] An implant-supported single crown is more prone to screw loosening compared to an implant-supported fixed partial dentures. While the incidence of screw loosening was 5.6% in the 5-year follow-up in fixed partial denture restorations, it reached 12.7% in single crowns.[12] Screw loosening may lead to mechanical problems such as loss of function due to excessive prosthesis displacement, loosening of other screws in a multi-unit prosthesis, fracture of screws due to fatigue, loss of restoration, and loss of the implant due to inadequate osseointegration, and biological problems such as microleakage, soft tissue irritation and peri-implantitis.[13,14]

Zirconia abutments have become popular in prosthetic treatments due to their superior optical properties compared to titanium and higher fracture resistance than alumina. *In vitro* studies have reported that the fracture resistance of zirconia abutments exceeds their maximum bite force of 90 to 370 N.[15,16] Unlike the classical failure models described for titanium systems, crack initiation and propagation caused by fatigue in zirconia due to plastic deformation of screw and implant parts cause fractures in thin parts of the ceramic structure.[11,17]

In this study, unlike other stress analysis studies, the biomechanical effects of different types of abutment and superstructure materials on the abutment screw with frequent complications were evaluated. Finite element analysis (FEA) method may contain dimensions and shapes, loads and support conditions suitable for clinical conditions, and despite the versatility of the analysis, the use of a single computer program is the reason for using this analysis method in the study.

The aim of this study was to assess the effects of different resin-based (PEEK and polymer-infiltrated resin ceramic) and ceramic (lithium disilicate and zirconia reinforced lithium silicate) superstructure materials and two different

abutment types (stock titanium abutment and zirconia abutment) on the stress distribution of the abutment screw using the three-dimensional finite element stress analysis method. The null hypothesis of this study was established by assuming that the force transmission of zirconia abutment and resin-based superstructure systems on the abutment screw would be low due to force absorption.

## MATERIALS AND METHODS

The geometric designs of 3D models were obtained for the implant, abutment, abutment screw, crown, and alveolar bone included in the study by using the Rhinoceros 4.0 (3670 Woodland Park Ave N, Seattle, WA 98103 USA) 3D modeling software and VRMesh Studio (Virtual Grid Inc, Bellevue) and Algor Fempro (ALGOR, Inc.150 Beta Drive Pittsburgh, PA 15238-2932 USA) analysis program. Ethical approval was acquired from the Clinical Research Ethics Committee of Afyonkarahisar Health Science University (decision date: 11.09.2020, ID number: 2020/407)

### *Implant model*

A three-dimensional (3D) model of the bone level threaded conical implant with a 3.75 mm diameter and a 10 mm long internal hexagonal connection (Parallel Conical Connection, Nobel Biocare) was designed.

### *Abutment models*

A zirconia abutment (Universal Base Conical Connection-Nobel Biocare) and titanium abutment (Universal Base Conical Connection-Nobel Biocare) were selected to be used in the study. Both abutments were designed as a narrow platform and flat with a gingival height of 3 mm, a crown length of 5 mm, and a total length of 8 mm (Figure 1).

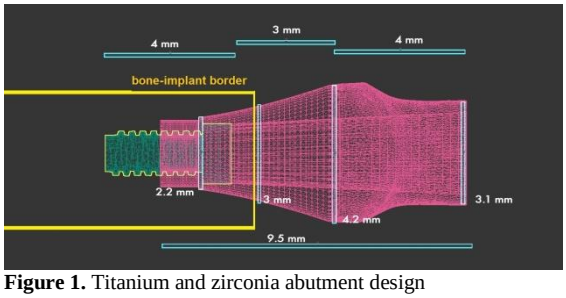


**Figure 1.** Titanium and zirconia abutment design

### *Abutment screw models*

A 9-threaded long screw with $Ti_6Al_4V$ alloy content with a 0.17 mm and 0.15 mm pitch and having a screw pitch with a length of 3.9 mm and a body thickness of 1.1 mm was designed (Figure 2).

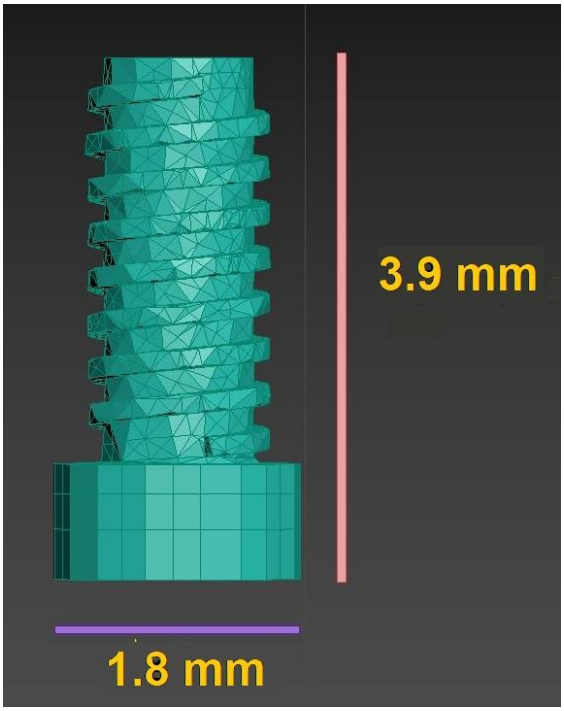


**Figure 2.** Titanium abutment screw design

### *Crown models*

Zirconia reinforced lithium silicate (ZLS) (Vita Suprinity, Vita Zahnfabrik, Germany), lithium disilicate (IPS e.max CAD, Ivoclar Vivadent, Germany), polymer-infiltrated resin ceramic (PICN) (Vita Enamic, Vita Zahnfabrik, Germany), and PEEK (JUVORA, Invibio/ Juvora Ltd., England) aesthetic materials were selected as crown materials. The crown suitable for the anatomy and morphology of the maxillary right first premolar with a crown width of 7 mm, a crown thickness of 9 mm, and a crown length of 8.5 mm was modeled (Figure 3).

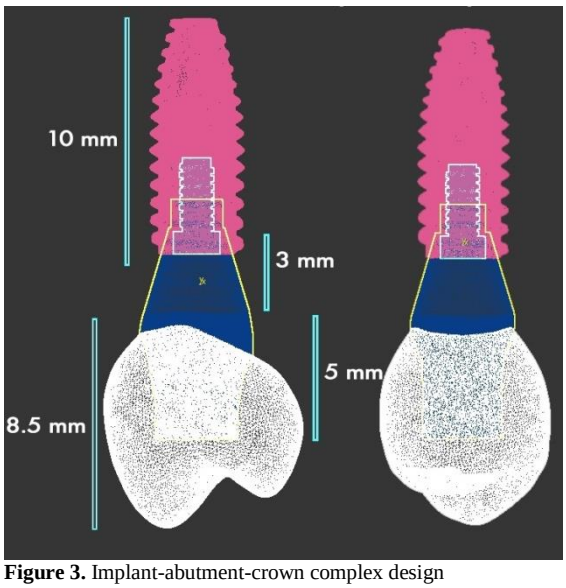


**Figure 3.** Implant-abutment-crown complex design

*Alveolar bone model*

A total bone model was obtained by modeling 2 mm thick Type 2 cortical bone in the form of a rectangular prism with a dimension of 40x30x20 mm and the trabecular bone 2 mm inside the borders of the prism in the maxillary right first premolar region and combining cortical and trabecular bone with the Boolean command.

The physical properties of the materials included during the geometric design of the 3D models are presented in Table 1.

**Table 1.** Physical properties of the materials used in the study

| | Elastic Modulus (MPa) | Poisson's ratio | Reference |
|---|---|---|---|
| Zirconia reinforced lithium silicate | 104 900 | 0.208 | 18 |
| Lithium disilicate | 102 700 | 0.215 | 18 |
| Polymer-infiltrated resin ceramic | 37 800 | 0.24 | 19 |
| PEEK | 3 500 | 0.36 | Manufacturer company information |
| Titanium | 110 000 | 0.35 | 20 |
| Zirconia | 210 000 | 0.30 | 21 |
| Cortical bone | 13 700 | 0.30 | 20 |
| Trabecular bone | 1 370 | 0.30 | 20 |

All models obtained were combined to form a total of 8 different simulations (4 different crown materials and 2 different types of abutment). The abutment body was tightened with the abutment screw with a torque of 30 Ncm in accordance with the company's recommendations, and friction between the implant internal threads and the abutment screw was ignored.

The discretization procedure involved forming the mesh and defining the elements with nodes and boundary conditions. An axisymmetric model of the implant was created, and the alveolar bone was assumed to have linear, homogeneous, and isotropic material properties. In the meshing process, the models were created as much as possible from 8-node (brick type) elements. The convergence study, which determines the minimum mesh size required to eliminate its effect on stress, was used to validate the finite element model. The total numbers of elements and nodes for each model are presented in Table 2.

**Table 2.** Numbers of elements and nodes of the created models

| | Number of Elements | Number of Nodes |
|---|---|---|
| Crown material *- zirconia abutment | 901 202 | 168 582 |
| Crown material *- titanium base abutment | 901 978 | 167 219 |
| Crown material *- titanium abutment | 901 202 | 168 582 |

* Zirconia reinforced lithium silicate, lithium disilicate, polymer-infiltrated resin ceramic and PEEK

The boundary conditions of the implants were modeled as part of the alveolar bone. The geometry of the alveolar bone model surrounding the implant was simplified by linear elastic description, both anterior and posterior regions of the bone were limited to represent the actual clinical condition, and the support at the bottom allowing the bending of the model was removed.

On the models prepared, loading was made on the lingual tubercle of the maxillary right first premolar crown at an angle of 30° with 150 N force obliquely in the buccolingual direction. Finite element stress analysis was performed using the VR Mesh Studio and Algor Fempro (ALGOR Inc) software. The von Mises stress was detected on the abutment screw due to the ductility characteristic of metallic materials.

## RESULTS

The stress distribution of all models is presented in Table 3. The higher von Mises stress values in the abutment screw were obtained in the models using a titanium abutment (on average 1336.24 MPa), and the lower stress values were obtained in the models using a zirconia abutment (on average 964.26 MPa).

**Table 3.** The von Mises stress values of abutment screws

| Numerical models (Crown material-Abutment type) | Abutment screw (MPa) |
|---|---|
| ZLS-Zr | 966.03 |
| ZLS-Ti | 1341.33 |
| PICN-Zr | 963.69 |
| PICN-Ti | 1331.01 |
| LD-Zr | 965.97 |
| LD-Ti | 1341.33 |
| PEEK-Zr | 961.36 |
| PEEK-Ti | 1331.32 |

***** ZLS: Zirconia reinforced lithium silicate, LD: Lithium disilicate, PICN: Polymer-infiltrated resin ceramic, PEEK: Polyether ether ketone, Zr: Zirconia abutment, Ti: Titanium abutment

When the prosthetic material used was changed, no significant difference was observed in the stress values on the abutment screw. However, the stress values in the model using the resin-based crowns and titanium abutment were approximately 10 MPa lower than the ceramic-based crowns. In all models, the highest von Mises stresses were concentrated on the head of the abutment screw (Figure 4).

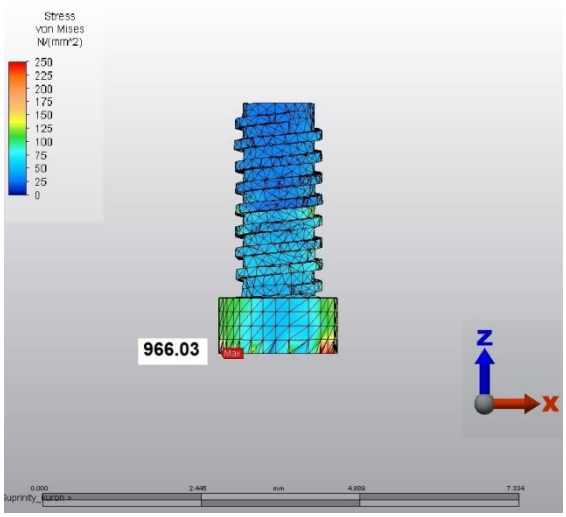


**Figure 4.** The von Mises stress on the abutment screw in the ZLS-Zr model

## DISCUSSION

Within the scope of the study results, the established hypothesis was partially accepted, and the abutment type had an effect on the stress values on the abutment screw. However, the effect of the crown material used was not observed.

In the literature, there was a limited number of studies revealing the stress change and failure types of abutment screws due to different abutment materials.[10,17,22-24] Nevertheless, studies conducted with two-piece titanium implants with metal or porcelain abutments revealed that abutment screws fractured regularly in case of metal abutment.[10,17] Porcelain abutments had complications due to screw loosening or deformation at a similar rate to metal abutments. However, abutment fracture was encountered before the screw fracture. While fractures in metal abutments are usually observed in the abutment screw, they occur in the abutment itself in ceramic abutments. Therefore, it is assumed that the fracture of the zirconia abutment occurs before the fracture of the abutment screw.[10]

Although previous studies mainly focused on the effects of titanium and zirconia abutments on stress distribution, research results were inconsistent.[22-24] The use of porcelain abutments has eliminated the aesthetic disadvantage of the titanium abutment, especially for the restoration of the maxillary anterior teeth. Studies on porcelain abutments with a proven aesthetic advantage reported some functional disadvantages.[25,26] Zirconia and alumina abutments have higher fragility and higher young modulus compared to conventional titanium abutment, thus affecting the screw preload and torque loss. The modulus of elasticity of the titanium implant and abutment screw is lower than that of the porcelain abutment. Thus, stress will be concentrated more on the implant and abutment screw, so it will increase the risk of implant fracture and screw loosening.[25] Dhingra *et al.*[27] showed that zirconia and titanium abutments had similar torque loss after cyclic loading. Debris between the zirconia abutment-abutment screw and implant-abutment can contribute to delaying torque loss and keeping the connection stable. Therefore, zirconia abutment may still be considered a choice for clinical applications.[26]

The tensile strength of the titanium screw was between 860-965 MPa.[28] When the stress values obtained were examined, it was observed that they remained in this range in the models using zirconia abutment and that stresses were higher than the physiological limit in the models with titanium abutment. Accordingly, while failure is not predicted in the zirconia abutment screw, failure may occur in the abutment screw in the titanium abutment.

Resin-based ceramics have low elastic modulus previous compared to many other ceramic materials. In the studies, it was concluded that PICN[29] and PEEK[30] materials absorbed the masticatory forces and reduced the stress values on the peripheral bone. In their study, Duan and Griggs[31] examined the stress distribution in resin nanoceramic and lithium disilicate crowns, and it was reported that resin nanoceramic materials had low-stress values under vertical loading. Similarly, in this study, resin-based crown

materials had slightly lower stress values than ceramic-based crown materials in the models with titanium abutments. However, this difference could not be detected in the models with zirconia abutment.

*In vitro* studies evaluating the effect of different prosthetic materials on stress distribution in peripheral bone structure and implants showed that prosthetic material changes did not lead to considerable differences or had only a minor effect on stress patterns.[20,32] Furthermore, In addition, Bassit *et al.*'s[33] *in vivo* study result were in line with these results. Although resin-based ceramics were recommended in implant-supported restorations due to their shock-absorbing properties[7-9], in this study, in parallel with these studies mentioned above, no considerable decrease in stress concentrations in the abutment screw was observed. Several layers or structures are involved in the transmission of masticatory forces to the abutment screw, including the prosthetic superstructure, the cement layer, or the prosthetic screw and abutment. It can be considered that some of the total energy transferred to the abutment screw is absorbed by the intermediate layers, which may explain the similar biomechanical effects of different superstructure materials on the abutment screw.

In this FEA study, it was assumed that all models were linear, homogeneous, and isotropic, and 100% osseointegration of the implant into the alveolar bone was assumed. In the model created with these assumptions, the diversity of stress and deformation rates was limited. Therefore, *in vivo* conditions could not be fully reflected. Furthermore, only internal hexagonal connection and static force were used in this study. However, the above-mentioned limitations do not considerably affect the accuracy and results of the FEA. The result of this FEA study can be used as a guide in clinical trials.

## CONCLUSIONS

Within the limitations of this study, it can be concluded that the effect of the prosthetic superstructure material on the stress distribution in the abutment screw was not significant. It was revealed that the zirconia abutment and the prosthetic materials used in the study did not have high stress values enough to cause a failure of the abutment screw. Considering that the abutment screw is the weakest component of the implant-system, zirconia abutments can be used reliably in the maxillary first premolar region where aesthetic expectations are high.

## ACKNOWLEDGEMENTS

There is no acknowledgement for this study.

## CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

***Farklı Materyallere Sahip Protetik Bileşenlerin Dayanak Vidası Üzerine Biyomekanik Etkilerinin Değerlendirilmesi***

### *ÖZ*

***Amaç:*** *Bu çalışmanın amacı; farklı rezin bazlı ve seramik üstyapı materyalleri ve farklı dayanak tiplerinin dayanak vidasının stres dağılımı üzerine etkilerini 3 boyutlu sonlu elemanlar stres analiz yöntemi ile değerlendirmektir.* ***Gereç ve Yöntemler:*** *3 boyutlu implant, dayanak (zirkonya ve titanyum), dayanak vidası, kuron (zirkonya ile güçlendirilmiş lityum silikat, lityum disilikat, polimer infiltre cam seramik ve PEEK) ve alveolar kemik, Rhinoceros 3 boyutlu modelleme yazılımı ve VRMesh Studio yazılımı kullanılıp tasarlanarak 8 simülasyon oluşturacak şekilde birleştirildi. Hazırlanan modeller üzerinde maksiller sağ 1. küçük azı diş kronun lingual tüberkülüne 30° açı ile bukkolingual yönde oblik olarak 150 N kuvvet uygulaması ile yükleme yapıldı. Dayanak vidasında elde edilen von Mises değerleri dayanak tipleri ve kuron materyallerine göre karşılaştırıldı.* ***Bulgular:*** *En yüksek von Mises stres değerleri titanyum dayanak kullanılan modellerde (1336,24 MPa), en düşük stres değerleri ise zirkonya dayanak kullanılan modellerde (964,26 MPa) elde edildi. Kullanılan protetik materyal değiştirildiğinde dayanak vidasındaki stres değerlerinde belirgin fark görülmedi.* ***Sonuçlar:*** *Dayanak vidasının implant sisteminin en zayıf halkası olduğu düşünüldüğünde estetik beklentinin yüksek olduğu maksiller 1. küçük azı bölgesinde zirkonya dayanak güvenilir olarak kullanılabilir.* ***Anahtar kelimeler:*** *Dental stres analizi, diş dayanakları, cam seramikler.*

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**Original research**

# COMPARING THE EFFECT OF PROBIOTIC *STREPTOCOCCUS SALIVARIUS* K12 AND M18 ON THE *STREPTOCOCCUS MUTANS* COUNT, SALIVARY PH AND BUFFER CAPACITY: A RANDOMIZED DOUBLE BLINDED CLINICAL TRIAL

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## ABSTRACT

**Aim:** There is a growing interest in the use of beneficial bacteria such as probiotics to modulate the oral microbiota. Literature reveals a number of studies on the key species *Streptococcus salivarius* K12 and M18 on prevention of dental caries. However, there is a paucity in the clinical studies on the effect of salivarius K12 on *S. mutans* count. In addition to this, the effect of salivarius K12 and M18 on the salivary pH and buffering capacity have also not been studied. Thus the aim of the present study was to evaluate and compare the effect of probiotic *Streptococcus salivarius* K12 and M18 on the *Streptococcus mutans* count, salivary pH and buffer capacity.

**Materials and method:** 146 subjects were screened for eligibility and 69 Subjects within the age group of 18-40 years were randomly allocated to three groups of 23 subjects each. Subjects enrolled in Group A received BLIS K12™, Subjects in Group B received BLIS M18™ and Subjects in Group C belonged to the control group and did not receive any form of probiotics. Unstimulated salivary samples were collected at baseline and after 30 days. The samples were analysed for *Streptococcus mutans* level, salivary pH and buffer capacity.

**Results:** Among the 69 subjects enrolled for the study, 6 subjects were lost to follow up and 63 subjects completed the trial. A statistically significant reduction in salivary *S. mutans* levels (p=0.001) and an increase in the salivary pH (p = 0.001) was observed after the use of probiotics when compared to the baseline. The buffer capacity remained unaltered following the use of both the probiotics. There was no change in the *S. mutans* count (p=0.065), salivary pH values (p value=0.242) and buffer capacity (p=0.87) for the subjects belonging to the control group.

**Conclusions:** Within the limitations of the present study it can be concluded that a 30day use of *Streptococcus salivarius* K12 and M18 resulted in a reduction in the *Streptococcus mutans* count while simultaneously improving the salivary pH.

**Keywords:** Dental caries, Oral health, Probiotics, Streptococcus salivarius.

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## INTRODUCTION

Dental plaque is a biofilm composed of various microbial species and their products, is attributed as a risk factor of oral infectious diseases such as periodontitis and dental caries.[1-3] Current literature attributes oral diseases to a complex interactions between virulent microorganisms, their products, frequency of carbohydrates consumption and also the host salivary composition.[4-6] Among the various pathogens studied, *Streptococcus mutans* (*S. mutans*) plays a vital role in pathogenesis of dental caries.[4,7] Whilst being only weakly competitive at neutral pH, this cariogenic bacteria dominates the dental biofilm under acidic conditions caused by catabolism of dietary carbohydrates.[8] With recent interest on the role of ecological imbalance and dental caries pathogeneisis, there is a growing interest in the use of beneficial bacteria such as probiotics thereby modulating the oral microbiota.[9-11]

Probiotics are defined as those "Live microorganisms which when administered in adequate amounts confer a health benefit to the host".[12] They are expected to accomplish certain functional requirements such as resistance to acid, adherence to epithelial surfaces and inhibitory activity against some pathogens.[13] Though most probiotics are gram positive bacteria, they belong to the genera Lactobacillus and Bifidobacterium.[14] However due to their limitations in terms of colonisation of oral tissues, a new generation of probiotic strains, Streptococcus salivarius, known to have low pathogenic potential is currently being extensively studied.[14-17]

Literature highlights a number of studies on the key species *Streptococcus salivarius* K12 and M18 on prevention of dental caries. Recent studies have revealed along with the safety and tolerability profiles, the capacity of this genera to reduce plaque formation and also to lower *S. mutans* counts in the oral cavity.[15,17] Pierro *et al.*[15] in his study demonstrated that in children who are at high risk for developing caries, when treated with a probiotic containing *Streptococcus salivarius* M18, they are less likely to develop dental caries. Burton *et al.*[17] in his study confirmed that *Streptococcus salivarius* M18 demonstrated a greater plaque reduction and a higher reduction in *S. mutans* count when compared to those subjects who were merely exposed to bacterial probiotics.

However, there is a paucity in the clinical studies on the effect of salivarius K12 on *S. mutans* count. In addition to this, the effect of salivarius K12 and M18 on the salivary pH and buffering capacity have also not been studied. Thus the current trial was undertaken to evaluate and compare the effect of probiotic *Streptococcus salivarius* K12 and M18 on the *Streptococcus mutans* count, salivary pH and buffer capacity.

## MATERIALS AND METHODS

### Study Design:

The objective of this study was to assess the variation in the *Streptococcus mutans* count, salivary pH and buffer capacity following the use of probiotic lozenges containing strains *Streptococcus salivarius* K12 and *Streptococcus salivarius* M18. The double blinded placebo controlled clinical trial adhered to the CONSORT statement and was conducted in the Department of Conservative Dentistry and Endodontics, Sri Venkateswara Dental College and Hospital, Chennai from December 2019 for a period of 30 days. This study was conducted in accordance with the 1964 Declaration of Helsinki and its subsequent amendments. Ethical clearance was acquired from the institutional ethical committee and informed consent was taken from all the participants enrolled in the current trial.

### Subjects and Criteria:

7 days prior to the commencement of the 30-day trial, 146 subjects were screened for eligibility. Based on the inclusion and exclusion criteria, 69 participants were enrolled for the trial. Subjects in the age group of 18-40 years, with a DMFT score of 4 and above were included in the trial. Subjects with systemic conditions, periodontal diseases, individuals who were on antibiotics in the last 2 weeks, individuals who had allergic history, pregnant and lactating women were excluded from the trial. During the trial period, subjects were instructed not to take probiotics in other forms for any other reason. A pilot study was conducted and based on the

*S.mutans* count in the pilot study, sample size was calculated using G Power Analysis (version 3.1.9.7). A power calculation indicated that 60 subjects were required to provide a 90% chance of detecting the effect size in a continuous outcome measure, assuming a significance level of 5%. The $\beta$ error was calculated at 0.10. Since an attrition rate of 15% was expected, a total of 69 subjects were enrolled for the study.

**Grouping of Subjects and Randomisation:**

*Streptococcus salivarius* that were marketed as probiotic lozenges in two forms as Teeth and Gums BLIS K12™ and Throat Health BLIS M18™ were used for the trial. According to the protocol, 69 subjects both male and female were recruited and randomly allocated to 3 groups of 23 subjects each. Subjects enrolled in Group A received BLIS K12™, Subjects in Group B received BLIS M18™ and Subjects in Group C belonged to the control group and did not receive any form of probiotics.

The subjects enrolled for the trial were allocated into 3 groups with the aid of a simple randomisation method. For allocation, a computer generated list of random numbers was used with the ratio of randomisation of 1:1:1 by using random allocation software (Version 2.0). Allocation sequences were concealed from the researchers who were a part of the study in order to reduce the selection bias.

**Treatment Protocol:**

Subjects enrolled in the 2 test groups that is Group A and Group B were instructed to take one lozenge of BLIS K12™ or BLIS M18 ™ for 30 consecutive days based on the group to which they belonged to. Subjects were instructed to slowly dissolve one lozenge on the tongue during bedtime, ideally after brushing. All subjects were asked to follow the regular oral hygiene procedures that were followed routinely. Subjects were also instructed not to eat or drink for at least 30 minutes' post ingestion. In order to evaluate the level of adherence to treatment protocol, the subjects were asked to return any unused product boxes and tablets. Acceptable adherence was considered to be administration of not less than 95% of the lozenges allocated. Subjects belonging to the control group (Group C) did not receive any treatment and were asked to followonly the regular oral hygiene procedures.

***Streptococcus mutans* count, Salivary pH and Buffer Capacity Testing:**

In order to obtain the samples for analysis of *S. mutans*, salivary pH and buffer capacity, Unstimulated whole saliva was collected from all the subjects enrolled for the study. The subjects were asked to refrain from talking and drop their head down so as to let the saliva run naturally to the front of the mouth. The subjects were also instructed not to cough up mucus as saliva is collected. They were made to spit into sterile bottles about once in a minute for up to 5 minutes until 3-4 millilitre of unstimulated saliva was collected. Samples were collected twice. Once at baseline that is before the start of the study and the second time after 30 days' use of probiotic lozenges. A pH meter (Digital pH meter, MIFA Systems Private Limited, Ahmedabad, Gujarat, India) was used to record the pH of the unstimulated saliva samples. About 0.1ml of the sample was taken, diluted and vortex mixed. 0.1 ml of this diluted sample was then taken to inoculate onto the Mitis Salivarius Agar base (HIMEDIA, Mumbai, Maharashtra, India). This followed by incubating the petri dishes at 37℃ with 3% $CO_2$ for 48 hours. The organisms were identified based on their colony morphology. The colony forming units were counted manually. Buffer capacity was evaluated using a handheld pH meter. Initially the pH sensitive electrode was calibrated for pH 4.0 and 7.0 using standard pH pellets. Two hundred and fifty microliters of lactic acid (pH 3, 1.5mM) were titrated into the test sample and mixed. pH value for the titrated sample was then noted using the handheld pH meter with digital reading display. The results were ranked as high (>5.8), medium (<5.7 and >4.8) or low (pH<4.7).

**Statistical Analysis**

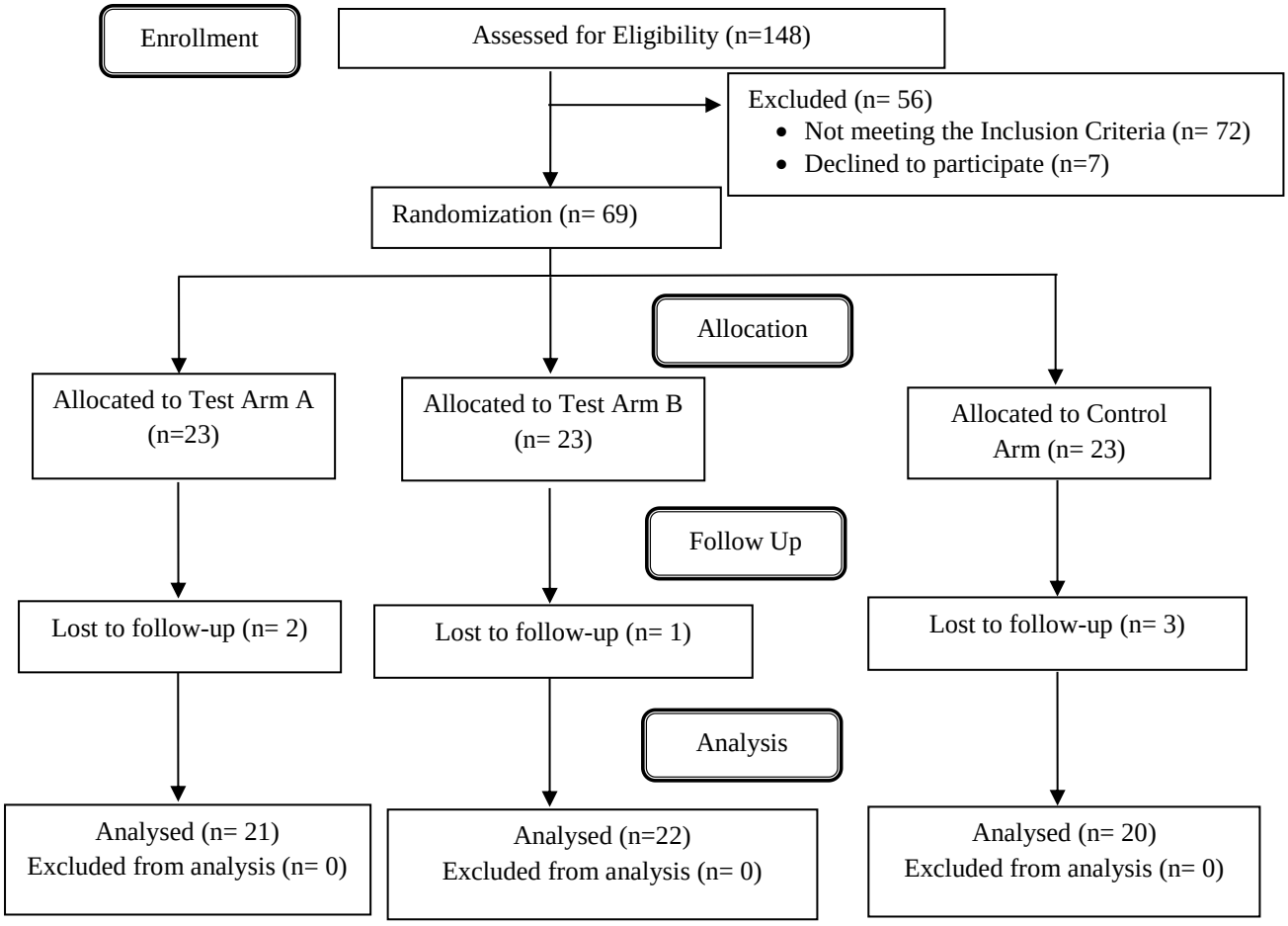
Data obtained at baseline and 30 days were recorded using Microsoft Office Excel 2016 and subjected to statistical analysis using SPSS Software version 22. Chi-square test was used to analyse the statistical difference in terms of Age

and sex among the subjects enrolled in the three group. Data was analysed for normal distribution of variables using One sample Kolmogorov–Smirnov test. The variables were found to be normally distributed for *S. mutans* count and salivary pH. Hence paired t- test was used for within the group comparisons and one-way ANOVA was used for inter group comparisons. Data on buffer capacity being ordinal in nature, Wilcoxon- signed rank test was used for within the group comparison and Kruskal Wallis test was used for inter group comparisons. The level of statistical significance was set at 5% ($p < 0.05$).

## RESULTS

This randomised controlled clinical trial has been carried out on 63 subjects. Though 69 subjects were enrolled for the study, 6 subjects were lost to follow. Figure 1 shows the total number of subjects enrolled for the study and the number of subjects in each group who completed the trial with details of the subjects excluded from the trial.

**Figure:** Flow Diagram showing information on excluded patients




Among the 63 subjects who completed the trial, 38 were males and 25 were females. The mean age of the subjects enrolled for the trial was 26.01±5.78 yrs. The mean DMFT score of subjects enrolled for the study was 5.9. As shown in Table 1, no statistical difference was observed between the three groups in terms of sex (p=0.739), age (p=0.072) and DMFT score (p=0.06). The dropout rate from the baseline was low and the compliance with the study protocol was found to be satisfactory.

**Table 1:** Mean age and Distribution of the study population based on their gender among the 3 groups

| | Gender | | Mean Age(SD) | Mean DMFT Score |
|---|---|---|---|---|
| | Male | Female | | |
| Group A | 14 | 7 | 24.71(5.68) | 5.6 |
| Group B | 13 | 9 | 28.59(5.45) | 6.4 |
| Group C | 11 | 9 | 24.55(5.49) | 5.7 |
| Total | 38 | 25 | | |
| p Value | | 0.739 | 0.072 | 0.06 |

The *Streptococcus mutans* count, salivary pH and buffer capacity values at baseline and at the end of the study (30$^{th}$ day) are demonstrated in Table 2. 30 days treatment with both BLIS K12$^{TM}$ and BLIS M18$^{TM}$ showed a reduction in the *S. mutans* count, while there was an increase in the salivary pH values. This difference for the subjects receiving BLIS K12$^{TM}$ (p=0.001) and BLIS M18$^{TM}$ (p=0.001) was statistically significant as shown in Table 2. In contrast to this there was no change in the buffer capacity of saliva in both the groups that received probiotic streptococcus. In addition to this, there was no statistically significant difference observed in the control group in terms of *S. mutans* count (p=0.065), salivary pH values (p=0.242) and buffer capacity (p = 0.87).

**Table 2:** Within the group comparison of S mutans count, pH and Buffer capacity. Chi-square test was used to analyze the statistical difference. Paired 't' test was used for comparing the S mutans count and Salivary pH values. Wilcoxon- signed rank test was used for comparing the Buffer capacity.

| Group | Time Intervals | N | Mean S mutans Count (in CFU) | p value | pH | p value | Buffer Capacity | p value |
|---|---|---|---|---|---|---|---|---|
| Group A | Baseline | 21 | 774190.48 | **0.001*** | 6.01(0.48) | **0.001*** | 21 | 1.00 |
| | 30$^{th}$ day | 21 | 160933.33 | | 6.52(0.47) | | 21 | |
| Group B | Baseline | 22 | 976281.82 | **0.001*** | 6.29(0.47) | **0.001*** | 22 | 0.269 |
| | 30$^{th}$ day | 22 | 248527.27 | | 6.62(0.54) | | 22 | |
| Group C | Baseline | 20 | 1033400.0 | 0.065 | 5.99(0.26) | 0.242 | 20 | 0.87 |
| | 30$^{th}$ day | 20 | 922550.00 | | 5.93(0.24) | | 20 | |

Table 3 shows comparison of S mutans count, pH and Buffer capacity among the three groups. Though there was no significant difference at baseline in the *S. mutans* count (p = 0.76), pH (p = 0.057) and buffer capacity (p value = 0.872), while analysis of data obtained after 30days showed that the number of *S. mutans* colonies were found to be higher in the control group than the two test groups and this difference was found to be statistically significant (p=0.001). However, at the 30th day, there was no significant difference between the *S. mutans* count and salivary pH levels obtained from the subjects enrolled in the two probiotic groups (p=0.607).

**Table 3:** Comparison of S mutans count, pH and Buffer capacity among the three groups at baseline and after 30 days. One Way ANOVA followed by Post Hoc Tukey test was used for comparing the S mutans count and Salivary pH values. Kruskal Wallis test was used for comparing the Buffer capacity.

| Time Intervals | | N | Mean S mutans Count (in CFU) | p value | pH | p value | Buffer Capacity | p value |
|---|---|---|---|---|---|---|---|---|
| Baseline | Group A | 21 | 774190.48 | 0.76 | 6.01(0.48) | 0.057 | 21 | 0.872 |
| | Group B | 22 | 976281.82 | | 6.29(0.47) | | 22 | |
| | Group C | 20 | 1033400.00 | | 5.99(0.26) | | 20 | |
| 30TH Day | Group A | 21 | 160933.33 | **0.001*** | 6.52(0.47) | **0.001*** | 21 | **0.001*** |
| | Group B | 22 | 248517.27 | | 6.62(0.54) | | 22 | |
| | Group C | 20 | 922550.00 | | 5.93(0.24) | | 20 | |

## DISCUSSION

*Streptococcus mutans* has been associated with dental caries due to its ability to metabolize the sugar substrates to produce acids that demineralizes the tooth structure.[18] Thus an increase in the *S. mutans* level in the saliva favours demineralisation.[19,20] Enhancing the salivary defence mechanism while diminishing the microbial levels seems to be a rational approach. Salivary pH and buffer are two salivary defence mechanisms that prevent demineralisation.[21] In the current era, focus has always been on caries prevention which is researched at large. There has always been a constant surge to identify preventive measures for dental caries. Thus evaluating the reduction of *Streptococcus mutans* counts along with change in salivary pH and buffer capacity is deemed necessary to establish the caries preventive effect for any intervention. Thus the current trial evaluated the impact of the probiotic strains on *S. mutans* count as well as salivary pH and buffer capacity.

*Streptococcus salivarius* is a predominant human commensal of the oropharynx and can constitute a large proportion of the total bacterial population inhabiting this region.[22] Burton *et al*.[23] have demonstrated the beneficial effect of S salivarius K12 and also highlighted the fact that this agent is well tolerated when regularly ingested in large numbers for 28 days. Salivarius K12 was first isolated from saliva of health child and being used in New Zealand for more than a decade,.[24] K12 produces salivaricin A2 and salivaricin B, two bacteriocins that effectively inhibit phylogenetically related bacterial species. In the human population, approximately 2% of children naturally possess salivarius strains that produce both salivaricin A and salivaricin B, which corresponds to the strain K12 bacteriocin profile. Due to its bacteriocin profile, BLIS K12™ was commercially developed and thus became the first Commercial K12 based oral probiotic to specifically target the oral tissues. Hence in our clinical trial one group of patients received BLIS K12™.

Another Strain of interest of our study is salivarius M18. This strain exhibits a very different bacteriocin profile when compared to K12. It effectively secretes the bacteriocins A2, 9, MPS and M. Due to its ability to inhibit cariogenic pathogens, BLIS M18™ utilizing an active strain of salivarius M18 was commercially developed.[25] For our study, subjects enrolled in the second group received BLIS M18™. In a study by Burton *et al.*[17], they have provided evidence to support the use of S.salivarius M18, a bacterium isolated from the human oral cavity and shown to have antibacterial activity against a number of clinically important human pathogens to reduce plaque accumulation in school children. Since there is a lack of clinical evidence in support of S. salivarius K12, the current study was embarked on to clinically compare the two strains K12 and M18.

Though most studies on probiotics and dental caries have evaluated the changes in MS counts, but only a few studies have assessed the impact on the pH and buffer capacity thus altering the overall chance to develop new carious lesions.[15] Hence this randomized double blinded clinical trial was undertaken to evaluate and compare the effect of probiotic *Streptococcus salivarius* K12 and M18 on the *Streptococcus mutans* count, salivary pH and buffer capacity. When K12 and M18 were first introduced in the oral cavity in the form of a probiotic lozenge, they were expected to colonize specific oral tissue and be tolerated by the human host. Studies have demonstrated that treatment dosing regimen of one lozenge per day, taken before bedtime was well tolerated by the individuals.[26]

A study by Power *et al*. showed that a lozenge containing streptococcus strain demonstrated an 80% effectiveness in colonizing the oral cavity among those who consume the probiotic while the controls ingesting powdered form showed a 33% decrease in the effectiveness. This reduced efficacy of the powdered form was attributed to a reduced oral exposure time when compared to the lozenge form of probiotic strain.[26] Thus the lozenge form of the *Streptococcus salivarius* K12 and M18 was used in the current trial.

Before salivarius K12 and M18 can exert their beneficial effects, they must individually colonize the oral cavity. To initiate their colonisation in the oral cavity, individual cells must adhere to oral epithelial cells and then rapidly reproduce to form colonies.[27] According to our results, 30days treatment with both BLIS K12™ and BLIS M18™ have shown reduction in the *S. mutans* count. This may be attributed to the ability of the salivarius K12 and M18 to colonize the oral tissues. Studies have shown that the higher levels of colonisation result in greater reduction of these caries causing bacteria in saliva and thus an overall reduction in the development of caries.[28] However, studies have also shown that the patterns of colonisation of the oral cavity by S. salivarius K12 and M18 are dose dependent.[27] When larger dosages are administered, an increased number of M18 bacteria are retained over longer periods of time. Thus further long term clinical trials comparing the two stains of the *Streptococcus salivarius* have to be undertaken to establish the dosage.

The results of the present trial have also demonstrated an increase in the salivary pH following a 30-day use of BLIS K12™ and BLIS M18™. Our results are in concordance with the results of Pierro *et al.*(15). Several microbial species belonging to the oral microbiota produce active urease as demonstrated by the *Streptococcus salivarius* and the urease from these species facilitates hydrolysis of urea.[29] This enzyme urease can increase the pH of saliva by breaking down urea into carbon dioxide and ammonia, thus increasing the salivary pH thereby preventing hydroxyapatite dissolution.[15] This could be an attributable reason for the increase in salivary pH following a 30-day use of both the probiotic strains. Though there was difference in the *S. mutans* count and salivary pH, the buffer capacity of saliva remained unaltered in all three groups. This shows that there is no impact of these probiotics on the buffer capacity of saliva.

To improve the validity and accuracy of the conclusion drawn from the research, more extensive research with larger sample size should be conducted. In the field of probiotic research, there is always a continuous demand for more information about this relatively novel branch of probiotics.

## CONCLUSIONS

Within the limitations of the present study it can be concluded that a 30day use of *Streptococcus salivarius* K12 and M18 resulted in a reduction in the *Streptococcus mutans* count while simultaneously improving the salivary pH. Thus it is very evident that the use of probiotic *Streptococcus salivarius* K12 and M18 for a short period will definitely provide anticariogenic benefits. With the ever changing scenario in the field of caries prevention, further trials are needed to establish the long term effects of these probiotics in terms of practical clinical application.

## COMPLIANCE WITH ETHICAL STANDARDS

The authors declared that the paper has complied with the ethical standards as per the guidelines of the journal.

## CONFLICT OF INTEREST

First author Dr Saravanan Poorni declares that she has no conflict of interest. Second Author Dr Nivedhitha M S declares that he has no conflict of interest. Third author Dr Manali Ramakrishnan Srinivasan declares that she has no conflict of interest.

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**Original research**

# COMPARISON TENSILE STRENGTH OF DIFFERENT SUTUR MATERIALS

Turan Emre Kuzu[1]

## ABSTRACT

**Objectives**: The purpose of this study is to compare the tensile strength of different types of surgical suture materials.

**Materials and Methods**: Current study, the tensile strengths of 4 different suture materials was compared. [(1-4/0 silk suture 2-4/0 propylene suture 3-4/0 Polyamide suture 4-4/0 Poly[Glycolide-Co-L-Lactide] (90%:10%) (PGLA) suture)] The tensile strength of a total of 40 samples was calculated, with 10 samples in each group. In the study, the tensile strength of the sutures was calculated using a universal tester, the sutures were tightened to both poles of the test device and fixed with a distance of 15 mm between the poles, tensile force was applied so that both poles of the test device moved away from each other at a speed of 25cm/min until the sutures broke, and the force value of the sutures at the moment of brake was recorded in Newton units (N) as the tensile strength of suture. Statistical analysis of the data was evaluated by One-Way ANOVA and Tukey HSD tests.

**Results:** In the statistical analysis of the tensile strength of the suture materials, the difference between the tension resistance of the PGLA and Polyamide suture was not statistically significant, but the difference between all other suture materials was statistically significant ($p<0.05$). Tensile strength of suture materials were determined as propylene, polyamide, PGLA and silk suture, respectively.

**Conclusions**: Within the limits of the current study, the tensile strength of the propylene suture was higher than that of PGLA, silk, and polyamide sutures.

