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## Journal Article in Print

1. Burrow MF, Tagami J, Negishi T. Early tensile bone strengths of several enamel and dentin bonding systems. J Dent Res 1994; 74: 522-8.

## Journal Article Electronic Publication Ahead of Print

2. McKeage K. Tobramycin inhalation powder: a review of its use in the treatment of chronic pseudomonas aeruginosa infection in patients with cystic fibrosis. Drugs 2013; [Epub ahead of print] Available from: http://link.springer.com/article/10.1007%2 Fs40265-013-0141-0

## Book

3. Mueller HJ, Freeman D. FT-IR spectrometry in materiolography. 2nd Ed., Ohio: American Society for Metal 1994, p.51-56.

## Chapter in a book

4. Alexander RG. Considerations in creating a beautiful smile. In: Romano R, editor. The art of the smile. London: Quintessence Publishing, 2005, p.187-210.
5. Hudson FB, Hawcroft J. Duration of treatment in phenylketonuria. In: Seakins J, Saunders R, editors. Treatment of inborn errors of metabolism. London: Churchill Livingstone, 1973, p.51-56.

## Thesis

6. Maden I. Effect Of Nd:YAG Laser Treatment In Addition To Scaling And Root Planning. Doctoral Dissertation, Istanbul University Institute of Health Sciences Periodontology Department, 2009.

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*Figure 1. Panoramic radiograph of the patient taken 6 months after surgery, note irregular borders of the lesion.*

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***Table 1.*** *Concise explanation of the table contents (SD: standard deviation, CTA: cartilage tissue area, NBA: new bone area ).*

| | Control group (Mean % ± SD %) | First group (Mean % ± SD %) | Second group (Mean % ± SD %) |
|---|---|---|---|
| CTA | 21.41 ± 4.2 | 2.5 ± 2.4 | 11.42 ± 4.2 |
| NBA | 11.48 ± 0.2 | 21.41 ± 14.22 | 11.41 ± 4.2 |

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Original research

# Evaluation of maxillary sinus expansion in children due to maxillary first molar extraction

**Sinem Kuru[1], Mustafa Mert Açıkgöz[2], Arzu Pınar Erdem[1], Gülsüm Ak[2], Tamer Lütfi Erdem[3], Gamze Aren[1], Elif Sepet[1]**

**Purpose**
This study is aimed at understanding the effects of maxillary first molar extraction on the expansion of maxillary sinus in children.

**Subjects and methods**
119 patients (aged 11-17 years) who had only one extracted maxillary first molar were included in the study. The superoinferior differences of the sinus floor position were measured in both dentate and edentulous sites on panoramic radiographs. The expansion of the maxillary sinus after maxillary first molar extraction was investigated in relation to fixed anatomic structures. The interorbital line (IL) and two zygomatic process lines (IZ) were used as a reference. The vertical distances between the IL and the inferior border of the maxillary sinus (IS) in edentulous ($IS_X$) and in dentate sites ($IS_T$) were measured. The data was analyzed statistically.

**Results**
The amount of maxillary sinus expansion in ISX was statistically significant in comparison to $IS_T$ ($p<0.001$). The most prominent sinus expansions were found in subjects with extractions over six months prior to analysis ($p<0.001$). A negative correlation was detected between the amount of maxillary sinus expansion and $IS_T$ ($r = -0.438$, $p<0.001$). There was a positive correlation between the amount of maxillary sinus expansion and $IZ_T$ ($r = 23.8$, $p< 0.009$).

**Conclusion**
This study showed that the extraction of one maxillary first molar resulted in a negligible amount of sinus expansion in children. The results could be attributed to there being only one tooth extraction and a transferring of functional forces to the area of the neighboring teeth.

***Keywords:*** *Maxillary sinus; sinus expansion; tooth extraction; panoramic radiography; children*

## Introduction

The expansion of paranasal sinuses begins at birth and continues with the development of the facial cranium (1). The maxillary sinus is the first sinus to develop, it starts growing in the fifth fetal month, reaches its final growth between 12-14 years of age and ends following the eruption of the 3rd molars (2,3).

Pneumatization of the sinus may be influenced by a number of factors including heredity and disuse atrophy. The dimensions of the maxillary sinus can be affected by the environmental factors, genetic diseases and infections (3). Wehrbein and Diedrich (4) reported a direct correlation between the amount of sinus expansion after tooth extraction and the projection length of roots into the sinus.

The extraction of the maxillary molar teeth results in dimensional changes with bone loss of the sinus floor (5). The rapid bone destruction can

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extend to the alveolar process, moreover, the bone doesn't regenerate to the preoperative level (5-7). Bone height can decrease alongside maxillary sinus extension into the alveolar process (8). Sinus expansion is clinically important during tooth extraction or an implant insertion (9).