**Keywords**: Sutures, Tensile, Break.

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## INTRODUCTION

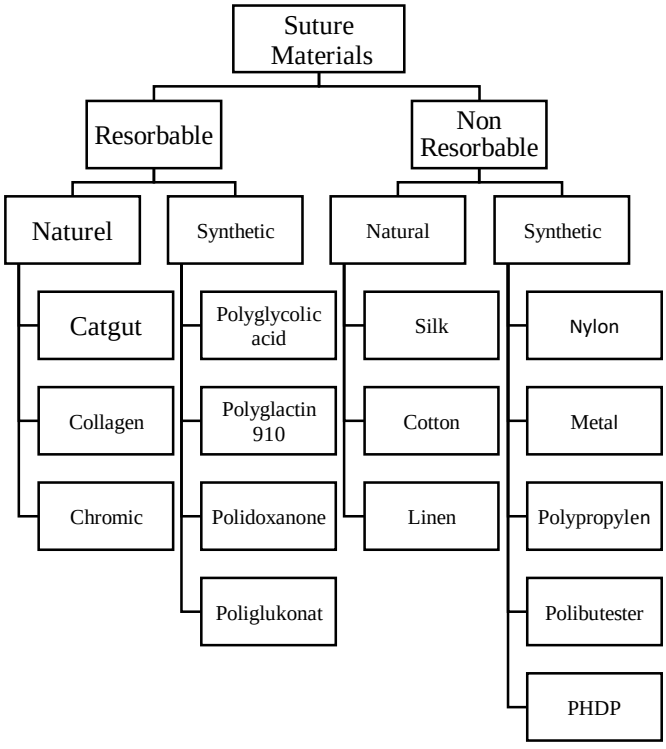
Suturing is performed in dentistry, implant treatment, impacted tooth extractions, minor surgical procedures or in cases where tissue integrity is disrupted due to trauma and similar reasons. Suturing are used sutures, tissue adhesives and staples in the clinic. Suture is the most frequently used material among them.[1] Which suture will be selected in the clinic; The suture's tensile strength, knot reliability, elasticity, capillarity, and tissue reaction should be evaluated.[2]

Tensile resistance; It is the condition that the suture material resists the tension on the wound edges enough to maintain the current position of during the wound healing process. Knot reliability; It is the condition of maintaining the stability of the knot against tissue tension, and this feature varies according to the capillarity feature of the suture, whether it is monofilament or multifilament. Capillarity in sutures is defined as the flow of absorbed fluids through the suture. This feature causes infection in the tissues around the suture by microorganisms in the mouth. Capillarity is higher in multifilament sutures than in monofilament sutures. The elasticity of an object is defined as the object's deformation with force and its ability to return to its original shape after the force is removed. Elasticity in sutures is defined as the return of the original dimensions after the pressure is removed, although the suture shows stretching at varying pressures in the tissue during healing. Flexibility in sutures varies according to the suture material. Foreign body reaction is that the suture causes inflammation in the tissue. When sutures are evaluated in terms of Foreign body reaction, multifilament sutures cause higher tissue reaction than monofilaments, and natural ones cause higher tissue reaction than synthetic sutures.[3]

The ideal suture material should provide the following properties; 1-It should have enough tensile strength to protect the primary closure during the healing process, 2-It should have sufficient knot reliability, 3-It should be easy to use clinically, 4-It should have low capillarity and it should cause minimal foreign body reaction in the tissues. Each suture material has some advantages and disadvantages.[4,5]

Suture materials are classified according to the material from which they are obtained (Table 1).

**Table 1**: Classificiation of suture materials



Our aim in this study is to investigate the tensile strength of different suture materials. The use of different suture materials provides clinical advantages. In this study, our aim is to compare the tensile strengths of different suture materials.

## MATERIALS AND METHODS

Our study was conducted with the approval of Nuh Naci Yazgan University non-interventional clinical research ethics committee dated 31.03.2021 and numbered 2021/1491.In our study, 4 different sutures were used. [(1-4/0 3/8 sharp black silk suture 16 mm Dogsan TURKEY, 2- 4/0, 3/8 sharp propylene suture 20 mm Dogsan TURKEY, 3- 4/0 3/8 sharp Polyamide, suture 20 mm Doğsan TURKEY, 4-Pegelak 4/0 1/2 round 20 mm poly (glycolide-co-lactide) (PGLA) suture, Doğsan TURKEY)]. This study, all sutures were used in 4/0 thickness for standardization and the type of suture to be used in the study was decided according to the frequency of use in the dentistry clinic.

The experimental stage of the study was carried out in Erciyes University Technology Research Application Center (TAUM). The number of samples in each group was determined as 10 (n=10). The tensile strength of all samples was calculated using a universal tensile-compression test device *(Shimadzu ag-xd 50kN Japan)* (Figure 1).


**Figure 1:** Tensile strenght measurement device (Shimadzu Ag-Xd 50kn Japan)

The sutures were fixed to the poles with a distance of 15 mm between then the poles moved away from each other at 25 cm/min and the tension force was provided on the sutures, the force was recorded at the moment break of the sutures as the tension resistance of that suture in Newtons (N) (Figure 2).

**Figure 2:** Fixing the suture on both poles of the tension resistance device

Statistical analysis of the obtained data was performed using SPSS 20 (SPSS Inc., Chicago, U.S.A) program. Variance homogeneity was examined using the Levene test. In evaluating the tensile strength analysis of variance (ANOVA) and Tukey's post hoc test were used to determine the differences between groups.

## RESULTS

Statistical results are as shown in Table 2 ,Table3 and Figure 3 The mean values of each group were found as follows, Propylene suture 19.252 N, Polyamide suture 17.446 N, PGLA 16.907 N, Silk suture 14.250 N. The difference between polyamide and PGLA sutures was not statistically significant ($p>0.05$), while the differences between all other groups were statistically significant ($p<0.05$).

**Table 2**: Statistical comparison of the study grops

| | Sum of squares | df | Mean of squares | F | Significant |
|---|---|---|---|---|---|
| Between groups | 128.038 | 3 | 42.679 | 24.989 | .000 |
| Within groups | 61.485 | 36 | 1.708 | | |
| Total | 189.523 | 39 | | | |

**The mean different is significant at the 0.05 level (One –Way ANOVA)*

**Table 3:** Statistical evaluation of the mean values of the all groups

| | n | Mean tensile strenght (Min-Max) | St. Dev. | p |
|---|---|---|---|---|
| **Silk** | 10 | 14.250 (12.25-15.92) | 1.54 [a] | p<0.05 |
| **PGLA** | 10 | 16.907 (13.85-19.85) | 1.77[b] | p>0.05 |
| **Polyamid** | 10 | 17.446 (16.8-19.16) | 0.772[b] | p>0.05 |
| **Propylene** | 10 | 19.252 (18.4-20.5) | 0.85[c] | p<0.05 |

*There is significant difference between the averages shown with the different letter. (Tukey HSD).*The mean different is significant at the 0.05 level*

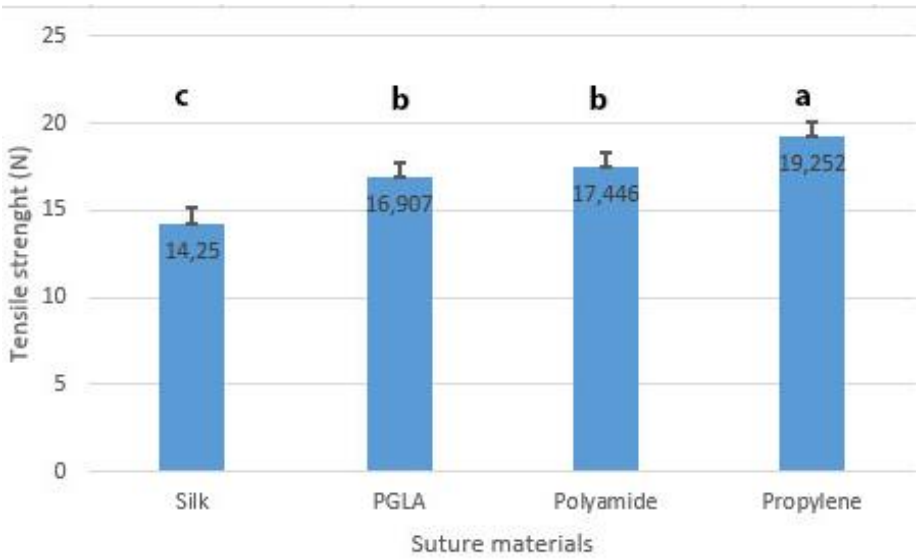



**Figure 3:** Graphical representation of the tensile strength averages of 4 different sutures. Different letters just above the columns in the graph indicate statistically significant difference.

## DISCUSSON

Today, in dentistry, especially in surgical operations suture selection and suturing methods are important parameters to consider for adequate healing and maintaining the integrity and continuity of soft tissues.[6] The tensile strength of suture materials is affected not only by the material from which the sutures are obtained, but it's being monofilament or multifilament, suture thickness, and the duration of use in oral tissues.[7] Monofilament sutures have strong shape memory but poor knot reliability. Multifilament suture materials are resistant to abrasion and have higher tensile strength, flexibility, elasticity and knot reliability than monofilament sutures.[8]

In our study, contrary to the current literature, it was observed that the silk suture less tension resistance than other sutures.[9,10]

In the current study, the tension resistance of the propylene suture was found to be higher than the silk suture, while Joshi *et al.* and Gemci R. *et al.*[1,10,11] the tension resistance of silk suture was found to be higher than that of porpylene sutures and other sutures.

Suture materials are classified according to their thickness. Two main standards are used in the classification made according to the suture thicknesses. These standards are USP (United States Pharmacopoeia) and EP (European Pharmacopoeia) standards and USP is more commonly used in suture classification than EP classification. In the USP classification, sutures are numbered with the metric system, such as 7/0, 6/0, 5/0, 4/0, 3/0, and as the number increases, the suture gets thinner and the tension resistance decreases.[12]

According to this classification, 7/0 and more thinner sutures are used in microsurgery and ophthalmology procedures, 6/0 and 5/0 sutures are used in aesthetic suturing and vascular surgery, 4/0 sutures are used in mucosa, extremity and tendon suturing, and 3/0 sutures are used in general surgery.[13] In our study, we chose 4/0 thickness because it is a commonly used suture thickness in dentistry clinical routine.

Arce *et al.*[14] compared the tension resistance of PGLA and silk sutures in their study and according to the result of the study, the tension resistance of PGLA Suture was found to be higher than silk suture. In our study, Arce *et al.* Similar to the results of the study, the tensile strength of the PGLA suture was found to be higher than the silk suture.

The rat study in which tissue tension was measured during the healing process soft tissue defects were created experimentally, then the tension resistance of the tissue was measured on different days during the healing process. The results of this study showed that the tissues resisted tension up to 8 Newtons in the 1st week, 11 Newtons in the 2nd week, and up to 15 Newtons in the 6th week during the healing process.[15]

When we compared that previous sentences study and our study. We can conclude that all the sutures used in our study can be used safely for up to 2 weeks, but the resistance of the silk suture is insufficient in procedures that require a longer healing period.

Chu *et al.*[16] reported that resorbable sutures lost 30% of their tensile strength during the resorption process. The results of Chu *et al.'s* study are consistent with the current literature, and resorbed sutures lose some of their tensile strength over time.

Another factor affecting the suture tension is the knot, knotting the suture causes a loss of approximately 1/3 of the tensile strength, especially in the knot area.[17] Polyamide sutures are produced in 2 different structures as monofilament and multifilament, the tensile strength of the monofilament is higher than the multifilament.[18] In our study, we preferred a monofilament polyamide suture. Results of the study, the second highest tensile strength was found in this suture, which is partially compatible with the literature.

In the present study, it was determined that there are 2 important limitations. The first limitation is that monofilament and multifilament sutures were used in the same study. The second limitation is that a single measurement value cannot be a clear result since the tension resistance of resorbable sutures decreases as long as they remain in the mouth.

## CONCLUSION

According to the results of the current study, propylene suture may be preferred in cases where primary closure of soft tissue is important, such as bone augmentation or mucogingival surgical procedures, since it is a suture with high tension resistance. However, the clinician should consider factors such as knot reliability, capillarity, and patient tolerance after the procedure when choosing sutures.

## CONFLICTS OF INTEREST STATEMENT

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.


## *ÖZ*

***Amaç**: Bu çalışmanın amacı elde edildikleri maddelere göre değişik yapıdaki sütür materyallerinin laboratuvar ortamında gerilime dirençlerinin karşılaştırılmasıdır.* ***Gereç ve Yöntem**: Çalışmamızda değişik materyalden üretilmiş 4 sütür materyalinin gerilim dirençleri karşılaştırılmıştır. [(1-4/0 siyah ipek sütür 2-4/0 propilen sütür, 3-4/0 Poliamid, sütür,4-4/0(PGLA )] her bir grupta*

*10 örnek olmak üzere toplamda 40 örneğin gerilim direnci hesaplandı. Çalışmada sütürlerin gerilim direnci üniversal bir test cihazı kullanılarak hesaplandı, sütürler test cihazının her iki kutbuna gergin ve kutuplar arası 15 mm mesafe olacak şekilde sabitlendi, gerilim kuvveti sütürler kopuncaya kadar test cihazının her iki kutbunun 25cm/dk hızla birbirinden uzaklaşması şeklinde uygulandı ve sütürlerin kopma anındaki kuvvet değeri o sütürün kopma direnci olarak Newton biriminde (N) kaydedildi. Verilerin istatistiksel analizi One –Way ANOVA ve Tukey HSD testleriyle değerlendirildi.* ***Bulgular:*** *Sütür materyallerinin gerilim dirençleri istatistiksel olarak analiz edildiğinde PGLA ve Poliamid sütürün gerilim dirençleri arasındaki fark istatistiksel olarak anlamlı değil iken, diğer tüm sütür materyalleri arasındaki fark istatistiksel olarak anlamlı bulunmuştur (p<0,05). Sütür materyallerinin gerilim direnci yüksekten düşüğe doğru sırasıyla propilen, poliamid, PGLA ve ipek sütür olarak belirlenmiştir.* ***Sonuçlar:*** *Bu çalışma sınırları dahilinde gerilim direncinin önemli olduğu durumlarda propilen sütürün PGLA, ipek veya poliamid sütüre göre daha avantajlı olabileceği belirtilmiştir. Bununla beraber, sütür seçiminde düğüm güvenilirliği, yabancı cisim reaksiyonu gibi faktörler de göz önünde bulundurulmalıdır.* ***Anahtar kelimeler****: Sütür, gerilim, kopma.*

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**Original research**

# THE EFFECT OF HUMIC ACID ON BONE REGENERATION IN RATS

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## ABSTRACT

**Objectives:** The objective of our study was to investigate the effect of the local humic acid application in addition to autogenous bone grafting on the premature new bone amount.

**Materials and Methods:** 12-week-old 24 Wistar rats were divided into 4 groups. Two parietal bone defects with 5-mm diameter were constituted in each animal. All defects in each group were treated with only autogenous bone graft (n=6), autogenous bone graft combined with 100 mg/kg (n=6) or 200 mg/kg humic acid (n=6). Defects in one group remained empty as control (n=6). The laboratory animals were sacrificed on the 28th day following the procedure. Morphological properties of all experimental defects were evaluated using micro-computed tomography (Micro- CT).

**Results:** The highest value among trabecular thickness (Tb.Th) and the ratio of the bone volume to the tissue volume (BV/ TV) was encountered in the autogenous graft group applied with 100mg/kg humic acid. The highest value in the bone volume (BV) variance was detected in the group to which only the autogenous graft was applied. A statistically significant difference was found between the control group and the only autogenous graft-applied group and the autogenous graft group applied with local 100mg/kg humic acid upon comparing the groups in pairs for bone surface area (BS) variance. BS/BV value was found higher in the autogenous graft group applied with local 200mg/kg humic acid than the autogenous group applied with local 100mg/kg humic acid.

**Conclusions:** Humic acid application in addition to autogenous bone grafting caused a decrease in the bone volume value. However, the positive effect of the humic acid application was observed in trabecular thickness and bone volume/tissue volume values, dosage increase negatively impacted the same. The dosage increase negatively affected the bone surface area value. No positive effect of the humic acid application to bone surface/bone volume value was observed. The statistical significance between the control group and the other groups is considered to be autogenous graft.

**Keywords:** Humic acid, autogenous bone graft, bone regeneration.

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## INTRODUCTION

Bone regeneration has been an important task in the field of dentistry. Considering aesthetical and functional demands, most clinicians aimed to obtain rapid bone augmentation with high quality. Therefore researches focus on the additional treatment modalities potentially accelerating the bone healing. The most frequently used surgical method in defect repair treatment is the bone grafting procedures.[1,2] Accordingly, the ideal bone graft material should have osteoinduction, osteoconduction and osteogenesis features, reportedly.[3] Autogenous bone grafts are accepted as the gold standard as the sole bone graft material possessing all these features, although they have disadvantages such as their being obtained at a limited amount, the observance of long-term postoperative disconfort.[4] Schlegel *et al*.[5] in their animal study, they used autogenous and bovine bone grafts in the sinus elevation operation. As a result of the study, they reported that the resorption rate of autogenous bone graft was 40% after 6 months, while this rate was 15% in bovine bone graft. Simion *et al*.[6] in their long-term retrospective study, they reported that autogenous bone particles used in guided bone regeneration are more prone to resorption.

Humic materials are organic carbon forms that already exist in nature. They represent many organic materials formed of earth, lignite, peat, mineral waters and precipitates. Humic materials are divided into three molecular forms: fulvic acids, humic acids and humin. Humic acids and fulvic acids disslove in an alkaline environment, while humans represent the unsolved residue. One of the essential parts of humic substances is humic acids.[7]

The naturally occurring humic acids are brown-black polymeric organic acids exist on earth and water surface.[8] Peat is an organic type of earth formed as a result of the humification of dead marsh plants. Peat has been used in the medical field for hundreds of years The organic part of the peat includes humin at the ratio of 90%, humic and fulvic acid (over 40%), lignin, polysaccharides, lipids, pectins, hemicellulose and cellulose.[9] Humic acids increase the vascularisation and thus accelerate the regeneration. Peat has been used for mud therapy (balneotherapy) for many years.[10] It is also used as an anti-inflammatory agent due to its antiinflammatory properties.[11,12] In addition, due to its antiviral, profibrinolytic, anti-inflammatory, estrogenic, antibacterial, anticancer, diuretic, and immunostimulant characteristics, it has been utilized for a variety of medical applications.[13-17] However, despite widespread research in the field of medicine, the research on the use of HA in the field of dentistry is insufficient .Peat-origin humic acid was used in this study.

Our aim in this study was to evaluate the effect of local humic acid use on insufficient new bone formation due to early period resorption, which is the disadvantage of autogenous bone grafts.

## MATERIAL AND METHOD

The approval of the Animal Testing Ethical Committee of Sivas Cumhuriyet University was obtained on 17.06.2020 with line no. 328. 12-week-old 24 Wistar Albino rats with a weighing of 230-250 g were used as subjects. The conditions such as overall wellness of the laboratory animals and performance of no study over them previously were considered. All rats were fed with water and a standard diet, observing 12 hours night/day, 21±1ºC temperature, and 40-60% humidity ratio. Rats were caged in metal cages for 10 days to prepare for their new living conditions before the study. The testing stage of our study was conducted in Sivas Cumhuriyet University School of Medicine Animal Laboratory. Micro-CT imaging was carried out in Erciyes University Scientific Research Laboratory.

### *Group Formation*

The laboratory animals were divided into 4 groups, and each group was formed of 6 laboratory animals. Those were determined as the control group, only autogenous graft-applied group, autogenous graft group applied with 100 mg/kg humic acid and autogenous graft group applied with 200 mg/kg humic acid (Table 1).

**Table 1:** Group formation

| Experimental and control groups | Number of animals per group | The number of repetitions | Total number of animals used |
|---|---|---|---|
| Control Group | 6 | 1 | 6 |
| Only Autogenous Graft-Applied Group | 6 | 1 | 6 |
| Autogenous Graft Group Applied With 100 mg/kg Humic Acid | 6 | 1 | 6 |
| Autogenous Graft Group Applied With 200 mg/kg Humic Acid | 6 | 1 | 6 |

***Surgical Method***

Rats were subjected to anesthesia through 30 mg/kg Ketamin-HCL (Ketas, Eczacıbaşı, Turkey) and 5 mg/kg Xylazine HCL (Rompun, Bayer, Germany) injection, the skin on the parietal area of the laboratory animals were shaved, and the operation zone was wiped using povidin iodine and covered with a sterile surgical cover. Then cranial cutaneous incision was applied, extending from the occipital to the frontal bone for about 2 cm. All tissues were dissected in a manner to include skin, subcutaneous tissue and periost, respectively, in order to reveal the parietal bone. Automax drill was used to obtain a 5mm-diameter bicortical bone fragment at critical size after the bone surface appeared. After exposing the bone surface, a critically sized 5mm diameter bicortical bone fragment was removed. The defect was formed under psychological saline solution irrigation at 600-1000 rpm. Then, the bone tissue formed as a particulate autogenous graft, which was obtained during the formation, was placed in the defected zone of the relevant test group. The skin flap was sutured to its original position using 5-0 polyglactin 910 suture following the closure of the under-skin fascia after the operation.

***Humic Acid Application***

The suitable humic acid concentration was prepared for the rats to which local humic acid would be applied; the amount of humic acid determined for the groups of local application was applied by being mixed with the autogenous graft.

***Termination of the Test***

The laboratory animals were sacrificed using 200mg/kg sodium pentobarbital (Petothal, Abbot, USA) on the 28th day following the process. Then, the grafted defect zone was removed with sufficient bones around it and placed in 10% formalin.

***Evaluation by Micro-Computerized Tomography (Micro-CT)***

Micro-CT was first started in 1989 by Feldkamp *et al*.[18] Tissue mineral density, bone mineral density and bone volume can be evaluated in Micro-CT. It is also accepted as a golden standard for evaluating the three-dimensional structure of trabecular bone. However, it is not successful in the evaluation of the microstructure of cortical bone.[18,19] The Micro-CT (SkyScan-1272, Bruker, Kontich, Belgium) device belonging to Erciyes University Faculty of Dentistry Research Laboratory was used in the study.

Micro-CT helps to analyze the microstructure of the bone trabecules and performing BMD (bone mineral density) measurement. Furthermore BV (new bone volume), BV/TV, BS (new bone surface), BS/BV (new bone surface/BV), Tb.Th (trabecular thickness), Tb. N (trabecular number) values were examined in the area of examination.[20,21]

***Statistical Method***

The data obtained in our study was loaded to SPSS (Ver 22.0) software and was obtained through measurement as the parametric test assumptions were performed in the evaluation of the data (Kolmogorov-Smirnov), Variance Analysis was used to compare the measurements obtained from more than two independent groups, and Tukey test was used to find the differentiating group and groups when the difference between groups was found significant at the end of the analysis. The level of significance was accepted as $p<0.05$.

## RESULTS

***Micro-CT analysis results***

The biopsy samples were subjected to radiologic examination in the micro-CT device. The result of the analysis is described in table 2.

**Table 2**: Intergroup comparison of micro-CT mean values

| Variables | Control group | Only autogenous graft-applied group | Autogenous graft group applied with 100 mg/kg humic acid | Autogenous graft group applied with 200 mg/kg humic acid |
|---|---|---|---|---|
| **BV($mm^3$)** | 3.23±1.31[a] | 8.91±2,47[b] | 7.33±1,88[b] | 6.70±2.11[b] |
| **BV/TV(%)** | 12.20±5.77[a] | 25.51±4.75[ab] | 28.88±4.44[b] | 20.69±6.77[ab] |
| **BS($mm^2$)** | 41.67±13.09[a] | 85.77±18.39[b] | 69.14±13.33[b] | 64.67±18.04[ab] |
| **BS/BV(%)** | 13.48±2.49[a] | 9.76±1.10[b] | 9.26±0.76[b] | 9.82±1.19[b] |
| **Tb.Th(mm)** | 0.29±0.06[a] | 0.39±0.02[b] | 0.41±0.02[b] | 0.40±0.03[b] |
| **Tb.N(1/mm)** | 0.41 ±0.16 | 0.63± 0.10 | 0.53± 0.22 | 0.50± 0.13 |

Data are expressed as mean ± standard deviation. Similar letters on the same line indicate the similarity between groups, and different letters indicate the difference between groups.

The highest value in BV variance was only detected in the autogenous graft-applied group (8.91±2.47$mm^3$), and the lowest was observed in the control group (3.23±1.31$mm^3$). Comparing the groups in pairs, the difference between the control group and the other groups was statistically significant ($p$<0.05). Likewise, the difference between the autogenous graft group applied with local 100mg/kg humic acid and the autogenous graft group applied with local 200mg/kg humic acid was not statistically significant; however, the BV value in the autogenous graft group applied with local 100mg/kg humic acid was found higher.

Considering the BS/BV rates, while the highest value was observed in the control group (13.48±2.49%), the lowest was detected in the autogenous graft group applied with local 100mg/kg humic acid (9.26±0.76%). In pairwise comparison of the groups, a statistically significant difference was found between the control group and the other groups ($p$<0.05). Similarly, the difference between the autogenous graft group applied with local 100mg/kg humic acid and the autogenous graft group applied with local 200mg/kg humic acid was not statistically significant; however, the BS/ BV rate in the autogenous graft group applied with local 200mg/kg humic acid was found higher.

The highest value was found in the local 100mg/kg humic acid-applied autogenous graft group (0.41±0.02mm), and the lowest was found in the control group (0.29±0.06mm) in Tb.The variance. Comparing the groups in pairs, a statistically significant difference was found between the control group and the other groups ($p$<0.05). In a similar manner, the difference between the autogenous graft group applied with local 100mg/kg humic acid and the autogenous graft group applied with local 200mg/kg humic acid was not statistically significant; however, the Tb. The value in the autogenous graft group applied with local 100mg/kg humic acid was found higher.

The highest value for BV/TV variance was detected in the local 100mg/kg humic acid-applied autogenous graft group (28.88±4.44%), and the lowest value was found in the control group (12.20±5.77%). Upon comparing the groups in pairs, When the groups are compared in pairs, a statistically significant difference was found between the control group and the local 100mg/kg humic acid-applied autogenous graft group ($p$<0.05). At the same time, also the difference between the autogenous graft group applied with local 100mg/kg humic acid and the autogenous graft group applied with local 200mg/kg humic acid was not statistically significant; however, the BV/TV value in the autogenous graft group applied with local 100mg/kg humic acid was found higher.

Upon comprising the groups in pairs for BS variance, a statistically significant difference was found between the control group and the only autogenous graft-applied group and local 100mg/kg humic acid applied autogenous graft group ($p$<0.05), the difference between the local 200mg/kg humic acid-applied autogenous graft group was not found statistically significant ($p$>0.05). At the same time, Furthermore the difference between the autogenous graft group applied with local 100mg/kg humic acid and the

autogenous graft group applied with local 200mg/kg humic acid was not statistically significant; however, the BS value in the autogenous graft group applied with local 100mg/kg humic acid was found higher.

Upon comparing in comparison, Tb/N (1/mm) variance groups, although no statistically significant difference was found among groups, the highest value was found in the only autogenous graft-applied group, and the lowest was found in the control group.

## DISCUSSION

The autogenous graft can be obtained from intraoral zones such as tuber maxilla, mandibular ramus, mandibular symphysis and the toothless zone or extraoral zones such as iliac bone, tibia and skull.[22] Although autogenous grafts are presented as the golden standard, their clinical activities are limited due to the scarcity of the obtained graft amount and resorption.[23] Given all these advantages and disadvantages, the autogenous graft has been the scope of our study similar to many other studies.[18,24]

Humic acids are the most widely available organic carbon forms, and they exhibit strong anti-inflammatory effects by inhibiting IL-1β ve TNF-α release, which is activated by leucocytes.[25] It was reported that humic acid significantly degrades liposaccharide-mediated adhesion molecules (ICAM-1, VCAM-1 and E-selectin) being cultured from human umbilical and endothelial cells.[26] This can be deemed among the methods to describe the possible effects of humic acid on the inflammatory process. In a study in which individuals suffering from HIV were treated using 2, 4, 6 and 8 grams of oxymate/day for 2 weeks, no sign of toxicity was encountered at the end of the period.[27]

In the study conducted by Van Rensburg *et al.*[25], the effects of potassium humins on TNF-α, IL-1β, IL-6 and IL-10 synthesis were obtained at a 40μg / ml concentration. In this concentration, it was reported that humins significantly inhibited TNF-α, IL-1β, IL-6 and IL-10 release produced by phytohemagglutinin (PHA), which is stimulated by mononuclear leukocytes (MNL).

It was reported that the oral use of the humin (leonardite) for 6 days, which is obtained from daily 61 mg /kg lignite compressed contact hypersensitivity in rats. Furthermore, no toxicity was observed as a result of the use of 1000 mg /kg leonardite human orally for 1 month in rats. The teratogenic effect was not observed as a result of the treatment of pregnant rats with 500 mg /kg humans between the 5th and 17th days of their pregnancy.[24]

Humic acid acts as a dilator increasing the cell wall permeability. This increasing permeability eases mineral transfer from blood to bone and cells. Furthermore, it was reported that humic acid increases the calcification of xenografts by 16%.[28]

We researched the effect of humic acid on the healing mechanism of bone when it is applied locally in the light of this information.

It was reported that the regenerative capacity of cranial defects in laboratory animal models was better than in human beings. Calvarium develops morphologically and embryologically from a membrane process and resembles other bones developing in a membranous way on the face. As calvarium has two cortical layers anatomically, it resembles a structure like a mandibula and the physiological structure of atrophic mandibula. Due to such reasons, calvaria is one of the most preferred test zones.[29,30] We preferred it due to reasons such as the simplicity of the operational intervention to the parietal bone, the low possibility of harming the relevant part of the animal following the operation, osteogenic potential of dura mater and periost. Besides these advantages, the fact that the bone is about 2 mm thick in the parietal zone, bleeding depending on the dura mater perforation during operation and brain damage constitutes the risk factors requiring us to be sensitive in our study.

Critical size defect is a size defect with no chance to heal itself both in terms of function and form as long as the bone tissue is alive. When the critical defect size is exceeded, healing occurs through connective tissue, not through bone tissue. The critical size of bone tissue wound is dependent on factors such as the type of the

defect, age, gender, zone, depth and systemic condition.[31-33]

Takagi and Urist[34], examined the bone defect of 8 mm in diameter, which they formed in rats after waiting for 6 months and reported that fibrous healing occurred at the end of this period. In another study, Mulliken and Glowacki[35,36], reported that a 2-mm defect on the parietal bone in rats is not an ideal for ossification after 6 months. As in these studies, defects with various diameters were formed, and different results were stated. Please take note that there are different factors together with defect diameter concerning the difference in results. It was reported that causes such as trauma, insufficient blood support, bone marrow deficiency, the infection might affect the result.[32,37]

As it is reported in the studies, it has been understood that there was no consensus about the critical defect size. In many studies conducted, it was reported that a defect of 5 mm in diameter was sufficient for a critical size defect.[38,39] In the light of this information, it was decided to take the defect size as 5 mm in diameter in our study.

Different sacrification times were expected upon examining bone healing in the studies conducted. Generally, sacrification occurred at 2-8 week intervals. Sacrification was performed in the 4th week in order to track premature bone formation in our study.[33]

Micro-CT examinations are also used in human studies. However, these practices mandates conducting biopsy on bone. It is a much more frequently used imaging method in animal studies. Histomorphometric and micro-CT evaluations give quite objective results by providing digital data in the micromorphological examinations of bone. Micro-CT imaging provides fast, simple and much more precise measurements compared to histomorphometric evaluations. At the same time, histomorphometric evaluations provide only 2-dimensional sections; however, micro-CT evaluations enable volumetric measurements through 3-dimensional images.[20,40] Micro-CT imaging method providing digital values through 3-dimensional volumetric measurements was used in our study.

There are many radiographically examined micro-CT evaluation studies related to bone healing and micromorphology in the literature.[41-43] No study evaluating the effect of humic acid on bone regeneration in bone defects formed in rats through Micro-CT examination is available in the literature. Our study may contribute to the literature in terms of giving opinions to the relevant dentists concerning the effect of the local humic acid application on bone regeneration in bone defects from this aspect.

Çalışır *et al*.[44] stated that the systemic effects of humic acid have potent anti-inflammatory and osteoblastic activity, may stimulate bone healing by causing an increase in the anti-inflammatory cytokine levels such as IL-10 however a decrease in proinflammatory cytokine levels such as IL-1β. Furthermore, they observed that daily oral 80 and 150 mg/kg humic acid application decreases alveolar bone loss and increases osteoblastic activity.

Tkachenko *et al.*[45] determined in their study that although a significant increase was observed in healing and formation of osteocytes when applied for one week on the fracture line formed experimentally, it caused a decrease in osteogenesis when application time was extended.

In the study conducted by Kel'ginbaev *et al.*[46] it was seen that humic acid had a positive effect on the regeneration of bone tissue.

Durmuş *et al*.[47] used 16 Wister Albino rats in the study in which they research local humic acid application to healing time in mandibula fractures. Rats were grouped as control group and local humic acid (0.3cc/zone) group. They applied a single dose of humic acid (0.3cc/region) locally to the bone surfaces of the fracture line by raising a full thickness flap in the subcondylar region and performing a surgical osteotomy. They reported that humic acid was not effective in the healing of bone fractures in local single dose application.

In our study, the highest value in the BV variable was found only in the autogenous graft

group (8.91±2.47mm3). At the same time, the difference between the autogenous graft group applied with local 100mg/kg humic acid and the autogenous graft group applied with local 200mg/kg humic acid was not statistically significant; however, the BV value in the autogenous graft group applied with local 100mg/kg humic acid was found higher. The highest value was found in Tb in the local 100mg/kg humic acid-applied autogenous graft group (0.41±0.02mm). The variance. At the same time, the difference between the autogenous graft group applied with local 100mg/kg humic acid and the autogenous graft group applied with local 200mg/kg humic acid was not statistically significant; however, the Tb.Th value in the autogenous graft group applied with local 100mg/kg humic acid was found higher. The highest BV/TV varisance value was detected in the local 100mg/kg humic acid-applied autogenous graft group (28.88±4.44%). At the same time, the difference between the autogenous graft group applied with local 100mg/kg humic acid and the autogenous graft group applied with local 200mg/kg humic acid was not statistically significant; however, the BV/ TV value in the autogenous graft group applied with local 100mg/kg humic acid was found higher. At the same time, the difference between the autogenous graft group applied with local 100mg/kg humic acid and the autogenous graft group applied with local 200mg/kg humic acid was not statistically significant; however, the BS value in the autogenous graft group applied with local 100mg/kg humic acid was found higher. In the light of these findings, it can be considered that local humic acid application increased bone regeneration at a limited level. However, a positive effect of increasing the locally applied humic acid ratio on bone regeneration was not found. More studies should be conducted regarding this matter.

## CONCLUSIONS

According to Micro-CT results:

- ❖ Humic acid application in addition to autogenous bone graftcaused a decrease in BV value.
- ❖ Although the positive effect of the humic acid application was observed in Tb.Th and BV/TV values, dosage increase negatively impacted the same.
- ❖ The dosage increase also negatively affected the BS value.
- ❖ No positive effect of the humic acid application to BS/BV value was observed.
- ❖ The reason behind the statistical significance between the control group and the test groups is attributed to high regenerative potential of autogenous graft, rather that humic acid.

Considering the limitations of animal study models, it is considered that humic acid may have a limited positive effect when applied in doses suitable to ossification.

This is the first study evaluating additional benefit of humic acid in bone regeneration in combination with autogenous bone grafts. We think that the results that we obtained from this study will contribute to the literature on the clinical use of humic acid based on the results that we obtained.

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## DECLARATIONS OF INTEREST

"No potential conflict of interest was reported by the authors."

### *Humik Asitin Kemik Rejenerasyonu Üzerine Etkisinin Araştırılması*

***ÖZ***

***Amaç:*** *Çalışmamızın amacı, otojen kemik grefti uygulamalarında lokal humik asitin erken dönem oluşan yeni kemik miktarı üzerine etkisinin araştırılmasıdır.* ***Gereç ve yöntemler:*** *Denek olarak 12 haftalık, ortalama ağırlıkları 230-250 g olan Wistar Albino cinsi 24 adet rat kullanılmıştır. Deney hayvanları 4 gruba ayrılmıştır. Bunlar kontrol grubu,*

*sadece otojen greft uygulanan grup, lokal 100 mg/kg humik asitle uygulanan otojen greft grubu ve lokal 200 mg/kg humik asitle uygulanan otojen greft grubu şeklinde belirlenmiştir. Deney hayvanlarının parietal kemiğinde kritik boyutta 5mm çapında bikortikal kemik fragmanı automax frez kullanarak serum fizyolojik irrigasyonu altında çıkarılmıştır. Defektli bölgeye oluşturulma esnasında elde edilen otojen greft ilgili deney gruplarına uygulanmıştır. Lokal uygulama yapılacak gruplar için belirlenen humik asit miktarı otojen greftle karıştırılarak uygulanmıştır. Deney hayvanları işlem sonrası 28.günde sakrifiye edilmiştir. Daha sonra greftlenen defekt bölgesi mikro-bilgisayarlı tomografi (Mikro- BT) ile değerlendirilmiştir.* ***Bulgular:*** *Trabeküler kalınlık (Tb.Th) ve kemik hacminin doku hacmine oranı (BV/ TV) değerlerinde en yüksek değer lokal 100mg/kg humik asitle uygulanan otojen greft grubunda bulunmuştur. Kemik hacmi (BV) değişkeninde en yüksek değer sadece otojen greft uygulanan grupta tespit edilmiştir. Kemik yüzey alanı (BS) değişkeni için gruplar ikişerli olarak karşılaştırıldığında kontrol grubuyla sadece otojen greft uygulanan grup ve lokal 100mg/kg humik asitle uygulanan otojen greft grubu arasında istatistiksel olarak anlamlı fark bulunmuştur. Lokal 100mg/kg humik asitle uygulanan otojen greft gruba göre lokal 200mg/kg humik asitle uygulanan otojen greft grubunda BS/BV değeri daha yüksek bulunmuştur.* ***Sonuçlar:*** *Humik asit uygulandığında kemik hacmi değerinde azalmaya sebep olmuştur. trabeküler kalınlık ve kemik hcmi/doku hacmi değerlerinde humik asit uygulaması olumlu etkisi görülmesine rağmen doz artırılması olumsuz etkilemiştir. Kemik yüzey alanı değerinde de doz artırılması olumsuz etkilemiştir. Kemik yüzey alanı/ kemik hacmi değerine humik asit uygulamasıın olumlu etkisi görülmemiştir. Kontrol grubuyla diğer gruplar arsındaki istatistiksel olarak anlamlı çıkmasının nedeni otojen greft olduğu düşünülmektedir.* ***Anahtar kelimeler:*** *Humik asit, otojen kemik grefti, kemik rejenerasyonu.*

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**Original research**

# THE EFFECT OF BLOOD CONTAMINATION ON SHEAR BOND STRENGTH OF CALCIUM SILICATE-BASED PULP CAPPING MATERIALS

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**ABSTRACT**

**Objectives:** The aim of this study was to examine the effect of different hardening times and blood contamination of MTA and Biodentine, which are widely used for pulp capping treatments in the market, on shear bond strength (SBS) with a self-etch adhesive resin, after different hardening times (24, 48, 72 and 96 hours).

**Materials and Methods:** Slots with a diameter of 5 mm and a height of 2 mm were prepared in 192 acrylic blocks for this study. Both ProRoot MTA and Biodentine were prepared according to the manufacturer's instructions, and half of the slots were filled with ProRoot MTA and the other half were filled with Biodentine. All the samples were divided into groups depending on four different hardening times and hardened. After the hardening process was completed, the group of each hardening time was divided into 2 subgroups (n:12) with and without contamination.

In the uncontaminated groups, a self-etch adhesive resin (Clearfil Liner Bond) and a resin-based composite (Filtek P60) were applied on the samples and polymerized. In the contaminated groups, the sample surfaces were contaminated with blood for 20 seconds. After washing and drying the samples, adhesive resin and composite were applied on them. After that, SBS tests were performed and the data were subjected to a 2-way ANOVA test analysis.

**Results:** In the uncontaminated groups, there was no significant difference in the SBS of each pulp capping material depending on different hardening times ($p>0.05$). ProRoot MTA showed statistically higher SBS than Biodentine in the 72 and 96 hour uncontaminated groups ($p<0.05$). Blood contamination caused a significant decrease in the SBS of ProRoot MTA and Biodentine ($p<0.05$).

**Conclusions:** In this study, it was determined that blood contamination reduces the SBS of pulp capping materials. Therefore, it is recommended to prolong the hardening times of the capping materials and to take clinical measures to prevent blood contamination as much as possible before restorative treatments are performed.

**Keywords:** Pulp Capping; MTA; Biodentine; Blood Contamination; Shear Bond Strength.

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## INTRODUCTION

Pulp capping treatments (PCT) aim to maintain the vitality of the pulp by directly covering the pulp tissue exposed for various reasons with a capping material.[1,2] Deep dentin caries, exposure of the pulp tissue due to mechanical or traumatic reasons are the most important factors that threaten the vitality of the pulp. In such cases, it is of great importance to allow the tooth to function in the mouth for a much longer time by maintaining the vitality of the pulp, in terms of contributing to phonation and aesthetics. It is aimed at maintaining the vitality of the pulp by ensuring the formation of tertiary dentin with the capping material applied over the exposed pulp tissue.[2,3]

Various pulp capping materials have been introduced to the market for use in PCT so far. Although calcium hydroxide (CH) has been used as the gold standard in PCTs for many years,[1] it has disadvantages such as high solubility and lack of adhesion to dental tissues. In PCT with CH, it has been reported that the dentin bridge formed by CH has tunnel defects and porosities[4,5], and CH has an inability of adhesion to the dentin surface, so it causes microleakage in the long term and does not protect the pulp sufficiently.[6]

As a result of the search for different materials due to the disadvantages of CH, ProRoot MTA (Densply, Tulsa Dental, USA), developed by Torabinejad *et al.*[7,8], at Loma Linda University in 1993, is the first calcium silicate-based (CSB) cement used in dentistry. MTA has advantages such as providing a more homogeneous (containing less tunnel defect) dentin bridge formation in a shorter time compared to CH, causing less inflammatory reaction, hardening in the presence of moisture, meeting high chewing pressure after hardening, and being biocompatible.[9] MTA is used for PCT, pulpotomy, repair of root perforations, bifurcation lesions, internal and external root resorption, apexification and as the apical plug material in endodontic surgery and deep cervical and radicular lesions.[10,11] Although MTA currently continues to be used successfully in PCT, it has maintained a different material trend in this area due to its disadvantages such as having long hardening time and not being economical.[7,12]

Biodentine (Septodont, France), developed in 2009, contains tricalcium silicate, calcium carbonate, calcium oxide and zirconium oxide in its powder and it is a CSB material containing modified polycarboxylate and calcium chloride components in its liquid. The main advantages of Biodentine are initial hardening time of approximately 12 minutes and enhanced physical properties according to the manufacturer's instructions.[13] Biodentine, which is a biocompatible material, is used in the treatment of pulp capping, amputation, apexification and root perforation repair and as retrograde root filling.[14,15] Biodentine stimulates odontoblast-like cells by forming mineralization nodules in dentin and provides dentin-like matrix formation.[16] It stimulates mineralization by releasing calcium ions and creates a mineral infiltration zone at the dentin-cementum interface, providing better coverage and reducing microleakage.[17] Biodentine is similar to MTA in terms of forming dentin bridge formation and causing lower inflammation in the pulp.[18] According to the manufacturer's instructions, the shorter hardening time of Biodentine is an advantage, for the restorative procedures can be performed in a shorter time compared to MTA, which has an initial hardening time of 3-4 hours.[7]

In PCT, after the exposed pulp tissue is covered with pulp capping materials, temporary restorations are frequently made on them to complete their hardening, and the capping material is expected to harden. After the pulp capping materials harden for a certain time period, the temporary filling is removed and permanent restorations are completed with adhesive and composite resins.

There are different literature data on the different hardening times of capping materials, affecting bond strength to adhesive resins.[19-22] In addition, it is often possible for the capping material to be exposed to oral fluids and blood contamination from the gingiva after hardening and prior to the construction of the upper restoration in clinical conditions. Moreover, an uncontaminated capping material has a different surface microstructure compared to the capping material exposed to blood contamination.[23] Therefore, blood contamination could affect the

Shear bond strength (SBS) of the capping material to adhesive resins. Hovewer, to the best of our knowledge, a study on the effect of blood contamination of the capping materials on the SBS to restorative materials after hardening is not available in the literature.

For this reason, our first aim in this study was to investigate the SBS of ProRoot MTA and Biodentine, which are CSB capping materials widely used in PCT, to a self-etch adhesive resin after different hardening times (24, 48, 72, 96 hours). Our second aim is to investigate the effect of blood contamination in vitro of ProRoot MTA and Biodentine after different hardening times (24, 48, 72, 96 hours) on SBS to a self-etch adhesive resin.

Our initial hypotheses in this study were as follows:

1. There is no difference between the SBS of ProRoot MTA and Biodentine to a self-etch adhesive resin after different hardening times (24, 48, 72, 96 hours).

2. Blood contamination of ProRoot MTA and Biodentine after different hardening times (24, 48, 72, 96 hours) does not affect the SBS to a self-etch adhesive.

## MATERIAL AND METHODS

### Ethics

Ethical approval was obtained from the Health Ethics Committee of Karadeniz Technical University, Faculty of Medicine, Scientific Research Ethics Committee in Trabzon, Turkey (ID: 2019/294 and decision date 18.11.2019).

In this in vitro study, the effect of two CSB pulp capping materials, ProRoot MTA (Densply, Tulsa Dental, USA) and Biodentine (Septodont, Saint-Maurdes, France) on SBS to a two-step self-etch adhesive, Clearfil Liner Bond F (Kuraray Noritake Dental Inc, Japan) and a microhybrid composite (Filtek P60 Posterior Composite, 3M ESPE, Dental Products, USA) after different hardening times (24, 48, 72, 96 hours) was investigated. Furthermore, the effect of blood contamination of these pulp capping materials after these different hardening times on SBS was also investigated.

The materials used in the study, their contents, production numbers and application instructions are shown in Table 1.

**Table 1.** Materials, Contents, Production Numbers and Application Instructions

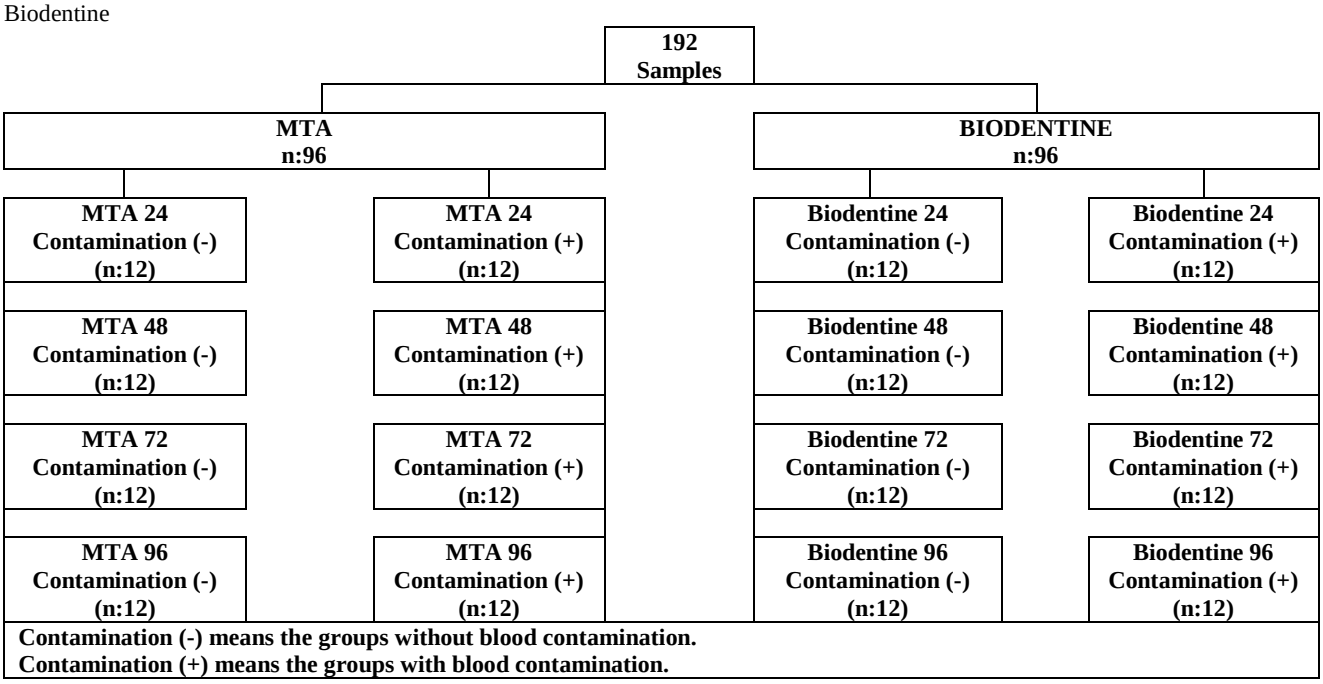
| MATERIALS | BATCH NUMBERS | CONTENTS | APPLICATION INSTRUCTIONS |
|---|---|---|---|
| **ProRoot White MTA** (Densply, Tulsa Dental, USA) | 0000249678 | **Powder:** Tricalcium aluminate, tricalcium silicate, silicate oxide and tricalcium oxide, iron oxide, magnesium and bismuth oxide<br>**Liquid:** distilled water, saline<br>Ph = 12-13 | Mix 1 pack of powder with 1 ampule of liquid for about 1 minute. After application, it is closed with damp cotton and temporary filling is made. After 96 hours, permanent filling is applied. |
| **Biodentine** (Septodont, Saint-Maur-desFosses, France) | B25453 | **Powder:** Tricalcium silicate, dicalcium silicate, calcium carbonate, iron oxide, zirconium oxide<br>**Liquid:** Calcium chloride, hydrosoluble polymer, water | The capsule is opened and 5 drops of liquid from the disposable liquid are dropped into the capsule. The capsule is closed and placed in the amalgamator and mixed for 30 seconds at 4500 rpm (rotation/min). The capsule is opened and the Biodentin material is applied with the appropriate handpiece. |

| | | | |
|---|---|---|---|
| **Clearfil Liner Bond F** (Kuraray Noritake Dental Inc., Japan) | 000019 | **Primer:** 10-Methacryloyloxydodecyl dihydrogen phosphate (MDP), 2-hydroxyethyl methacrylate (HEMA), Hydrophilic aliphatic dimethacrylate, dl-camphorquinone, N,N-di-ethanol-p-tolidine, Water. **Ph≈ 1.9** **Bond:** 10-Methacryloyloxydodecyl phosphate (MDP), 2-hydroxyethyl methacrylate (HEMA), Bisphenol A digicidyl methacrylate (Bis-GMA), Colloidal silica, Hydrophobic aliphatic dimethacrylate, dl-camphoroquinone, N,N-diethanol p-tolidine | 1. The primer is applied to the tooth surface and waited for 20 seconds. Oil-free air is squeezed to allow solvents to evaporate and disappear. 2. Bond is applied to the tooth surface and oil-free air is gently squeezed to form a uniform layer. 3. The adhesive is cured with a dental light device that emits light at a wavelength of 400-515 nm for 10 seconds. |
| **Filtek P60 Posterior Composite** (3M ESPE, Dental Products, USA) | NA36198 | It contains Zirconia / Silica and BiS-GMA, UDMA and BIS-EMA resin. The inorganic filler content is 61% by volume. | It is applied with the incremental technique in 2 mm masses and polymerized with a dental light device for 20 seconds. |

**Preparation of Test Samples**

A total of 192 cylindrical acrylic blocks with a diameter of 30 mm and a height of 10 mm were prepared by forming 16 groups (n:12) for 4 different hardening times and contamination groups for each CSB capping material. Slots with a diameter of 5 mm and a height of 2 mm were prepared in these 192 acrylic blocks. For each setting time of each pulp capping material, 24 samples were prepared and half of them were used in experiments with blood contamination and the other half in experiments without blood contamination (Table 2).

**Table 2:** Experimental Groups Prepared According to Different Hardening Times and Contamination Conditions of MTA and Biodentine




ProRoot MTA and Biodentine were applied to the slots prepared in acrylic blocks according to the manufacturer's instructions. The surface of the MTA samples was covered with moist cotton and all the prepared samples were hardened for the planned times (24, 48, 72, 96 h) by storing them at 37°C and 100% humid environment.

***In uncontaminated groups (without blood contamination),*** after certain hardening times, Clearfil Liner Bond F, a two-step self-etch

adhesive system, was applied on the pulp capping materials according to the manufacturer's instructions and polymerized with light (Elipar S10, 3M ESPE) for 20 seconds. After polymerization, a 3 mm high composite resin (Filtek P60, 3M ESPE) was applied on the capping materials in layers of 1.5 mm thickness each, using a mold and each layer was polymerized with light for 20 seconds again.

***In the contaminated groups (with blood contamination),*** after certain hardening times, the surfaces of the pulp capping materials were contaminated with fresh blood for 20 seconds. After that, the surfaces were washed with water for 10 seconds and then dried by wiping once with a cotton roll. After the contamination and drying processes, adhesive resin and composite applications were performed and then all the samples were kept in a 100% humid environment at 37 degrees for 24 hours.

**Shear Bond Strength Test**

SBS test was applied to all the samples with a Universal Tension/Compression Testing Machine (Instron 3382, USA) at a speed of 0.5 mm/min. After the SBS test, the results were converted to Megapascals (MPa). The fracture surfaces of the samples were examined with an optical microscope (Olympus Metallurgical Microscope) under X30 magnification, and different failure types (adhesive, cohesive and mixed) were determined.

**Statistical analysis**

SPSS for Windows 17.0 (SPSS Inc., Chicago, USA) program was used for statistical analysis. Normal distribution analysis of the data was performed with the Shapiro Wilk test. Two-way ANOVA test and Fisher's LSD test were used for repetitive samples and paired samples test was used for time-dependent variations. A $p<0.05$ level was considered statistically significant in all the analyses.

## RESULTS

**Shear Bond Strength Test Results**

Interactions are reported in Table 3. According to the interaction table, SBS test values were significantly influenced by both time (p: 0.029) and time x material (p: 0.007) whereas no effects were found for interactions (time x contamination), (p: 0.341) and (time x material x contamination), (p: 0.274).

**Table 3.** Two-way ANOVA interaction effects for shear bond strength

| Source | Value | F | Hypothesis df | Error dF | Sig. p |
|---|---|---|---|---|---|
| Time | .192 | 3.3 | 3 | 42 | .029 |
| Time* Material | .249 | 4.6 | 3 | 42 | .007 |
| Time* Contamination | .076 | 1.1 | 3 | 42 | .341 |
| Time* Material* Contamination | .087 | 1.3 | 3 | 42 | .274 |

df: degrees of freedom.

* $p< 0.05$; level was considered statistically significant.

***1- Comparisons of SBS Results of Pulp Capping Materials Hardened at Different Times within Groups***

Table 4 shows the mean and standard deviation values of SBS according to the different hardening times (24, 48, 72, 96 h) and contamination status of the capping materials used in the study, as well as within-group differences.

**Table 4.** Mean and Standard Deviation Values of Shear Bond Strength (MPa) and Comparisons within Groups

| PULP CAPPING MATERIALS / HARDENING TIME | 24 HOURS | 48 HOURS | 72 HOURS | 96 HOURS |
|---|---|---|---|---|
| **MTA** | $20.46 \pm 3.97^{A}$ | $22.50 \pm 4.52^{A}$ | $23.75 \pm 5.81^{A}$ | $22.65 \pm 4.11^{A}$ |
| **CONTAMINATED MTA** | $10.43 \pm 2.42^{A}$ *p‹0.05 ( with contMTA48) *p‹0.05 (with contMTA72) *p‹0.05 (with contMTA96) | $14.92 \pm 5.20^{B}$ | $16.80 \pm 2.52^{B}$ | $17.14 \pm 3.38^{B}$ |
| **BIODENTINE** | $18.61 \pm 5.51^{A}$ | $20.32 \pm 3.04^{A}$ | $18.01 \pm 3.46^{A}$ | $18.55 \pm 3.40^{A}$ |
| **CONTAMINATED BIODENTINE** | $13.25 \pm 3.74^{A}$ | $11.72 \pm 3.25^{A}$ | $13.40 \pm 6.06^{A}$ | $12.26 \pm 1.97^{A}$ |

*According to Bonferroni correction, a significant difference in shear bond strength of each pulp capping material at different hardening times (in the same row) is shown in superscript. Different letters indicate statistical differences in rows (p<0.05).*

**When the SBS values were examined statistically,**

There was no statistically significant difference between the groups in terms of SBS after different hardening times (24, 48, 72, 96 hours) for the non-contaminated MTA group (p>0.05). Contaminated (Cont) MTA24 group showed statistically significantly lower bond strength compared to Cont MTA48, 72, 96 hour groups (p<0.05).

There was no statistically significant difference between the groups after different setting times (24, 48, 72, 96 hours) for the uncontaminated Biodentine group (p>0.05). For the Contaminated (Cont) Biodentine group, there was no statistically significant difference between the groups after different setting times (24, 48, 72, 96 hours) (p>0.05).

***2- Comparison of the Shear Bond Strength Test Results of Pulp Capping Materials Among the Groups within the Same Hardening Time***

Comparison of the SBS values of contaminated and uncontaminated MTA and Biodentine groups with each other in the same hardening time group is given in Table 5.

**Table 5.** Comparison of the SBS Values Among the Groups within the Same Hardening Time

| PULP CAPPING MATERIALS | Hardening Times | | | |
|---|---|---|---|---|
| | **24 Hours** | **48 Hours** | **72 Hours** | **96 Hours** |
| **MTA** | **20.46** *p‹0.01 (with Cont.MTA24) | **22.50** *p‹0.01 (with Cont.MTA 48) | **23.75** *p‹0.05 ( with Cont. MTA 72) **p‹0.05 (with BD72) | **22.65** *p‹0.01 (with Cont. MTA 96) **p‹0.05 (with BD 96) |
| **CONTAMINATED MTA** | **10.43** | **14.92** | **16.80** | **17.14** **p‹0.01 (with Cont.BD 96) |
| **BIODENTINE** | **18.61** * p=0.002; p‹0.01 (with Cont.BD 24) | **20.32** *p‹0.01 (with Cont.BD 48) | **18.01** *p‹0.05 (with Cont.BD 72) | **18.55** *p‹0.01 (with Cont.BD 96) |
| **CONTAMINATED BIODENTINE** | **13.25** | **11.72** | **13.40** | **12.26** |

* : It refers to the significant differences in the same hardening time (in the same column) of the contaminated and uncontaminated groups of the same pulp capping material.
** : It refers to the significant difference in the same hardening time of two different materials (MTA-Biodentine). Different letters indicate statistical differences in rows (p<0.05).
BD is used as an abbreviation for Biodentine.

**When the shear bond strength values of MTA and Biodentine groups were compared with each other in the same hardening time,**

There was no statistically significant difference between SBS at the end of the 24-hour and 48-hour hardening times of the uncontaminated MTA and Biodentine groups (p>0.05).

However, the SBS of MTA72 group (23.75 MPa) was found to be statistically higher than that of the Biodentine72 group (18.01 MPa) at the end of the 72 hour hardening period (p<0.05). Similarly, at the end of the 96 hour hardening time, the mean SBS of the MTA group (22.65 MPa) was statistically significantly higher than

the mean SBS of the Biodentine group (18.55 MPa) ($p<0.05$).

**When the Contaminated MTA and Contaminated Biodentine groups were compared,**

At the end of the 24, 48, 72 hour setting time, there was no statistically significant difference between the SBS values of the Cont. MTA24, 48, 72 groups and those of the Cont. Biodentine 24, 48, 72 groups ($p>0.05$). At the end of the 96-hour hardening period, SBS values of the Cont. MTA96 group were found to be significantly higher than the SBS values of the Cont. Biodentine96 group ($p<0.05$).

The Bar Graphic showing the Contamination Status of the Pulp Capping Materials and the S values according to the different setting times is as in Figure 1.

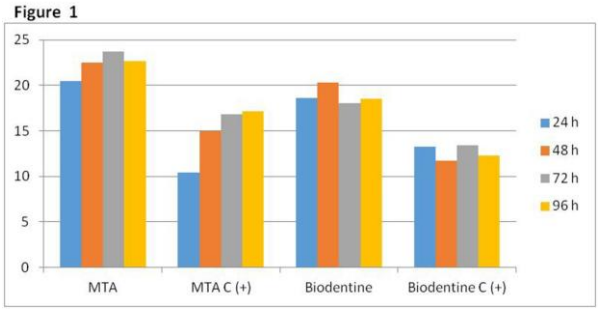



**Figure 1.** Bar graphic of shear bond strength values by capping material/contamination conditions and hardening time.

**Evaluation of Stereomicroscope Images of Fractured Surfaces**

In Figure 2, in the uncontaminated MTA groups, the failures were predominantly cohesive, but the adhesive failures increased as the hardening time increased.

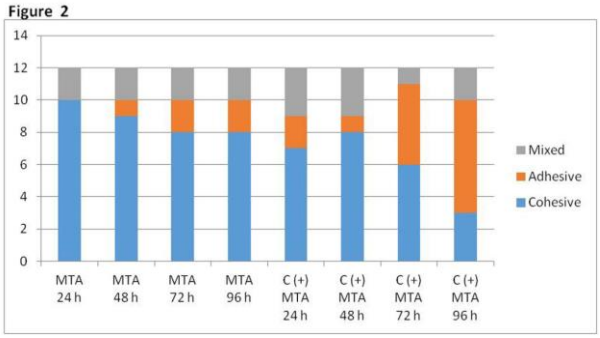



**Figure 2**. Distribution of failure types at different hardening times of MTA. C(+) means blood contamination.

In the Cont. MTA groups, the group with the highest rate of adhesive failure was the group hardening for 96 hours.

As seen in Figure 3, cohesive failures were mostly observed in the uncontaminated Biodentine groups. Adhesive failure type was more commonly seen in the Cont. Biodentine groups, particularly in the Cont. Biodentine 24 group.

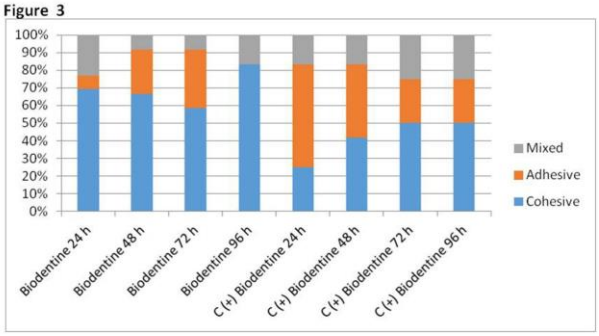



**Figure 3.** Distribution of failure types at different hardening times of Biodentine. C(+) means blood contamination.

## DISCUSSION

Although different materials have been used in PCT in the literature to date, MTA and Biodentine are CSB materials preferred in PCT due to their advantages such as biocompatibility, high level of sealing properties, and forming reparative dentin formation depending on cell differentiation.[14,15,18,24-26] Studies have shown that these materials stimulate the release of TGF-β1 and that this growth factor provides reparative dentin regeneration from pulp stem cells and the dentin bridge, which is formed by the effect of both MTA and Biodentine, is less porous.[27,28]

PCT should be followed by an impermeable upper restoration that prevents bacterial invasion at the restoration and dentin interface. That is because the prevention of microleakage and the high bond strength between the pulp coating material and the restoration are also factors that ensure the continuity of the vitality and function of the tooth.[29] For this reason, the upper restoration made in capping treatments is expected to show sufficient bond strength to the capping material used in the deepest part of the cavity, as well as to the tooth tissues.

Today, adhesive resins are the most preferred permanent restorative materials due to their aesthetic properties. Although there are many studies in the literature investigating the bond strength of direct pulp capping materials to dental

adhesives, different and contradictory results have been obtained in the bond strength of capping materials to adhesive resins after different hardening times in different studies.[19,22,30,31]

However, there are different factors that can affect the bond strength of composite resins to capping materials in clinical conditions. During the restorative treatments in the clinical conditions, leakage of saliva and blood into the cavity during the different stages of restorative material applications will cause contamination of the surface and pose a risk for the bond strength of the restoration.[32,33] Although there have been studies examining the bond strength of MTA and Biodentine to various adhesives, as far as we know, we have not come across any study on the effect of blood contamination of these materials on SBS.

Therefore, in this study, the effect of different hardening times (24, 48, 72, 96 h) and blood contamination of CSB capping materials (ProRoot MTA and Biodentine) on SBS to a two-step self-etch adhesive resin (Clearfil Liner Bond F) and a mycrohybrid composite resin (Filtek P60) was investigated in-vitro. According to the findings of this study, there was no statistically significant difference between the SBS values of uncontaminated ProRoot MTA at different hardening times ($p>0.05$). There was no statistical difference between the bond strength values of the uncontaminated Biodentine at different hardening times ($p>0.05$). The bond strength values of both MTA and Biodentine groups after all hardening times were found to be equal to or higher than the clinically expected bond strength (17-20 MPa) (Table 4).

When the uncontaminated MTA and Biodentine groups at the same hardening time were compared, no statistically significant difference was observed between the SBS at 24 and 48 hours, while the SBS of MTA groups hardening for 72 and 96 hours were found to be statistically significantly higher than the Biodentine72 and Biodentine96 groups ($p<0.05$), (Table 5). Therefore, the first hypothesis of our study, “There is no difference between the SBS of ProRoot MTA and Biodentine at different setting times (24, 48, 72, 96 h)" was partially accepted.

Atabek *et al.*[19] compared the bond strength of ProRoot MTA to adhesive systems after different hardening times (2h 45 min, 24h, 48h, 72h and 96h) using different adhesives and showed the highest bond strength with a 2-step total etch system (One-Step Plus) in 96 hours in an in vitro study. It was suggested that the acetone content of the adhesive increases the bond strength to MTA as a result of increasing monomer diffusion due to its high vapor pressure.[19,34] It was also suggested that early bonding failure occurs during initial hardening, due to the possible high water content of MTA. When the bond strength values at all hardening times were examined, they stated that the performing of an adhesive restoration on MTA should be postponed for at least 96 hours in order for MTA to achieve sufficient physical properties.[19]

In our study, although the highest SBS values were obtained for ProRoot MTA at 72 and 96 hours, the bond strength values were found to be higher compared to this study. The reason for this difference may be different monomer systems in the adhesive used. The Clearfil Liner Bond F used in our study contains 10-MDP monomer in both the primer and the adhesive. It has been reported that self-etch adhesive systems containing 10-MDP monomer increase the bond strength by chemically binding the 10-MDP monomer it contains with Ca ions in MTA and Biodentine.[21,35,36]

In another study, the SBS values of ProRoot MTA were found to be higher than Angelus MTA with a one-step adhesive system (Adhese One F) compared to total etch and self-etch systems.[37] The reason reported for this is that ProRoot MTA has fewer large particles and more homogeneous distribution compared to Angelus MTA. They assumed that these structures are calcium silicate and calcium silicate hydrate, and it was suggested that they form the basis of the binding phases in a hydrated Portland cement–based material.[38,39] It was reported that acid application on the MTA surface has an erosive effect on the MTA surface and causes cracks on the surface, but self-etch

primer application does not create cracks on MTA surface.[37] In addition, it was suggested that the content with 5% filler ratio of Adhese One F, which is a one-step adhesive system, causes a lower shrinkage stress and increases the bond strength.[40] Furthermore, it was reported that potassium fluoride increases the surface strength of Portland cement in several engineering reports.[41,42] An increase in surface strength can also result in reduced cohesive failure and increased bond strength. The fluoride content of Clearfil Liner Bond F may also contribute to its high bond strength in our study.

In a study by Odabaş *et al*., no significant difference was observed between the SBS of different adhesive systems after Biodentine's hardening time of 12 minutes and 24 hours. In addition, the highest SBS was obtained with Clearfil SE Bond (19.5 MPa) at a hardening time of 24 hours, and Clearfil SE Bond was the only adhesive system that provided sufficient SBS among all adhesives used. There was no significant difference in SBS between Clearfil SE Bond and Clearfil S3 Bond groups at different hardening times. Furthermore, the bond strength value of Clearfil SE Bond (16.9 MPa) after the hardening time of 12 minutes was found to be higher than the other 12-minute groups, although there was no statistically significant difference.[43] In this study too, it was noted that 10-MDP monomer which formed chemical adhesion with the Ca ions in the structure of Biodentine increased the bond strength of Clearfil SE Bond.[21]

Moreover, Keleş and Şimsek examined the SBS of different adhesive systems to Biodentin at the end of the 12-minute hardening time. The SBS of Clearfil SE Bond (14.1 MPa), a two-step self-etch adhesive used in the study, was found to be higher than other adhesive systems (a single-step self-etch; Clearfil Universal Bond, a two-step total etch; Prime&Bond NT).[30] Çolak *et al.*[36] examined the SBS of different adhesive systems to Biodentine hardened for 9 minutes and 48 hours and reported that the hardening time did not affect the SBS values, but the adhesive containing 10-MDP (Clearfil S3 Bond) showed higher bond strength.

Aksoy and Ünal obtained the lowest SBS value in the group set for 12 minutes by applying different universal adhesive systems in both self-etch and total-etch modes at the end of five different setting times (12 min, 24 h, 48 h, 72 h, 96 h) of Biodentine. They also noted that there was no significant difference between self-etch and total-etch modes of universal adhesives in all the groups. They reported that the setting time of 24 hours is enough to obtain sufficient SBS and that the universal adhesives provide higher SBS regardless of the application mode of the adhesive systems used in the study.[44] In our study, Biodentine showed sufficient bond strength in 24 hours, and there was no significant difference between the groups in which the hardening time was increased.

On the other hand, Nekoofar *et al.* examined the bond strength of several different resin restorative materials with Biodentine, dividing Biodentine samples into groups according to three different hardening times (12 minutes, 1 week and 1 month). Biodentine samples hardening for 1 week were found to have significantly higher micro-SBS than those with 12-minute hardening time. In the study, it was noted that the bond strength of Biodentine, which is porous and low-resistant in the early stage, may decrease with the application of adhesive agent and polymerization shrinkage of the composite resin. It was reported that CSB materials that harden with hydration are not only weak in crystallized structures at the early stage, but also porous, but these materials complete their maturation after a certain period of time. However, in this study where the etch-rinse adhesive system was used, there was no significant difference in bond strength values between Biodentine samples hardened for one week and one month. The universal adhesive material (All Bond Universal) in self-etch mode applied to the Biodentine samples after 12 minutes of hardening exhibited significantly higher average micro-SBS compared to the other groups (Clearfil SE Bond, Adper Single Bond 2). In addition, it was reported that higher SBS values can be achieved with all adhesives if a longer waiting time can be planned after mixing

Biodentine.[45] In our study too, Biodentine showed sufficient SBS in four different setting times (24h, 48h, 72h, 96h). Differences in study findings may be due to differences in material method.

Hashem *et al.* investigated the bond strength of Biodentine in the early (0 min., 5 min., 20 min. and 24 hours) and late period (2 weeks, 1 month, 3 months and 6 months) with total etch and self-etch systems. In the study, the highest μSBS in the early period was obtained in the 24-hour group.[46] However, in clinical applications, it has been suggested to extend the hardening time of Biodentine, since placing Biodentine in cavities with high C factor will increase polymerization stress. A 2-week waiting period is recommended for Biodentine to fully mature and reach maximum physicochemical properties.[47] As a result of the study, while there was no significant difference between all hardening times in the late period, it was noted that μSBS values were significantly higher than the early period. Moreover, there was no significant difference between the 24-hour hardening time and the late period. In this study, ScotchBond Universal adhesive containing MDP monomer was used in self-etch mode.[46] Similarly, in our study, there was no significant difference between the SBS value of Biodentine in the 24-hour hardening time and the groups with the other hardening times (48h, 72h, 96h). The possible reason for obtaining similar results to this study may be the use of adhesive containing MDP monomer in our study too.

In the study performed by Tulumbacı *et al.*[48], the SBS of MTA was found to be higher than Biodentine using Prime&Bond NT after 72 hours of hardening time for both capping materials. In our study, however, there was no significant difference between SBS after 72 hours of hardening in both groups, and the highest bonding values were obtained. The use of different adhesive systems in this study and the differences in sample preparation for the SBS test may also have caused differences in the study results.

In the study where Cantekin and Avci hardened Biodentin and ProRoot MTA in two different periods of 15 minutes and 96 hours and then All-Bond 2 total etch adhesive system was applied, the Biodentine group showed the highest SBS value (17.7 MPa). They noted that the reasons for the high SBS value were the low level of free radical monomer and polymerization shrinkage of the methacrylate-based composite resin (Aelite All Purpose Body, Bisco Inc) they used. In addition, it was suggested that the restorative treatment be delayed for 72 hours to 96 hours in order for MTA to reach the most suitable physical properties in the capping process with MTA.[22] In our study, MTA showed sufficient SBS at all hardening times. Moreover, in our study, the highest SBS value was obtained in the MTA72 and then in the MTA96 groups, which is consistent with the recommendation in this study.

In a study by Abdel-Rhman *et al.*[31], it was proposed that MTA provided the desired impermeability after at least 72 hours and recommended waiting for 72 hours before the restoration. It was also reported that although the initial setting reaction of Biodentin takes 12 minutes after mixing powder and liquid, Biodentin gains its full physical properties after 2 weeks.[49] In our study, however, there was no statistically significant difference between the SBS values of Biodentin at different hardening times, and it was observed that the SBS was similar in all setting times (24h, 48h, 72h, 96h). Again in our study, although there was no statistically significant difference in the MTA group, the highest bond strength was obtained after 72 hours.

Although there are different results regarding the bond strength of MTA and Biodentine to restorative materials, it is important for the durability of bond strength that these materials have reached sufficient maturation before restoration. Additionally, the bonding of capping materials to dentin as well as restorative materials is very important in terms of both sealing and durability of the restoration in the long term. As a matter of fact, there are many studies on the bond strength of these materials to dentin.[51-53]

In the study by Kaup *et al*., in which ProRoot MTA and Biodentine's bond strength to dentin was examined, the SBS of the groups with 2 days,

7 days and 14 days was evaluated and the SBS of MTA was found to be significantly lower than Biodentine in the groups hardened for 2 days. In the study, it was noted that the SBS values of MTA and Biodentine, which were hardened for 7 days and 14 days, increased significantly compared to the 2-day values. Biodentine was found to be superior to ProRoot MTA in terms of the SBS values.[50] In the study by Jantarat *et al.*[51], the SBS of MTA and Biodentine to dentin was evaluated at the end of 60 minutes and 24 hours, and the SBS values of MTA and Biodentine hardened for 24 hours were found to be significantly higher. Atmeh *et al.*[11] reported that when Biodentine is applied on dentin, it forms alkaline bonding with its high pH value and forms Biodentine lattices in the dentinal tubules, which shows dentin-like adhesion-like structures.

Additionally, in clinical conditions, there is a high risk of contamination of capping materials with blood or saliva during the applying of adhesive resins. It is known that contamination reduces the bond strength of restorative materials to dentin.[32,33,54] Contamination of capping materials with blood are mostly studies evaluating the bond strength to dentin.[52,55-57]

To the best of our knowledge, there is no study in the literature on the effect of blood contamination of pulp capping materials on the SBS to adhesive resins before permanent restoration. Therefore, in this study, the effect of blood contamination on SBS after different hardening times was investigated.

According to the findings of this study, blood contamination decreased the SBS value at different hardening times in all MTA groups ($p<0.01$ for Cont. MTA24, 48, 96; $p<0.05$ for Cont MTA72). In the Biodentine groups too, blood contamination significantly decreased the bond strength at all hardening times ($p<0.01$ for Cont Biodentine 24, 48, 96; $p<0.05$ for Cont Biodentine72). Therefore, the second hypothesis of our study, "Contamination of ProRoot MTA and Biodentine with blood after different hardening times (24, 48, 72, 96 hours) does not affect the shear bond strength of a self-etch adhesive", was not accepted. We could not discuss the findings of this phase of our study, as we could not find a study examining the SBS of capping materials to adhesive resins after contamination.

In the literature, there are studies on the bond strength of contamination to dentin tissue at different stages of restorations.[33,54] Although the bond strength to dentin was not investigated in our study, it has been reported in the studies that blood contamination reduces the bond strength to dentin and that it especially adversely affects the bond strength of dentin before and after the application of adhesive systems.[56,57] Procedures such as washing and drying with cotton to remove blood contamination during restorative treatments could also create effects that reduce bond strength. Therefore, since exposure to blood is inevitable when applying CSB capping materials to the exposure surface in clinical conditions, prolonging the hardening time to at least 72 to 96 hours could increase the bond strength of pulp capping materials to both dentin and composite restoration.

In this study, the SBS of both MTA and Biodentine to adhesive resins after different hardening times and after they were contaminated with blood was investigated, and a decrease in SBS bond strength was observed in all hardening groups compared to the uncontaminated groups. In addition, the SBS values of Cont MTA48, Cont MTA72, Cont MTA96 groups in contamination groups are between and very close to clinically acceptable bond strength values. The longest hardening time within the limits of this study was 96 hours.

Whether longer hardening times would affect the bond strength could be investigated in future studies. Moreover, studies in which saliva or both contamination materials are used in addition to blood contamination may be more clinically meaningful. In this study, the SBS of ProRoot MTA and Biodentine with only one self-etch adhesive was examined, and differences that may occur with different adhesive systems were not observed. Furthermore, in this study, the early SBS values of MTA and Biodentine were examined. In vitro and clinical studies, in which

different adhesive resins of these CSB capping materials, which are widely used in the clinic, are used and long-term study results and early-term bond strength values are compared, will also contribute to the literature.

## CONCLUSIONS

In this in vitro study, the effects of different hardening times (24h, 48h, 72h, 96h) and blood contamination of CSB pulp capping materials (Biodentine, MTA) on the SBS to a self-adhesive (Clearfil Liner Bond F) were investigated. Although hardening MTA and Biodentine at different times did not have a negative effect on the SBS, contamination with blood negatively affected the SBS of both capping materials. However, SBS values increased with the prolongation of the hardening time of MTA (72 and 96 hours) in the contaminated groups.

Beyond this study, which examines the hardening times of different capping materials and the effect of contamination on the bond strength, there is a need for studies examining the effect of longer hardening times and different contamination agents on the bond strength of both restorative materials and dentin tissue with different adhesive systems.

### *Kan Kontaminasyonun Kalsiyum Silikat Esaslı Kuafaj Materyallerinin Makaslama Bağlanma Dayanımına Etkisi*


### *ÖZ*

***Amaç:*** *Bu çalışmanın amacı piyasada yaygın olarak kullanılan kuafaj materyalleri olan MTA ve Biodentin'in farklı sertleşme süreleri (24, 48, 72 ve 96 saat) sonrasında kanla kontaminasyonunun ve sertleşme sürelerinin bir self etch adeziv rezinle makaslama bağlanma dayanımına (MBD) etkisinin incelenmesidir.* ***Gereç ve Yöntemler:*** *Bu çalışma için 192 adet akrilik bloğa 5 mm çapında, 2 mm yüksekliğinde yuvalar hazırlandı. Blokların yarısı üretici talimatlarına göre hazırlanan ProRoot MTA ve diğer yarısı ise Biodentin ile dolduruldu. Tüm örnekler 4 farklı sertleşme süresine göre gruplara ayrıldı ve sertleştirildi. Sertleşme süreci tamamlandıktan sonra, herbir sertleşme süresinin grubu kontaminasyonlu ve kontaminasyonsuz olarak 2 alt gruba (n:12) ayrıldı. Kontamine olmayan gruplarda, örnek yüzeylerine bir self etch adeziv rezin (Clearfil Liner Bond) ve bir rezin esaslı kompozit (Filtek P60) uygulandı ve polimerize edildi. Kontamine olan gruplarda ise örnek yüzeyleri 20 saniye kanla kontamine edildi. Örnekler yıkanıp kurulandıktan sonra adeziv rezin ve kompozit uygulaması yapıldı. MBD testlerinden sonra elde edilen veriler 2-yönlü ANOVA testine tabi tutuldu.* ***Bulgular:*** *Kontamine olmamış gruplarda her bir kuafaj materyalinin zamana bağlı olarak farklı sertleşme süreleri arasında bağlanma dayanım değerleri arasında anlamlı bir fark görülmedi ($p>0,05$). 72 ve 96 saatlik kontamine olmayan gruplarda ProRoot MTA, Biodentin'e göre istatistiksel olarak daha yüksek makaslama bağlanma dayanımı gösterdi ($p<0,05$). Kan kontaminasyonu, ProRoot MTA ve Biodentine'in MBD değerlerinde önemli oranda azalmaya neden oldu ($p<0,05$).* ***Sonuçlar:*** *Bu çalışmada kan kontaminasyonunun kuafaj materyallerinin makaslama bağlanma dayanımını azalttığı tespit edilmiştir. Bu nedenle restoratif tedavilerden önce, kuafaj materyallerinin sertleşme sürelerinin uzatılması ve kanla kontaminasyonu mümkün olduğu kadar önleyici klinik tedbirlerin alınması önerilir.* ***Anahtar Kelimeler:*** *Pulpa Kaplaması; MTA; Biodentin; Kan Kontaminasyonu; Makaslama Bağlanma Dayanımı.*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**Original research**

# THE EFFECT OF COATING MATERIAL ON THE COLOR STABILITY OF GLASS-IONOMER-BASED RESTORATIVE MATERIALS: AN *IN-VITRO* STUDY

Burak Çarıkçıoğlu[1]

## ABSTRACT

**Objectives:** The aim of this study was to determine the effect of coating on the color stability of different glass ionomer cements (GICs) after immersed in different children beverages.

**Materials and Methods**: Four different GIC and a glass-ionomer coating material were used in this study. Disc shaped sixteen specimens of each GICs were done and divided into two groups, uncoated and coated (n=8). For color change, the specimens were immersed 7 day in the four solutions of cola, orange juice, chocolate milk and water. The color measurements were carried out before immersed solutions and at the end of the 1st and 7th days, using a spectrophotometer. The color change $\Delta E_{00}$ was calculated using the CIEDE2000 formula. Data were analyzed by Kruskall-Wallis and Wilcoxon test ($p<0.05$).

**Results**: The $\Delta E_{00}$ values for all coated and uncoated GICs showed an increase in all solutions after 7 days of immersion. All the $\Delta E_{00}$ values obtained from all the specimens immersed in cola were higher than the acceptability threshold. There was no statistical difference in terms of $\Delta E_{00}$ values between the coated and uncoated specimens of the same GICs at same time periods.

**Conclusions**: Coating of GICs exhibited relatively good color stability and protect from the discoloration. The staining effect of GICs should be carefully considered when selecting dental materials in pediatric dentistry.

**Keywords:** Surface coating agent, glass-ionomer cement, staining.

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## INTRODUCTION

The glass ionomer cements (GICs), compomers, and composites are commonly used restorative materials in the paediatric dentistry.[1,2] Besides the treatment necessities, these dental materials are also preferred for their esthetics.[3] Their esthetically pleasing results, anti-cariogenic potentials depending on flouride release, biocompatibilities, and chemical bonding to dental tissues made GICs a more qualified material group when compared to other restorative materials.[4] Besides that, GICs also have weak physical and chemical characteristics such as sensitivity to dehydration and moisture contamination during initial hardening reaction, a prolonged setting reaction time, and a rough surface texture.[5] In order to overcome these disadvantages, enhance handling characteristics, increase working times, and improve esthetic properties, materials such as high-viscosity GIC (HVGIC), resin-modified GIC (RMGIC), and reinforced GIC (RFGIC) were developed by changing the formulations of several GIC-based restorative materials.[1,6]

In order to achieve the ideal esthetic, the restorative materials should be able to mimic the tooth in terms of color, translucency, and surface texture and they should exhibit long term color stability.[7] The discoloration of restorative materials are explained with absorption and adsorption mechanisms of staining agents due to intrinsic and extrinsic factors.[8] The intrinsic discolorations are described as the discoloration of restorative material arising from the oxidation between resin matrix and filler components.[9] The exogenous discoloration however, depends on the type of staining particles, the surface roughness, the type of restorative material, and staining solution exposure time.[10] Exogenous discoloration occurs generally as a result of the consumption of colored beverages.[8]

In order to prevent the possible negative results in physical features due to liquid contamination, manufacturer's recommend the use of GICs together with surface coating resin. It was reported that the applying coating resin to the GICs gives the material gloss, prevents the decrease in the translucence of material in the course of time, provides a smooth surface by filling the gaps arising from material and finishing process, decreases the moisture sensitivity in the short term, increases the fracture and wear resistance of restoration and improves the structural properties of the material.[11] The effects of coating resin on the mechanical properties of GICs have been previously reported[12-14] but the studies on the effect of coating resin application on the coloration mainly remained limited to the composite resins.[15-17]

This study aimed to evaluation of the color stability of coated and uncoated 4 different GIC-based restorative materials after immersed in various staining solutions. The null hypotheses were (1) GIC type has no effect on color stability; (2) different staining solutions and immersion period would not show significant difference on the color stability; (3) coating of GICs would not change the staining susceptibility.

## MATERIALS AND METHODS

This study was designed as an in vitro research. The types and contents of dental restorative materials used in the present study are presented in *Table 1* and the details of solutions in *Table 2*.

**Table 1:** Description of the materials.

| Material (Code) | Manufacturer | Type | Composition | Batch Number |
|---|---|---|---|---|
| ***Riva Light Cure (RLC)*** | SDI, Victoria, Australia | RMGIC | Fluoro-aluminosilicate glass/ Polyacrylic acid Aluminium-fluoro-silicate | K1111265EG |
| ***Chemfil Rock (CR)*** | Dentsply, DeTrey, Konstanz, Germany | Zinc RFGIC | Calcium-aluminium-zinc-fluorophosphor silicate glass, iron oxide pigments, titanium dioxide pigments/Polycarboxylic Acid, water, tartaric Acid | 1903000819 |

| | | | | |
|---|---|---|---|---|
| ***Riva Self Cure HV (RSC)*** | SDI, Victoria, Australia | GIC | Fluoro-aluminosilicate glass/ Polyacrylic acid/Tartaric acid | C1114854F |
| ***Zirconomer (Z)*** | SHOFU Inc, Kyoto, Japan | Zirconia RFGIC | Alumino-fluoro-silicate glass, zirconium oxide/Polyacrylic acid, deionized water, tartaric acid | 07183080 |
| ***Riva Coat*** | SDI, Victoria, Australia | Light-cured resin coating | Acrylic monomer | 190107 |

**Table 2**: Immersion solutions used in the study.

| Product | Material type | Manufacturer | pH |
|---|---|---|---|
| **Cola** | Soft drink | The Coca-Cola Co., Istanbul, Turkey | 2.5 |
| **Orange juice** | Fruit juice | Dimes, Istanbul, Turkey | 3.9 |
| **Chocolate milk** | Milk | Danone, Istanbul, Turkey | 6.6 |
| **Distilled water** | Water | Polifarma, Tekirdağ, Turkey | 6.4 |

**Specimen Preparation**

Specimens were made following the manufacturers' directions, placed into teflon moulds (2mm-thickness and 5mm-diameter) and held between glass slabs with polyester-mylar strip onto both sides under finger pressure to form a smooth surface. The specimens in the Chemfil Rock, Riva Self Cure and Zirconomer group were kept in the mold during the initial cement setting. Only the specimens in the Riva Light Cure group were polymerized from each surface according to the manufacturer's directions with a LED polymerization light (Elipar Freelight 2, 3M ESPE, Germany, 1.200mW/cm$^2$). Polymerization device was tested by a radiometer (Hilux UltraPlus, Benlioglu Dental, Ankara, Turkey). All specimens were taken out from the mold and a total of 256 specimens (64 from each GIC group) were prepared. A caliper was used to measure the final thickness of the specimens (UltraCal V, Fowler, Sylvac, Swiss) (2±0.1 mm). Each different GIC specimens were divided randomly into two subgroups as 32-coated and 32-uncoated specimens. Thirty-two of the specimens prepared using the same GIC were coated with Riva Coat according to manufacturers' directions using the same LED light curing device. Before color measurement the specimens were immersed in distilled water for 24 hours at 37°C.[18]

**Discoloration measurements and immersion cycles**

The spectrophotometer (VitaEasyshade V, Vita Zahnfabrik, Bad Sackingen, Germany) used in the present study and calibrated before every color measurement. The color measurements of specimens were performed on standard neutral gray background (Munsell N7 neutral gray color) by the same operator (BÇ) with D65 illuminant light. After drying the specimens using a tissue paper, the measurements were repeated 3 times on the same surface of each specimen and the mean L*, a*, and b* values were measured. The initial color measurement values ($L_o$, $a_o$, $b_o$) were obtained from specimens immersed in distilled water at 37°C for 24 hours. The coated and uncoated specimens were randomly divided into 4 groups (n=8) and immersed in 4 different solutions (cola, orange juice, chocolate milk, water) then incubated in an airproof and dark environment at 37°C. The immersion solutions were refreshed every day during the 7-day test period. The color measurements were repeated on the 1$^{st}$ and 7$^{th}$ days as explained before.

The color change values were obtained at the end of the 1$^{st}$ and 7$^{th}$ days by using CIEDE2000 formula and recorded for assessment periods t1 (0$^{th}$-1$^{st}$ day) and t2 (1$^{st}$- 7$^{th}$ day), respectively. The formula used is below:

$$\Delta E_{00} = \left[ \left( \frac{\Delta L'}{K_L S_L} \right)^2 + \left( \frac{\Delta C'}{K_C S_C} \right)^2 + \left( \frac{\Delta H'}{K_H S_H} \right)^2 + R_T \left( \frac{\Delta C'}{K_C S_C} \right) \left( \frac{\Delta H'}{K_H S_H} \right) \right]^{\frac{1}{2}}$$

$\Delta L'$, $\Delta C'$ and $\Delta H'$ in the CIEDE formula represent the differences in parameters lightness, chroma, and hue, respectively. $S_L$, $S_C$, and $S_H$ are the weighting functions for the $L^*$, $a^*$ and $b^*$ coordinates, respectively. The parametric factors ($K_L$, $K_C$ and $K_H$) for changes in experimental conditions were set to 1:1:1. And finally, the interaction between chroma and hue differences in the blue region is accounted for by $R_T$ (a rotation function).[19] According to CIEDE2000, the threshold values for perceptibility and acceptability were set as 0.8 and 1.8, respectively.[20]

### Statistical Analysis

Data were analysed using the statistical package of the SPSS for Windows, Version 19.0 (IBM, Chicago, IL, USA). Comparison between GICs color stabilities by the solutions was performed using Kruskal-Wallis test and paired comparisons were performed using Dunn-Bonferroni test. The comparisons between assessment periods for each group were performed using Wilcoxon test. p-values <0.05 was considered significant.

## RESULTS

$\Delta E_{00}$ values of the GICs after immersed in various beverages are presented in *Table 3*. Cola was the solution that stained RLC and RLC+ at the highest level in t1 and t2. Moreover, the chocolate milk stained RLC+ in t2 at the highest level. Cola and chocolate milk stained CR in t1 at the highest level, whereas cola colored it at the highest level in t2. Different solutions had no significant effect on the color change of CR+ in t1 and t2. Cola was the solution stained RSC in t1 and t2 at the highest level. Chocolate milk stained RSC+ at the highest level in t2, whereas cola was the solution stained in t1 at the highest level. Chocolate milk stained Z at the highest level in t1 and orange juice was the solution stained in t2 at the highest level. Different solutions had no significant effect on the color change of Z+ in t1 and t2 (*Table 3*).

**Table 3**: Minimum-maximum (median) of color change values ($\Delta E_{00}$) of glass ionomer-based restorative materials (GICs) after immersion in different solutions in same time interval.

| Materials | Immersion period | Staining Solutions | | | | *p value* |
|---|---|---|---|---|---|---|
| | | Cola | Orange juice | Chocolate milk | Water | |
| ***Riva Light Cure*** | **t1** | 5.7-15.9 (11.3)$^{A}$ | 1-6.8 (3.3)$^{B}$ | 1.4-5 (2.4)$^{B}$ | 1.2-3 (2.7)$^{B}$ | ***<0.001*** |
| | **t2** | 3.2-11.5 (10.3)$^{A}$ | 0.6-4.9 (2.3)$^{B}$ | 0.7-2.8 (1.5)$^{B}$ | 0.6-2 (1)$^{B}$ | ***<0.001*** |
| ***Riva Light Cure+*** | **t1** | 1.8-8.6 (5.1)$^{A}$ | 0.3-5.2 (2.4)$^{AB}$ | 0.9-5.1 (1.8)$^{B}$ | 1.5-2.5 (2.2)$^{AB}$ | ***0.023*** |
| | **t2** | 5.1-13.5 (8.1)$^{A}$ | 1.3-2.7 (2)$^{B}$ | 7.5-11.6 (10.4)$^{A}$ | 1.2-2.2 (1.3)$^{B}$ | ***<0.001*** |
| ***Chemfil Rock*** | **t1** | 1.2-6.5 (2)$^{A}$ | 0.9-3.9 (1.5)$^{AB}$ | 1-5.6 (1.4)$^{A}$ | 0.2-1.2 (0.7)$^{B}$ | ***0.002*** |
| | **t2** | 1.2-7 (2.5)$^{A}$ | 1.2-3.7 (2.1)$^{AB}$ | 0.3-3.1 (0.8)$^{B}$ | 1.1-2.3 (1.8)$^{AB}$ | ***0.021*** |
| ***Chemfil Rock+*** | **t1** | 0.3-4.1 (2.6) | 0.4-6.8 (2) | 0.4-5 (2.3) | 0.2-1.3 (0.8) | *0.73* |
| | **t2** | 0.6-8.3 (2.9) | 0.5-6.3 (1.7) | 0.5-4.9 (0.8) | 1.2-1.5 (1.3) | *0.102* |
| ***Riva Self Cure*** | **t1** | 1.6-4.7 (2.3)$^{A}$ | 0.7-4.5 (0.9)$^{B}$ | 0.4-4.3 (2.5)$^{AB}$ | 1-2 (1.2)$^{AB}$ | *0.042* |
| | **t2** | 4.5-46.4 (22.7)$^{A}$ | 0.9-4.1 (1.6)$^{AB}$ | 0.1-1.8 (0.6)$^{B}$ | 0.5-1.8 (1.3)$^{B}$ | ***<0.001*** |
| ***Riva Self Cure+*** | **t1** | 0.6-7 | 0.5-7.3 | 0.7-3.1 | 0.2-1 | ***0.002*** |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | (2.8)$^{A}$ | (1.2)$^{AB}$ | (1.9)$^{A}$ | (0.5)$^{B}$ | |
| | **t2** | 1-13.3 (4)$^{AB}$ | 0.5-4 (1.2)$^{B}$ | 6.2-11.2 (8.6)$^{A}$ | 1.1-1.8 (1.5)$^{B}$ | ***<0.001*** |
| **Zirconomer** | **t1** | 0.5-35.3 (5.6)$^{AB}$ | 1.4-15.8 (9)$^{AB}$ | 4.6-35.1 (12)$^{A}$ | 1.1-2.4 (1.9)$^{B}$ | ***0.006*** |
| | **t2** | 1.1-23.8 (2.6)$^{AB}$ | 2.6-19.8 (12.4)$^{A}$ | 1-34.5 (5)$^{AB}$ | 1.2-2.4 (1.7)$^{B}$ | ***0.017*** |
| **Zirconomer+** | **t1** | 1.4-5.8 (3.2) | 0.7-4.2 (1.8) | 1.7-5.1 (2.1) | 3.3-4 (3.7) | *0.194* |
| | **t2** | 1-7.5 (3.2) | 0.5-7.1 (2.3) | 0.6-3.4 (1.4) | 1.2-2.3 (2) | *0.269* |

+: *refers to the specimens coated with Riva Coat*
*Different uppercase letters (A, B) indicate statistically significant differences (in a row) (p<0.05)*

All the $\Delta E_{00}$ values obtained from all the specimens immersed in cola were higher than the acceptability threshold ($\Delta E_{00}$=1.8). The immersion solutions did not show any significant color change in the CR+ and Z+ at any time period (*Table 3*).

The $\Delta E_{00}$ values of GICs used in the present study in different immersion periods are presented in *Table 4*. In both periods, the group showing the highest $\Delta E_{00}$ value was Z, the coating in this group slightly reduced the discoloration but yielded no significant difference. RSC+ group showed the minimum discoloration in t1 but $\Delta E_{00}$ values significantly increased after the 1$^{st}$ day ($p$<0.001). There was no statistical differences with regard to $\Delta E_{00}$ values between coated and uncoated specimens of the same GICs evaluated between themselves at same time periods.

**Table 4**: Minimum-maximum (median) of color change values ($\Delta E_{00}$) of glass ionomer-based restorative materials (GICs) after different immersion periods.

| **Materials** | **Immersion Period** | | |
|---|---|---|---|
| | **t1** | **t2** | ***p** |
| ***Riva Light Cure*** | 1-15.9 (3.1)$^{a}$ | 0.6-11.5 (1.7)$^{ab}$ | ***0.001*** |
| ***Riva Light Cure +*** | 0.3-8.6 (2.3)$^{abc}$ | 1.2-13.5 (3.9)$^{a}$ | ***0.006*** |
| ***Chemfil Rock*** | 0.2-6.5 (1.3)$^{bc}$ | 0.3-6.9 (1.9)$^{ab}$ | 0.568 |
| ***Chemfil Rock +*** | 0.2-6.8 (1.6)$^{bc}$ | 0.5-8.3 (1.3)$^{b}$ | 0.866 |
| ***Riva Self Cure*** | 0.4-4.7 (1.7)$^{bc}$ | 0.1-46.4 (1.5)$^{ab}$ | 0.272 |
| ***Riva Self Cure +*** | 0.2-7.3 (1.2)$^{c}$ | 0.5-13.3 (3.1)$^{ab}$ | ***<0.001*** |
| ***Zirconomer*** | 0.5-35.3 (5.3)$^{a}$ | 1-34.5 (2.8)$^{a}$ | 0.080 |
| ***Zirconomer +*** | 0.7-5.8 (3.4)$^{ab}$ | 0.5-7.5 (2)$^{ab}$ | 0.084 |
| ****p*** | ***<0.001*** | ***<0.001*** | |

+: *refers to the specimens coated with Riva Coat*
**Wilcoxon Signed Rank Test, **Kruskall Wallis Test (p<0.05)*
*Different lowercase letters (a, b, c) indicate statistically significant differences (in a column)*

## DISCUSSION

This study, color stability of four different GIC-based restorative materials, on which a surface coating was applied or not, after exposure to different immersion solutions was investigated. According to the results obtained, it was found that type of restorative material, type of staining solution, and immersion time had effect on the color stability of GICs; thus, the first two hypotheses have been rejected and the third partially accepted.

In addition to all the positive features of GICs, they are often preferred as a restorative material in paediatric dentistry due to their ease of clinical application.[3,4] Therefore, the importance of color stability in GIC is parallel to the increase in esthetic expectation. In paediatric dentistry, long-term color stability should not only be considered for aesthetic reasons and loss of

money from repeated restorative procedures but also for the fact that more frequent visits to dentist might cause dental anxiety and behavior management problems among children.[2]

In recent years, the consumption of soft drinks significantly increased among children and youth. Especially flavors such as chocolate, banana, and strawberry added to milk encourage consumption.[21] Although there are studies in the literature about the coloration of dental materials in different solutions[3,6,8,10,21,22], studies evaluating the effects of surface coating application on the color stability of GICs is still limited.[23,24] The present study was designed since there was no study evaluating the effect of surface coating application on the color stability of GICs, which are the materials widely used in dental treatments of children, after exposure to beverages commonly consumed by children.

The immersion time used in the present study was designed by taking reference from the study of Guler *et al*.[22] In their study, authors assumed the average consumption duration of a beverage to be 15 minutes and average daily consumption to be 3.2 cups. Thus, 1-day immersion simulates the amount of beverage consumed in approximately 1 month. In this study, color change values at t1 ($0^{th}$-$1^{st}$ day) and t2 ($1^{st}$-$7^{th}$ day) were compared. Thus, it was aimed to evaluate the short and long term effect of coating on the color change of GICs.

In the present study Riva Coat was chosen as a coating material. Because two GIC materials (RLC and RSC) were produced by the Riva Coat's manufacturer and there was no study evaluating the efficiency of Riva Coat surface coating resin material on GICs in the literature.

In this study, the color changes were measured using a spectrophotometer. When the literature was examined, CIE Lab was generally used to detect discoloration of restorative materials stored in various beverages. However, the International Commission on Illumination has developed the CIEDE2000 formula, which is more accurate in terms of perceptibility and acceptability due to the inadequacy of the CIE Lab formula.[25] The CIEDE2000 formula was also used in this study, as it proved to be more up-to-date and appropriate for detecting color changes.[26]

Differences in composition and setting of GICs may affect their staining susceptibility.[27] The water content of GICs is an also important determinant for color stability.[8] Compared to RMGICs, GICs contain more water, therefore their water absorption capacity is reduced, resulting in less staining.[23] When t1 and t2 time periods were compared, there was no significant differences in $\Delta E_{00}$ values of the uncoated RSC, while a statistically significant increase was observed in the $\Delta E_{00}$ value when the RSC was coated. In RLC, $\Delta E_{00}$ values showed a significant decrease after the $1^{st}$ day when uncoated but a significant increase was obtained when coated. There are many studies supporting that surface coating resin should be applied on the surface of GICs and RMGICs.[24,28] But the manufacturer of RLC does not recommend the use of surface coating but it is recommended to not eat anything for a minimum of 1 hour after the material hardens. In this study, one of the materials showing the highest color change was RLC. This result is in parallel with the previous studies reporting that GIC has higher color stability than RMGICs.[29] Zimmerli *et al*.[30] was reported that the surface coatings have the potential of decreasing the surface porosity and they might reduce the color change by protecting the material from external factors but they tend to break up from the surface. This in vitro study was completed within 7 days, simulating approximately 7-month of staining, so no separation from the surface was occurred in the coating material. Using a surface coating is not recommended by the manufacturer of CR but in a previous study, it was stated that coating the CR enhanced the mechanical properties.[31] The manufacturer of Z recommends cocoa butter as a coating material. However, this material can cover the restoration surface for a short time and it may fall short in moisture control. In our study, coating material was also applied on the surfaces of CR and Z specimens, included in the RFGICs, and no significant differences were found between the $\Delta E_{00}$ values of the coated and uncoated specimens at the same

time interval or within the coated and uncoated specimens at different time intervals. The coated CR and Z did not show any significant differences in discoloration when immersed in different immersion solutions in t1 and t2. It has been observed that the effects of the solutions on color change are protected with when CR and Z were coated. The consumption of acidic drinks is very common among all the adolescent and children. Acidity of these beverages might damage the properties of restorative materials.[32] In a study evaluating the effect of acidic drinks on bacterial adhesion in GICs, it was reported that cola caused the deterioration of GICs surface layer and consequently, an increase in bacterial adhesion. Even if the use of surface coating material decreased the bacterial adhesion, it caused an increase in bacterial adhesion due to the rapid dissolution of coating material under the acid effect.[33] In this study, only the color changes of GICs were compared, bacterial adhesion was not evaluated. Among the solutions used in the present study, cola was found that caused in the highest level of color change in coated and uncoated specimens. This might be because it has a lower pH than other solutions. In addition, cola is a brown carbonated beverage which gains its color by adding caramel that displays colors ranging from pale yellow to deep brown.[34] Thus, clinicians should warn the patients, who consume cola at high amounts, about the discoloration of GIC-based dental restorations. In this study, $\Delta E_{00}$ values of specimens in cola in all times were found to be higher than the acceptability threshold. Following cola, chocolate milk was the solution yielding the second-highest color change. Although pH value of chocolate milk is more alkaline than the other solutions, it is thought that the staining particles in solution might cause discoloration. These results are in parallel with the previous studies stated that the color changes of restorative materials may vary depending on the size of particles in the staining solution and the restorative material's components.[35]

As expected, the lowest color change at t1 and t2 was observed in distilled water in all GIC groups. Consequently, it was found that the color change was not related to whether the GICs were coated, but with color change being associated with GIC type and immersion time. Since this study is in-vitro, it cannot fully provide clinical applications. Saliva generally acts as a buffer for pH of beverage and it dilutes the concentration of beverages in the mouth. However, in this study, the specimens were stored in undiluted beverages for 7 days. Moreover, only limited time effects of the coating were investigated. Given the fact that longer protection is needed in the oral environment, long-term studies are needed. Besides, the absence of previous studies testing similar GICs and methodology together made it difficult for comparison.

## CONCLUSIONS

Within the limitations of present study, all GIC groups exhibited less color stability than other solutions when immersed in cola. RFGICs showed more stability in terms of color change in the long term than other GICs. Determining the role of coatings in the color stability and which GIC material is sensitive to the color changes are very important for in estimating the lifetime of the restoration.

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None to declare.

## CONFLICT OF INTEREST

Author confirm that he has no conflict of interest.

## *ÖZ*

***Amaç:*** *Çalışmanın amacı, cam iyonomer içerikli (CIS) farklı restoratif materyallerde yüzey örtücü uygulamasının, çocukların sıklıkla tükettiği içeceklerin renk stabilitesi üzerine etkisini değerlendirmektir.* ***Gereç ve Yöntemler:*** *Bu çalışmada dört farklı CIS ve bir yüzey örtücü kaplama materyali kullanıldı. Her bir CIS 'den disk şeklinde on altı örnek yapıldı ve kaplanmamış ve kaplanmış olmak üzere iki gruba ayrıldı (n=8). Renklenmeleri için örnekler yedi gün boyunca kola, portakal suyu, çikolatalı süt ve su solüsyonlarında bekletildi. Renk ölçümleri, solüsyonlarda bekletilmeden önce başlangıçta ve 1. ve 7. günlerin sonunda spektrofotometre kullanılarak yapıldı. Renk değişimleri ($\Delta E_{00}$), CIEDE2000 formülü kullanılarak hesaplandı. Veriler Kruskall-Wallis ve Wilcoxon testi ile analiz edildi ($p<0.05$).* ***Bulgular:*** *Kaplanan ve kaplanmayan tüm CIS'lerde $\Delta E_{00}$*

*değerleri, yedi günlük bekletilme sonrasında tüm solüsyonlarda artış gösterdi. Kolada bekletilen tüm örneklerden elde edilen $\Delta E_{00}$ değerleri, kabul edilebilirlik eşiğinin üzerinde olduğu tespit edildi. Aynı CIS'lerde, aynı zaman dilimlerinde kaplanmış ve kaplanmamış örnekleri arasında $\Delta E_{00}$ değerleri açısından istatistiksel bir fark bulunamadı.* ***Sonuçlar:*** *CIS'lerin kaplanması, nispeten iyi bir renk stabilitesi sergiledi ve renklenmeye karşı koruma sağladı. Çocuk diş hekimliğinde CIS içerikli dental materyaller tercih edilirken renklenme etkileri de göz önünde bulundurulmalıdır.* ***Anahtar kelimeler:*** *Yüzey örtücü ajan, cam-iyonomer siman, renklenme.*

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**Original research**

# HOW IMPORTANT ARE THE IMPLANT INCLINATION AND THE INFRASTRUCTURE MATERIAL USED IN IMPLANT SUPPORTED FIXED PROSTHESES?

Afra Eda Karadayı Yüzükcü[1]
*Kaan Yerliyurt[2]

## ABSTRACT

**Objectives:** The aim of this study is to evaluate the stress, which is caused by the fixed prosthesis under oblique forces around dental implants and bone by using different infrastructure materials and different inclusions, by 3-dimensional (3D) finite element analysis (FEA) method.

**Materials and Methods:** 3D-FEA models of mandible, dental implants and prostheses were designed. The anterior and posterior implants were designed 10 mm in length and 4.3 mm in diameter. The anterior implant was placed parallel to each model. Posterior implant designed to make inclinations those mesial 17°, distal 17°, buccal 17°, lingual 17°. Implant supported fixed restorations were divided into 3 main groups according to the infrastructure materials. These materials were; chromium-cobalt, zirconia, polyetheretherketone (PEEK). In each model, a total of 500 N oblique force was applied from the buccal tubercle crests to the buccolingual direction at an angle of 30 degrees to the long axis of the tooth. Maximum principal (tensile) stress and minimum principal (compressive) stress values in the bone models were taken. In addition, von Mises stress values were obtained from implants and substructure materials.

**Results:** When the stress findings in the mandible during oblique loading were evaluated, it was found that the stresses on the cortical bone were higher than the stresses on the trabecular bone. It was observed that the highest stress values occurred in the implants.

**Conclusions:** It is thought that chromium-cobalt and zirconia-based ceramic bridge restorations are more positive in terms of stress distribution than PEEK-based ceramic bridge restorations.

**Keywords:** Finite element analysis, implant-supported dental prosthesis, polyetheretherketone, zirconia.

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## INTRODUCTION

Dental implant applications have become a current treatment option in dental clinics due to the increase in success rates.[1,2] Implant-supported dental prosthesis are divided into two classes as fixed or removable. The biggest advantage of implant-supported fixed dental prosthesis is that patients are psychologically satisfied and have a longer life span compared to implant-supported partial prostheses. Implant-supported fixed prostheses have increased function, stabilization and more satisfactory results compared to partial removable prostheses.[3]

In order to achieve functional and aesthetically satisfactory results with successful implant placement in the mandibular region, appropriate angulation and positioning are required.[4] The direction of implant placement is closely related to the transmission of occlusal loads. The placement angle or inclination of the implant is very important in terms of biomechanics.[5,6]

In implant-supported fixed prosthesis, the infrastructure material plays an important role in stress transmission to implant and the bone around the implant. It has been reported that zirconium, which is used as a infrastructure thanks to its aesthetic properties, provides very good marginal compatibility and sufficient durability with the implant. In addition, since metals are not used as a substructure material, there are no disadvantages such as the emergence of toxic and allergic reactions caused by ion release.[7-9] PEEK; which is a high performance polymer, has been used as an alternative to metal alloys in many industries since the late 1970s. There are many areas of use in dentistry such as endocrones, infrastructures of fixed prostheses, implant materials and parts, and removable prosthetic skeleton.[10-14] The mechanical properties of PEEK are similar to dentin and enamel, making this material more advantageous and positive than alloy and ceramic restorations.[15] Chrome-cobalt alloys are resistant to abrasion and corrosion. They are biocompatible, high modulus of elasticity that do not stain easily. Their high elastic modulus allows them to be prepared thinner.[16] Among the prosthetic restorations on implants, porcelains with metal substructure are still the most preferred materials today. Their biggest advantages are their durability, good bonding to porcelain, cheap and easy access. However, metal-based porcelains have many disadvantages. Corrosion and allergy are the main disadvantages.[17]

The aim of this study is to evaluate the stresses around dental implants and bone which is caused by the oblique forces on 3-member fixed prosthes which was made by different infrastructure materials [chrome-cobalt (Cr-Co), zirconia, polyetheretherketone (PEEK)] and different implant inclusions (mesial 17°, distal 17°, buccal 17°, lingual 17°) by using 3D-FEA method.

## MATERIALS AND METHODS

Ethical approval was granted by the University of Tokat Gaziosmanpaşa, Ethics Committee of the Clinical Research (Project no: 21-KAEK-266). 3D-FEA model of the edentulous mandible is designed. While modeling the mandibular bone, Division - A bone with a width of more than 5 mm in the bucco-lingual direction and a vertical bone dimension of 10 mm was chosen. The NobelActive (Nobel Biocare, Gothenburg, Sweden) implants were used in the models of the study. The anterior and posterior implants were 10 mm in length and 4.3 mm in diameter. Implants were placed in teeth 45-47 position. The posterior implant was placed in 4 different directions with 17° inclination (mesial 17° , distal 17°, buccal 17° and lingual 17°). Anterior implant was placed in parallel in each model (Figure 1).

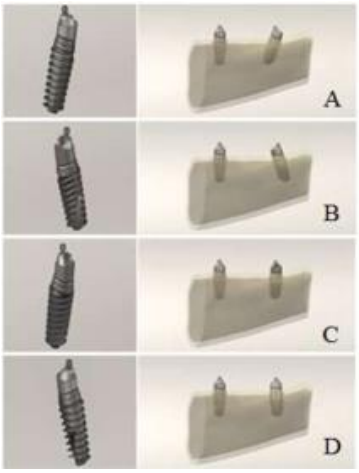


**Figure 1.** 17° inclined implant to mesiale **(A)**, 17° inclined implant to distale **(B)**, 17° inclined implant to buccal **(C)**, 17° inclined implant to lingual **(D)**.

The distance between implants were positioned to be 16 mm. Cr-Co alloy, zirconia, PEEK material

was used as infrastructure during the modeling of implant prostheses. Three different infrastructures were designed and the connector thickness was 2.5 mm. (Figure 2).

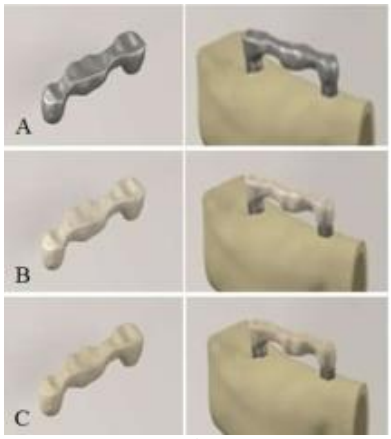


**Figure 2.** Cr-Co infrastructure **(A)**, Zirconia infrastructure **(B)**, PEEK infrastructure **(C)**.

The implant and prosthesis parts supplied in the study were scanned in 3D optical scanner (Activity 880-Smart Optics, Sensortechnik GmbH, Bochum, Germany). The models obtained in.stl format were sent to Rhinoceros 4.0 (Robert McNeel & Associates, Seattle, USA) 3D modeling software. With the Boolean method in Rhino software, harmonization was made between prosthesis upper and lower parts, implant screws and bone tissues and force transfer was achieved.

Thanks to this modeling technique, it has been tried to create the highest quality network structure with the highest possible node elements in order to facilitate the calculation. Young's modulus and Poisson's ratios of the materials and tissues that make up our study models are given in Table 1.

**Table 1.** Young's modulus (elasticity modulus) and Poisson's ratios of the materials used in the study

| MATERIALS | Young's Modulus (MPa) | Poisson's Ratio |
|---|---|---|
| **Cortical bone** | 13700 | 0.30 |
| **Trabecular bone** | 1370 | 0.30 |
| **Titanium** (implant and screws) | 110000 | 0.35 |
| **Zirconia** (infrastructure) | 205000 | 0.22 |
| **Chrome-Cobalt** (infrastructure) | 218000 | 0.33 |
| **PEEK** (infrastructure) | 4000 | 0.36 |
| **Acrylic resin** | 3000 | 0.35 |
| **Feldspathic porcelain** | 82800 | 0.35 |

As a result, 4 subgroups were created according to the posterior implant angulation. Three different subgroups were created according to the infrastructure materials used in implant-supported bridge prosthes. In total, 12 finite element analyzes were performed. In each model, 500 N total oblique force was applied at an angle of 30 degrees from the buccal tubercle crests to the long axis of the tooth in the buccolingual direction. 100 N to the second premolar tooth, 200 N to the first molar and 200 N to the second molar (Figure 3).

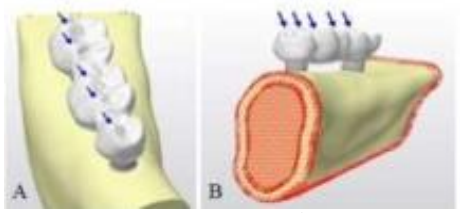


**Figure 3.** Oblique loads loaded from the buccal tubercle crests of restorations at an angle of 30° (view from the mesial) **(A)** , Oblique loads and red color in the meshed model the peripheral points are given zero degrees of freedom **(B)**.

The highest tensile stress and compressive stress values those occur in cortical and trabecular bone were analyzed. In addition, von Mises stress values were obtained from implants and infrastructures.

## RESULTS

When the stress findings in the mandible during oblique loading were evaluated, it was found that the stresses on the cortical bone were higher than the stresses on the trabecular bone. It was seen that the highest stress values occurred in implants. When the stress values formed in the infrastructures were examined, it was seen that lower stress values occurred in the PEEK infrastructure models. When the stress values in posterior implants were examined, it was seen that higher stress values occurred in PEEK models.

Maximum principal (tensile) stress (σmax) distributions in cortical bone during oblique loading

are shown in Figure 4. In oblique loading, the maximum principle stres ($\sigma_{max}$) findings in cortical bone are shown in Figure 5.

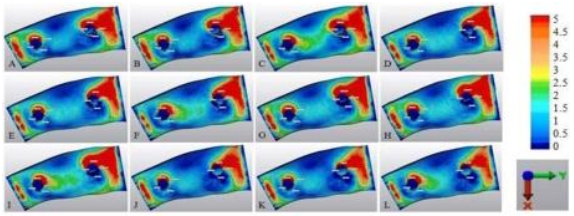

**Figure 4.** Maximum principal (tensile) stress ($\sigma_{max}$) distributions in cortical bone during oblique loading. Cr- Co / mesial 17° **(A)**, Zirconia/mesial 17° **(B)**, PEEK/mesial 17° **(C)**, Cr-Co/distal 17° **(D)**, Zirconia/distal 17° **(E)**, PEEK/distal 17° **(F)**, Cr-Co / buccal 17° **(G)**, Zirconia / buccal 17° **(H)**, PEEK / buccal 17° **(I)**, Cr- Co / palatinal 17° **(J)**, Zirconia / palatinal 17° **(K)**, PEEK / palatinal 17° **(L)**,

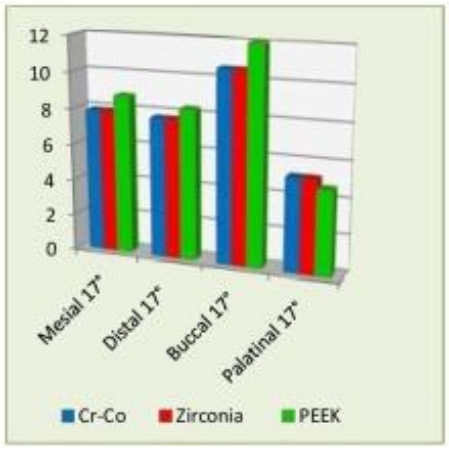


**Figure 5.** Maximum principal (tensile) stress ($\sigma_{max}$) values in cortical bone around the implant in oblique loading.

In this installation; the highest value in the selected node points in the cortical bone around the implant was 12 MPa in the PEEK model with a 17° inclination to the buccal, and the lowest value was 4.69 MPa in the PEEK model with a 17° inclination to the palatinal.

The findings of the minimum principle (compressive) stresses occurring in the cortical bone in oblique loading are shown in Table 2. In this installation; the highest value in the selected node points in the cortical bone around the implant was found to be -30.74 MPa in the mesiale 17° inclined PEEK model and the lowest value was found as -18.35 MPa in the palatinal 17° inclined Cr-Co model.

**Table 2.** Stress values in oblique loading (MPa)

| | **Maximum principal (tensile) stresses ($\sigma_{max}$)** | **Minimum principal (compressive) stresses ($\sigma_{min}$)** | | **Von Mises stresses** | |
|---|---|---|---|---|---|
| **GROUP** | **Cortical bone** | | **Infrastructure** | **Implant** (Posterior) | **Implant** (Anterior) |
| Cr – Co / mesial 17° | 7.88 | -27.58 | 17.27 | 162.37 | 155.34 |
| Zirconia / mesial 17° | 7.88 | -27.58 | 16 | 162.24 | 155.30 |
| PEEK / mesial 17° | 8.77 | -30.74 | 8 | 182.33 | 152.18 |
| Cr – Co / distal 17° | 7.70 | -21.42 | 14.76 | 180.43 | 148.80 |
| Zirconia / distal 17° | 7.70 | -21.43 | 14.47 | 180.41 | 148.83 |
| PEEK / distal 17° | 8.32 | -23.06 | 8.19 | 202.25 | 155.49 |
| Cr – Co / buccal 17° | 10.60 | -20.45 | 18.38 | 155.97 | 163.06 |
| Zirconia / buccal 17° | 10.59 | -20.45 | 18.08 | 156.02 | 163.03 |
| PEEK / buccal 17° | 12 | -22 | 8.23 | 193.16 | 155.88 |
| Cr – Co / palatinal 17° | 5.18 | -18.35 | 13.44 | 198.24 | 137.61 |
| Zirconia / palatinal 17° | 5.18 | -18.35 | 13.14 | 198 | 137.68 |
| PEEK / palatinal 17° | 4.69 | -18.59 | 8.25 | 207.11 | 150.02 |

Von Mises stress distributions observed in infrastructures during oblique loading are shown in Figure 6. In oblique loading, the highest von Mises stress findings occurring in the infrastructure are shown in Figure 7. In this installation; the highest value was found to be 18.38 MPa in the group of Cr-Co buccal-17° inclination and the lowest value was found in the group of PEEK mesial-17° inclination as 8 MPa.

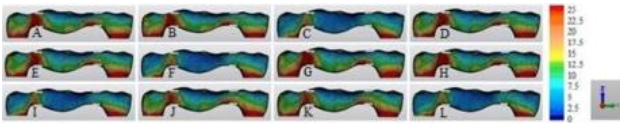

**Figure 6.** Von Mises stress distributions in infrastructures during oblique loading. Cr- Co / mesial 17° (A), Zirconia / mesial 17° (B), PEEK / mesial 17° (C), Cr- Co / distal 17° (D), Zirconia / distal 17° (E), PEEK / distal 17° (F), Cr- Co / buccal 17° (G), Zirconia / buccal 17° (H), PEEK / buccal 17° (I), Cr- Co / palatinal 17° (J), Zirconia / palatinal 17° (K), PEEK / palatinal 17° (L).

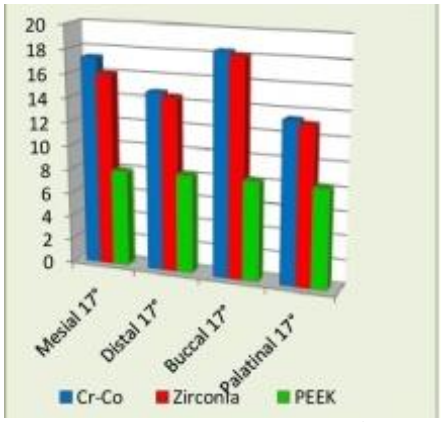


**Figure 7.** Von Mises stress values in infrastructures during oblique loading.

The von Mises stress distributions on implants are shown in Figure 8. The highest von Mises stress values in posterior implant during oblique loading are shown in Figure 9.

**Figure 8.** Von Mises stress distributions in anterior and posterior implants during oblique loading. Cr- Co / mesial 17° **(A)**, Zirconia / mesial 17° **(B)**, PEEK / mesial 17° **(C)**, Cr- Co / distal 17° **(D)**, Zirconia / distal 17° **(E)**, PEEK / distal 17° **(F)**, Cr- Co / buccal 17° **(G)**, Zirconia / buccal 17° **(H)**, PEEK / buccal 17° **(I)**, Cr- Co / palatinal 17° **(J)**, Zirconia / palatinal 17° **(K)**, PEEK / palatinal 17° **(L)**.

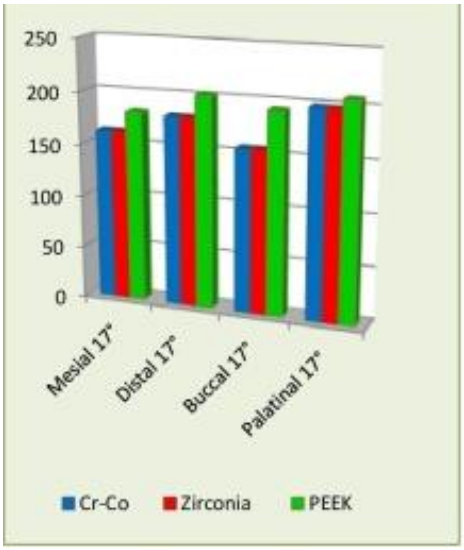


**Figure 9.** Von Mises stress values in posterior implant during oblique loading.

When the stress values were calculated, except for the groups with inclusion in the buccal direction, posterior implant values were found to be higher than the stress values in the anterior implant. In this installation; the highest value was found in the group of PEEK palatinal - 17° inclination posterior implant (207.11 MPa) and the lowest value was found in the group of Cr-Co palatinal - 17° inclination anterior implant as 137.61 MPa (Table 2).

## DISCUSSION

It is impossible to determine the effect of biomechanical factors on the success of implant-supported prostheses by in vivo studies alone. In addition, it is very difficult to achieve standardization of *in vitro* and *in vivo* studies. Standardization can be achieved with finite element stress analysis and stress distributions can be determined digitally.[18] At the same time, finite element analysis allows analysis to be carried out by changing only the determined factors and keeping all other factors constant.[19] Due to these advantages, our study was carried out with finite element stress analysis.

Bone; although it is an inhomogeneous, anisotropic, viscoelastic structure, it is assumed that trabecular and cortical bone are homogeneous, isotropic and linear elastic in order to complete the analysis by simplifying the model.[20] Therefore, in this study, trabecular and cortical bone was assumed to be homogeneous, isotropic and linear elastic, as in other studies.[21-23]

In our study we used the values for the Young's modulus (elastic modulus) and Poisson's ratios that which were used the mostly in the literature.[23-25]

Sarot *et al.*[10] said that oblique forces have a more destructive effect than vertical forces. Contrary to this, no study reporting was found. In our study, 200 N oblique force was applied to the molars and 100 N to the premolar tooth, in total 500 N oblique force. An angle of 30 degrees was preferred to use for oblique force.

Lee *et al.*[26] compared PEEK material with titanium and zirconia as a substructure material for implant-supported prostheses in a study they conducted with finite element analysis. As a result of this study, they found that the stress absorbing effects of the low elastic modulus substructure were limited in some areas and the stiffer substructure material showed a positive stress distribution in the components of the prosthesis.

In another study in the literature; cobalt-chrome, titanium and zirconia prosthetic infrastructures were compared. Regardless of the treatment concept, harder materials such as cobalt-chromium and zirconia showed better biomechanical results; they created lower levels of stress on the bone, implant, abutment, abutment screw.[21]

In a finite element analysis study; 2 different 3-member fixed prostheses were designed as porcelain on metal infrastructure and particulate composite coating on fiber reinforced composite infrastructure. After all; It was reported that while lower stress values were observed in the prosthesis parts in the composite content group, higher stress values were obtained in the implant - abutment parts.[27]

In a study in which a finite element analysis was performed using titanium, zirconium and gold as an implant fixed prosthesis infrastructure; As a result of splinting implants with titanium or zirconia infrastructures, less stress values were observed around the implant and bone compared to gold.[28]

In our study, when the stresses on bone tissues and implants were examined, the highest values were observed in models using PEEK, and similar stresses were observed in models using zirconia and Cr-Co materials compared to PEEK, and it is seen that these results are compatible with the results of the above mentioned studies.

Zampelis *et al.*[29] in their study, they evaluated the effect of the connection of distal inclined implants with a fixed restoration on the stress distribution with two dimensional finite element analysis. In this study, 45° distally inclined implants were compared with those placed vertically, and no significant increase was observed in bone stress in the neck region of the implant.

Satoh *et al.*[5] prepared working models by inclining the implants placed in the mandibular bone to 10° and 20° mesial. The researchers used straight cylinder implants in their models, and the force applied in the study was applied parallel to the long axis of the tilted implant. The results showed that inclined placement of the implants did not adversely affect bone stress.

In our study, maximum principle stress value in the cortical bone was observed in the PEEK buccal 17 degrees inclined group, the lowest cortical bone tensile stress value was observed in the PEEK palatinal 17 degrees inclined group. The highest values observed in the buccal may be due to the application of oblique forces from the buccal direction.

## CONCLUSIONS

PEEK material has the highest tensile and compressive stress values in cortical bone. When stresses occurring in implants are evaluated, the highest stresses occurred in models using PEEK material. Similar stresses were found at lower levels in models using Cr-Co and zirconia material. When the von Mises stresses occurring in the substructures were evaluated, the highest stresses occurred in Cr-Co and zirconia models, and the lowest stresses occurred in PEEK models.

In the light of the results we have obtained; It is thought that Cr-Co based ceramic bridge restorations and zirconia supported ceramic bridge

restorations applied with the correct indication in fixed prostheses on implants will be more successful in terms of stress distribution compared to PEEK infrastructure restorations. It is thought that different inclination directions at the same angle have no important effect on stress values. Besides the advantages of the finite element analysis method, it also has some limitations. Therefore, the results obtained need to be supported by clinical studies.

**ACKNOWLEDGEMENTS**

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**CONFLICT OF INTEREST**

The authors declare to have no conflict of interest.

***İmplant Destekli Sabit Protezlerde İmplant Eğimi ve Kullanılan Altyapı Malzemesi Ne Kadar Önemlidir?***

***ÖZ***

***Amaç:*** *Bu çalışmanın amacı, sabit protezlerin farklı altyapı malzemeleri ve farklı inklüzyonlar kullanarak dental implantlar ve kemik etrafındaki eğik kuvvetler altında neden olduğu stresi 3 boyutlu (3B) sonlu elemanlar analizi (SEA) yöntemi ile değerlendirmektir.* ***Gereç ve Yöntemler:*** *Mandibulada dental implant ve protezlerin 3B-SEA modelleri hazırlandı. Anterior ve posterior implantlar 10 mm uzunluğunda ve 4,3 mm çapında tasarlandı. Anterior implant her modele paralel olarak yerleştirildi. Posterior implant; mesiale 17°, distale 17°, bukkale 17°, linguale 17° eğimli olacak şekillerde tasarlandı. İmplant destekli sabit restorasyonlar alt yapı malzemelerine göre 3 ana gruba ayrıldı. Bu malzemeler; krom-kobalt, zirkonya, polietereterketon (PEEK). Her modelde bukkal tüberkül tepelerinden bukkolingual yöne dişin uzun eksenine 30 derecelik bir açıyla toplam 500 N eğik kuvvet uygulandı. Kemik modellerinde maksimum asal (çekme) gerilme ve minimum asal (basma) gerilme değerleri alındı. Ayrıca implant ve alt yapı malzemelerinden maksimum von Mises stres değerleri elde edildi.* ***Bulgular:*** *Oblik yükleme sırasında mandibulada meydana gelen stres bulguları değerlendirildiğinde, kortikal kemik üzerindeki streslerin trabeküler kemik üzerindeki streslerden daha yüksek olduğu bulundu. En yüksek stres değerlerinin implantlarda meydana geldiği görüldü.* ***Sonuçlar:*** *Krom-kobalt ve zirkonya esaslı seramik köprü restorasyonlarının, stres dağılımı açısından PEEK esaslı seramik köprü restorasyonlarından daha olumlu oldukları düşünülmektedir.* ***Anahtar kelimeler:*** *Sonlu elemanlar analizi, implant destekli diş protezi, polietereterketon, zirkonya*

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**Original research**

# RELATIONSHIP BETWEEN THE DEGENERATIVE CHANGES IN THE MANDIBULAR CONDYLE AND ARTICULAR EMINENCE INCLINATION, HEIGHT, AND SHAPE: A CBCT STUDY

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## ABSTRACT

**Objectives:** This study aimed to analyze any relationship between the articular eminence inclination, height and shape and degenerative condylar changes using cone-beam computed tomography (CBCT).

**Materials and Methods:** The assessments were established on CBCT images of 566 temporomandibular joints (TMJ) that were included from the archive. Age and sex were recorded for all individuals. Degenerative changes were examined on the articular surface of the condyle. The articular eminence (AE) inclination and height measurements were performed on central parasagittal slices of the TMJs. The shape of the AE was classified as box-shaped, sigmoid, flattened, and deformed.

**Results:** The prevalence of degenerative changes in the condyle was higher in males, but no significant difference was found ($p>0.05$). Mean AE inclination and height were greater in males than females ($p<0.05$). Reduced mean eminence inclination and height values were detected in the +50-year-old group ($p<0.05$). Sigmoid and box-shaped articular eminence morphologies were more common. The eminence with a deformed shape was related to two or more degenerative alterations in the condylar head.

**Conclusion:** The eminence inclination and height are associated with the presence and types of degenerative condylar changes. There are significant relationships between sex-AE morphology and age-AE morphology.

**Keywords:** Articular eminence, Cone-beam computed tomography, Degenerative change, Mandibular condyle, Temporomandibular joint.

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## INTRODUCTION

The temporomandibular joint (TMJ) is known as the most complex articular system in the human organism, and connects the mandible to the temporal bone.[1] It has the capability of moving in the three planes of space, and the structures of the TMJ are important to sustain a balanced stomatognathic system. The mandibular condylar process constitutes the inferior bone part and the glenoid fossa constitutes the superior bone part of the TMJ.[2]

The articular eminence (AE) is a part of the temporal bone on which the mandibular condyle and the articular disc complex slides in the course of the opening and closing cycles of the mouth. The morphology of the AE enables the path of the condylar movements to flow naturally; it varies within the population and can also change due to age, sex, and masticatory function.[2,3-6] The eminence inclination is an essential component in the entire masticatory system and biomechanics of the TMJ.

In TMJ disorders, several changes can be detected in the subarticular surfaces of the condyle and the glenoid fossa.[7] Increased loading of the TMJ generally results in degenerative bone changes of the articular surface of the condyle and fossa.[8] Condylar bone changes such as loss of articular cortex, various degrees of flattening, erosion of the articular surfaces, osteophyte formation, and sclerosis may be correlated with the AE inclination, as close relationships exist between these structures.[7]

Several imaging techniques or different modalities have been used to analyze the eminence inclination, such as computed tomography (CT),[7] dry skull measurements,[8] conventional radiography[9] and tomography,[10] and magnetic resonance imaging (MRI).[11,12]

The Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) state that scanning is the reference standard for the diagnosis of degenerative joint disease.[13] Recently, cone-beam computed tomography (CBCT) has been accepted as an alternative to conventional CT to diagnose hard tissue components of the dentomaxillofacial region. CBCT allows for a shorter scanning time and lower radiation dose, reduced cost, smaller machine, and easier access than conventional CT.[14] CBCT is the imaging modality of choice to visualize the bony elements and pathologies of the TMJ in all three dimensions without superposition and structural distortion.[15]

The current study firstly aimed to specify the association between the eminence inclination and height with AE shapes, and the degenerative condylar changes. Secondly, factors that may affect these variables, such as sex and age groups, were evaluated.

## MATERIAL AND METHODS

### *Study design*

The Institutional Ethical Review Board of X University, Faculty of Dentistry approved this retrospective multicenter study with decision number: 14/3 (Ref: 36290600/124), and the study followed the Declaration of Helsinki on medical protocol and ethics.

The G*POWER 3.1 (Heinrich-Heine University of Dusseldorf, Germany) program was performed to determine the sample size. A power analysis revealed that a minimum of 532 cases would provide >80% power to detect significant differences with an effect size of 0.34 at a significance level of $\alpha = 0.05$.

CBCT images of patients who were referred to the clinics of three university hospitals for several reasons such as orthodontic therapy, TMD, impacted teeth, and airway evaluation without a history of any systemic diseases during the period from 2013 to 2019 were retrospectively evaluated.

The inclusion criteria were images with completely visible TMJs, all posterior teeth present and degenerative bone changes of the mandibular condyle in at least one TMJ in CBCT images. Low quality images were not evaluated. The radiologic evidence of bone disease (especially osteoporosis), noticeable periodontal diseases, skeletal asymmetries or trauma, developmental or congenital disorders, systemic diseases that may affect joint morphology such as rheumatoid arthritis, prosthetic restorations, a

history of surgery as well as any tumor or malignancy were exclusion criteria for the study. The final sample was narrowed to 289 cases and 566 TMJs were analyzed in this study.

### *Imaging procedures*

The same standardized scanning protocols were performed for acquiring CBCT images. Technical parameters and dedicated software of the CBCT machines used are summarized in Table 1.

**Table 1:** Technical parameters and dedicated software of used CBCTs

| | | CBCT Units | | |
|---|---|---|---|---|
| | | **ProMax®3D Max** | **NewTom 3G®** | **Veraviewepocs 3D®** |
| **Technical parameters** | **FOV sizes** | 90x100 mm, 230x160 mm | 12-inch | 40x80 mm |
| | **voxel size** | 0.200 $mm^3$, 0.400 $mm^3$ | 0.300 $mm^3$ | 0.125 $mm^3$ |
| | **kVp** | 96 | 120 | 90 |
| | **mA** | 8-12 | 3-5 | 5 |
| | **scan time (s)** | 9-15 | | 9.4 |
| **Software programs** | | Romexis 3.7[a] | NNT 3.0[b] | 3D Tomo X[c] |
| **Monitor** | | NEC MultiSync[d] 21.3-inch flat-panel 2048 x 2560 pixel resolution | Nio Color 3MP[e] 2048 x 1536 pixel resolution | EIZO RadiForce[f] MS230W 23 inch LCD monitor |

*CBCT* cone beam CT, *FOV* field of view, *kVp* Kilovoltage peak, *mA* milliampere, *s* second
Promax 3D Max by Planmeca, Helsinki, Finland;
NewTom 3G by Quantitative Radiology, Verona, Italy;
Veraviewepocs 3D by J Morita MFG Corp., Kyoto, Japan
[a]Romexis 3.7, by Planmeca Oy, Helsinki, Finland; [b]NNT 3.0 by Quantitative Radiology, Verona, Italy; [c]3D Tomo X by IORB, Brasilia DF Brazil
[d]NEC MultiSync by Munchen, Germany; [e]Nio Color 3MP by Barco, Kortrijk, Belgium; [f]EIZO RadiForce MS230W by Eizo Nanao Corporation, Ishikawa, Japan

### *CBCT evaluations*

The images were analyzed by one informed and calibrated oral radiologist (CG with six years of experience, Mİ and SA with over ten years of experience) at each center by using the scanner's software programs. Before the evaluation, a standard positioning was defined and each image was placed in that position by the examiners.

In the axial view, the patient's sagittal median plane was adjusted to the vertical reference line. In the sagittal view, the hard palate was positioned so that the investigator could view the anterior nasal spine and the posterior nasal spine and was then tilted to overlap the horizontal reference line. Thus, the reference line was aligned with the palatine plane (PP).

For secondary reconstruction, the axial view on which the condylar processes were seen with their widest mediolateral extent was used as a reference view. The paracoronal slices (1 mm thick) were made along the long axis of the condyle, and the parasagittal slices were obtained perpendicular to the paracoronal plane (Figure 1).

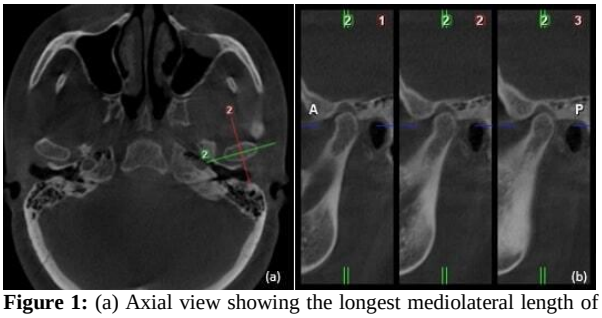


**Figure 1:** (a) Axial view showing the longest mediolateral length of the condyle. Paracoronal slice of the condyle (green line) and parasagittal slice of the condyle (red line). (b) Parasagittal reconstruction of the temporomandibular joint in maximum intercuspation.

Age, sex, condylar bone changes, articular eminence shapes, and measurements were noted on an evaluation sheet for each case. To prevent misinterpretation, the observed degenerative changes had to be detected in at least two consecutive slices. The image excluded any doubt about which classification choice was decisive. The three examiners were asked to assess the following radiographic characteristics:

### *Diagnostic Classification for Condylar Bone Change*

The degenerative changes of the condyles were classified according to previously reported definitions as follows:[16] flattening (Figure 2a);

sclerosis (Figure 2b); erosion (Figure 2c); osteophytes (Figure 2d); and combination of two or more degenerative condylar changes (Figure 2e).

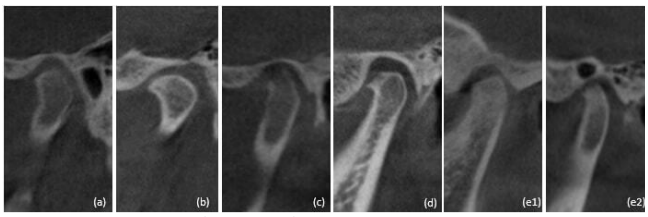


**Figure 2:** The classification of the degenerative condylar bone changes (a) flattening; (b) sclerosis; (c) erosion; (d) osteophyte; (e) combination of two or more degenerative changes. e1: flattening and sclerosis, e2: flattening, sclerosis, and erosion.

### *Measurements*

All measurements were performed on the central parasagittal slice of the TMJ. The eminence height was measured by tracing two parallel lines that were parallel to the PP, one tangent to the highest point of the glenoid fossa and another tangent to the lowest point of the articular eminence. Between those two lines, the distance was measured as the eminence height (Figure 3a).

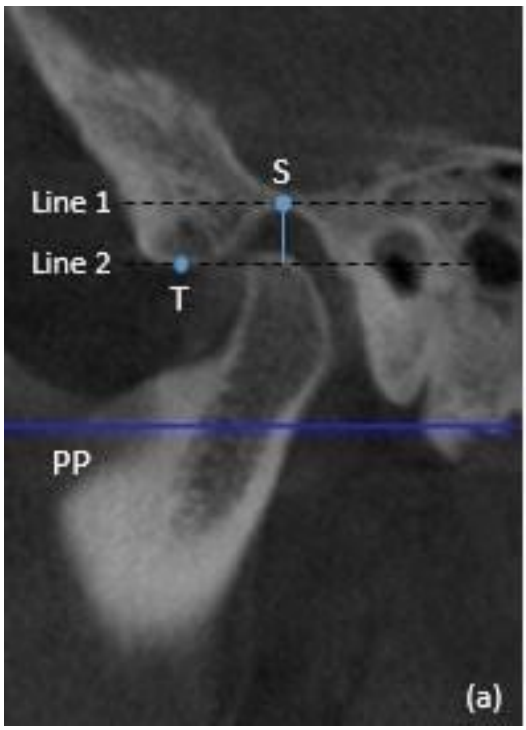


**Figure 3a:** Articular eminence height.

To detect the eminence inclination, the same parallel lines were used as reference. The internal angle formed between line A (passing through the highest point in the roof of the glenoid fossa and the lowest point at the crest of the articular eminence) and line B (parallel to the palatine plane) was used to define the value of AE inclination (Figure 3b).

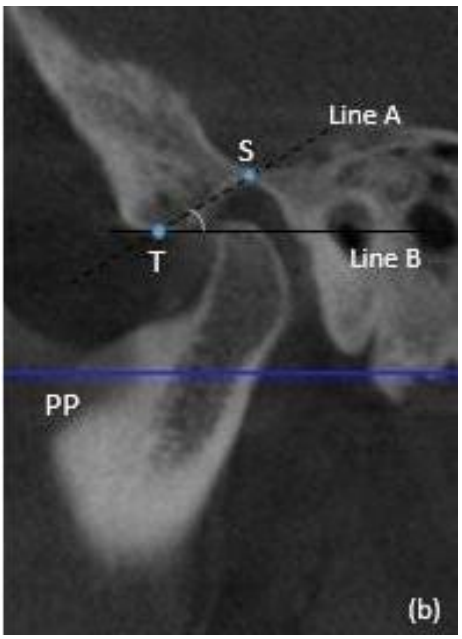


**Figure 3b:** Articular eminence inclination. Line 1: the parallel line to the PP passing through the highest point of the glenoid fossa, Line 2: the parallel line to the PP passing through the lowest point of the articular eminence. *PP* palatal plane, *S* highest point of glenoid fossa, *T* lowest point of articular eminence.

### *Articular Eminence Morphology*

The AE morphology was classified into four types, according to the classification of Kurita *et al*. [17] (2000): box-shaped, sigmoid, flattened or deformed. In the closed-mouth position, the central parasagittal TMJ slices were evaluated. The box shape represents a deep fossa with a steep posterior articular eminence inclination, while the sigmoid shape represents a continuously S-shaped slope. The flattened eminence has a smooth eminence and therefore a shallow fossa. If the eminence morphology failed to fit one of these three categories, it was classified as deformed (Figure 4).

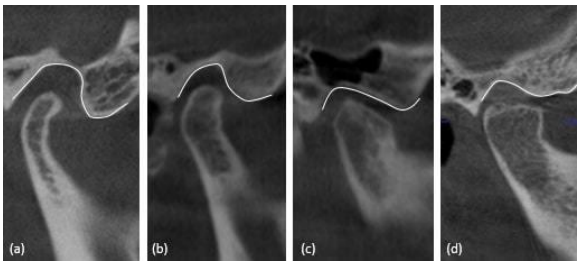


**Figure 4:** Articular eminence shapes. CBCT images and outline drawings to represent the four shapes of the eminence classified in this study. (a) box-shaped, (b) sigmoid, (c) flattened, (d) deformed.

### *Statistical Analysis*

Data analysis was performed using the IBM SPSS Statistics 21.0 (Statistical Package for Social Sciences) program. The Student *t*-test and/or Mann-Whitney U test were performed for the comparison of two independent groups.

Comparison among three or more groups was performed by analysis of variance (ANOVA test) and/or Kruskal-Wallis H test. The categorical variables were analyzed using the Chi-square test. A probability level of less than 0.05 ($p$<0.05) was considered to be significant.

## RESULTS

Degenerative condylar changes were observed in 480 of the 566 joints. Eighty-six TMJs had no changes. The distributions of the presence and types of the degenerative changes in TMJs according to sex and age groups are presented in Figure 5 and Figure 6.

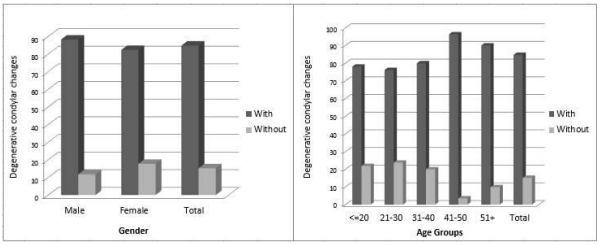


**Figure 5:** Bar graphs show the distribution of the presence of degenerative condylar changes according to sex and age groups.

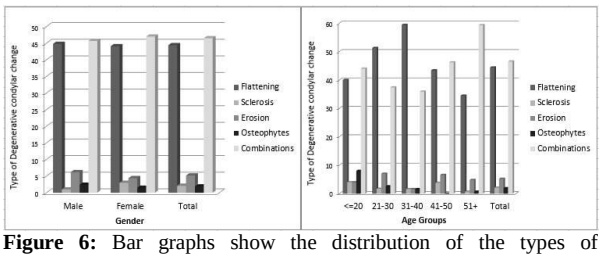


**Figure 6:** Bar graphs show the distribution of the types of degenerative condylar changes according to sex and age groups.

Regarding the age groups, individuals aged 30 years and older showed higher prevalence of degenerative condylar changes and this difference was statistically significant ($p$ = 0.001). For sex, the prevalence of degenerative findings was higher in males (88.2%) than in females (82.3%) but, this difference was not statistically significant ($p$=0.053). The prevalence of the degenerative change types was higher for combination (46.5%) followed by flattening (44.4%), erosion (5.2%), sclerosis (2.1%), and osteophytes (1.9%).

The mean results of eminence inclination and height according to sex and age groups are shown in Table 2. There was a significant difference between the metric variables of the AE according to sex. The eminence inclination and height in males were higher than those of females ($p$ = 0.0001). Additionally, the mean values of eminence inclination and height in the +50-year-old group were found to be statistically lowest among age groups ($p$=0.0001).

**Table 2:** The mean eminence inclination and eminence height values according to sex and age groups

| | | n | Eminence inclination mean ± sd | *p* value | Eminence height mean ± sd | *p* value |
|---|---|---|---|---|---|---|
| **Sex** | Female | 328 | 33.6 ± 9.3 | 0.0001* | 6.22 ± 1.96 | 0.0001* |
| | Male | 238 | **37.0 ± 8.6** | | **6.87 ± 1.75** | |
| | **Total** | 566 | 35.0 ± 9.2 | | 6.49 ± 1.90 | |
| **Age Groups** | ≤20 | 64 | 35.5 ± 9.5 | 0.001** | 7.16 ± 2.07 | 0.0001** |
| | 21-30 | 151 | 35.5 ± 9.5 | | 6.79 ± 1.91 | |
| | 31-40 | 80 | 36.6 ± 8.5 | | 6.67 ± 1.67 | |
| | 41-50 | 110 | 36.7 ± 9.1 | | 6.80 ± 1.89 | |
| | 51≤ | 161 | **32.4 ± 8.6** | | **5.65 ± 1.68** | |
| | **Total** | 566 | 35.0 ± 9.2 | | 6.49 ± 1.90 | |

* Statistically significant differences ($p$< 0.05), *sd* standard deviation, *n* number of TMJs ,by Student's *t*-test
**Statistically significant differences (p<0.05), sd standard deviation, n number of TMJs, by one-way ANOVA

The most common AE shape was the box-shape (223 cases; 39.4%), followed by sigmoid (191 cases; 33.7%), flattened (111 cases; 19.6%), and deformed (41 cases; 7.2%). Concerning the variations in the shape of the AE, there was a significant difference between sexes ($p$>0.05). Box-shaped eminences were observed most frequently in males; sigmoid-shaped eminences were observed most frequently in females. The prevalence of AE morphology according to sex and age groups are shown in Table 3.

**Table 3:** Distribution of the articular eminence morphology with sex and age groups

| **Articular Eminence morphology** | **Sex** | | | **Age Groups** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | Total | ≤20 | 21-30 | 31-40 | 41-50 | 51≤ | Total |
| | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) |
| Box | 83 | **140** | 223 | 27 | 68 | 24 | 40 | 64 | 223 |
| | (34.9) | **(42.7)** | (39.4) | (42.2) | (45.0) | (30.0) | (36.4) | (39.8) | (39.4) |
| Sigmoid | **101** | 90 | 191 | 19 | 45 | 37 | 44 | 46 | 191 |
| | **(42.4)** | (27.4) | (33.7) | (29.7) | (29.8) | (46.3) | (40.0) | (28.6) | (33.7) |
| Flattened | 32 | 79 | 111 | 13 | 29 | 16 | 20 | 33 | 111 |
| | (13.4) | (24.1) | (19.6) | (20.3) | (19.2) | (20.0) | (18.2) | (20.5) | (19.6) |
| Deformed | 22 | 19 | 41 | 5 | 9 | 3 | 6 | 18 | 41 |
| | (9.2) | (5.8) | (7.2) | (7.8) | (6.0) | (3.8) | (5.5) | (11.2) | (7.2) |
| **Total** | 238 | 328 | 566 | 64 | 151 | 80 | 110 | 161 | 566 |
| | (100.0) | (100.0) | (100.0) | (100.0) | (100.0) | (100.0) | (100.0) | (100.0) | (100.0) |
| ***p* value** | | 0.0001* | | | | 0.168 | | | |

* Statistically significant differences (p< 0.05), n number of TMJs, by the Chi-square test

No association was found between the AE shape and age groups. ($p$>0.05). Additionally, the metric variables of the AE were significantly higher in sigmoid and box-shaped groups compared to other groups ($p$ = 0.0001) (Table 4).

**Table 4:** The mean eminence inclination and eminence height values according to articular eminence morphology

| | | **n** | **Eminence inclination mean ± sd** | ***p* value** | **Eminence height mean ± sd** | ***p* value** |
|---|---|---|---|---|---|---|
| **Articular Eminence morphology** | Box | 191 | **41.3 ± 7.0** | 0.0001* | **7.67 ± 1.57** | 0.0001* |
| | Sigmoid | 223 | **34.2 ± 7.3** | | **6.17 ± 1.65** | |
| | Flattened | 111 | 27.4 ± 8.4 | | 5.33 ± 1.87 | |
| | Deformed | 41 | 30.8 ± 9.2 | | 5.93 ± 1.83 | |
| | **Total** | 566 | 35.0 ± 9.2 | | 6.49 ± 1.90 | |

* Statistically significant differences (p<0.0001), sd standard deviation, n number of TMJs, by one-way ANOVA

The mean values of eminence inclination and height were analyzed, and the results are presented according to presence and types of the degenerative changes (Table 5).

**Table 5:** The eminence inclination and eminence height values according to presence and types of degenerative condylar changes

| | | **n** | **Eminence inclination mean ± sd** | ***p* value** | **Eminence height mean ± sd** | ***p* value** |
|---|---|---|---|---|---|---|
| **Degenerative Condylar Changes** | *Without* | 86 | 36.9 ± 8.7 | | **6.90 ± 1.71** | 0.049* |
| | *With* | 480 | 34.7 ± 9.2 | 0.073 | 6.42 ± 1.93 | |
| | **Total** | 566 | 35.0 ± 9.2 | | 6.49 ± 1.90 | |
| **Types of Degenerative Condylar Changes** | Flattening | 213 | 36.0 ± 9.2 | 0.015** | 6.60 ± 1.90 | 0.089 |
| | Sclerosis | 10 | **38.6 ± 9.0** | | 7.07 ± 2.0 | |
| | Erosion | 25 | 35.0 ± 11.6 | | 6.41 ± 2.21 | |
| | Osteophytes | 9 | 35.0 ± 4.7 | | 7.10 ± 1.82 | |
| | Combinations | 223 | **33.2 ± 8.9** | | 6.20 ± 1.91 | |
| | **Total** | 480 | 34.7 ± 9.2 | | 6.42 ± 1.93 | |

* Statistically significant differences (p<0.05), sd standard deviation, n number of TMJs, by the Mann-Whitney U test
** Statistically significant differences (p<0.05), sd standard deviation, n number of TMJs, by the Kruskall-Wallis H test

No significant differences were observed between the eminence inclination and condyles either with bone change or without ($p$>0.05). Considering the eminence height values, there was a significant difference according to the presence or absence of degenerative alterations ($p$=0.049), which showed higher values in condyles *without* change group (Table 5). The mean values of eminence

inclination were significantly higher in the sclerosis-type degenerative change group compared to other groups and were significantly lower in the combination-type group compared to the flattening, erosion and osteophyte groups ($p$<0.05). There was no statistical relationship between the eminence height and types of degenerative condylar change.

In terms of the AE morphology, sigmoid and box shapes were the most common forms, although no statistically significant difference was found between both groups ($p$ > 0.05) (Table 6). Additionally, the combination of two or more degenerative alterations in the condyle was associated with the deformed form, but flattening was observed most frequently in the box-shaped and sigmoid forms (Table 7).

**Table 6:** Distribution of the articular eminence shapes on with and without degenerative changes groups.

| Articular Eminence morphology | *Presence of Degenerative condylar changes* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Without | | With | | Total | | | |
| | n | % | n | % | n | % | $\chi^2$ | *p* value |
| Box | 30 | 15.7 | 161 | 84.3 | 191 | 100.0 | | |
| Sigmoid | **47** | 21.1 | **176** | 78.9 | 223 | 100.0 | | |
| Flattened | 9 | 8.1 | 102 | 91.9 | 111 | 100.0 | 17,6 | 0.0001* |
| Deformed | 0 | 0.0 | 41 | 100.0 | 41 | 100.0 | | |
| **Total** | 86 | 15.2 | 480 | 84.8 | 566 | 100.0 | | |

* Statistically significant differences (p<0.0001), n number of TMJs, by the Chi square test

**Table 7:** Distribution of the AE morphology according to types of degenerative change present in the condyles

| Articular Eminence morphology | *Types of Degenerative Bone Changes* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Flattening | Sclerosis | Erosion | Osteophytes | Combinations | Total | *p* value |
| | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | |
| Box | 78 (36.6) | 7 (70.0) | 9 (36.0) | 2 (22.2) | 65 (29.1) | 161 (33.5) | 0.0001* |
| Sigmoid | 86 (40.4) | 2 (20.0) | 12 (48.0) | 4 (44.4) | 72 (32.3) | 176 (36.7) | |
| Flattened | 44 (20.7) | 0 (0.0) | 4 (16.0) | 1 (11.1) | 53 (23.8) | 102 (21.3) | |
| Deformed | 5 (2.3) | 1 (10.0) | 0 (0.0) | 2 (22.2) | 33 (14.8) | 41 (8.5) | |
| **Total** | 213 (100.0) | 10 (100.0) | 25 (100.0) | 9 (100.0) | 223 (100.0) | 480 (100.0) | |

* Statistically significant differences ($p$< 0.0001), $n$ number of TMJs, by the Chi square test with Monte Carlo simulation

## DISCUSSION

In the literature, the two main ways to measure the AE inclination are defined as follows: the *best-fit line method*, which involves adjusting a line drawn to the posterior slope of the eminence, and the *top-roof line method*, which involves connecting the highest point of the glenoid fossa and the lowest point of the eminence. The angle is established by measurement between the selected line and a horizontal reference plane. Both techniques have been used in various studies and result in similar values for AE angulation.[18-20] It has been suggested that the top-roof line method considers the position of the eminence crest relative to the glenoid fossa roof, whereas the other method considers the posterior surface of the AE. Thus, the actual condylar path can be determined by the best-fit line method, while the top-roof line figures out the morphology of the eminence better. The current study aimed to analyze the TMJ morphology, so the top-roof line technique was preferred.[21]

The inclination of the AE is described as the angle between the posterior wall of the eminence and a horizontal reference plane such as the Frankfort horizontal plane, occlusal plane or palatal plane.[22] The Frankfort horizontal plane has been commonly preferred in the previous studies.[12,18,21] In this study, the evaluations were made to refer to the palatal plane, since some of the FOV sizes of images did not cover the anterior part of the orbital floor. Also, the PP is a useful indicator for image orientation in the axial and sagittal slices.[22]

It is important to use standardized protocols that can make accurate and reliable measurements in the best representation of the evaluated structure. Sülün *et al*.[12] (2001) and Ren *et al*.[23] (1995) performed the measurements of the AE on central, lateral, and medial slices. The central sagittal section of the condylar process is the steepest part of the AE, and therefore the most appropriate slice for analyzing. Several studies used this section for obtaining accurate results.[18,21,22] In our analysis, measurements were made on a single parasagittal section.

The TMJ allows a large range of mandibular movements and exposes the functional loads from different types of activities, and provides the transmission of forces and loads to the cranial base.[24] The AE is an important structure in the biomechanics of the TMJ and consists of thick and dense bone, which is suitable for mechanical forces and loads.[1] The relationship between the AE inclination and several factors such as TMD or internal derangement[12,17,25], sex[19,20], age[19,22], malocclusion[6], and changes in dentition[24] has been evaluated in previous studies. However, there is limited data related to degenerative condylar changes and the eminence inclination.

In a study with dry human skulls, Pirttiniemi *et al*.[27] (1990) confirmed a functional dependence relationship between the mandibular condyle and articular eminence. In an animal experiment with mouse models, it was suggested that glenoid fossa growth was initiated, but that the continuous development of this structure could not be sustained in the case of absence or dislocation of the condyle.[28]

In the literature, the AE inclination and height have been assessed according to the presence of osteoarthritic changes. Some investigators reported that AE inclination in condyles with osteoarthritic changes was significantly lower than without changes. Sa *et al*.[22] (2017) mentioned that condylar changes did not affect the value of eminence inclination, but an average reduction in eminence inclination was detected when combination-type degeneration was present. Similarly, we could not find any significant difference between the AE inclination and presence of condylar change. However, steeper inclination was observed in the sclerosis-type condylar change group than in other groups. In the combination-type (two or more condylar changes) group, more shallow inclination was detected, a result that may be due to the more serious effects of two or more bone changes.

In this study, the mean eminence height value was lower in individuals with degenerative condylar change ($p<0.05$). The eminence height in cases with osteophytes had higher values, while in the combination-type group, a reduced eminence height value was detected. However, no significant difference was found when all types were evaluated together ($p=0.089$).

Previous studies evaluated the influencing factors for stress distribution in the condylar region and concluded that morphological alterations in the head of the condyle may change the mechanical loading in the roof of the fossa.[19] The same relation could be established between the AE and mandibular condyle. In addition to this, Lee *et al*.[29] (2019) hypothesized that osteoarthritic changes can develop in the articular eminence after condylar changes when osteoarthritic alterations are more advanced. The greater values in individuals with sclerotic and osteophytic changes in the articular surface of the condyle may be related to mechanical stimulation. Sclerosis and osteophytes are advanced stages of degenerative changes, reflecting the body's adaptation to repair the TMJ. These degenerative alterations can increase the bone thickness in the articular eminence, as well as change in the stress distribution. Following this, when the combination of two or more changes occurs in the mandibular condyle, adaptation capacity may be insufficient and morphologic alteration in the AE can be detected.

The comparison between the eminence shapes revealed no significant differences in the presence of degenerative changes ($p>0.05$). Nevertheless, eminence forms were mainly related to the types of alterations in the condyle. In agreement with this study, Kurita *et al*.[17] (2000) stated that greater eminence height and inclination

values were related to box-shaped eminences and lower values were related to flattened eminences.

It should be noted that the architectural features of the AE and mandibular condyle are different. The eminence has thick cortices with transversely oriented trabeculae, while the mandibular condyle has vertically oriented fine bony trabeculae. Therefore, these two structures of the TMJ may be affected by the same movements and muscle activities differently.[30]

In the literature, some of the studies associated the articular eminence morphology and internal derangements of the TMJ. Several authors have reported the eminence as a predisposing factor for internal derangement.[12,25] In contrast, Ren *et al*.[23] (1995) concluded that a steeper eminence was detected in symptom-free individuals than in patients with internal derangement. It is also suggested that condylar bone change is more related to the eminence inclination than to the disc displacement condition. The more advanced the disc displacement present, the more frequent bone changes become. Kurita *et al*.[17] (2000) observed flattened eminence in TMJs with disc displacement. Nevertheless, whether a greater eminence could be an effect of internal disorders or whether flattened eminence could be a result is still controversial.

It has been reported that morphologic changes can occur in the AE with advanced age, which results from flattening of the eminence in the long term.[12,17] When analyzing the eminence inclination and height in different age groups, we verified that both mean eminence inclination and height significantly decreased in cases aged over 50 years. In contrast, some authors found no correlation between advanced age and eminence morphometry.[8,19,20]

In the present study, the frequency of degenerative alterations was higher in males than in females, but no significant difference was detected. We suggested that the finding could be due to sex differences in willingness to seek help. The rate of seeking treatment may be lower in men than women, and men may only refer to the hospital at an advanced stage of the disease. These results can be attributed to the fact that the study was designed with randomized subjects at a time interval. Furthermore, the analysis of sex differences in degenerative change frequency resulted in a borderline *p*-value ($p$=0.053, small effect size=0.0814). However, this difference was limited due to the low number of subjects in the study groups.

The relationship between sex and morphometric measurements of the AE has been evaluated in earlier research studies. Some of these stated a relationship between the eminence inclination and sex[20,23], while others did not.[4,19] Authors who suggested that the inclination changed with sex revealed that males presented higher inclination values. Many studies affirmed a higher eminence height in males compared with females.[4,20,21] These results agree with those of our study. For the AE morphometry, sex had a statistically significant influence, the mean results being higher for males ($p$<0.05). The shapes of condylar pathways were also significantly different between females and males. The box shape was significantly more common in males, while in females the most prevalent eminence shape was detected as sigmoid-type. The box shape represents a larger articular eminence or a deeper articular fossa than sigmoid shapes, and the box-shaped eminence presents high AE inclination and height values. The greater results and morphology in males may be relevant to the relatively larger cranio-caudal sizes in males.

The discrepancy between results may be caused by racial/ethnic diversity of populations or methodological differences in the studies, such as the diagnostic criteria and techniques used, sample size, measurement methods, and age range. Furthermore, decreased adaptive capacity of the articular elements or excessive or sustained loading in the TMJ are predisposing factors in the development of disorders.[31,32] Even if the biomechanical behaviors are within physiological ranges, ageing, systemic disorders and hormonal changes can affect the remodeling of the TMJ. Mechanical factors, including parafunction, trauma, unstable occlusion, and functional

overloading affect TMJ internal derangement and osteoarthrosis. These factors can exist alone or be interrelated, interdependent, and/or coexistent.[31-33]

The present study is not free of limitations. Firstly, the soft tissue component of the TMJ, which can play a role in the articular eminence morphology, was not evaluated. Secondly, due to the observational design of the study, the long-term relationship between articular eminence morphology and degenerative changes of the condylar articular surface was not analyzed directly. In addition, this observational design limits the degree of cause-and-effect relationships. Further longitudinal and stratified research with larger sample sizes is necessary to resolve this issue.

## CONCLUSIONS

The presence of two or more degenerative changes in the mandibular condyle resulted in reduced eminence inclination and height and it was more prominent in the deformed eminence shape. The AE inclination and height were influenced by age and sex. It is believed that further studies on this subject will provide a better understanding of the relationships and more definitive conclusions.

## ACKNOWLEDGEMENTS

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## INFORMED CONSENT

For this type of study, formal consent is not required.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## FINANCIAL DISCLOSURE

The authors declared that this study received no financial support.

***Mandibular Kondildeki Dejeneratif Değişiklikler ile Artiküler Eminensin Eğimi, Yüksekliği ve Şekli Arasındaki İlişki: Bir KIBT Çalışması***

***ÖZ***

***Amaç:*** *Bu çalışma, konik ışınlı bilgisayarlı tomografi (KIBT) kullanılarak artiküler eminens (AE) eğim ve yüksekliğini analiz etmeyi, elde edilen sonuçları eminens şekilleri ve dejeneratif kondiler değişiklikler ile ilişkilendirmeyi amaçlamaktadır.* ***Gereç ve Yöntemler:*** *Toplam 566 temporomandibular eklemin (TME) KIBT görüntüleri değerlendirildi. Tüm bireylerin yaşları ve cinsiyetleri kaydedildi. Kondil yüzeyindeki dejeneratif değişiklikler incelendi. Artiküler eminens eğim ve yükseklik ölçümleri TME'nin santral parasagital kesitleri üzerinde yapıldı. AE'nin şekli kutu, sigmoid, düz ve deforme olarak sınıflandırıldı.* ***Bulgular:*** *Kondildeki dejeneratif değişikliklerin prevalansı erkeklerde daha fazlaydı, ancak cinsiyet ile kondilin dejeneratif değişiklikleri arasında anlamlı bir fark bulunamadı (p>0,05). AE eğim ve yükseklik ortalamaları erkeklerde daha fazlaydı (p<0,05). Elli yıl üzeri yaş grubunda diğer yaş gruplarına göre eminens eğim ve yüksekliğinin ortalama değerlerinin azalmış olduğu tespit edildi (p<0,05). Sigmoid ve kutu şekilli artiküler eminens morfolojileri diğerlerine göre daha yaygındı. Deforme eminens şekilli grupta kombinasyon tip kondiler dejeneratif değişiklikler daha fazla bulundu.* ***Sonuç:*** *Kondildeki dejeneratif değişikliklerin varlığı ve tipleri ile eminensin ortalama eğim ve yükseklik sonuçları arasında anlamlı farklılıklar tespit edilmiştir. AE morfolojisi cinsiyet ve yaşa göre istatistiksel olarak anlamlı seviyede değişmektedir.* ***Anahtar Kelimeler:*** *Artiküler eminens, konik ışınlı bilgisayarlı tomografi, dejeneratif değişiklik, mandibular kondil, temporomandibular eklem.*

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**Original research**

# THE EFFECT OF MENOPAUSE ON NADPH OXIDASE LEVELS AFTER NON-SURGICAL PERIODONTAL TREATMENTS ON PATIENTS WITH PERIODONTITIS

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## ABSTRACT

**Objectives:** This study evaluated the clinical parameters [plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL)] and the levels of Nadph Oxidase (NOX-1) in gingival crevicular fluid (GCF) samples of pre-menopausal and post-menopausal periodontally healthy and periodontitis patients.

**Materials and Methods:** Study included pre-menopausal periodontitis 15, post-menopausal periodontitis 15, pre-menopausal periodontally healthy 15 and post-menopausal periodontally healthy 15; a total of 60 individuals were included.

**Results:** Clinical periodontal evaluation indices in the 6th week after treatment were found to be statistically significantly lower than the initial values of PI, GI, PPD, CAL in the periodontitis groups($p<0.05$). Pre-treatment baseline NOX-1 values were singificantly higher in both periodontitis groups than healthy groups ($p<0.05$). While the pre-menopausal and post-menopausal periodontitis groups before treatment had similar PI, PPD and CAL values, the GI was found to be significantly higher in the post-menopausal periodontitis group($p<0.05$). While NOX-1 values in pre-treatment GCF samples were similar in pre-menopausal and post-menopausal periodontitis groups, NOX-1 values in the post-menopausal periodontitis group at the 6th week after treatment were found to be statistically higher than the pre-menopausal periodontitis group ($p<0.05$).

**Conclusions:** According to the results of our study, oxidative stress that increases with menopause may negatively affect the healing potential after periodontal treatment. Accordingly, antioxidant supplementation can be predicted with hormone replacement during this period.

**Keywords:** Periodontitis, Nox-1, Menopause, Oxidative Stress.

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**INTRODUCTION**

Periodontal disease is a chronic inflammatory disease occurring as a result of the complex interactions between the periodontal pathogen bacteria and host immune response, characterized by periodontal tissue destruction and tissue loss, in which environmental and genetic factors play a role in its etiopathogenesis and progress.[1]

Although the primary etiological agent in periodontal diseases is gram-negative anaerobic or facultative bacteria in microbial dental plaque, abnormal contact responses formed against such organisms and products cause periodontal tissue destruction.[2]

It is reported that the chronic inflammatory state is inducted and inflammation becomes intensified due to the joining of some cytokine and hormones secreted systematically, bacteria and bacterial products to the blood circulation.[3] It was determined that there was a rise in saliva, GCF and inflammatory mediators in serum due to the periodontal destruction in individuals with periodontitis.[4]

Environmental, genetic and acquired factors may change the host response that forms against pathogen and modify the amount of destruction in periodontal tissues.[5]

There are two mechanisms responsible for periodontal tissue destruction. The first mechanism is the direct effect of the bacteria through the toxic materials that it produced, proteases and endotoxins; the second mechanism is the indirect effect mediated the inflammatory mediators produced by the host.[6] The difference of inflammatory response for every individual causes difference in the rate of progression and severity of the destruction.[4]

It is shown that oxidative stress plays a major role in many diseases such as obesity, periodontitis, rheumatoid arthritis, diabetes mellitus, cardiovascular diseases, hemolytic anemia, systemic lupus erythematosus, multiple sclerosis, Behcet disease and Guillain-Barre syndrome.[7-13]

NOX enzymes are proteins linked to the membrane, and their main function is transferring electrons from the plasma membrane to molecular oxygen. $O_2$- is formed by the transfer of the electrons to molecular oxygen. It causes the formation of $O_2$-, $H_2O_2$, and OH radicals and leads to reactive oxygen species (ROS).[14,15]

The principal center of the ROS formation in the cell occurs in the plasma membrane and mitochondrion. Cytokines activate NOX through growth factors and hormones. The activated NOX causes $O_2$- production. One to two percent of the electrons transferred in a manner to follow each other in the electron transfer chain is directly transferred to molecular oxygen through leakage, resulting in the production of $O_2$- radical.[16]

It is determined that NOX enzymes are essential in the immune system, protein translation, cellular signal transmission and gene expression, furthermore in the modulation of the redox-sensitive signal pathway related to functions such as growth, differentiation, migration and proliferation of cells.[15]

The protein regulating NOX enzyme activity comprises two sub-units and four cytosolic proteins bound to the membrane. Two cytochrome heterodimer components (gp91phox and p22phox) bound to membrane and four cytosolic components (p47phox, p67phox, p40phox) and RAC2 activate NOX to produce $H_2O_2$. Activation starts with combining the membrane-located gp91phox and p22phox of NOX at the rate of 1:1, and cytochrome b588 occurs. This activation combines the cytosolic components of the NOX enzyme (p47, p67, p40) with cytochrome b558 and the joining of GTP- protein to this complex as an activator. Lack of any of the four components of NOX (gp91,p22, p47, p67) and GTP binding protein (Rac2) terminates NOX activity.[17]

Some NOX enzymes are located higher in some organs and tissues. For example, NOX-1 is present in the colon, NOX-2 is present in phagocytes, NOX-3 is present in the inner ear, NOX-4 is present in the kidney, NOX-5 is present in testicles lymphoid tissues, and DUOX-1 and DUOX-2 are present in thyroid.[18]

NOX-1 is expressed in endothelial and smooth muscle and cells. Many studies showed that NOX-1 is localized in cell membranes, especially in the plasma membrane.[15] NOX-1 activity requires its sub-units being p22phox, NoxO1 (or p47phox), NoxA1 and GTPase Rac. It is shown that NOX-1 dependent ROS production has a significant role in cell signaling, cell growth, angiogenesis and cell mobility.[19]

NOX-1 mediates vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMPS) expressions; it can increase ROS formation and support NOX-1 angiogenesis and tumorigenesis.[20] Furthermore NOX-1 plays a role in cell proliferation, emerging and development of angiogenesis and hepatic fibrosis.[21]

It is shown that Nox-1 / Nox-2 plays a role in developing endothelial dysfunction, hypertension, and inflammation.[22,23] The pro-atherogenic roles of NOX-1, NOX-2 have emerged, and it was determined that the most significant sub-unit is p47phox.[24]

Neutrophils and monocytes show antibacterial features with cooperating enzymatic (non-dependent to oxygen) and non-enzymatic (oxygen-dependent) mechanisms in periodontal defense mechanisms. Oxygen-dependent, i.e., Non-enzymatic mechanism, is related to non-mitochondrial oxidative burst and increased ROS production; and causes tissue damage in host besides its antimicrobial effect against pathogens. The normally inactive NOX enzyme in the plasma membrane of neutrophils is activated by this mechanism and forms $O_2$- and is used against bacteria in the phagosome. The formed $O_2$- forms $H_2O_2$ is automatically dismutated at a low pH level in the phagosome. This phenomenon occurs as intense radical accumulation within phagosome is named as "respiratory burst" or "oxidative burst." This process is detrimental to host cells besides its antimicrobial contribution.[25]

Various pro-inflammatory cytokines (TNF-α, IL-8, IL-1, IL-6), growth factors and lipopolysaccharides exhibit triggering features for the respiratory burst of neutrophils. ROS, which is formed through these interactions, leads to proteoglycans in periodontal tissues, lysis in epithelial cells, osteoclast activation, carbohydrate, lipid and protein catabolism and causes periodontal destruction.[26]

Tissue destruction occurs through the stimulation of cytokines through NF-κB activation; release of pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and prostaglandin E2 generation through lipid peroxidation and $O_2$ -. Furthermore, ROS activity plays a role in an inflammatory state caused by periodontal diseases on other organs at the systemic level.[27]

According to the World Health Organization (WHO) definition, menopause is the permanent termination of menstruation as a result of the loss of ovary activity.[28]

It is stated that changes in female gender hormones may affect the severity of periodontal diseases.[29] A decrease in the amount of estrogen occurring by menopause causes osteoporosis systematically and in alveolar bone, increase in the CAL, and increase in the inflammation in gingival tissues.[30]

Besides the systematic changes in menopause, also intra-oral changes may occur. The flow rate of the saliva that secreted by submandibular and sublingual salivary glands, decreased. The incidence of decay and formation of periodontitis increase due to the decrease in the number of salivary glands. Deterioration of flavor, senile atrophic gingivitis, menopausal gingivostomatitis can also be seen in the menopause period.[31] Changes such as puberta gingivitis, pregnancy gingivitis are seen in situations such as puberta and pregnancy in which female gender hormones change, and it is mentioned that periodontal situation is frequently affected negatively.[29] It is stated that gingivitis in periods such as pregnancy and puberta may be due to vasodilatation, increase in vascular permeability and increase in mast cells around vessels caused by estrogen on vascular system.[32]

Estrogen decreases the formation of inflammatory cytokines essential in osteoclastic activation, and the lack of estrogen causes a more

gingival severe inflammation and osteonecrosis during periodontitis.[30]

Furthermorethe osteoporosis table, alveolar osteonecrosis occurs systemically in the menopause period. Osteonecrosis is seen most dense in the first year following menopause and increases its rate in a subsequent process. The history of osteoporosis in the family, premature menopause, lacking physical activity, vitamin D and calcium deficiency, old age, regular smoking and drinking alcohol may increase the risk of osteoporosis.[33,34]

Oral atrophic mucosa is frequently seen in females in menopause; burning mouth syndrome can also be seen as a more severe symptom. Burning mouth syndrome is characterized by automatically starting burning and pain.[35] Tongue, lips, palate, tooth-supporting tissues and gingiva are also affected. Dense burning and pain are present, although no underlying pathological lesion is seen. Moreover, bad taste, desert mouth, difficulty in swallowing may also be experienced. Estrogen supplement psychological support and use of tricyclic antidepressant are present in its treatment.[36]

No study concerning how menopause affected biochemical marker levels related to NOX-1 among oxidative stress enzymes in females with periodontitis has been seen in the literature search. Therefore we aimed to review the relationship between the NOX-1 enzyme levels in females in the premenopausal and post-menopausal period and some clinical periodontal parameters showing the periodontal health conditions.

## MATERIALS AND METHODS

Approval was obtained from Cumhuriyet University, School of Medicine Researches Ethical Committee on 17.12.2019 with decision no. 2019-12/05 for the study. Systemically healthy 30 individuals with periodontitis and 30 periodontally healthy individuals admitting to Cumhuriyet University Faculty of Dentistry Department of Periodontology Clinic between 2019-2020 participated in our research. The patients signed a voluntary consent form showing that they participated in the study voluntarily. The current classification, named as 2017 Classification of Periodontal and Peri-implant Diseases and Conditions, was used to describe the study groups to be studied in the research.

### Study Groups

**GROUP PRP:** Pre-menopause periodontitis patients who did not enter into menopause (15 individuals)

**GROUP POP:** Post-menopause periodontitis patients who entered into menopause (15 individuals)

**GROUP PRH:** Pre-menopause periodontally healthy individuals who did not enter into menopause (15 individuals)

**GROUP POH:** Post-menopause periodontally healthy patients who entered into menopause (15 individuals)

### GROUP PRP

Periodontitis patients who stated that they did not enter into menopause in their verbal medical history, with probing depth (PPD) of ≥4 and <7 at least at 2 non-adjacent teeth, with bleeding in probing, inflammation symptoms, bone loss extending to the middle of the root or apical trio.

Stage III periodontitis, degree B.

### Group POP

Periodontitis patients stating in the verbal medical history taken that a time longer than 1 year has passed as of the starting of menopause, PPD of ≥4 and <7 at least at 2 non-adjacent teeth, with bleeding in probing, inflammation symptoms, bone loss extending to the middle of the root or apical trio.

Stage III periodontitis, degree B.

### Group PRH

Periodontitis patients who stated that they did not enter into menopause in their verbal medical history; who are periodontally healthy with the whole mouth bleeding score below 10%, with an PPD not exceeding 3 mm in teeth, with Decreased or Non-Deteriorated Periodontal Health.

**Group POH**

Individuals stating in the verbal medical history taken that a time longer than 1 year has passed as of the onset of menopause, who are periodontally healthy with the whole mouth bleeding score below 10%, with an PPD not exceeding 3 mm in teeth, with Decreased or Non-Deteriorated Periodontal Health.

In periodontitis patient groups 2 non-adjacent teeth with PPD ≥4, 33% periodontal bone loss was determined by sampling GCF before the initial periodontal treatment. The GCF samples were taken from any 2 teeth among the maxilla incisor teeth on each half jaw in periodontally healthy individuals.

**Clinical Evaluation**

A single researcher (M.K) carried out clinical evaluation indices, GI, PI, PPD and CAL analyses in patients were carried out by a single researcher (M.K) throughout the research.

Periodontal clinical treatment including PPD, CAL, PI and GI measurements in periodontitis patients was carried out initially and in the 6th week.

The initial periodontal treatment, including subgingival curettage and root planning processes by Gracey curettes (Hu-Friedly, Chicago, IL, USA) following oral hygiene training and scaling and periodontal treatment of each patient was completed within 14 days.

All clinical periodontal measurements and GCF samples were taken from the individuals in periodontitis patient groups 2 times in total (before periodontal treatment, 6th week) throughout the study; they were taken only once from the individuals in the control group.(Figure 1)

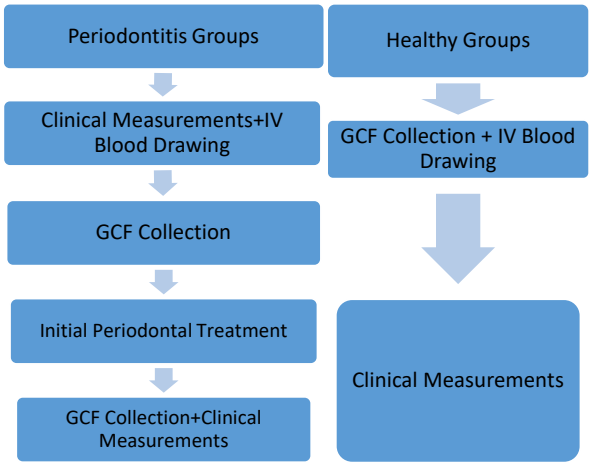


**Figure 1:** Study Plan Work Flow Chart

**Collection of Samples**

The patients were asked whether they entered menopause or not, and the information on the onset of menopause in the patients stating that they entered menopause was obtained. Approximately 15 ml venous blood sample was taken from patients in order to establish a final diagnosis**.** The samples were centrifuged for 10 minutes at 15000 x g speed to decompose serum, and then the serum was decomposed to eppendorf tubes. Serum samples were kept at - 80 degrees until the day of analysis. The measurements were in the central laboratory of Cumhuriyet University School of Medicine Biochemical Department on the day of analysis. Two patients stated that they entered menopause in their medical histories, with FSH hormone levels at 40 -60 IU/L and E2 levels at 20-22 µg/ml were removed from postmenopausal patient groups as they might be in the perimenopausal period. 5 individuals among the patients stating that they did not enter menopause were removed from the study group because they might be in the perimenopausal period as their serum E2 levels were at 20 - 22 µg/ml and their FSH levels were at 40-60 IU/L.

The GCF samples were taken from periodontitis patients a week following their clinical measurements and in the morning (09:00-11:00) in periodontally healthy patients using Periopaper (OraFlow Inc, Amityville, NY, USA). The PPD 4-6 mm-areas were preferred at the anterior maxilla area to mitigate the risk of slobbering in the GCF collection. Periopapers were intrasulcularly positioned in the gingival crevice. It was held for 30 seconds in the crevice

to establish standardization in each sample, and GCF was retaken in periopapers contaminated by blood. Periopapers were put into eppendorf tubes and were held at -80 °C until the day of analysis.

**Laboratory Studies**

The GCF samples collected were used by NOX-1 ELISA kits as per the suggestions of the manufacturer company (Fine Test, Wuhan, China) in Cumhuriyet University School of Medicine Biochemical Department.

**Statistical Analysis of Data**

Descriptive statistics, regular distribution analysis (Kolmogorov-Simirnov), Dependent groups t-test, Independent groups t-test, Pearson correlation analysis were conducted using IBM SPSS 22.0 suit.

**RESULTS**

15 pre-menopause periodontitis patients (Group PRP), periodontally healthy 15 pre-menopause patients (Group PRH), 15 post-menopause periodontitis patients (Group POP), periodontally healthy 15 post-menopause patients (Group POH) were included in the study (Table 1).

**Table 1:** Demographic situations of the individuals participating in the study

| **Patients Groups** | GRUP PRP n:12 | GRUP PRH n:13 | GRUP POP n:14 | GRUP POH n:14 |
|---|---|---|---|---|
| Age | 43.30 (±2.73) | 42.43 (±2.30) | 48.80 (±1.26) | 49.88 (±1.37) |
| Age intervals | 40-49 | 40-48 | 48-52 | 48-53 |
| Menopause(years ago) | - | - | -2.72 | -2.85 |

**Clinical Findings**

The difference between the pre-treatment PI, PPD and CAL values PRP and POP groups was not statistically significant ($p>0.05$). However, GI values are higher in a POP group than PRP group, and the difference was statistically significant ($p<0.05$)

The difference in PRP and POP groups in PI, GI, PPD, CAL values in the post-treatment 6th-week results was not statistically significant ($p>0.05$).

Pre-treatment initial PI, GI, PPD values of PRP and PRH group were higher in the PRP group than PRH group, and the difference was statistically significant ($p<0.05$)

Pre-treatment initial PI, GI, PPD values of POP and POH group were higher in a POP group than POH group, and the difference was statistically significant ($p<0.05$).

Upon comparing the pre-treatment and post-treatment 6th-week results of PRP group patients PI, GI, PPD, CAL pre-treatment values were higher than the post-treatment 6th-week values, and the difference was found statistically significant ($p<0.05$).

Upon the comparison the pre-treatment and post-treatment 6th-week results of POP group patients PI, GI, PPD, CAL pre-treatment values were higher than post-treatment 6th-week values, and the difference was found statistically significant ($p<0.05$). (Table 2)

**Table 2:** Clinical and Laboratory Results

| Parameters | PRP | | POP | | PRH | POH |
|---|---|---|---|---|---|---|
| | Baseline | 6th Week | Baseline | 6th Week | Baseline | Baseline |
| **Plaque Index** | **1.91±0.05[b]** | **1.22±0.20[a]** | **1.96±0.11[c]** | **1.16±0.08[a]** | **0.81±0.09** | **0.86±0.11** |
| **Gingival Index** | **1.97±0.09[b]** | **1.11±0.11[a]** | **2.11±0.09[c,d]** | **1.12±0.08[a]** | **0.68±0.07** | **0.85±0.08** |
| **Probing Pocket Depth** | **4.69±0.41[b]** | **3.54±0.15[a]** | **4.83±0.28[c]** | **3.68±0.27[a]** | **2.36±0.13** | **2.60±0.08** |
| **Clinical Attachment Level** | **6.69±0.74[b]** | **5.28±0.57[a]** | **6.39±0.49[c]** | **5.18±0.43[a]** | **-0** | **-0** |
| **NOX-1** | **7.19±2.94[b]** | **1.89±1.58[a]** | **8.12±2.54[c]** | **3.78±2.24[a,d]** | **2.73±1.21** | **2.75±1.17** |

[a]significantly different from periodontitis groups baseline ($p<0.05$)
[b]significantly different from the PRHgroup.($p<0.05$)
[c] significantly different from the POH group.($p<0.05$)
[d] significantly different from the PRP group.($p<0.05$)

### Laboratory Findings

Upon comparing the periodontal pre-treatment and post-treatment NOX-1 values in PRP and POP groups, including periodontitis patients, pre-treatment initial values were higher than the 6th-week results, and the difference was statistically significant ($p<0.05$).

Upon comparing pre-treatment NOX-1 values of the PRP and POP groups, the initial NOX-1 values of the POP group were higher; however, the difference was not found statistically significant ($p>0.05$).

The NOX-1 values according to the post-treatment 6th-week results were found higher in POP group compared to PRP group and the difference was statistically significant ($p<0.05$).

The pre-treatment initial NOX-1 values of the PRP and PRH group were found higher in the PRP group than PRH group, and the difference was statistically significant ($p<0.05$)

The pre-treatment initial NOX-1 values of the POP and POH group were higher in the POP group than the POH group, and the difference was statistically significant ($p<0.05$).

The pre-treatment NOX-1 values of periodontitis patients correlated to the pre-treatment initial NOX-1 values PI, GI and PPD values in all PRP and POP group patients in the correlation analysis ($p<0.05$).

The PI was found correlated to the GI and PPD ($p<0.05$).

The GI was found correlated to PPD ($p<0.05$).

The NOX-1 values of the post-treatment 6th-week result in PRP and POP patient groups correlated to the 6th week PI and GI values ($p<0.05$).

The post-treatment 6th-week values correlated to the PRP and POP patient groups ($p<0.05$). (Table 2)

## DISCUSSION

It was stated that the analysis of the oxidative stress products was complex due to the short half-life of ($O_2$-,$H_2O_2$,OH-) them and their replacement with the oxidative stress enzymes in the pathogenesis of periodontal diseases advantageous method.[37] We preferred NOX-1 member of the NOX family among the oxidative stress mechanism enzymes, which plays a role in inflammation development.

In our study, the NOX-1 enzyme among the oxidative stress markers was found higher in both the pre-menopause periodontitis and post-menopause periodontitis groups than the periodontally healthy groups ($p<0.05$). When examined current literature there are a lot study proving that oxidative stress markers are higher in the patients with periodontitis than the periodontally healthy patients.[38–41] Our study

results comply with the studies showing that the oxidative stress products are higher in the patients with periodontitis than the periodontally healthy patients.[38–41]

Upon comparison of the pre-treatment and post-treatment 6th-week NOX-1 levels of our PRP and POP group patients among periodontitis group patients, it was seen that the post-treatment NOX-1 levels significantly decreased in both groups ($p<0.05$). Upon reviewing the literature, the clinical studies evaluating the pre-treatment and post-treatment period reveal that the initial periodontal treatment decreased the oxidative stress levels in periodontitis patients.[39,42,43] The decreasing oxidative stress level in the post-treatment periodontitis patients in our study complies with similar studies in the literature.[39,42,43]

Any studies using the NOX-1 enzyme could not be found among the studies in the literature showing the relationship between periodontitis and oxidative stress. Many studies worked NO, malondialdehyde (MDA), lipid peroxidation level, and total antioxidant capacity as oxidative stress markers. The oxidative stress markers evaluated in these studies were found higher in periodontitis groups than the healthy groups.[38,39,41]

Tsai *et al*.[39] found lipid peroxidation levels higher in the periodontitis group than the healthy group in the study conducted on the saliva and gingival crevicular fluid samples taken from periodontitis periodontally healthy patients. Furthermore, they found glutathione level, an antioxidant enzyme, lower in the group with periodontitis than in the healthy group. In Accordance with the healing of periodonal tissues, decreases in periodontal parameters (PI, GI, PPD, CAL) were determined. [39] The decrease in the oxidative stress level compared to the post-treatment initial values and the post-treatment improvement in the clinical parameters PI, PPD, GI, CAL values are similar to our results.

Batista *et al*.[44] reported a positive correlation between the PI, GI, and nitric oxide synthase (INOS) expression degree in the study conducted. As a result of these studies, it was concluded that INOS enzyme level might be a marker showing the periodontal disease activity [44]. In our study, pre-treatment and post-treatment NOX-1 levels were found correlated to PI and GI. Our study results have similarities with the study conducted by Batista *et al*.

There are various techniques for sampling GCF, which can be preferred following the result of the study, and each technique has its advantage and disadvantage. GCF sampling was conducted according to the shallow intracrevice method Rudin *et al*.[45] using standard paper strips to prevent irritation. The sampling time was determined as 30 seconds, as it is in many studies, to prevent the bleeding and irritation caused by the paper strips and provide a standardization.[46] GCF is among the most frequently used methods as it includes host-caused enzymes playing a role in the pathogenesis of periodontitis, tissue destruction products and inflammatory markers.[47]

There are various techniques in which the gingival crevice measurements are conducted 1 week before[48], and 2 weeks before GCF sampling[49] or after GCF sampling[49] with the concern that measuring periodontal crevice depth with periodontal drilling may cause irritation and change GCF amount. In our study, GCF sampling from the sampling area was conducted a week after periodontal drilling to minimize the irritation.

A typical ideal GCF collection time is not available in all studies. Sampling times were applied in various time ranges such as 3 sec., 5 sec., 15 sec., 20 sec., 25 sec., 30 sec., 1 min., 90 sec., 2 mins., and 3 mins in the literature. However, the general opinion is that extending the sampling time increases the mechanical irritation risk.[50] 30 seconds GCF collection method was preferred in our study.

Many clinical studies were conducted concerning periodontitis, menopause and osteoporosis. Menopause, postmenopausal hormone treatment, estrogen level and periodontitis relation, its impact on the osteoporosis and periodontium, changes in GCF

are the most studied subjects in studies concerning menopausal period.[51–53]

The periodontal effects of the hormones in females are tracked in periods such as pregnancy, puberta and menopause in which such changes are observed.[54] Periodontal tissues including receptors belonging to gender hormones.[55]

Studies show that the decreases in menopause and estrogen levels affect the host response and may increase the damage in the periodontal tissues by causing an increase in response to the local irritants.[56,57]

The studies indicate that the decrease in the postmenopausal estrogen levels may be bound to cytokines such as interleukin-1, IL-6, IL-8, IL-10, TNF-α. This increase activates the mature osteoclasts and causes systemic and alveolar bone loss by unbalancing bone formation and destruction.[58]

The decrease of estrogen level through the menopausal period increases the estrogen receptor activity in osteoclasts and decreases the receptor activity in osteoblasts.[55]

According to the results of our study, pre-treatment oxidative stress enzyme NOX-1 levels of the Group PRP and Group POP patients were not statistically different; their post-treatment 6th week NOX-1 levels were observed as statistically higher in Group POP than Group PRP ($p<0.05$). Those results suggest the effect of estrogen levels decreasing after menopause. It supports the results of the studies reporting that estrogen decreases the ROS, contributes to the antioxidant defense system, and compresses the release of cytokines.

Baltacıoğlu *et al*.[59] found that the patients serum and GCF total antioxidant capacity levels were higher in the pre-menopause group than in the post-menopause group in the studies they conducted on pre-menopause and post-menopause periodontitis patients and healthy patients. The total antioxidant capacity was found the most in the pre-menopause healthy group and the least in the post-menopause chronic periodontitis group.[59] When the impact of the antioxidant enzymes over the ROS is considered, the NOX-1 enzyme levels, which are significantly higher in the patients in the post-treatment postmenopausal period than in the pre-menopause group, can be related to the decrease of the protective role of the total antioxidant capacity, which decreases following menopause. Furthermore, the total antioxidant capacity found more in the healthy group by Baltacıoğlu *et al*. may explain that NOX-1 levels in the healthy groups, which we found in our study results, were significantly lower than the levels in the periodontitis groups.

PI, GI, PPD and CAL values of the group PRP and group POP patients in the 6th week after the treatment did not show statistical difference according to the results of our study ($p>0.05$). Upon reviewing the literature, it can be seen that studies are showing that the PI, GI and CAL values in the postmenopausal patients were higher compared to the premenopausal patients[30,52,60] or finding the values of the premenopausal and postmenopausal similar.[61–63]

It was shown that estrogens decreased ROS production and therefore prevented oxidative stress formation.[64] It is considered that the antioxidant contribution of estrogen arises from its stimulating effect on the natural antioxidant enzymes.[65]

## CONCLUSIONS

NOX-1 enzyme levels were significantly higher in the post-treatment 6th-week postmenopausal periodontitis group than in the premenopausal periodontitis group ($p<0.05$). We can say that the antioxidant role of the estrogen decreases, and the postmenopausal oxidative stress increases upon the decreasing estrogen following menopause.

No other study could be found concerning the evaluation of the effect of menopause on the pre- and post-periodontal treatment NOX-1 levels of the females who entered/did not enter into menopause. Concerning the matter, the clinical researches to be conducted with more participants in the periodontitis groups in which periodontitis is present in various severity may enable a better understanding of the effect of oxidative stress enzyme NOX-1 and menopause on the periodontitis pathogenesis.

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**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

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**Original research**

# EFFICACY, TOOTH SURFACE MICROHARDNESS AND ROUGHNESS AFTER TREATMENT WITH COMBINATION OF LASER AND HOME BLEACHING PROTOCOL: AN IN VITRO STUDY

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## ABSTRACT

Teeth whitening or bleaching has pleased people with aesthetic concerns for long. To improve the efficacy of bleaching and reduce the ill effects on the tooth surface, a combination of power bleaching (PB) and home bleaching (HB) can be used.

**Objective:** To compare the efficacy and effects of combinations of PB/laser bleaching (LB) and HB protocol on human natural tooth structure.

**Materials and methods:** Eighty-eight permanent maxillary central incisors were randomly divided into four groups (n=22). After staining, teeth were bleached using different bleaching protocols: Group 0 (control), Group 1 (Laser White 20 46% hydrogen peroxide (HP) + Opalescence PF 20% carbamide peroxide (CP) – HB sequence for 3 days), Group 2 (Laser White 20 46% HP + Opalescence PF 20% CP – HB sequence for 7 days), Group 3 (Opalescence PF 20% CP – HB sequence for 14 days). Colorimetric measurement was performed, enamel surface roughness and microhardness were measured. Scanning Electron microscope (SEM) evaluation was done to compare the surface topography. Pre and post bleaching data were analysed using paired *T*-test. Multiple comparison between groups was carried out using one-way ANOVA complemented by Post hoc test (Bonferroni), with significance level set at $P<0.05$.

**Results:** All protocols demonstrated significant efficacy to whiten stained enamel. All groups demonstrated significant increase in hardness ($P<0.05$). Surface roughness reduced significantly in Group 1 and 2. SEM showed that Group 1 had a similar microscopic surface appearance as unbleached enamel, while Group 3 had accentuated irregularities.

**Conclusions:** LB followed by HB for 3 days is the most effective in whitening stained teeth with positive effects on tooth surface hardness while maintaining surface topography of dental hard tissues.

**Keywords:** Home bleaching, Laser bleaching, Combination bleaching, Teeth whitening, tooth surface hardness.

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## INTRODUCTION

Vital tooth bleaching or whitening has gained popularity in recent years, to fulfil patient demands for health and aesthetics.[1] A study assessing the impact of teeth on personal aesthetic satisfaction found that dental variables (including tooth colour) were more important than orthodontic variables, suggesting that appearance of the teeth was a greater contributing factor to an aesthetic smile than their position within an arch.[2] Several methods have been described in the literature for improving dental aesthetics. However, the bleaching technique is widely used because of its ease, effectiveness[3], non-invasive nature and affordability for treating discoloured teeth when compared to veneers and crowns.[4,5]

There are three well-known techniques of vital tooth bleaching: professionally supervised nightguard (home) bleaching, in-office or PB and over the counter bleaching products.[6] A successful HB technique was first described by Klusmies in 1968 however, it received worldwide acceptance when it was described by Haywood and Heymann in 1989.[7-9]

In office bleaching involves the use of HP (30-35%)[10] as bleaching solution, with or without light activation to treat severely discoloured or difficult bleaching cases to produce immediate results.[11] A further modification to the PB system was the use of an argon laser and a variety of light sources.[10,12] PB was frequently combined with HB systems to maximize the bleaching effect and give a kick start to the whitening procedure before the patient continued with nightguard vital bleaching at home.[13] Generally, the efficacy of LB and HB are approximately the same when compared at providing effective tooth whitening.[4]

Several studies have been carried out to measure the microhardness of enamel and dentine after application of short regimes of 10-22% of carbamide peroxide. They have found that it does not significantly alter the hardness.[14-17] It was suggested that an increase in enamel erosion caused by bleaching treatment may facilitate fluoride penetration and consequently result in re-hardening the enamel after bleaching.[18,19]

Most studies concentrate on comparing the effectiveness of various bleaching agents, that is, either comparing their concentration or the method of practical application and duration of application to prove the most reliable product that can give the best aesthetic result with minimal side effects on the tooth.[20-23] However, the results are still inconclusive with regards to the efficacy and effects of treatment with various bleaching agents. To the best of our knowledge, there is no previous study that has compared the combination of LB and HB over a period of different days. Thus, to take advantages of the efficacy of home bleaching and reduce its ill-effects on the tooth surface, a combination of LB and HB can be used. Hence, the purpose of this study was to investigate and determine whether the combination of LB and HB treatment will give an optimal result while minimizing the ill-effects on dental hard tissues.

To the best of our knowledge, no study has been carried out to determine the effectiveness and effects on the enamel surface after laser and home bleaching combination treatment. Thus, this study will assess the efficacy of a combination of laser and home bleaching treatment and its effects on microhardness, roughness and morphological changes on the enamel surface. The results of this study will serve as a basis for determining whether the use of a combination of laser and home bleaching can help in achieving optimum bleaching efficacy while causing minimum ill-effects to the tooth structure.

The null hypotheses tested were, that there was no significant difference in colour changes of teeth, microhardness of teeth, enamel surface roughness, and enamel surface morphology after treatment with combinations of laser bleaching and different durations of home bleaching protocol.

## MATERIALS AND METHODS

Eighty-eight permanent maxillary central incisors were collected based on inclusion and exclusion criteria. Sound human permanent maxillary central incisors extracted due to periodontal problems and other reasons, were included, whereas a tooth that had caries, cracks, fracture, or restorations, were excluded from this study.

The teeth were kept in saturated thymol solution until preparation for testing. Human blood that was used for staining teeth was obtained from Hospital Universiti Sains Malaysia blood bank, which had been heparinized to avoid coagulation. The ethical approval for conducting this study was obtained from the Human Research Ethics Committee of Universiti Sains Malaysia (JEPem code: USM/JEPem/17050252).

**Specimen Preparation**

The collected teeth were cleaned with pumice and prophylaxis brush to remove any debris and stains. The cleaned teeth were stored in chlorhexidine solution to avoid dehydration. The roots were cut 1mm below the cementoenamel junction. Each tooth was embedded in self-cure acrylic resin with the enamel surface facing the mould base. The enamel surface was then exposed by trimming acrylic with an acrylic bur. Each specimen had a surface area of 14mm x 8mm (±1mm).[7]

**Staining Procedure**

Enamel of specimens were etched with 37% phosphoric acid for 15 seconds to remove the smear layer. Human blood that was obtained from the blood bank was used to stain the specimens. The blood was centrifuged at 10,000 rpm for 10 mins and the serum discarded. Then, 40mL of distilled water was added to 60mL of the precipitated blood and this mixture was centrifuged at 10,000 rpm for 20 mins. The teeth specimens were immersed in this solution for 4 days, where a centrifugation cycle of 10,000 rpm for 20 mins was performed at every 24 hours. After 4 days, the specimens were removed, washed with distilled water, dried with absorbent paper and kept in an incubator at 37ºC at 100% relative humidity for 15 days.[7] The Sanyo MIR 253 Cooled Incubator (Sanya, MA, USA) was used. It has the following specifications: provides large capacity, accurately controlled environment over a wide range of temperatures. This refrigerated incubator has a temperature range between -10ºC to +50ºC, controlled from the keypad interface with digital screen set at the top of the instrument. From this control panel users can set their desired temperature, activate auto defrost, set and silence alarms, and define up to 3 programs each with up to 99 steps. Users of this Sanyo Incubator can choose to repeat their program multiple times, up to 99, set their independent high/low temperature limit, and choose their duration up to 99.5 hours. This cooling incubator is upright with a brightly lit, 9 cu ft interior, with fan circulation and adjustable wire shelving, lined with easy to clean stainless steel.

**Bleaching Treatment**

After staining, the specimens were randomly assigned to four different groups (n=22); Group 0 (Staining with no bleaching - control), Group 1 (Laser White 20 46% HP + Opalescence PF 20% CP – HB sequence for 3 days), Group 2 (Laser White 20 46% HP + Opalescence PF 20% CP – HB sequence for 7 days), Group 3 (Opalescence PF 20% CP – HB sequence for 14 days). The samples were dried with cotton wool pellets and care was taken to not let the specimen desiccate.

For Group 1, a thin layer of Laser White 46% HP (Biolase Technology, CA, USA) whitening gel was applied all over the exposed tooth surface evenly with a brush applicator tip to about 1mm thickness. The Ezlase 810nm system was prepared and laser-activated. The whitening handpiece was held in place near (~1mm) to the tooth surface coated with bleaching material for the duration of laser delivery (200 J or approximately 30 seconds). The Ezlase 810nm system was allowed to rest for one (1) minute and the tooth was exposed to laser irradiation for the second time as per the manufacturer's instructions. The gel was then allowed to remain on the teeth for a minimum of 5 minutes after the second laser cycle. The gel was then removed using high-speed suction and flushed with an air and water spray to remove any residual gel. For the next 3 days, the Opalescence PF 20% CP (Ultradent Co., South Jordan, UT, USA) was used according to the manufacturer's instruction where the exposed teeth surfaces were covered by a layer of 1mm of the whitening gel for two hours daily in a humid atmosphere at 37ºC. The specimens were stored in artificial saliva, in an incubator at 37ºC with 100% relative humidity in between the HB treatments. The artificial saliva was replaced daily.

The teeth in group 2 were also bleached following the procedure described in group 1, however, the Opalescence PF 20% CP (Ultradent Co., South Jordan, UT, USA) was applied for 7 days.

In Group 3, only Opalescence PF 20% CP (Ultradent Co., South Jordan, UT, USA) was applied for 14 days following the procedure as described for Group 1.

Colorimetric measurement, surface hardness and surface roughness on sample surfaces were taken before (baseline) and after the application of bleaching agent in each group. Each measurement was taken three times for each sample before and three times after the application of the bleaching agent. Contents and properties of the two bleaching systems can be seen in table 1. Specifications of the Biolase diode laser system can be seen in table 2.

**Table 1:** Contents and properties of bleaching systems used in this study

| BLEACHING SYSTEMS | Laser White 20 46% HP | Opalescence PF 20% CP – HB |
|---|---|---|
| **PROPERTIES** | **1.** LaserWhite20 is a proprietary dental whitening gel used in conjunction with a BIOLASE diode laser system.<br>**2.** The laser, through a specialized handpiece and delivery system, activates the LaserWhite20 whitening gel to accelerate the whitening process.<br>**3.** The Base Gel contains a 45% concentrated hydrogen peroxide as an active ingredient.<br>**4.** The Activator is formulated with a proprietary dye that activates by absorbing laser energy in the specific BIOLASE diode laser system wavelengths.<br>**5.** The unique mixing syringe system ensures freshness for each application and precise dosing of the activator.<br>**6.** When mixed, the LaserWhite20 whitening gel results in a 35% hydrogen peroxide. | **1.** This system is intended to be dentist-supplied and supervised.<br>**2.** The whitening agents are clear, flavoured, high-viscosity, sticky, peroxide containing gels.<br>**3.** The agents are available in 10%, 15%, and 20% carbamide peroxide, and in 35% and 45% carbamide peroxide equivalent gels.<br>**4.** Opalescence whitening take home products feature sustained release action and adhesive properties.<br>**5.** All Opalescence whitening products are gluten-free and kosher.<br>**6.** The increase in microhardness is due to potassium nitrate and sodium fluoride.<br>**7.** They also contain significant water content to help prevent tooth dehydration and shade relapse. |
| **CONTENTS** | **1.** LaserWhite20 whitening gel<br>**2.** Base Gel<br>**3.** Activator<br>**4.** BIOLASE diode laser | **1.** Whitening agents (e.g. carbamide peroxide containing gels)<br>**2.** Potassium Nitrate<br>**3.** Sodium Fluoride<br>**4.** Water |

**Table 2:** Specifications of Biolase Diode Laser System

| BIOLASE DIODE LASER SYSTEM | |
|---|---|
| **GENERAL** | |
| Frequency | 50 / 60 Hz |
| External Fuses | None |
| **Weight** | 2 lbs. (1.0 kg) |
| **Dimensions** | |
| W x H x D | (3.5” x 7.0” x 2.5”) (8.5 x 18 x 6cm) |
| **ELECTRICAL** | |
| Operating Voltage | 100 to 240 ~ at 2A |
| **Main Control Power Switch** | On / Off Controls Keypad Button, Emergency Stop |
| Remote Interruption | Remote Interlock Connector |
| **LASER** | |
| Laser Classification | IV (4) |
| Medium | GaAlAs, InGaAsP |
| Wavelength | 810 ± 15 nm or 940 ± 15 nm |
| **Max Output Power** | 7 Watts @ 940nm, 4.5 Watts @ 810nm |
| **Power Accuracy** | ± 20% |

| | |
|---|---|
| **Power Modes** | Continuous, Pulse Modulation |
| **Pulse Length** | 0.06 ms - 10 sec |
| **Pulse Interval** | 0.06 ms - 10 sec |
| **Pulse Repetition** | Rate up to 10 KHz |
| **Fiber Tips Diameter** | 200, 300, 400 μm |
| **NOHD** | 11.8 meters |
| **Beam Divergence** | 8 – 22 degrees per side angle |
| **Fiber Cable Length** | 5 feet (1.524 meters) |
| **OTHER LIGHT SOURCES** Aiming Beam Laser Diode | max 3 mW, 630-670nm, class 3B |

### Colorimetric Measurement

Colorimetric measurements were done using Vita Easy Shade ® Advance 4.0 (VITA Zahnfabrik, Bad Säckingen, Germany) on sample surfaces before (baseline) and after the application of bleaching agent in each group. Calibration was done on Vita Easy Shade followed manufacturer instructions before measurement was performed. Calibration was repeated after every 5 samples. The measurements were taken three times for each sample before and three times after the application of the bleaching agent). Then their mean was calculated and recorded.[4] The colour difference was calculated using the formula.

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]1/2,$$

$\Delta L$ = final L–initial L, $\Delta a$ = final a–initial a, $\Delta b$ = final b–initial b

Even though the room lighting did not have an effect on measurements, since the 4th generation Vita Easy Shade® was utilized which has its own built-in light, but still the measurements were performed in a room with bright day light and a black background. The time of the day was fixed for taking all measurements to ensure standardization. A black coloured matte cardboard sheet was used as a background to avoid scattering of the light and shadowing. Since the colour black absorbs all the incoming light, so its' use is advantageous, as it helps in producing clear and shadowless images.[49] Colour recordings were performed by one experienced clinician using a Vita Easyshade Advance 4.0 spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany) according to the manufacturer's instructions. This digital shade matching device uses a D-65 illumination. In order to mimic a standardized daylight, all measurements were performed in a room, which was exposed to daylight conditions.[47]

As per manufacturer's details for Vita Easy Shade ® Advance 4.0 (Vita Zahnfabrik, Bad Säckingen, Germany), it is the 4th generation electronic shade taking device, which is ideal as it ensures fast, reliable and objective tooth shade determination. In a matter of seconds, this device allows you to determine and verify all tooth shades based on the internationally renowned tooth shade standards VITA classical A1 – D4 and VITA SYSTEM 3D-MASTER. The device is accurate as the human eye, and is equipped with its own light source so that daylight is no longer a relevant factor. Further technical details for Vita Easy Shade ® Advance 4.0, can be seen in table 3.

**Table 3:** Technical data for Vita Easy Shade ® Advance 4.0

| | **Vita Easyshade Advance 4.0®** |
|---|---|
| **Technical data** | **1.** Spectrophotometer, measurement range 400 - 700 nm.<br>**2.** Inductive charging concept with long-life AA batteries.<br>**3.** Output of all tooth shades in the established standard shade systems VITA classical A1-D4 and VITA SYSTEM 3D-MASTER, as well as indication of the VITABLOCS shades and bleached index, in accordance with the American Dental Association.<br>**4.** Display of lab and LCh values.<br>**5.** Bluetooth interface for wireless communication with the VITA Assist PC software and VITA mobileAssist app.<br>**6.** Reliable and economical, thanks to precise tooth shade information in the VITA shade standards for reliable shade reproduction and reduced shade corrections.<br>**7.** Simple and intuitive, thanks to the easy-to-use touchscreen and software.<br>**8.** Efficient, digital communication for exchanging information about tooth shade and images between the dental practice and the laboratory. |

**Microhardness Testing**

Each specimen was divided into two surface area using pencil subjected to indentation before and after bleaching treatment. Specimens were positioned perpendicularly to the long axis of the indentor for 15 seconds to record the Vickers hardness number (VHN). A 5 kg load Vickers indentor (Future Tech, Tokyo, Japan) attached to a hardness tester (VM-50, Fuel Instruments, and Engineers, Maharashtra, India) was used. Their mean values were calculated and recorded.[24]

**Surface Roughness Testing**

A profilometer (Surface Texture Measuring Instrument – Surfcom Flex 50A, Tokyo Seimitsu Co., Ltd, Japan) was used to measure the surface roughness. The resolution for this device was 0.00016 μm/±4 μm to 0.016 μm/±400 μm. The measuring range was 400 μm vertically and 50 mm horizontally. This procedure was done according to the manufacturer's instructions. Each specimen was positioned on a flat surface where the pickup of the profilometer came in contact with the pattern on the calibration side of the specimen. The stylus was then applied and the meter was run at the evaluation length of 7mm at a measured speed of 0.15mm/s. Three (3) measurements were taken and their mean values were calculated and recorded.[24]

**Morphological changes evaluation**

Two samples from each group were selected randomly for morphological evaluation using scanning electron microscope (SEM) (FEI QuantaTM 450 FEG, ThermoFisher Scientific, Oregon, USA). The specimen was gently air-dried, dehydrated with alcohol and then dried at the critical point (a method used to minimize specimen distortion due to drying tensions). The samples were then sputter-coated with gold-palladium and examined under a SEM. Serial SEM microphotographs of the surface of each specimen at 5,000X and 10,000X original magnification were obtained to evaluate enamel texture changes. The surface morphology of enamel in each picture was examined by two operator compared and determine whether any difference in the enamel texture could be seen after different treatment modalities.[25]

**Statistical Analysis**

Data collected were analysed using SPSS version 26.0. Data for colour changes, hardness testing, and surface roughness pre and post bleaching were analyzed using paired T-test. Multiple comparison between groups was carried out using one-way ANOVA complemented by Post Hoc (Bonferroni procedure). The level of significance was set at P =0.05.

## RESULTS

### Colour changes

Table 4 and 5 show the mean colour changes of human tooth post bleaching procedure with three different bleaching protocols. Paired t-test revealed that there were significant changes in all treatment groups except the control group. One-way ANOVA test showed that there was a significant difference in the color changes when the comparison was made between groups with $p < 0.05$. Furthermore, posthoc Benferroni analysis revealed that colour changes produced by a combination of laser and HB were superior compared to HB alone ($p<0.05$). However, there was no significant difference in colour changes between both groups of laser and HB combination ($p>0.05$) (Table 6).

**Table 4:** Colorimetric reading before and after using combination of laser and home bleaching protocol

| Groups | N | Before bleaching | | | Post-bleaching | | | ΔE Mean difference (SD) | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| | | L* | a* | b* | L* | a* | b* | | |
| Control | 22 | 52.96 | 7.75 | 37.89 | 61.70 | 6.62 | 43.00 | 11.10 (4.481) | 0.068 |
| LB + HB 3days | 22 | 59.54 | 7.29 | 36.43 | 89.30 | -0.81 | 28.41 | 32.06 (3.459) | **0.001*** |
| LB + HB 7days | 22 | 58.05 | 5.78 | 34.31 | 89.70 | -0.08 | 28.01 | 33.12 (6.191) | **0.001*** |
| HB 14 days | 22 | 66.26 | 4.57 | 32.65 | 90.81 | -0.19 | 28.53 | 25.82 (5.825) | **0.001*** |

Paired T-test; *statistically significant $p<0.05$

**Table 5:** The comparison of colour changes (ΔE) after using combination of laser and home bleaching protocol

| Groups | N | ΔE Mean (SD) | F statistic[a] (df) | *p*-value[b] |
|---|---|---|---|---|
| Control | 22 | 11.10 (4.481) | 86.75 (3) | **<0.001*** |
| LB + HB 3days | 22 | 32.06 (3.459) | | |
| LB + HB 7days | 22 | 33.12 (6.191) | | |
| HB 14 days | 22 | 25.82(5.825) | | |

[a]ANOVA test *statistically significant $p<0.05$
[b] the mean score with significant P=value were tested for multiple comparisons using Post hoc test (Bonferroni)

**Table 6:** Multiple comparisons of colour changes (ΔE) after bleaching regime between groups (n=88)

| Between groups | | Mean difference (95% CI) | *p*-value |
|---|---|---|---|
| Control | LB+ HB 3days | -20.96 (-25.120, -16.800) | **<0.001*** |
| Control | LB + HB 7days | -22.01 (-26.173, -17.852) | **<0.001*** |
| Control | HB 14 days | -14.71 (-18.874, -10.554) | **<0.001*** |
| LB+ HB 3days | LB + HB 7days | -1.05 (-5.213, 3.108) | 1.000 |
| LB + HB 3days | HB 14days | 6.25 (2.086, 10.406) | **0.001*** |
| LB + HB 7days | HB 14days | 7.30 (3.139, 11.459) | **<0.001*** |

Post hoc test (Bonferroni); *statistically significant $p<0.05$

### Hardness Test

Table 7 shows that there was a significant change in surface hardness (VHN) before and after bleaching protocols in all treatment groups. Meanwhile Table 8, ANOVA test demonstrated that all treatments group had a significant increase in hardness as compared to control. However, no significant difference was noted among treatment groups ($P>0.05$), as depicted in Table 9.

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**Review**

# AN EXPLORATORY REVIEW OF CURRENT TRENDS IN NANODENTISTRY

## ABSTRACT

Nanotechnology is a cutting-edge concept that is evolving manifolds in various fields of science and medicine and is by no means exceptional to dentistry. Nanotechnology is popularly known as the 'science of the small' that deals with particles of size 1-10nm. Methods like top-down or bottom-up approaches are used in manufacturing nanoparticles and nanorobots, catering to the needs of medical diagnostics and therapeutics. Nanorobotics advances medicine through miniaturization from microelectronics to nanoelectronics. Nanotechnology can be applied to all fields of dentistry such as to create nano implants, nano-drug delivery systems, nanocomposites and nano impression materials. Additionally, it helps in orthodontic tooth movement, alleviating hypersensitivity, and effective anesthesia. This paper highlights the various applications of nanotechnology in dentistry and also mentions the clinical trials performed to have a more focused approach to practicing nanodentistry. Apart from this the paper briefly explains the benefits of integrating artificial intelligence and nanotechnology for creating more personalized treatment options and also its role in Covid 19 vaccines.

**Key words:** Nanotechnology, artificial intelligence, nanoparticles, nanocomposite.

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## INTRODUCTION

Nanotechnology is popularly known as the 'science of the small' that deals with particles of size 1-10nm.[1] Richard Feynman introduced nanotechnology in the year 1959 and is considered the father of nanotechnology.[2] Nanomedicine is a field that is transforming the approach in diagnosis, treatment, prevention of diseases, alleviating pain, improving human health by using the molecular tools and molecular knowledge of the human body.

Nanoparticles in medicine can enter living cells effortlessly as they are much smaller than those used in chemical and industrial applications. There are two approaches to fabricate nanomaterials. The bottom-up approach creates nanoparticles from molecular components that self-assemble via molecular recognition, and the top-down approach builds nano-objects from larger entities.[3] Table 1 gives examples of nanomaterials fabricated through the two approaches.

**Table 1.** Nanomaterials fabricated through top-down and bottom-up approach.

| Top-down approach | Bottom-up approach |
|---|---|
| Nanocomposites | Dentinal hypersensitivity |
| Nanosolutions | Nanodentifrices |
| Nanocapsulation | Local anesthesia |
| Bone replacements | Tooth repair |
| Impression materials | Tooth positioning |
| | Oral cancer diagnosis |

### *Nanotechnology and dentistry*

Similar to nanomedicine, nanodentistry has evolved multi-folds and has diverse applications in all fields of dentistry. It holds a good promise of providing personalized treatment options with improved efficacy and reduced side effects. In the past few years, numerous reviews have been done in the literature on nanodentistry. However, clinical trials focusing on the practice of nanodentistry are limited due to factors like cost, technique sensitivity, and obtaining similar results to other contemporary materials. Thus, we look forward to highlighting the clinical trials undertaken recently in the different fields of Dentistry in this exploratory review to have a more focused approach to practicing Nanodentistry rather than seeing it as a future perspective. Thus, Nanodentistry is not the future but is happening now, and let's look forward to becoming nanodentists.

### *Nanoparticles*

Nanoparticles include particulate substances, which have a size of 100nm at least in one dimension. Nanoparticles are composed of three layers; the surface layer, shell, and core layer.[4] A variety of small molecules, metal ions, surfactants, and polymers make up the surface layer. The shell layer is a chemically different material from the core in all aspects, and the core is the central portion of the nanoparticle.[5] Based on the chemical and physical properties they can be classified as carbon-based nanoparticles, metal-based nanoparticles, ceramic-based nanoparticles, polymeric-based nanoparticles, lipid-based nanoparticles, semiconductor-based nanoparticles.[6] (Figure 1)

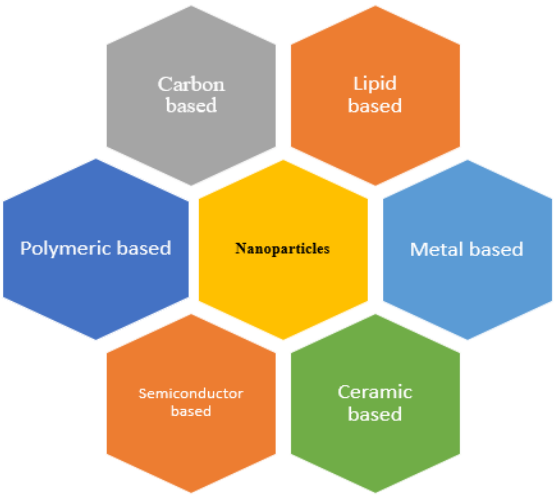


**Figure 1.** Classification of nanoparticles based on physical and chemical properties.

### *Nanorobots*

Nanorobotics pioneered by Adriano Cavalcani is a technology which converts nanoparticles into miniature nanorobots.[7] These Nanorobots consist of carbon atoms in a diamondoid structure which

include parts like Manipulator's gripper, Telescopic macromanipulator, Biomolecular Sensor, Acoustic Sensor, Antenna, Connector, and others as portrayed in figure 2 Nanorobots have inert properties, which evades the reaction of the immune system, thereby allowing them to have an unimpeded function.[1]

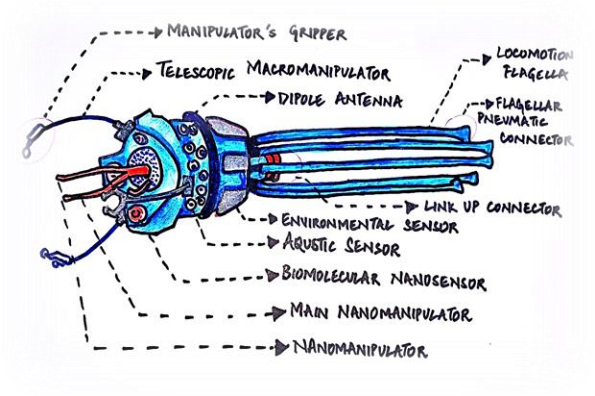


**Figure 2.** schematic diagram of a Nanorobot

They use glucose or natural body sugars and oxygen as a source for propulsion.[8] Each nanorobot could be modified with specific functions depending on the biochemical stimulus provided.[1] Nanorobots are expected to provide advances in medicine through miniaturization from microelectronics to nanoelectronics.[9] The application of nanotechnology in the field of Artificial intelligence is the future of science and this combined technology when integrated in the field of medicine would cater to improvise various setbacks in the diagnosis and the therapeutic aspects of medicine as well as in dentistry which has also been briefly explained in this article.

**Recent Advances in Nanodentistry**

Broadly materials used in medicine have been classified into 3 main avenues - diagnostics, drug delivery and bone grafts and implants.[10]

***Nanodiagnostics***

Oral fluid nanosensor test (OFNASET): has been recently introduced in the market to detect cancers through salivary biomarkers. OFNASET uses bionanotechnology, cyclic enzymatic amplification, and microfluidics self-assembled monolayers (SAM) which gives accurate results.[11]

**Nanoscale cantilever:** Another nanodiagnostic device that helps in the rapid detection of cancer-related molecules that are flexible in build and resemble rows of divided boards. The cancer-related molecules bind to the sensors in the device and cause conformational changes in shape.[12]

**Nanopores and nanotubes:** Nanopores help in efficient DNA sequencing by acting as a filter for DNA strands. Nanotubes are made of carbon that helps in detecting altered genes.[12]

**Quantum dots (QD):** They are nanocrystals that fluoresce when illuminated by ultraviolet light, made of semiconductor materials. They bind to proteins associated with cancer cells and therefore help with the detection of cancer. These fluorophores have unique photophysical properties that also overcome the limitations of using conventional dyes. They are also known to detect metastasis of cancer.[13]

**Optical Nanobiosensor:** It is a fiber-optic-based, compact analytical system that detects substances by producing a signal that is proportionate to the concentration of the measured substance. They contain a biological element such as an enzyme, protein, nucleic acid, or a receptor that recognizes the substance and passes it to the inbuilt optical transducer which in turn produces a signal. They are highly sensitive and specific devices and are cost-effective.[14]

**Lab-on-a-chip methods:** These are silicon chips with chemically activated beads embedded in them. They help in analysing and diagnosing various diseases by detecting biomarkers. They are cost-effective and require a small sample size.[15]

**Nano CT:** Nano CT one of the Diagnostic methods like micro CT have been used in dental applications like evaluation of various materials like composite using resolution of around 450 nm compared to micro CT evaluation which has a resolution of 2 µm. This clearly shows us that greater details of the ultrastructural properties could be evaluated. Thus nano diagnostics could be useful in effective and critical evaluation of biomaterials as well as their action in the respective sites could be studied in detail. In soft tissue evaluation contrast -enhanced nano CT reveals soft tissues like the ones stained with

Haemotaoxylin and Eosin stain of odontoblasts. The nano contrast CT involves the use of a specific contrast dye which depends on the tissue type and volume. One of the most important applications of nano CT is the 3D visualization of the dental tissues and subsequent spatial analyses, both descriptive and quantitative.[16]

### *Nano Drug Delivery systems*

Oral infections are very complex diseases, thus requiring antimicrobial nano drug delivery systems to combat the same which aid in site-specific wound healing.

Nanoelectromechanical systems (NEMS) are devices integrating electrical and mechanical functionality on the nanoscale level.[17] They make use of motors, pumps or mechanical actuators to form a functional, biological and chemical nano systems. Bio-nanoelectromechanical systems (BioNEMS) are bio-hybrid systems that combine biological and structural elements on a nanoscale level. They include DNA or proteins combined with mechanical nanostructures. It can develop low-cost, biocompatible, and controlled drug delivery devices. It is available in both sustained and pulsatile delivery of the drug. It works as multiple reservoir-based devices, simultaneously delivering multiple drugs.[18] Figures 3 and 4 show drug delivery devices that work on an osmotic pump and spring-powered pump.

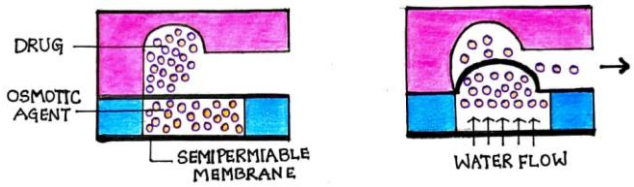


**Figure 3.** Osmotic pump

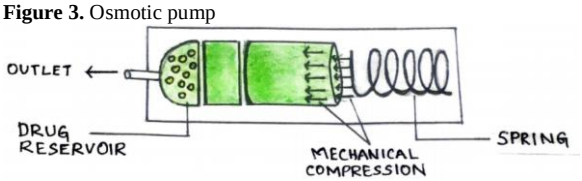


**Figure 4.** Spring powered pump

### *Nanoneedles and nanotweezers*

Nano needles made of silicon are now being developed to aid in effective and sustained drug delivery. They are fabricated using various procedures such as Vapour– liquid–solid (VLS) growth of nanoneedles, Metal- assisted chemical etch of nanoneedles and Focused ion beam etch of nanoneedles. There are many types of nano needles such as solid, porous and hollow nano needles based on the loading and release characteristics of the needle.[19] Nowadays needles with nanosized stainless steel crystals incorporated into them are available commercially (Sandvik Bioline 1RK91™, Sandvik, smt.sandvik.com).[12]

Nanotweezers are nano devices which help in the construction of the human cell. Recent studies reported in literature shows that nanotweezers could be used to manipulate single organelles by trapping and extracting them from various parts of the body. These tweezers effectively interact with single cells thus helping us to understand their signaling pathways of interaction during an external stimuli.[20]

### *Nano coated Dental Implants*

Dental implants have undergone various advances, however, share few drawbacks too. A major disadvantage for titanium alloy as oral implant material is relatively poor wear resistance. To overcome this drawback, nanostructured ceramic coatings such as TiN (titanium nitride) (Is this a ceramic material?), ZrO2/Al2O3, Si3N4/ TiO2, and ZrO2/SiO2 are used.[21]

Many studies have demonstrated increased function of osteoblasts on nanoparticles compared to conventional materials (Figure 5).

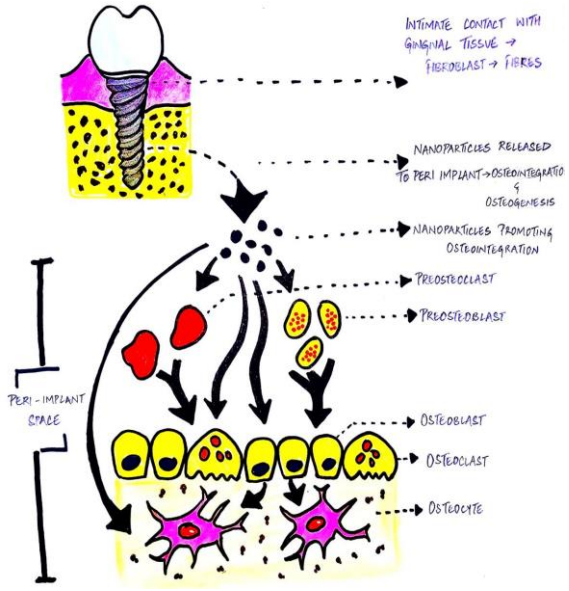


**Figure 5.** Nanoparticles promoting osseointegration.

Yao *et al.* created nanometer surface features on titanium and Ti6Al4V implants by anodization, a

quick and relatively inexpensive electrochemical method. The results showed that the anodized surfaces had higher root-mean-square roughness at nanoscale dimensions than the unanodized Ti-based surfaces.[22] In addition blood clot retention and osseointegration is improved. Anodized Ti6Al4V dental implants show better results when placed in regions with poor bone quality or in cases with immediate loading protocol.[23]

The versatility of nanomaterials may allow the fabrication of implants with various porosities, pore shapes, and mechanical properties that can mimic the complex architecture of bone-specific sites to optimize bone tissue regeneration.[24] Table 2 summarizes the clinical trials done on nano implants.

**Table 2.** Clinical Trials on Dental Implants

| Sl.no | **year** | **Author** | **Nanomaterial used** | **Clinical trial done** |
|---|---|---|---|---|
| 1 | 2018 | Zia Arshad Khan[25] | Nanopore surface implant | **Evaluation of peri-implant tissues around nanopore surface implants with or without platelet-rich fibrin: a clinicoradiographic, randomized clinical study** showed that the mean difference in the probing depth between the two groups was insignificant. Thus, it was concluded that additional graft materials would not be required when using a nanopore surface implant. |
| 2 | 2010 | Luigi Canullo [26] | e-PTFE membrane and nanostructured Mg-HAP | **Early implant loading after vertical ridge augmentation (VRA) using e-PTFE titanium reinforced membrane and nanostructured hydroxyapatite a 2- year prospective study** aimed to evaluate survival of implants, loaded 14 weeks after vertical ridge augmentation (VRA). It was concluded that VRA around rough surface implants using e-PTFE membrane and nanostructured Magnesium-Hydroxyapatite can be successful even in cases with early loading. |

***Graft materials***

Nanotechnology enhances the characteristics of bone grafts by improving the uptake, biocompatibility, proliferation of the tissues, and pore size. An optimal interconnected porous network of micro and nanoscale features allows increased bone formation, cellular migration, proliferation and vascularization.[27]

Bone grafts essentially consist of scaffold, cells and extracellular matrix. Incorporation of carbon nanotubes and nanofibers enables the flexibility and strength, thus providing enhanced mechanical properties. Cellular adhesion through adsorption of proteins like fibronectin causes bone formation. The extracellular matrix thus formed shows increased vascularization, osteoblastic adhesion and also the resultant osseointegration providing a potential bone healing property.

Polyvinyl alcohol shows increased mechanical strength, excellent biocompatibility and less metallic impurities.[28]

Bionanocomposites have found application as scaffold matrix by way of their increased surface area to volume ratio, close contact to surrounding tissues, increased biocompatibility, osteoconductivity, cell adhesion properties, cellular proliferation.

Alginate infused with bioactive glass has been found to have increased biomineralization, protein adsorption properties, cellular adhesion and proliferation of human periodontal ligament fibroblasts.[29]

Other interesting facts were that of increased ALP activity of the human PDL fibroblast cells cultured on the scaffold.

Gelatin has shown to have excellent cell adhesive properties, high biocompatibility, biodegradability, low immunogenicity. In periodontal regeneration, scaffolds incorporated with nanoparticle on gelatin in the concentration of 2.5% gel /2.5% HA and 2.5% gel/ 5% HA have been shown to have properties such as cell attachment, proliferation of PDL fibroblast cells.[30]

Chitosan another nanobiocomposite has shown very minimal foreign body reaction. The positive cell surface charge has enabled cell growth, attachment, and cell differentiation.

A major advantage of chitosan is that when placed in hydrated environments, chitosan turns into a flexible material which is more rigid than materials like PLA, PGA. Studies have shown that use of a combination of chitosan along with bioactive glass nanoparticles have shown improved bioactivity, promoted human PDL cells metabolic activity. This suggests its use as a guided tissue regeneration membrane.[31]

Different surface morphologies are required to enhance the proliferation of osteoblasts and fibroblasts. By maintaining some fundamental constraints regarding pore size and interconnectedness the response of osteogenic cells and their precursors to micro and nanotextured morphologies on planar surfaces can be reproduced on three-dimensional scaffolds.[24]

Documented disadvantages of scaffolds include inflammation, limited cell turnover, and growth factor expression.[14]

Clinical reports, highlight the use of nanofibers that release antimicrobial particles, hence paving the way for use as a scaffold for tissue engineering purposes.[32] The self-assembly property of the nanomaterials at body temperature directs bone growth; combating multifactorial diseases like periodontal disease and Osteomyelitis.[33] Self-assembling peptides like RAD16-I when transplanted, can assist with new callus formation and inhibit demineralization.[34]

***Nano Antihypersensitivity Agents***

Hypersensitivity has become a common problem encountered in dental setup. In hypersensitive teeth, the dentinal tubules have surface densities eight times more than nonsensitive teeth.[35] Nanotechnology has effectively addressed this problem by creating nanorobots that traverse through the dentinal tubules and occlude them providing relief to the patient.[36] Also, the nanoparticles have remineralizing property which helps in reducing sensitivity.[37] Table 3 shows the clinical trials conducted using nanomaterials for treating dentinal hypersensitivity.

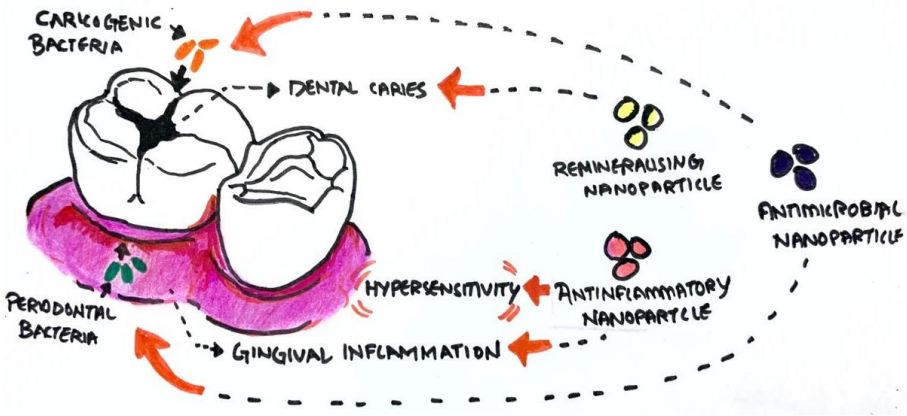


**Figure 6.** Remineralization, antimicrobial and anti-inflammatory properties of nanoparticles.

**Table 3.** Clinical Trials conducted using nanomaterials for treating dentinal hypersenitivity.

| Sl.no | Year | Author | Nanomaterial | Clinical trial outcome |
|---|---|---|---|---|
| **1** | 2019 | James R Fernando[38] | $SnF_2$ and CPP-ACP nanocomplexes | **Self-assembly of dental surface nanofilaments and remineralization by SnF 2 and CPP-ACP nanocomplexes.** This uses in vitro studies and a double-blind, randomized controlled, cross-over design in situ clinical trial and shows that SnF2 and CPP-ACP interact to form a nanofilament coating on the tooth surface and that together they are superior in their ability to promote dental remineralization and reduce hypersensitivity. |
| **2** | 2014 | Michele Vano[39] | Nanohydroxyapatite | **Effectiveness of nano-hydroxyapatite toothpaste in reducing dentin hypersensitivity: a double-blind randomized controlled trial.** The findings of the study encourage the application of nano-hydroxyapatite in fluoride-free toothpaste as an effective desensitizing agent providing quick relief from symptoms after 2 and 4 weeks. |

***Nano aided Anesthesia***

Navigation technology in the form of nanorobots also termed as nanobots induces oral analgesia. When these nanorobots reach the pulp, it results in reversible and temporary loss of pain and sensitivity in the tooth or area of interest. They can be computer-controlled by the dentist, and this property helps to reverse or restore the anesthetic activity.[40]

Nanotechnology-modified liposomal nanoformulations are small nanovesicles encased in a phospholipid bilayer.[26] Which has effective pain control and aiding quick recovery.[41,42] They also reduce the side effects of the anesthetic; for example, liposomal bupivacaine maintains a minimum concentration needed to deliver the therapeutic effect for 3 days without increasing the concentration of the active constituent in the body. In conclusion, liposomal bupivacaine has reduced cardiac toxic effects.[43]

***Nano aided Orthodontics***

Nanoelectromechanical systems (NEMS) have their broader application in orthodontics recently. Electrical energy generates mechanical forces which enhance tooth movement as shown in animal studies.[44] These animal studies reveal that electric stimulation using low amperes enzymatic micro battery (15-20 microamps) enhances cellular enzymatic phosphorylation activities, which lead to accelerated bone remodeling. This device uses glucose as the fuel for the micro battery and is placed on the gingiva near the alveolar bone to facilitate orthodontic tooth movement. (Figure 7) But several issues have to be considered, such as the effect of food, pH, and biocompatibility.[44]

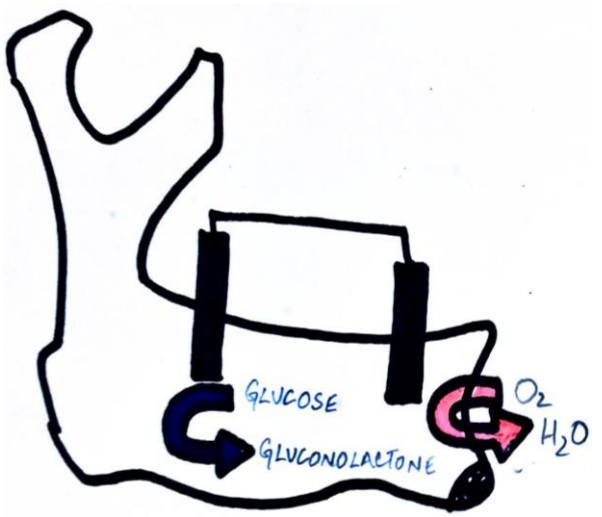


**Figure 7.** Oral biocatalytic fuel cell. The following reaction generating electricity for enhancing orthodontic tooth movement occurs: Glucose+O2→gluconolactone+H2O/H2O2

Temporary anchorage devices manufactured with smooth titanium surfaces when aided with titanium nanotubes enhance initial osseointegration and serve as an interfacial layer between the newly formed bone and the

temporary anchorage devices during orthodontic tooth movement.[45]

***Nanotechnology in the field of restorative dentistry and endodontics has seen major research in 4 main types of material- composites, GIC, adhesives and endodontic materials.***

***NanoComposites***

Incorporating nanofillers in composites improved material characteristics like the smoothness of surfaces resulting in better aesthetics. The remanent minute irregularities formed during finishing and polishing are way smaller than the wavelength of visible light (0.4-0.8 micrometers) thereby minimizing the reflection of light thus warranting good optic properties. They also improve the strength of the composite and reduce polymerization shrinkage.[46]

A rechargeable nano-amorphous calcium phosphate (nACP) filled composite resin (smart material) helps neutralize the acids produced by the bacteria preventing secondary caries. Additionally, the levels of calcium and phosphorus are also maintained.[47] (Figure 8)

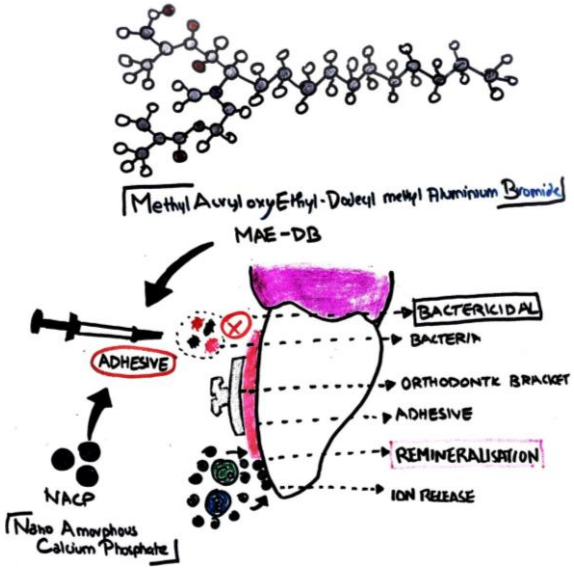


**Figure 8.** Remineralization and bactericidal effects of nACP.

Dental nanocomposites (bionanocomposites) contain nanofillers and nanofibers with a photopolymerizable resin matrix. The distribution of nanofillers is such that there is increased filler load which in turn increases the viscosity leading to better mechanical properties and reduced polymerization shrinkage. Due to reduced particle size, load-bearing stress is reduced, which results in inhibition of crack propagation.

Nanoparticles of dicalcium phosphate anhydrous- (DCPA-) whiskers, tetracalcium phosphate- (TTCP: Ca4(PO4)2O-) whiskers, kaolinite (Al2Si2O5(OH)4), and calcium fluoride are incorporated, which resulted in the highest release of calcium, phosphorous and fluoride owing to their reduced particle size. In addition, calcium and phosphorus release was increased six times in acidic conditions. Due to the unique structure of kaolinite (large surface area), the adsorption of fluoride is higher and therefore can also provide sustained release of fluoride.[48] Table 4 represents the clinical trials done on nanocomposite.

**Table 4.** Clinical trials on nanocomposites

| Sl. no | year | Author | Nanomaterial | Clinical trial |
|---|---|---|---|---|
| **1** | 2014 | Mary A S Melo[49] | Nanostructured Hybrid Fluoridated Restorative Composites. | In Situ Response of Nanostructured Hybrid Fluoridated Restorative Composites on Enamel Demineralization, Surface Roughness, and Ion Release. |
| **2** | 2014 | Jan W V van Dijken[50] | Nanohybrid composite | A randomized 10-year prospective follow-up of Class II nanohybrid and conventional hybrid resin composite restorations<br>This 10 year follow-up study showed good clinical effectiveness of nanohybrid composite in extensive class 2 restorations. |
| **3** | 2013 | Wei Qin[51] | | Two-year clinical evaluation of composite resins in non-carious cervical lesions<br>Both restorative materials exhibited acceptable clinical performance in class 5 non carious lesions 2 years post restoration. |
| **4** | 2013 | Umit Candan [52] | fiber-reinforced nanofilled resin composite | Clinical performance of fiber-reinforced nanofilled resin composite in extensively carious posterior teeth of children: 30-month evaluation<br>13 month evaluation of nanofilled resin composite applied with or without glass-fiber layering showed similar and good results in large cavities of posterior permanent teeth in children. |
| **5** | 2012 | Lei Cheng.[53] | amorphous calcium phosphate and silver nanocomposites | Effect of amorphous calcium phosphate and silver nanocomposites on dental plaque microcosm biofilms<br>Novel amorphous calcium phosphate(NACP) and silver nanoparticles (NAg) nanocomposites possess good mechanical and antibacterial composites reducing biofilm viability and lactic acid production. Thus promising for good dental restorations with remineralizing and antibacterial capabilities. |
| **6** | 2011 | Dina Gamal Taha[54] | Ormocer, Nanofilled, and Nanoceramic composite | Fracture resistance of maxillary premolars with class II MOD cavities restored with Ormocer, Nanofilled, and Nanoceramic composite restorative systems<br>Teeth with microhybrid, ormocer, and nanofilled composite restorations had lower cuspal fracture resistance than those with nanoceramic composite restorations. |

***Nano- GIC (Nanoionomers)***

Glass ionomer cement (GIC) is one of the most versatile materials used in dentistry that has undergone various modifications. Nanotechnology has played a major role in modifying GIC, improving its mechanical properties and the esthetics offered. The first nano-GIC was developed for Ketac™ Nano (3M ESPE, 3mespe.com) with fluor aluminum-silicate technology.[55]

In a study conducted by Alatawi RA et.al in 2019, they produced GIC mixed with hydroxyapatite nanoparticles, this mixture resulted in increased fluoride release and enhanced mechanical properties. In addition, they studied the antibacterial effect of 8% HA wt% against S.mutans, which resulted in a bacterial inhibition zone of about 8.6 mm.[56]

***Nano adhesives***

These are bonding agents, when incorporated with nanoparticles-show better bond strength, marginal seal, fluoride release, stress absorption, and long shelf-life due to the well-homogenized consistency of the adhesive.[12]

***Nano aided Endodontics***

Nanoparticles play a major role in material aspects of the endodontic sealers and obturation materials. It improves the handling and physical properties. Additionally, it has antimicrobial properties due to increased pH and improved sealing ability.

Bioceramic-based sealer EndoSequence BC Sealer™ and Silicon-based sealer containing gutta-percha powder and silver nanoparticles (GuttaFlow® 2, Coltene Whaledent) have been introduced. Recently antibacterial quaternary ammonium polyethyleneimine (QPEI) nanoparticles have also been incorporated into other sealers.[57,58]

Recently diamond nanoparticles were incorporated into gutta-percha, and the obturation viewed under digital radiography and micro-computed tomography revealed better adaptation to canal walls and less void formation.[59]

***Nano Impression materials***

Research in prosthetic impression materials based on nanotechnology is basically of two types: one is to create new inorganic nanomaterials and the other is to improve the surface characteristics of the existing materials by incorporating nanoparticles on the surface helping us to overcome the disadvantage of traditional impression materials which are known to be brittle and low ductility. Thus, incorporation of nanoparticles into ceramic, resin and metals paves the way to attaining better mechanical and structural properties of impression materials.

Nanoceramics have superplasticity and show good toughness, ductility, hardness, and strength which is four to five times higher than those of the traditional materials. For example, at 100°C the microhardness of nano-TiO2 ceramics is 13,000 kN/mm2, while that of ordinary TiO2 ceramics is lower than 2,000 kN/mm2.[60]

Poly methyl methacrylate (PMMA) is one of the dental materials that is indispensable and the incorporation of nanotechnology has improved its characteristics multifold. Carbon Nanotubes (CNT) and carbon nanofibrils have been used as additives in impression materials to improve the properties of PMMA. Studies show better impact strength of PMMA matrix, when prepared using with even smaller amounts of single-wall nanotubes as additives using a dry powder mixing method.[61]

**Recent applications in other fields of medicine**

We would also like to highlight the recent applications of nanotechnology in other fields of medicine like artificial intelligence, cancer detection and covid 19 vaccines. Though these topics are not within the scope of the article, yet we would like to bring to the readers the diverse applications which could be further explored.

***Artificial intelligence and nanomedicine***

Artificial intelligence (AI) is one of the newfangled technologies being researched extensively in the field of medicine.

Integrating artificial intelligence and nanotechnology is instrumental in the field of nanomedicine and dentistry. It helps in enhancing patient data acquisition and improves the design of nanomaterials and diagnostic and therapeutic efficacy. AI bridges the gap of heterogeneous patient treatment modalities by providing custom-made treatment options by analyzing the patient requirements.[62] It aids in making appropriate combinations of drugs and the nanomaterial to be used for carrying the drug by using pattern analysis and classification algorithms.

***Cancer detection through Integration of Artificial Intelligence and Nanotechnology***

Artificial intelligence has improved cancer detection and treatment in many ways. IBM Watson for use in oncology helps in providing a more personalized therapy to cancer patients. It collects information from medical journals, textbooks, clinical data and analyses patient medical records, along with the oncologist's expertise, which could render the best treatment to the patient. Microsoft's Hanover project and

Google's DeepMind are other platforms working on AI in medicine.[63]

In a recent study, Wang *et al*. developed feedback system control, a widely used platform for unmodified and nanotechnology-modified therapeutic optimizations. The authors used AI to standardize drug dose combinations that would produce maximum cytotoxicity. They studied nanodiamond-doxorubicin, nanodiamond-mitoxantrone, nanodiamond-bleomycin, and unmodified paclitaxel combinations on many breast cancer cell lines. The results showed that when compared to randomly selected nanomedicine combinations, AI-optimized nanomedicine drug combinations gave a better performance.[64]

**Application of nanotechnology for covid-19 vaccines**

Covid - 19 vaccines have become the need of the hour, and nanotechnology can invariably play a role in vaccine development. Research in a Nano ImmunoEngineering, University of California, Faculty proposed the idea of a plant virus using a peptide-based approach, which could be fabricated as COVID-19 Nano-vaccine patch and microneedle that could be painlessly self-administered by patients. It can efficiently deliver antigens, serve as adjuvant platforms and mimic viral structures.[65]

WHO in January 2021 considered BNT162b2 as an emergency vaccine which is a lipid nanoparticle-formulated, nucleoside-modified RNA vaccine that encodes a prefusion stabilized, membrane-anchored SARS-CoV-2 full-length spike protein.[66]

**Safety issues:**

The extensive use of nanomaterials in medical applications has raised concerns about the safety and toxicity levels. This is because of the associated rate of increased absorption into the cells of the skin, Lung mucosa, digestive tract cells, and other parts of the body. Toxic effects include DNA damage which when evaluated in a study, showed that copper oxide and titanium dioxide nanoparticles were highly toxic compared to iron oxide, zinc oxide, carbon nanotubes.[12]

## CONCLUSIONS

Nanotechnology will soon become the key aspect of the development of any diagnostic aid or treatment option and is inevitably the most emerging interdisciplinary field in medicine and dentistry. It has brought the treatment approach to the level of the size of the cells of the body and can as well render treatment that is more specific and personalized to the patient. It can be integrated with other disciplines of science and technology such as artificial intelligence and improve the efficiency of diagnosis and treatment multifold. Clinical trials in the field of Nanodentistry have been evolving at a very slow pace, in this review, we presented some of the recent developments in terms of clinical trials and incorporate high quality restorative and disease prevention strategies. Further, we like to conclude by Nanodentistry is definitely a promising field and when incorporated into our day-to-day practice could enhance the quality of interdisciplinary treatment modalities. Thus, let's march towards a change and aim for precision-based Dentistry which could be positively obtained using the perfect material of choice "the Nanos" in all the dental fraternity branches.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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**Table 7:** The comparison of surface hardness (VHN) before and after bleaching with combination of laser and home bleaching protocol

| Groups | n | Pre-bleaching Mean (SD) | Post-bleaching Mean (SD) | Mean difference (95% CI) | *t*-statistics (df) | *p*-value |
|---|---|---|---|---|---|---|
| Control | 22 | 320.23 (33.242) | 296.86 (51.911) | 23.36 (-1.643, 48.370) | 1.943 (21) | 0.066 |
| LB + HB 3 days | 22 | 310.41 (31.367) | 413.23 (35.232) | -102.82 (-120.258, -85.378) | -12.260 (21) | **<0.001** |
| LB + HB 7 days | 22 | 299.86 (33.901) | 415.18 (38.575) | -115.32 (-134.780, -95.856) | -12.322 (21) | **<0.001** |
| HB 14 days | 22 | 311.05 (27.825) | 423.32 (18.334) | -112.27 (-126.251, -98.294) | -16.703 (21) | **<0.001** |

paired T-test, *statistically significant $p<0.05$

**Table 8:** The comparison of change in surface hardness (VHN) after bleaching with combination of laser and home bleaching protocol

| Groups | N | VHN Mean (SD) | F statistic[s] (df) | *p*-value[b] |
|---|---|---|---|---|
| Control | 22 | 46.09 (39.081) | 15.467 (3) | **<0.001*** |
| LB+ HB 3days | 22 | 102.82 (39.335) | | |
| LB+ HB 7days | 22 | 115.32 (43.895) | | |
| HB 14 days | 22 | 112.27 (31.528) | | |

[a]ANOVA test * statistically significant $p<0.05$
[b] the mean score with significant P=value will be tested multiple comparison Post hoc test (Bonferroni)

**Table 9:** Multiple comparisons of microhardness differences after bleaching regime between groups (N=88)

| Between groups | | Mean difference (95% CI) | *p*-value |
|---|---|---|---|
| Control | LB + HB 3 days | -56.73 (-88.270, -25.185) | **0.001*** |
| Control | LB + HB 7 days | -69.23 (-100.77, -37.685) | **<0.001*** |
| Control | HB 14 days | -66.18 (-97.724, -34.640) | **<0.001*** |
| LB + HB 3 days | LB + HB 7 days | -12.50 (-44.042, 19.042) | 1.000 |
| LB + HB 3 days | HB 14 days | -9.45 (-40.997, 22.088) | 1.000 |
| LB + HB 7 days | HB 14 days | 3.05 (-28.497, 34.588) | 1.000 |

Post hoc test (Bonferroni procedure); *statistically significant $p<0.05$

## Surface roughness

Table 10 shows that there was a significant reduction in surface roughness after treatment with combination bleaching procedures while there was no significant change with HB alone. Table 11 and 12 shows a significant difference in surface roughness change in LB + HB 3 days and HB groups as compared to the control but no significant difference between treatment groups.

**Table 10:** The comparison of surface roughness (Ra) before and after bleaching in each group

| Groups | n | Pre-bleaching Mean (SD) | Post-bleaching Mean (SD) | Mean difference (95% CI) | t-statistics (df) | *p*- value |
|---|---|---|---|---|---|---|
| Control | 22 | 2.18 (0.587) | 2.49 (0.703) | -0.31 (-0.593,-0.027) | -2.277 (21) | **0.033*** |
| LB+ HB 3days | 22 | 2.51 (0.506) | 2.35 (0.430) | 0.16 (0.018, 0.299) | 2.349 (21) | **0.029*** |
| LB+ HB 7days | 22 | 3.04 (0.657) | 2.71 (0.421) | 0.32 (0.129, 0.520) | 3.448 (21) | **0.002*** |
| HB 14 days | 22 | 2.53 (0.565) | 2.41 (0.433) | 0.12 (-0.100, 0.335) | 1.121 (21) | 0.275 |

paired T-test, *statistically significant p<0.05

**Table 11:** The comparison of change in surface roughness (μm) after bleaching with combination of laser and home bleaching protocol

| Groups | n | Roughness differences Mean (SD) | MS | F Statistics[a] (df) | *p*-value[b] |
|---|---|---|---|---|---|
| Control | 22 | 0.59 (0.373) | 0.452 | 3.592 (3) | **0.017*** |
| LB+ HB 3days | 22 | 0.28 (0.207) | | | |
| LB+ HB 7days | 22 | 0.04 (0.398) | | | |
| HB 14 days | 22 | 0.01 (0.403) | | | |

[a]ANOVA test * statistically significant p<0.05
[b] the mean score with significant P=value will be tested multiple comparison Post hoc test (Bonferroni)

**Table 12:** Multiple comparisons of roughness differences after bleaching regime between groups (N=88)

| Between groups | | Mean difference (95% CI) | *p*-value |
|---|---|---|---|
| Control | LB+ HB 3days | 0.31 (0.021, 0.599) | **0.029*** |
| Control | LB+ HB 7days | 0.22 (-0.069, 0.509) | 0.257 |
| Control | HB 14 days | 0.30 (0.008, 0.585) | **0.041*** |
| LB+ HB 3days | LB+ HB 7days | -0.09 (-0.379, -0.199) | 1.00 |
| LB+ HB 3days | HB 14 days | -0.01 (-0.302, 0.276) | 1.00 |
| LB+ HB 7days | HB 14 days | 0.08 (-0.212, 0.366) | 1.00 |

Post hoc test (Bonferroni); *statistically significant p<0.05

**Morphological changes evaluation using SEM.**

SEM analysis revealed that enamel specimens in the control group presented with regular surface morphology, pores and also some superficial irregularities in a certain area. (Figure 1). Effect of bleaching was randomly distributed on enamel surface after combination treatment with morphological changes characterized by waviness, depression, porosities and superficial irregularities of different degree of severity, together with some areas of relatively smooth enamel as seen in Figures 2 and 3. However, morphological surface changes became much more pronounced after HB treatment for 14 days, showing increased depth of irregularities and a number of porosities (Figure 4).

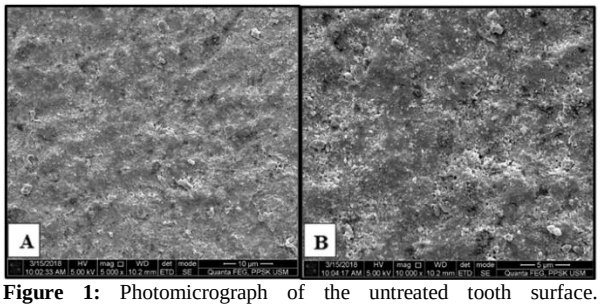


**Figure 1:** Photomicrograph of the untreated tooth surface. (Magnification: left 5000x; right 10000x)

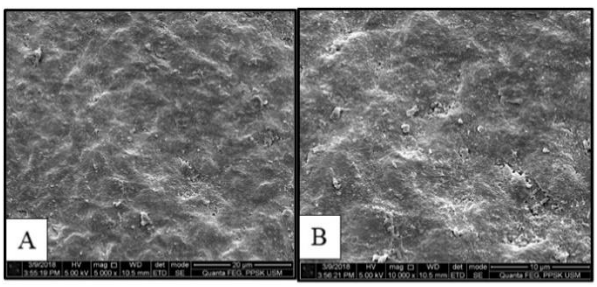


**Figure 2:** Photomicrograph of enamel after laser bleaching and home bleaching for 3 days. (Magnification: left 5000x; right 10000x)

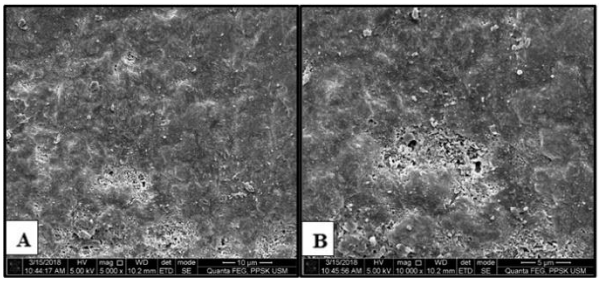


**Figure 3:** Photomicrograph of enamel after treatment with laser and home bleaching for 7 days (Magnification: left 5000x; right 10000x)

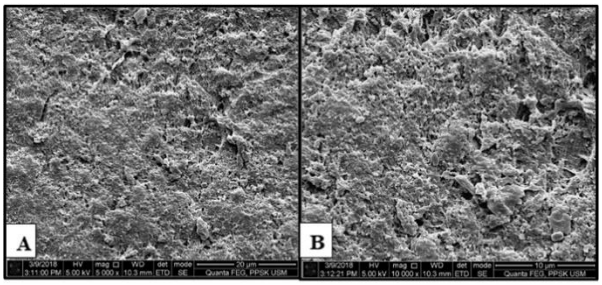


**Figure 4:** Photomicrograph of altered enamel after home bleaching procedures. (Magnification: left 5000x; right 10000x)

## DISCUSSION

Bleaching has been used for a very long time for patients wanting to have clear bright white teeth. As we know, LB and HB is very commonly used now a days. In our study an interest spiking factor is the use of a combination of the two types of bleaching methods. A combination of these two types of bleaching could not only save chairside time, but could also provide us with satisfactory results within a shorter than expected period of time.

In the current study, the rejected null hypotheses, were as follows: it was seen that there was a significant difference in colour changes of teeth, microhardness of teeth, and in enamel

surface morphology after treatment with combinations of laser bleaching and different durations of home bleaching protocol. The accepted null hypothesis was that there was no significant difference seen in enamel surface roughness with combinations of laser bleaching and different durations of home bleaching protocol.

In this study, colorimetric analysis was done using spectrophotometer (Vita esay shade guide). A spectrophotometer is an instrument used for measuring the amount of light absorbed by a bleached tooth sample. It helps in determing the "lightness", "chroma" and "hue".[26] L* values represent the lightness or brightness of the samples, whereby low L* value represent dark or black objects. The highest mean difference in colourimetric assessment for L* value pre and post bleaching treatment were recorded in group LB + HB 7 days (-31.65). In this study, it was found that L* values increased significantly in all groups, which implies that teeth in all treatment groups became whiter after bleaching. Thus, all bleaching procedures in this study proved to be effective at whitening as was found in some previous studies.[5,27] Hence, the null hypthesis was rejected. It is worth noting that, a change in the colour was also found in the control group, nevertheless it was not significant. This result may be due to the storage of control specimens in artificial saliva after staining. Artificial saliva may have washed away the blood or stain molecules. A study by Zeczkowski, M. *et al.*[28], (2015) which evaluated the effect of different storage materials on colour variation, also reported a slight increase in L* value and speculated that it might have occurred because of the constant contact of specimens with saliva. Hence, a minor change that may have occurred in the colour of control group was detected by the spectrophotometer. The pH value of the solution also plays an important role in the degree of discolouration.[29] Low pH is responsible for an increase in tooth staining. While normal blood pH is tightly regulated between 7.35 and 7.45, to reduce the effect of pH on the degree of discolouration and to produce close resemblance to the main cause of tooth discolouration, that is intrinsic staining by oxidation of haemoglobin.

However, no significant difference was found between the two combination groups in this study. The objective of LB is to achieve the process by using the most effective energy source while avoiding or reducing the adverse effects. A laser beam activates HP quickly and thus helps in achieving satisfactory whitening of teeth. A study by Son, J. et al, (2012) indicated that diode lasers have a greater penetration depth compared to other laser systems.[10] Biolase diode laser (Ezlase 810nm) system was used in this study to activate HP, and it was effective in tooth whitening. The results of this study are generally in agreement with other studies on LB, which showed that LB is very efficient.[30-32] It is believed that 46% HP will produce some degree of porosity in the enamel, thus allowing the oxygen free radicals to be trapped in the dental hard tissues for longer periods, eventually facilitating further enhanced bleaching with the application of CP at home. This explains why combination bleaching protocols were found to be significantly more effective than HB alone.

Changes in organic and inorganic content after bleaching treatment affect the mechanical properties of enamel. Microhardness test is one method used to evaluate these changes in mechanical properties.[33] In this study, after all bleaching regimens, enamel surface presented with an increase in microhardness ($P<0.05$). These results are contradictory with some studies[18,19,34-36] but are in agreement with others.[16,37-39] Hence, the null hypothesis was rejected. Determination of loss or gain in mineral contents of enamel after the experiment can be obtained from the differences among the baseline and final microhardness values. A slight reduction in the microhardness in the control group was noted. This is because it was isolated with very low microhardness values and this might be due to the removal of outer hypomineralised enamel layer (often containing fluoroapatite).[37]

Alterations in the mineral content of enamel and dentine are expected to occur during the bleaching procedure due to the acidic properties

of the materials used. However, mineral content in the enamel may not be affected with the use of bleaching agents that contain fluoride, thus preventing demineralization or loss of enamel.[38,40] The 0.11% fluoride ions in the Opalescence PF HB formulation used in the current study, most likely resulted in remineralisation of the enamel. Thus, resulting in maintaining or increasing its microhardness. Hence, post bleaching re-hardening of enamel surface was noted in this study which is supported by the use of fluoridated gel as compared to non-fluoridated gels.[15,17,20]

However, another study found a decrease in microhardness of enamel exposed even to a 10% CP whitening gel.[41] Another factor that may attribute to a loss of mineral content is pH of the bleaching agent. Application of high pH bleaching agent or phosphoric acid before bleaching procedure may enhance the bleaching effect and cause further microhardness reduction.

Therefore, an increase in the concentration, duration and frequency of exposure of tooth structure to HP, fluoridated bleaching gel, and use of saliva as storage medium play a proportional role on the bleaching action and associated sequelae such as effects on enamel microhardness. Hardness reflects the mechanical properties of natural teeth.[19] Although no clinical studies or case reports in the literature have documented macroscopically relevant tissue destruction, it is important to minimize the risk of even minor damage to ensure life-long integrity of dental hard tissues.[15]

The key factors that affect tooth whitening efficacy by peroxide-containing agents are its concentration and time.[42] In this study, surface roughness reduced significantly post-treatment with combination bleaching therapy. However, there was no significant difference between the groups. Hence, the null hypothesis was accepted. Furthermore, in this study, LB + 3 days HB significantly reduced the surface roughness compared to the non-treated group. This result is in line with some studies that compared the effect of diode laser activation on the crystalline structure of enamel.[10,38,41] The study reported that the arrangement of the enamel rods did not break down and was maintained or improved after exposure to laser radiation thus, preventing the roughening of enamel during bleaching. These results lead to the understanding that the damage of tooth structure occurs during the whitening process while using HP. The damage can be significantly reduced or improved by laser exposure and the shorter periods utilized for bleaching treatment.[10,41] Furthermore, the remineralizing effect exerted by the artificial saliva and fluoride presence enhance the effect.[39] A study showed that changes in the surface roughness has an increased risk of microbial adhesion to the tooth surface. Thus, this increases susceptibility to the development of caries and staining.[43]

The chromophores existing in the laser-activated gels can absorb the narrow wavelength of diode lasers, thus improving the efficacy of bleaching with lesser heat generation.[23] It may also be assumed that HP invades into the enamel surface only at the earlier stage and that HP no longer enters into the enamel surface due to the change in property of the whitening gel.[10] However, these studies[10,23,41] did not use artificial saliva as a storage medium. As described in previous studies, the opening of diffusion channels on demineralized enamel facilitates diffusion of fluoride and other ions into deeper enamel layers and enhances remineralization near to non-bleached enamel by blocking the surface pores of enamel. All this justifies the results of this study whereby surface roughness was reduced in all treated groups.

Enamel layer of the specimens was etched with 37% phosphoric acid for 15 seconds to remove the smear layer. Human blood that was obtained from the blood bank was used to stain the specimens like in other studies.[7,48] Some studies also utilized rat blood for the same purpose. The blood was centrifuged at 10,000 rpm for 10 mins and the serum discarded. Then, 40mL of distilled water was added to 60mL of the precipitated blood and this mixture was centrifuged at 10,000 rpm for 20 mins like previous studies.[7,48]

Thus, in this study professional whitening treatment with HP combined with diode laser irradiation and HB with 20% CP containing fluoride did not alter or reduce enamel surface structure. This demonstrated that simulated intraoral conditions, such as temperature, presence of saliva and shorter bleaching treatment result in different outcomes.

The specimens were evaluated for a variety of surface characteristics. In this study, two specimens from each experimental and control groups were evaluated using SEM. Fragments of each tooth were analyzed by comparing to other groups under 5000x and 10000x magnification. Although the number of samples was considered low to represent each group, SEM analysis was done to support the profilometer readings in this study.

Most investigators have shown that the bleaching agent is able to make changes on enamel surface texture, such as topographical alterations, decalcification and porosities.[34,44] Nevertheless, in the current investigation, specimens in the control group did not reveal completely smooth enamel surface morphology, showing pores and surface irregularities, similar to another study.[45] These findings may have resulted due to preparation of samples for microhardness analysis and the variation of the teeth.

In this study, specimens of the bleached group with laser showed relatively smooth enamel surface area, which is in agreement with some studies[10,23,30] that described a reduction and prevention of loss of mineral compositions in enamel.[46] Thus, results of this study clearly demonstrate that laser irradiation during the whitening process not only improves the brightness of a tooth but also prevents enamel structure from deformation. Hence, the null hypothesis was rejected.

Within the limitations of this study, it is rather fair to mention, that a larger sample size could have been used and also, since this was an in vitro study, patient cooperation was not required. Further reseach, such as clinical trials should be untertaken in the future to apprehend findings of this study.

## CONCLUSIONS

LB followed by HB for 3 days was found to be the most effective in whitening stained teeth with positive effects on tooth surface microhardness while maintaining surface topography of enamel. Within the limitations of this study, all bleaching protocols tested were effective in whitening discoloured tooth and all groups showed a significant increase in enamel microhardness.

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## CONFLICTS OF INTEREST STATEMENT

There was no conflict of interest among the authors.

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**Case Report**

# ROOT CANAL FILLING WITH NEOMTA PLUS IN SECOND PRIMARY MOLAR TEETH WITH MISSING SUCCESSOR: TWENTY-FOUR MONTHS OF FOLLOW-UP

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## ABSTRACT

Hypodontia of the mandibular second premolar teeth is one of the most frequent anomalies of tooth development, which reveals a unique hardship for clinicians. Retaining the primary second molars helps to maintain arch integrity until facial growth is complete. This case series investigates the potential improvement and longevity of retained primary mandibular second molars obturation of the root canal system using a mineral trioxide aggregate when successors are missing. Neo MTA Plus (Avalon Biomed Inc, Bradenton, FL) recently introduced calcium silicate-based cement that may have some potential as a root canal obturating material. Nevertheless, no study using Neo MTA Plus as a root canal filling material was found in the literature. Five female patients with a decayed, necrotic second primary molar tooth without a successor were selected and performed a root canal treatment with Neo MTA Plus. Patients were scheduled for clinical and radiographic evaluation at 6, 12, 18, and 24 months. They were followed up for twenty-four months. According to the present study, it can be concluded that NeoMTA Plus may be a proper material for use in the root canal treatment of primary molars with a missing successor.

**Keywords:** Root Canal Filling, Neo MTA Plus, Pulpectomy, Primary Molar, Tooth Agenesis.

***Highlights of the study***

- The complex anatomical irregularities of the primary molars root canal system present technical difficulties for the complete cleaning and obturation of the root canal.
- It was concluded that NeoMTA Plus might be a suitable obturation material in primary molars' pulpectomy with missing successors, while treatment with gutta-percha may reveal some difficulties.
- NeoMTA Plus was not used as a root filling material in previous studies. This case series is the first study that used NeoMTA Plus as a root filling material with successful clinical results.

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## INTRODUCTION

Hypodontia, one of the most common anomalies of dentition, is defined as the congenital absence or agenesis of one or more primary or permanent teeth, excluding the third molars.[1] Agenesis of the mandibular second premolar teeth is one of the most frequent anomalies of tooth development. It reveals a hardship to clinicians in terms of diagnosis and management.[2] Several studies in different populations have reported these teeth to account for more than 40% of all hypodontia cases, making mandibular second premolars the second most common congenitally missing teeth after the third molars.[2] The second premolar agenesis prevalence was reported as 2.4% to 4.3%.[3]

In cases of primary molars without a permanent successor, they remain in place beyond the time of regular exfoliation. Treatment preferences for these over-retention cases comprise preservation of the primary molar as a long-term temporary resolution until the completion of facial growth or extraction of the primary molar. Space closure includes various methods and depends on patient-related conditions such as age, occlusion, and the primary molar tooth and alveolar bone condition.[3,4] These teeth frequently exhibit a tendency to caries owing to prolonged retention in the mouth. Furthermore these teeth include a thin enamel layer than permanent teeth. Furthermore, owing to their large pulp horns, pulpal involvement is frequently observed.[1]

Primary teeth are significantly different from permanent teeth regarding the cellular content of their undifferentiated mesenchymal stem cells. They contain an abundant supply of stem cells in their dental pulp compared to permanent teeth. Mesenchymal cells may give rise to odontoclastic cells in response to either the caries process or the pulp-capping material, resulting in the exaggerated inflammatory response and, consequently, the internal resorption in the primary teeth. Pathologic root resorption is the most common cause of premature tooth loss in primary dentition.[5,6] If the treatment preference is to preserve a primary molar with deep caries lesion including pulpal invasion, it includes a pulpotomy or root canal treatment. Zinc oxide eugenol and calcium hydroxide with iodoform pastes are resorbable when used in pulpectomies. They demonstrate resorption rates similar to the physiologic root resorption rates of primary molars with successors. However, their use might not be ideal in the case of extremely slow root resorption of necrotic primary molars without successors.[7]

If pulpectomy is essential, filling the root canal with gutta-percha is the standard treatment method and the treatment of permanent teeth.[8] non-resorbable root canal filling material application aims to keep the primary molar in dentition as long as possible without root resorption to maintain adequate bone, which has a crucial role in an implant surgery requirement after the patient's bone development comes up to an end.[3,4] Nevertheless, anatomical disorders such as curved fragile primary molar roots with complex internal anatomy may prevent complete removal of the necrotic tissue. That would eventually result in a suboptimal root filling in the root canal systems of primary teeth compared to permanent teeth. As a result, performing root filling with gutta-percha may be more difficult.[9]

Modern pediatric dentistry seeks novel methods for the regeneration of remaining dental tissues to preserve primary teeth and maintain their developmental, esthetic, and functional capabilities. For this purpose, biocompatible materials such as bone morphogenetic proteins, osteogenic protein–1, demineralized dentin, and mineral trioxide aggregate have previously been studied.[10] More recently, mineral trioxide aggregate (MTA) has been recommended for root canal obturation in retained primary teeth due to its capacity to provide a biocompatible material. It has been revealed that MTA has excellent sealing ability, and it stimulates hard-tissue healing in lateral root perforations, internal resorption, furcal perforations, and as a root-end filling.[10] MTA is a dental material used extensively for vital pulp therapies (VPT), protecting scaffolds during regenerative endodontic procedures, apical barriers in teeth with necrotic pulps and open

apices, perforation repairs, as well as root canal filling and root-end filling during surgical endodontics.[11] MTA has been used for many other applications, including primary teeth pulp capping and pulpotomy.[12] Several case reports have been published describing the use of MTA as an alternative root canal filling material for primary teeth without successors.[13]

However, long-setting, tooth discolouration, high cost, and complex handling characteristics have emerged as potential drawbacks.[10] Recently, new bioactive materials have been introduced and one of them was NeoMTA Plus. This silicate-based hydraulic tricalcium contains tantalum oxide as a radiopacifying agent in place of bismuth oxide, thus avoiding discolouration and can enhance the mineralization potential by inducing differentiation into mineralsecreting cells.[14] Furthermore, it has been reported that NeoMTA Plus has a capacity to release calcium, to prevent bacterial leakage, to reveal adequate radio-opacity, and it has good sealing ability. Thanks to these features of MTA, it can be used as an endodontic sealer or cement repair.[15]

## METHODS

### Case Series

*Preoperative Findings*

In this study, following orthodontic consultations, only patients with minimal crowding (1-3 mm) or perfect alignment according to the Little's Irregularity Index in the lower arch and those with no class II molar relationship, mandibular retrusion, and other orthodontic anomalies were included. Patients with infraocclusion and ankylosis were also excluded. Moreover, patients were followed up periodically for infraocclusion and ankylosis since either infraocclusion or ankylosis greater than 1 mm would indicate extraction. Infraocclusion or ankylosis would affect bone height and require bone grafting for any future implant restoration. However, infraocclusion or ankylosis was not observed among the participants.

A total of five healthy patients were referred to the Department of Pediatric Dentistry of the Afyonkarahisar Health Science University Faculty of Dentistry in Afyonkarahisar, Turkey, for endodontic and restorative management of primary mandibular second molars with missing permanent successors. All affected teeth presented with deep carious lesions or failed restorations and sinus tracts in the absence of sensitivity to percussion and tenderness to palpation. Following consultations with the department of orthodontics, a treatment plan involving endodontic and restorative treatment of the molars was made to prevent malocclusion and maintain the existing alveolar dimensions. Molars had healthy occlusal relationships, with no sign of ankylosis or infraocclusion. A radiographic examination revealed periradicular and interradicular radiolucency without internal or external root resorptions and approved the congenital absence of second premolars.

*Treatment Protocol*

All molars received the same two-visit root canal protocol. Pulpectomies were performed as described below. At the first visit, an inferior alveolar nerve block was performed by administering 2% lidocaine with 1:100 000 adrenaline (Astra Pharmaceutical Products, Westboro, Mass., USA); caries was removed using a tungsten-carbide bur in a slow speed handpiece was used to excavate caries after using a water-cooled aerator. Following the removal of the coronal pulp, the diagnosis of pulpal status was determined. If the radicular pulp presented continuous bleeding, the diagnosis was “irreversible pulpitis”; however, if no pulp tissue remained when the pulp chamber was accessed or in cases suppuration or purulence presented, the diagnosis was “pulp necrosis.” The working length was determined using a size 15 sterile K-file to 2 mm short of the radiographic apex. Intra-canal tissue was extirpated using a barbed broach (Dentsply/Maillefer, Ballaigues, Switzerland), and the canals were prepared with Protaper Gold (PTG, Dentsply Maillefer, Ballaigues, Switzerland) until a master file size of F2 File was reached. Each root canal orifice was irrigated between instruments with 2 mL of 5.25% sodium hypochlorite (NaOCl) (Promida 5.25%, Promida, Eskişehir, Turkey) using a 27-gauge dental irrigation needle with round close end & round

open. It was positioned approximately 2–3 mm below the root canal orifice and final irrigation with 5 mL of sterile saline was performed. The canals were dried with pre-measured paper points up to 2 mm from the root apices and were dressed with non-setting calcium hydroxide for one week (Metapaste, Meta Biomed, Chungbuk, Korea).

At the second visit, after the removal of calcium hydroxide, NeoMTA Plus (Avalon Biomed, Bradenton, FL, USA) under aseptic conditions, NeoMTA Plus were mixed according to the manufacturers' instructions, dispense one scoop (1 gr) of NeoMTA Plus powder on a glass slab done one drop of MTA Plus Gel as a streak 0.5–0.75 inch long next to the powder. After that, a putty-like consistency material was placed in the canal via MTA carrier (Sybro Endo, Orange, CA, USA) and compacted using endodontic pluggers. The MTA was allowed to set entirely by placing a cotton pellet moistened with sterile water inside the pulp chamber for 15 minutes and sealing the access cavity with glass ionomer cement (Fuji IX, GC, Tokyo, Japan). Furthermore, the cavities were permanently restored with composite resin incrementally and final restoration was done by stainless steel crown (Clearfil Majesty Posterior A2, Kuraray, Osaka, Japan) as a final restoration (Figure).

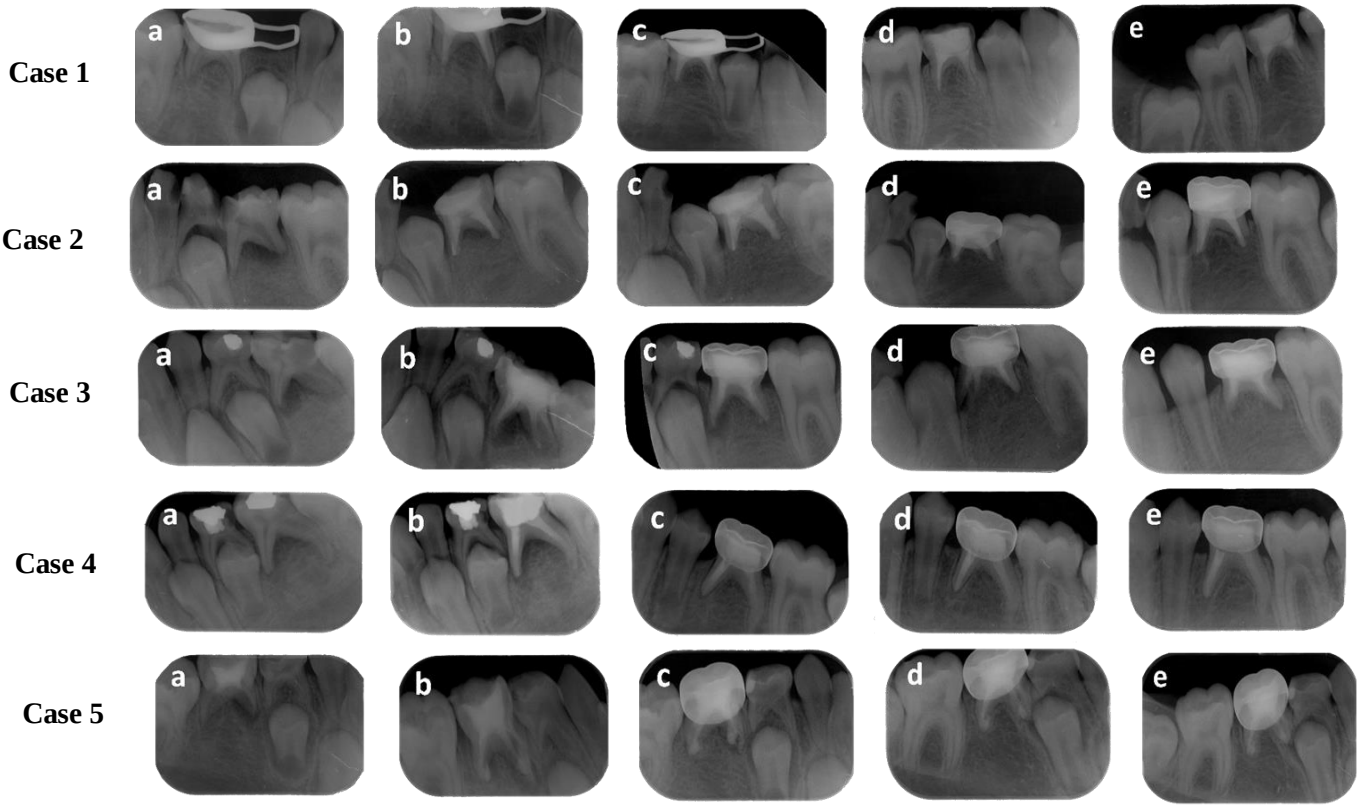


**Figure:** Root Canal Filling with NeoMTA a: Preoperative radiograph of the tooth. b: 6 months of follow-up. c: 12 months of follow-up. d: 18 months of follow-up. e: 24 months of follow-up

## DISCUSSION

The treatment preference for agenesis of the mandibular second premolar teeth depends on the patients' specific conditions and the second primary molar tooth. In adult patients, treatment options include single-tooth implants, autotransplantation, and prosthetic restoration. Long-term follow-up studies in pediatric patients have provided evidence that properly-maintained primary molars without successors remain until middle age in some cases.[3,4] Mandibular second premolar agenesis treatment preferences should merely be considered after orthodontic consultation.[7] Early removal of the primary second molar initiates a series of changes, including a reduction in arch length, an inclination of adjacent teeth, alveolar bone resorption, extrusion of the antagonist's tooth, and alterations in tongue posture, which may all be challenging to

treat orthodontically.[3,6] However, it is recommended to maintain the second primary molar in cases of class II occlusion, mandibular retrusion, polydiastema and non-crowded dental arches.[7] While the latter option poses the risks of infraocclusion and ankylosis if the patient is followed up closely, retaining primary second molars in a restorable condition can be the best space and alveolar bone maintainers, representing a semi-permanent solution in non-crowded dental arches until alveolar development is complete. After this stage, implant therapy can be performed.[3]

Two features of root canal filling materials that may dramatically affect treatment prognosis are sealing ability and antibacterial effectiveness, considering that MTA has been revealed to possess superior biocompatibility and sealing ability. When used for repair of perforations, VPT, root-end fillings, and when used as an apical plugin, many laboratories, animal and clinical studies, including those examining bacteria and endotoxin leakage.[10,16] MTA has been revealed to possess antibacterial features useful against bacteria comprising *Enterococcus faecalis*, suggesting that any bacteria remaining in the ramifications of the root canal system may have been eliminated or at least dramatically reduced.[16] Tunc and Bayrak[13] emphasized that white MTA treatment in primary molar teeth has been successful in the 3 years. Teeth with irreversible pulpitis represent a significantly higher treatment success rate than teeth with necrotic pulp.[17] A two-stage treatment method with the use of calcium hydroxide as an inter-appointment dressing has been proposed as a standard for infected teeth in order to maximize bacterial reduction and enhance treatment outcomes.[18]

Moreover, in histological studies, it has been emphasized that using an antibacterial agent between sessions in teeth with apical periodontitis showed better outcomes. The two-stage treatment method with calcium hydroxide dressing was used in all the cases in this study. No significant difference in success rates was found between one and two-stage treatment methods.[19]

If a hermetic seal cannot be maintained through obturation, tissue fluids may enter the canal and provide additional nutrition to any remaining bacteria.[20] Application of stainless steel crowns (SSC) has been recommended after the pulpal treatment of primary teeth.[8] However, in this study, four of the treated teeth were restored with composite resins and SSCs. (one of the teeth had a fixed space maintainer, previously).

This study had some limitations. We did not use rubber-dam clamps for all patients because some could not tolerate the rubber-dam clamps pressure and latex frame. Additionally, we could not use SSC to increase the survival rate and bite blocks in some patients in order to achieve standardized radiographs for endodontic treatment follow-up. Although the use of bite blocks is frequent, this method was not an option in the present study because the study subjects were in the mixed dentition stage, characterized by alterations in occlusal development.[20]

## CONCLUSIONS

The good results obtained with NeoMTA Plus can be attributed to the material's sealing ability, antimicrobial and anti-inflammatory properties. In this study, successful results was observed in the cases treated with NeoMTA Plus. Within the limitations of this study, it can be concluded that NeoMTA Plus can be recommended for use in the root canal treatment of primary second molars without successors based on radiographic evidence.


## *ÖZ*

*Mandibular ikinci premolarda görülen hipodonti, diş gelişimindeki en sık görülen anomalilerden biridir ve klinisyenler için nadirde görülse zorluklar ortaya çıkarır. Süt ikinci azıların ağızda tutularak idame edilmesi, yüz gelişimi tamamlanana kadar ark bütünlüğünün korunmasına yardımcı olur. Bu olgu serisinde, yerine gelecek daimi dişin olmadığı ve kök kanal sistemlerinin mineral trioksit agregat (MTA) ile kapatıldığı durumlarda, süt mandibular ikinci molarların potansiyel gelişim ve ömürleri incelendi. NeoMTA Plus (Avalon Biomed Inc, Bradenton, FL) yeni tanıtılan, kök kanal dolum materyali olarak kullanılabilen kalsiyum silikat bazlı bir simandır. Bununla birlikte literatürde NeoMTA Plus'ın kök kanalı*

*dolum materyali olarak kullanıldığını belirten herhangi bir çalışma bulunmamaktadır. Altında daimi diş germi olmayan, çürük veya nekrotik süt ikinci molara sahip beş hasta seçilip, bu hastalara NeoMTA Plus ile kök kanal tedavisi uygulandı. Hastalar klinik ve radyografik değerlendirmeler için 6, 12, 18 ve 24 ay sonra kontrol seanslarına çağırıldı. Hastalar 24 ay süresince takip edildi. Günümüzdeki çalışmaya göre NeoMTA Plus'ın, altında daimi diş olmayan süt molarların kök kanal tedavisinde kullanılmak üzere uygun bir materyal olduğu sonucuna varılmıştır.*
***Anahtar kelimeler:*** *Kök Kanal Dolumu, NeoMTA Plus, Pulpektomi, Süt Molar, Diş Agenezisi.*

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**Review**

# AN EXPLORATORY REVIEW OF CURRENT TRENDS IN NANODENTISTRY

## ABSTRACT

Nanotechnology is a cutting-edge concept that is evolving manifolds in various fields of science and medicine and is by no means exceptional to dentistry. Nanotechnology is popularly known as the 'science of the small' that deals with particles of size 1-10nm. Methods like top-down or bottom-up approaches are used in manufacturing nanoparticles and nanorobots, catering to the needs of medical diagnostics and therapeutics. Nanorobotics advances medicine through miniaturization from microelectronics to nanoelectronics. Nanotechnology can be applied to all fields of dentistry such as to create nano implants, nano-drug delivery systems, nanocomposites and nano impression materials. Additionally, it helps in orthodontic tooth movement, alleviating hypersensitivity, and effective anesthesia. This paper highlights the various applications of nanotechnology in dentistry and also mentions the clinical trials performed to have a more focused approach to practicing nanodentistry. Apart from this the paper briefly explains the benefits of integrating artificial intelligence and nanotechnology for creating more personalized treatment options and also its role in Covid 19 vaccines.

**Key words:** Nanotechnology, artificial intelligence, nanoparticles, nanocomposite.

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## INTRODUCTION

Nanotechnology is popularly known as the 'science of the small' that deals with particles of size 1-10nm.[1] Richard Feynman introduced nanotechnology in the year 1959 and is considered the father of nanotechnology.[2] Nanomedicine is a field that is transforming the approach in diagnosis, treatment, prevention of diseases, alleviating pain, improving human health by using the molecular tools and molecular knowledge of the human body.

Nanoparticles in medicine can enter living cells effortlessly as they are much smaller than those used in chemical and industrial applications. There are two approaches to fabricate nanomaterials. The bottom-up approach creates nanoparticles from molecular components that self-assemble via molecular recognition, and the top-down approach builds nano-objects from larger entities.[3] Table 1 gives examples of nanomaterials fabricated through the two approaches.

**Table 1.** Nanomaterials fabricated through top-down and bottom-up approach.

| Top-down approach | Bottom-up approach |
|---|---|
| Nanocomposites | Dentinal hypersensitivity |
| Nanosolutions | Nanodentifrices |
| Nanocapsulation | Local anesthesia |
| Bone replacements | Tooth repair |
| Impression materials | Tooth positioning |
| | Oral cancer diagnosis |

### *Nanotechnology and dentistry*

Similar to nanomedicine, nanodentistry has evolved multi-folds and has diverse applications in all fields of dentistry. It holds a good promise of providing personalized treatment options with improved efficacy and reduced side effects. In the past few years, numerous reviews have been done in the literature on nanodentistry. However, clinical trials focusing on the practice of nanodentistry are limited due to factors like cost, technique sensitivity, and obtaining similar results to other contemporary materials. Thus, we look forward to highlighting the clinical trials undertaken recently in the different fields of Dentistry in this exploratory review to have a more focused approach to practicing Nanodentistry rather than seeing it as a future perspective. Thus, Nanodentistry is not the future but is happening now, and let's look forward to becoming nanodentists.

### *Nanoparticles*

Nanoparticles include particulate substances, which have a size of 100nm at least in one dimension. Nanoparticles are composed of three layers; the surface layer, shell, and core layer.[4] A variety of small molecules, metal ions, surfactants, and polymers make up the surface layer. The shell layer is a chemically different material from the core in all aspects, and the core is the central portion of the nanoparticle.[5] Based on the chemical and physical properties they can be classified as carbon-based nanoparticles, metal-based nanoparticles, ceramic-based nanoparticles, polymeric-based nanoparticles, lipid-based nanoparticles, semiconductor-based nanoparticles.[6] (Figure 1)

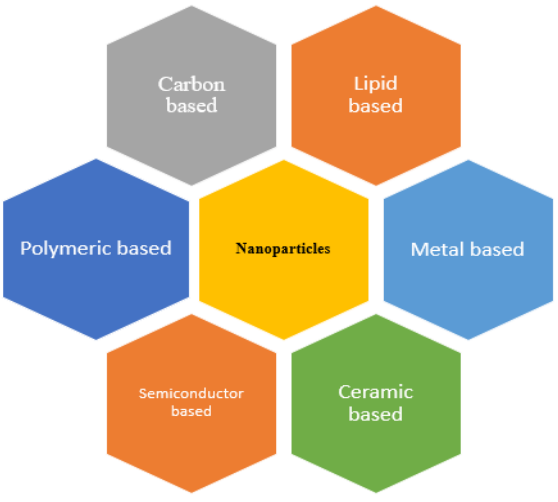


**Figure 1.** Classification of nanoparticles based on physical and chemical properties.

### *Nanorobots*

Nanorobotics pioneered by Adriano Cavalcani is a technology which converts nanoparticles into miniature nanorobots.[7] These Nanorobots consist of carbon atoms in a diamondoid structure which

include parts like Manipulator's gripper, Telescopic macromanipulator, Biomolecular Sensor, Acoustic Sensor, Antenna, Connector, and others as portrayed in figure 2 Nanorobots have inert properties, which evades the reaction of the immune system, thereby allowing them to have an unimpeded function.[1]

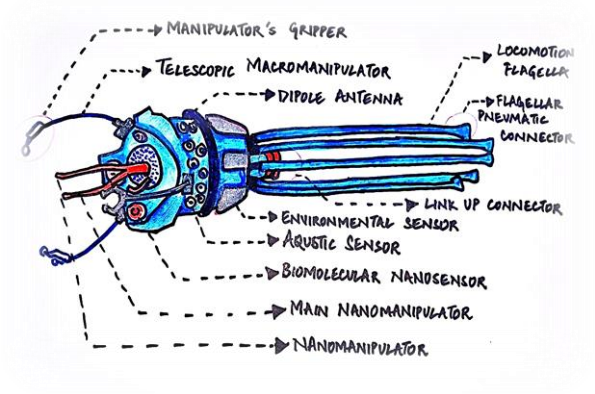


**Figure 2.** schematic diagram of a Nanorobot

They use glucose or natural body sugars and oxygen as a source for propulsion.[8] Each nanorobot could be modified with specific functions depending on the biochemical stimulus provided.[1] Nanorobots are expected to provide advances in medicine through miniaturization from microelectronics to nanoelectronics.[9] The application of nanotechnology in the field of Artificial intelligence is the future of science and this combined technology when integrated in the field of medicine would cater to improvise various setbacks in the diagnosis and the therapeutic aspects of medicine as well as in dentistry which has also been briefly explained in this article.

**Recent Advances in Nanodentistry**

Broadly materials used in medicine have been classified into 3 main avenues - diagnostics, drug delivery and bone grafts and implants.[10]

***Nanodiagnostics***

Oral fluid nanosensor test (OFNASET): has been recently introduced in the market to detect cancers through salivary biomarkers. OFNASET uses bionanotechnology, cyclic enzymatic amplification, and microfluidics self-assembled monolayers (SAM) which gives accurate results.[11]

**Nanoscale cantilever:** Another nanodiagnostic device that helps in the rapid detection of cancer-related molecules that are flexible in build and resemble rows of divided boards. The cancer-related molecules bind to the sensors in the device and cause conformational changes in shape.[12]

**Nanopores and nanotubes:** Nanopores help in efficient DNA sequencing by acting as a filter for DNA strands. Nanotubes are made of carbon that helps in detecting altered genes.[12]

**Quantum dots (QD):** They are nanocrystals that fluoresce when illuminated by ultraviolet light, made of semiconductor materials. They bind to proteins associated with cancer cells and therefore help with the detection of cancer. These fluorophores have unique photophysical properties that also overcome the limitations of using conventional dyes. They are also known to detect metastasis of cancer.[13]

**Optical Nanobiosensor:** It is a fiber-optic-based, compact analytical system that detects substances by producing a signal that is proportionate to the concentration of the measured substance. They contain a biological element such as an enzyme, protein, nucleic acid, or a receptor that recognizes the substance and passes it to the inbuilt optical transducer which in turn produces a signal. They are highly sensitive and specific devices and are cost-effective.[14]

**Lab-on-a-chip methods:** These are silicon chips with chemically activated beads embedded in them. They help in analysing and diagnosing various diseases by detecting biomarkers. They are cost-effective and require a small sample size.[15]

**Nano CT:** Nano CT one of the Diagnostic methods like micro CT have been used in dental applications like evaluation of various materials like composite using resolution of around 450 nm compared to micro CT evaluation which has a resolution of 2 µm. This clearly shows us that greater details of the ultrastructural properties could be evaluated. Thus nano diagnostics could be useful in effective and critical evaluation of biomaterials as well as their action in the respective sites could be studied in detail. In soft tissue evaluation contrast -enhanced nano CT reveals soft tissues like the ones stained with

Haemotaoxylin and Eosin stain of odontoblasts. The nano contrast CT involves the use of a specific contrast dye which depends on the tissue type and volume. One of the most important applications of nano CT is the 3D visualization of the dental tissues and subsequent spatial analyses, both descriptive and quantitative.[16]

### *Nano Drug Delivery systems*

Oral infections are very complex diseases, thus requiring antimicrobial nano drug delivery systems to combat the same which aid in site-specific wound healing.

Nanoelectromechanical systems (NEMS) are devices integrating electrical and mechanical functionality on the nanoscale level.[17] They make use of motors, pumps or mechanical actuators to form a functional, biological and chemical nano systems. Bio-nanoelectromechanical systems (BioNEMS) are bio-hybrid systems that combine biological and structural elements on a nanoscale level. They include DNA or proteins combined with mechanical nanostructures. It can develop low-cost, biocompatible, and controlled drug delivery devices. It is available in both sustained and pulsatile delivery of the drug. It works as multiple reservoir-based devices, simultaneously delivering multiple drugs.[18] Figures 3 and 4 show drug delivery devices that work on an osmotic pump and spring-powered pump.

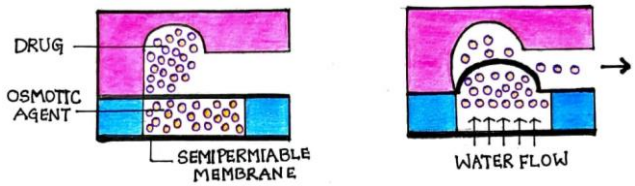


**Figure 3.** Osmotic pump

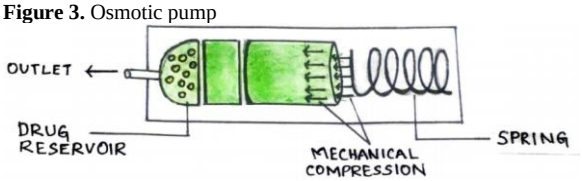


**Figure 4.** Spring powered pump

### *Nanoneedles and nanotweezers*

Nano needles made of silicon are now being developed to aid in effective and sustained drug delivery. They are fabricated using various procedures such as Vapour– liquid–solid (VLS) growth of nanoneedles, Metal- assisted chemical etch of nanoneedles and Focused ion beam etch of nanoneedles. There are many types of nano needles such as solid, porous and hollow nano needles based on the loading and release characteristics of the needle.[19] Nowadays needles with nanosized stainless steel crystals incorporated into them are available commercially (Sandvik Bioline 1RK91™, Sandvik, smt.sandvik.com).[12]

Nanotweezers are nano devices which help in the construction of the human cell. Recent studies reported in literature shows that nanotweezers could be used to manipulate single organelles by trapping and extracting them from various parts of the body. These tweezers effectively interact with single cells thus helping us to understand their signaling pathways of interaction during an external stimuli.[20]

### *Nano coated Dental Implants*

Dental implants have undergone various advances, however, share few drawbacks too. A major disadvantage for titanium alloy as oral implant material is relatively poor wear resistance. To overcome this drawback, nanostructured ceramic coatings such as TiN (titanium nitride) (Is this a ceramic material?), ZrO2/Al2O3, Si3N4/ TiO2, and ZrO2/SiO2 are used.[21]

Many studies have demonstrated increased function of osteoblasts on nanoparticles compared to conventional materials (Figure 5).

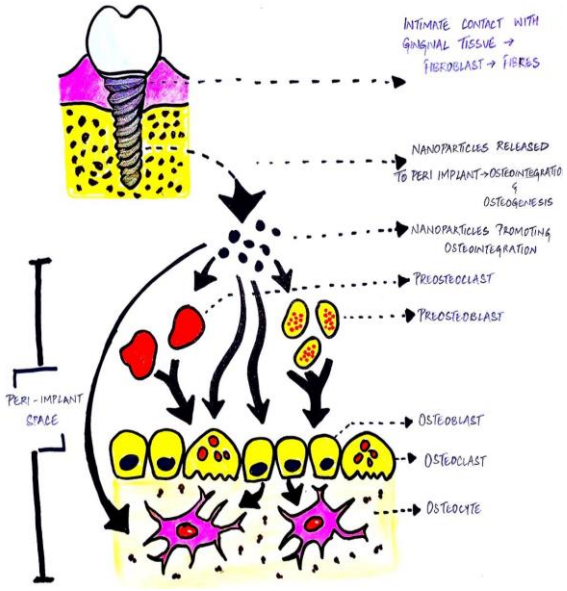


**Figure 5.** Nanoparticles promoting osseointegration.

Yao *et al.* created nanometer surface features on titanium and Ti6Al4V implants by anodization, a

quick and relatively inexpensive electrochemical method. The results showed that the anodized surfaces had higher root-mean-square roughness at nanoscale dimensions than the unanodized Ti-based surfaces.[22] In addition blood clot retention and osseointegration is improved. Anodized Ti6Al4V dental implants show better results when placed in regions with poor bone quality or in cases with immediate loading protocol.[23]

The versatility of nanomaterials may allow the fabrication of implants with various porosities, pore shapes, and mechanical properties that can mimic the complex architecture of bone-specific sites to optimize bone tissue regeneration.[24] Table 2 summarizes the clinical trials done on nano implants.

**Table 2.** Clinical Trials on Dental Implants

| Sl.no | **year** | **Author** | **Nanomaterial used** | **Clinical trial done** |
|---|---|---|---|---|
| 1 | 2018 | Zia Arshad Khan[25] | Nanopore surface implant | **Evaluation of peri-implant tissues around nanopore surface implants with or without platelet-rich fibrin: a clinicoradiographic, randomized clinical study** showed that the mean difference in the probing depth between the two groups was insignificant. Thus, it was concluded that additional graft materials would not be required when using a nanopore surface implant. |
| 2 | 2010 | Luigi Canullo [26] | e-PTFE membrane and nanostructured Mg-HAP | **Early implant loading after vertical ridge augmentation (VRA) using e-PTFE titanium reinforced membrane and nanostructured hydroxyapatite a 2- year prospective study** aimed to evaluate survival of implants, loaded 14 weeks after vertical ridge augmentation (VRA). It was concluded that VRA around rough surface implants using e-PTFE membrane and nanostructured Magnesium-Hydroxyapatite can be successful even in cases with early loading. |

***Graft materials***

Nanotechnology enhances the characteristics of bone grafts by improving the uptake, biocompatibility, proliferation of the tissues, and pore size. An optimal interconnected porous network of micro and nanoscale features allows increased bone formation, cellular migration, proliferation and vascularization.[27]

Bone grafts essentially consist of scaffold, cells and extracellular matrix. Incorporation of carbon nanotubes and nanofibers enables the flexibility and strength, thus providing enhanced mechanical properties. Cellular adhesion through adsorption of proteins like fibronectin causes bone formation. The extracellular matrix thus formed shows increased vascularization, osteoblastic adhesion and also the resultant osseointegration providing a potential bone healing property.

Polyvinyl alcohol shows increased mechanical strength, excellent biocompatibility and less metallic impurities.[28]

Bionanocomposites have found application as scaffold matrix by way of their increased surface area to volume ratio, close contact to surrounding tissues, increased biocompatibility, osteoconductivity, cell adhesion properties, cellular proliferation.

Alginate infused with bioactive glass has been found to have increased biomineralization, protein adsorption properties, cellular adhesion and proliferation of human periodontal ligament fibroblasts.[29]

Other interesting facts were that of increased ALP activity of the human PDL fibroblast cells cultured on the scaffold.

Gelatin has shown to have excellent cell adhesive properties, high biocompatibility, biodegradability, low immunogenicity. In periodontal regeneration, scaffolds incorporated with nanoparticle on gelatin in the concentration of 2.5% gel /2.5% HA and 2.5% gel/ 5% HA have been shown to have properties such as cell attachment, proliferation of PDL fibroblast cells.[30]

Chitosan another nanobiocomposite has shown very minimal foreign body reaction. The positive cell surface charge has enabled cell growth, attachment, and cell differentiation.

A major advantage of chitosan is that when placed in hydrated environments, chitosan turns into a flexible material which is more rigid than materials like PLA, PGA. Studies have shown that use of a combination of chitosan along with bioactive glass nanoparticles have shown improved bioactivity, promoted human PDL cells metabolic activity. This suggests its use as a guided tissue regeneration membrane.[31]

Different surface morphologies are required to enhance the proliferation of osteoblasts and fibroblasts. By maintaining some fundamental constraints regarding pore size and interconnectedness the response of osteogenic cells and their precursors to micro and nanotextured morphologies on planar surfaces can be reproduced on three-dimensional scaffolds.[24]

Documented disadvantages of scaffolds include inflammation, limited cell turnover, and growth factor expression.[14]

Clinical reports, highlight the use of nanofibers that release antimicrobial particles, hence paving the way for use as a scaffold for tissue engineering purposes.[32] The self-assembly property of the nanomaterials at body temperature directs bone growth; combating multifactorial diseases like periodontal disease and Osteomyelitis.[33] Self-assembling peptides like RAD16-I when transplanted, can assist with new callus formation and inhibit demineralization.[34]

***Nano Antihypersensitivity Agents***

Hypersensitivity has become a common problem encountered in dental setup. In hypersensitive teeth, the dentinal tubules have surface densities eight times more than nonsensitive teeth.[35] Nanotechnology has effectively addressed this problem by creating nanorobots that traverse through the dentinal tubules and occlude them providing relief to the patient.[36] Also, the nanoparticles have remineralizing property which helps in reducing sensitivity.[37] Table 3 shows the clinical trials conducted using nanomaterials for treating dentinal hypersensitivity.

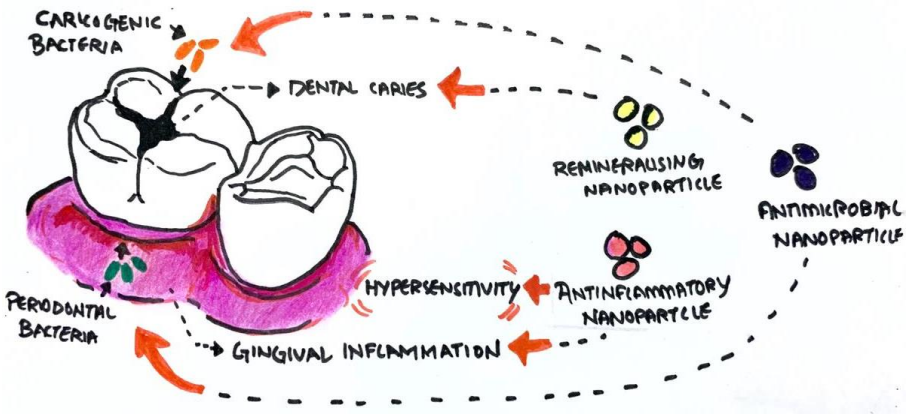


**Figure 6.** Remineralization, antimicrobial and anti-inflammatory properties of nanoparticles.

**Table 3.** Clinical Trials conducted using nanomaterials for treating dentinal hypersenitivity.

| Sl.no | Year | Author | Nanomaterial | Clinical trial outcome |
|---|---|---|---|---|
| **1** | 2019 | James R Fernando[38] | $SnF_2$ and CPP-ACP nanocomplexes | **Self-assembly of dental surface nanofilaments and remineralization by SnF 2 and CPP-ACP nanocomplexes.** This uses in vitro studies and a double-blind, randomized controlled, cross-over design in situ clinical trial and shows that SnF2 and CPP-ACP interact to form a nanofilament coating on the tooth surface and that together they are superior in their ability to promote dental remineralization and reduce hypersensitivity. |
| **2** | 2014 | Michele Vano[39] | Nanohydroxyapatite | **Effectiveness of nano-hydroxyapatite toothpaste in reducing dentin hypersensitivity: a double-blind randomized controlled trial.** The findings of the study encourage the application of nano-hydroxyapatite in fluoride-free toothpaste as an effective desensitizing agent providing quick relief from symptoms after 2 and 4 weeks. |

***Nano aided Anesthesia***

Navigation technology in the form of nanorobots also termed as nanobots induces oral analgesia. When these nanorobots reach the pulp, it results in reversible and temporary loss of pain and sensitivity in the tooth or area of interest. They can be computer-controlled by the dentist, and this property helps to reverse or restore the anesthetic activity.[40]

Nanotechnology-modified liposomal nanoformulations are small nanovesicles encased in a phospholipid bilayer.[26] Which has effective pain control and aiding quick recovery.[41,42] They also reduce the side effects of the anesthetic; for example, liposomal bupivacaine maintains a minimum concentration needed to deliver the therapeutic effect for 3 days without increasing the concentration of the active constituent in the body. In conclusion, liposomal bupivacaine has reduced cardiac toxic effects.[43]

***Nano aided Orthodontics***

Nanoelectromechanical systems (NEMS) have their broader application in orthodontics recently. Electrical energy generates mechanical forces which enhance tooth movement as shown in animal studies.[44] These animal studies reveal that electric stimulation using low amperes enzymatic micro battery (15-20 microamps) enhances cellular enzymatic phosphorylation activities, which lead to accelerated bone remodeling. This device uses glucose as the fuel for the micro battery and is placed on the gingiva near the alveolar bone to facilitate orthodontic tooth movement. (Figure 7) But several issues have to be considered, such as the effect of food, pH, and biocompatibility.[44]

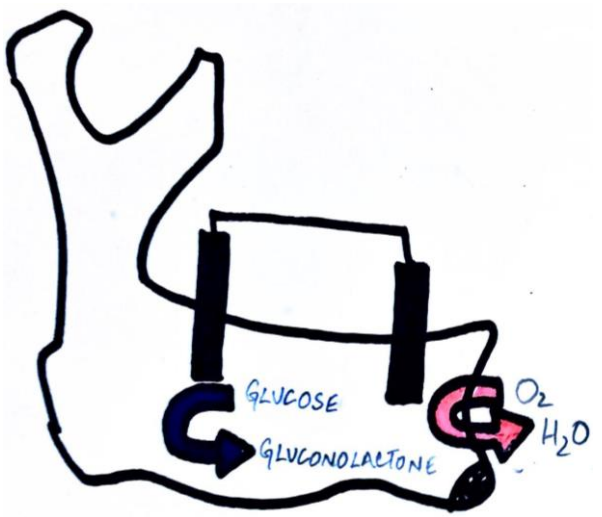


**Figure 7.** Oral biocatalytic fuel cell. The following reaction generating electricity for enhancing orthodontic tooth movement occurs: Glucose+O2→gluconolactone+H2O/H2O2

Temporary anchorage devices manufactured with smooth titanium surfaces when aided with titanium nanotubes enhance initial osseointegration and serve as an interfacial layer between the newly formed bone and the

temporary anchorage devices during orthodontic tooth movement.[45]

***Nanotechnology in the field of restorative dentistry and endodontics has seen major research in 4 main types of material- composites, GIC, adhesives and endodontic materials.***

***NanoComposites***

Incorporating nanofillers in composites improved material characteristics like the smoothness of surfaces resulting in better aesthetics. The remanent minute irregularities formed during finishing and polishing are way smaller than the wavelength of visible light (0.4-0.8 micrometers) thereby minimizing the reflection of light thus warranting good optic properties. They also improve the strength of the composite and reduce polymerization shrinkage.[46]

A rechargeable nano-amorphous calcium phosphate (nACP) filled composite resin (smart material) helps neutralize the acids produced by the bacteria preventing secondary caries. Additionally, the levels of calcium and phosphorus are also maintained.[47] (Figure 8)

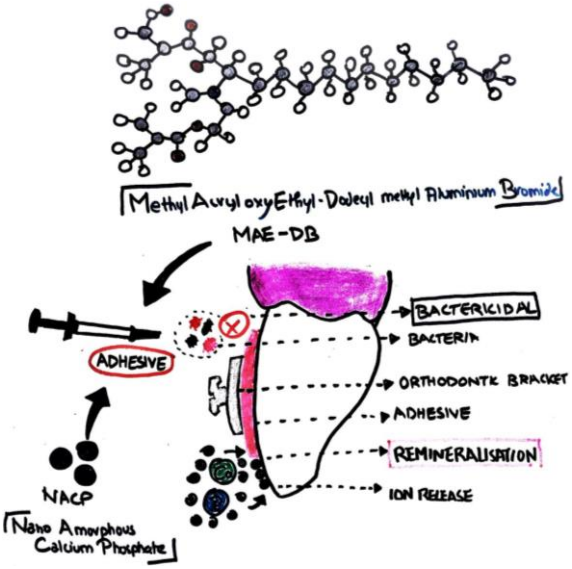


**Figure 8.** Remineralization and bactericidal effects of nACP.

Dental nanocomposites (bionanocomposites) contain nanofillers and nanofibers with a photopolymerizable resin matrix. The distribution of nanofillers is such that there is increased filler load which in turn increases the viscosity leading to better mechanical properties and reduced polymerization shrinkage. Due to reduced particle size, load-bearing stress is reduced, which results in inhibition of crack propagation.

Nanoparticles of dicalcium phosphate anhydrous- (DCPA-) whiskers, tetracalcium phosphate- (TTCP: Ca4(PO4)2O-) whiskers, kaolinite (Al2Si2O5(OH)4), and calcium fluoride are incorporated, which resulted in the highest release of calcium, phosphorous and fluoride owing to their reduced particle size. In addition, calcium and phosphorus release was increased six times in acidic conditions. Due to the unique structure of kaolinite (large surface area), the adsorption of fluoride is higher and therefore can also provide sustained release of fluoride.[48] Table 4 represents the clinical trials done on nanocomposite.

**Table 4.** Clinical trials on nanocomposites

| Sl. no | year | Author | Nanomaterial | Clinical trial |
|---|---|---|---|---|
| **1** | 2014 | Mary A S Melo[49] | Nanostructured Hybrid Fluoridated Restorative Composites. | In Situ Response of Nanostructured Hybrid Fluoridated Restorative Composites on Enamel Demineralization, Surface Roughness, and Ion Release. |
| **2** | 2014 | Jan W V van Dijken[50] | Nanohybrid composite | A randomized 10-year prospective follow-up of Class II nanohybrid and conventional hybrid resin composite restorations<br>This 10 year follow-up study showed good clinical effectiveness of nanohybrid composite in extensive class 2 restorations. |
| **3** | 2013 | Wei Qin[51] | | Two-year clinical evaluation of composite resins in non-carious cervical lesions<br>Both restorative materials exhibited acceptable clinical performance in class 5 non carious lesions 2 years post restoration. |
| **4** | 2013 | Umit Candan [52] | fiber-reinforced nanofilled resin composite | Clinical performance of fiber-reinforced nanofilled resin composite in extensively carious posterior teeth of children: 30-month evaluation<br>13 month evaluation of nanofilled resin composite applied with or without glass-fiber layering showed similar and good results in large cavities of posterior permanent teeth in children. |
| **5** | 2012 | Lei Cheng.[53] | amorphous calcium phosphate and silver nanocomposites | Effect of amorphous calcium phosphate and silver nanocomposites on dental plaque microcosm biofilms<br>Novel amorphous calcium phosphate(NACP) and silver nanoparticles (NAg) nanocomposites possess good mechanical and antibacterial composites reducing biofilm viability and lactic acid production. Thus promising for good dental restorations with remineralizing and antibacterial capabilities. |
| **6** | 2011 | Dina Gamal Taha[54] | Ormocer, Nanofilled, and Nanoceramic composite | Fracture resistance of maxillary premolars with class II MOD cavities restored with Ormocer, Nanofilled, and Nanoceramic composite restorative systems<br>Teeth with microhybrid, ormocer, and nanofilled composite restorations had lower cuspal fracture resistance than those with nanoceramic composite restorations. |

***Nano- GIC (Nanoionomers)***

Glass ionomer cement (GIC) is one of the most versatile materials used in dentistry that has undergone various modifications. Nanotechnology has played a major role in modifying GIC, improving its mechanical properties and the esthetics offered. The first nano-GIC was developed for Ketac™ Nano (3M ESPE, 3mespe.com) with fluor aluminum-silicate technology.[55]

In a study conducted by Alatawi RA et.al in 2019, they produced GIC mixed with hydroxyapatite nanoparticles, this mixture resulted in increased fluoride release and enhanced mechanical properties. In addition, they studied the antibacterial effect of 8% HA wt% against S.mutans, which resulted in a bacterial inhibition zone of about 8.6 mm.[56]

***Nano adhesives***

These are bonding agents, when incorporated with nanoparticles-show better bond strength, marginal seal, fluoride release, stress absorption, and long shelf-life due to the well-homogenized consistency of the adhesive.[12]

***Nano aided Endodontics***

Nanoparticles play a major role in material aspects of the endodontic sealers and obturation materials. It improves the handling and physical properties. Additionally, it has antimicrobial properties due to increased pH and improved sealing ability.

Bioceramic-based sealer EndoSequence BC Sealer™ and Silicon-based sealer containing gutta-percha powder and silver nanoparticles (GuttaFlow® 2, Coltene Whaledent) have been introduced. Recently antibacterial quaternary ammonium polyethyleneimine (QPEI) nanoparticles have also been incorporated into other sealers.[57,58]

Recently diamond nanoparticles were incorporated into gutta-percha, and the obturation viewed under digital radiography and micro-computed tomography revealed better adaptation to canal walls and less void formation.[59]

***Nano Impression materials***

Research in prosthetic impression materials based on nanotechnology is basically of two types: one is to create new inorganic nanomaterials and the other is to improve the surface characteristics of the existing materials by incorporating nanoparticles on the surface helping us to overcome the disadvantage of traditional impression materials which are known to be brittle and low ductility. Thus, incorporation of nanoparticles into ceramic, resin and metals paves the way to attaining better mechanical and structural properties of impression materials.

Nanoceramics have superplasticity and show good toughness, ductility, hardness, and strength which is four to five times higher than those of the traditional materials. For example, at 100°C the microhardness of nano-TiO2 ceramics is 13,000 kN/mm2, while that of ordinary TiO2 ceramics is lower than 2,000 kN/mm2.[60]

Poly methyl methacrylate (PMMA) is one of the dental materials that is indispensable and the incorporation of nanotechnology has improved its characteristics multifold. Carbon Nanotubes (CNT) and carbon nanofibrils have been used as additives in impression materials to improve the properties of PMMA. Studies show better impact strength of PMMA matrix, when prepared using with even smaller amounts of single-wall nanotubes as additives using a dry powder mixing method.[61]

**Recent applications in other fields of medicine**

We would also like to highlight the recent applications of nanotechnology in other fields of medicine like artificial intelligence, cancer detection and covid 19 vaccines. Though these topics are not within the scope of the article, yet we would like to bring to the readers the diverse applications which could be further explored.

***Artificial intelligence and nanomedicine***

Artificial intelligence (AI) is one of the newfangled technologies being researched extensively in the field of medicine.

Integrating artificial intelligence and nanotechnology is instrumental in the field of nanomedicine and dentistry. It helps in enhancing patient data acquisition and improves the design of nanomaterials and diagnostic and therapeutic efficacy. AI bridges the gap of heterogeneous patient treatment modalities by providing custom-made treatment options by analyzing the patient requirements.[62] It aids in making appropriate combinations of drugs and the nanomaterial to be used for carrying the drug by using pattern analysis and classification algorithms.

***Cancer detection through Integration of Artificial Intelligence and Nanotechnology***

Artificial intelligence has improved cancer detection and treatment in many ways. IBM Watson for use in oncology helps in providing a more personalized therapy to cancer patients. It collects information from medical journals, textbooks, clinical data and analyses patient medical records, along with the oncologist's expertise, which could render the best treatment to the patient. Microsoft's Hanover project and

Google's DeepMind are other platforms working on AI in medicine.[63]

In a recent study, Wang *et al.* developed feedback system control, a widely used platform for unmodified and nanotechnology-modified therapeutic optimizations. The authors used AI to standardize drug dose combinations that would produce maximum cytotoxicity. They studied nanodiamond-doxorubicin, nanodiamond-mitoxantrone, nanodiamond-bleomycin, and unmodified paclitaxel combinations on many breast cancer cell lines. The results showed that when compared to randomly selected nanomedicine combinations, AI-optimized nanomedicine drug combinations gave a better performance.[64]

**Application of nanotechnology for covid-19 vaccines**

Covid - 19 vaccines have become the need of the hour, and nanotechnology can invariably play a role in vaccine development. Research in a Nano ImmunoEngineering, University of California, Faculty proposed the idea of a plant virus using a peptide-based approach, which could be fabricated as COVID-19 Nano-vaccine patch and microneedle that could be painlessly self-administered by patients. It can efficiently deliver antigens, serve as adjuvant platforms and mimic viral structures.[65]

WHO in January 2021 considered BNT162b2 as an emergency vaccine which is a lipid nanoparticle-formulated, nucleoside-modified RNA vaccine that encodes a prefusion stabilized, membrane-anchored SARS-CoV-2 full-length spike protein.[66]

**Safety issues:**

The extensive use of nanomaterials in medical applications has raised concerns about the safety and toxicity levels. This is because of the associated rate of increased absorption into the cells of the skin, Lung mucosa, digestive tract cells, and other parts of the body. Toxic effects include DNA damage which when evaluated in a study, showed that copper oxide and titanium dioxide nanoparticles were highly toxic compared to iron oxide, zinc oxide, carbon nanotubes.[12]

## CONCLUSIONS

Nanotechnology will soon become the key aspect of the development of any diagnostic aid or treatment option and is inevitably the most emerging interdisciplinary field in medicine and dentistry. It has brought the treatment approach to the level of the size of the cells of the body and can as well render treatment that is more specific and personalized to the patient. It can be integrated with other disciplines of science and technology such as artificial intelligence and improve the efficiency of diagnosis and treatment multifold. Clinical trials in the field of Nanodentistry have been evolving at a very slow pace, in this review, we presented some of the recent developments in terms of clinical trials and incorporate high quality restorative and disease prevention strategies. Further, we like to conclude by Nanodentistry is definitely a promising field and when incorporated into our day-to-day practice could enhance the quality of interdisciplinary treatment modalities. Thus, let's march towards a change and aim for precision-based Dentistry which could be positively obtained using the perfect material of choice "the Nanos" in all the dental fraternity branches.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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