The maxillary sinus may be accidentally opened during difficult tooth extractions (10). Sinus expansion has been investigated by some clinical studies with various results (11-15). Some studies have found an increase in size after molar extractions (11,15), while other studies have reported no difference in sinus size between dentate and edentulous subjects (12,16,17).

This study is aimed at understanding the effect of upper first molar extraction on the expansion of maxillary sinus in children. The main null hypothesis tested in the present study is that first molar extraction in children has no effect on maxillary sinus expansion.

## Subjects and methods

*Study design*

The Ethics Committee of Istanbul University, Faculty of Medicine approved this study (2011/2108-895) and the subjects parents gave informed consent for them to participate in the study. The guidelines of the Helsinki Declaration were followed in this investigation.

Panoramic radiographs were taken from patients who received dental treatment at Istanbul University's Pediatric Dentistry and Oral-Maxillofacial Surgery Departments, Istanbul, Turkey. The panoramic radiographs were all obtained using a Kodak 8000 Digital Panoramic Machine (Carestream Health, Inc., Rochester, NY, USA) at kVp 65-68; mA range varies between 2-3,2 for infants; 5-6.5 for adolescents. The subjects' selection criteria were as follows: (1) extraction only of one maxillary first permanent tooth, (2) no history of sinus diseases.

*Study population*

119 patients (aged 11-17 years) who had only one extracted maxillary first molar were included in the study. The superoinferior differences of the sinus floor position were measured both in dentate and edentulous sites on the panoramic radiographs and were obtained at least 6 months after tooth extraction.

*Image analysis*

We used the method which previously described by Sharan and Madjar (15) for evaluating vertical distances on panoramic radiographs. Expansion of the maxillary sinus after maxillary first molar extraction was investigated in relation to fixed anatomic structures. The interorbital line (IL) and two zygomatic process lines (IZ) were used as a reference. The reference lines were marked on the radiographs with a pencil then they were scanned and digitized. Adobe Photoshop 7.0 software was used to measure the distance between the interorbital line and the zygomatic process lines on images magnified by 200%. The vertical distances between the IL and the sinus floor (IS) in edentulous (ISX) and in dentate sites (IST) were measured. The lengths of the vertical distances between the IL and IZ on both sides (IZX, IZT) of the jaw were also measured (Figure 1).

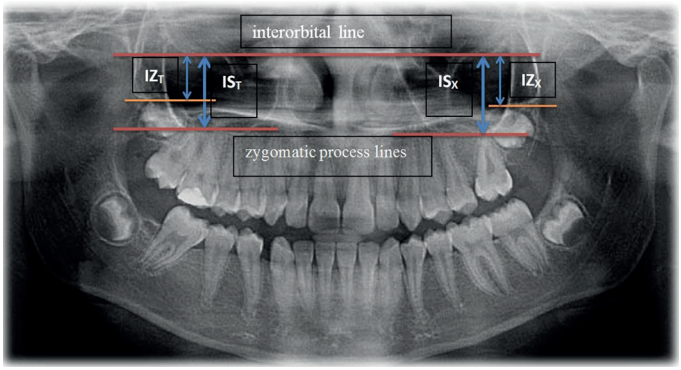


***Figure 1.*** *Interorbital line and two zygomatic process lines used as reference lines on panoramic radiographs.*

The ratio: IS/IZ was used to express the position of the sinus floor. The maxillary sinus pneumatization is calculated using the formula ISX- (IST*IZx/IZT). A positive value was evaluated as an inferiorly located sinus floor on the edentulous site. We repeated the measurements three times and used the mean value for statistical analysis.

*Statistical analysis*

Statistical analyses were performed using Statistical Package for Social Scince (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp, USA). Shapiro–Wilk, Mann-Whitney U and Spearman's Rho correlation tests were applied. Data was expressed as means and medians of the study groups. Confidence interval was set to 95% and p values less than 0.05 were considered statistically significant.

## Results

The number of patients, according to age and sex are presented in Table 1. The mean age of the subjects included in the study (69 boys, 50 girls) was 13.06±1.54. Evaluation of ISX and IST parameters is presented in Table 2. There was no statistically significant differences between the investigated parameters. The mean values for maxillary sinus expansion according to time are presented in Table 3. The average length of time following tooth extraction

**Table 1.** *Number of patients stratified by age and sex.*

| Age (years) | Male | Female | Total |
|---|---|---|---|
| 11 | 15 (21.7%) | 7 (14%) | 22 (18.5%) |
| 12 | 17 (24.6%) | 8 (16%) | 25 (21%) |
| 13 | 12 (17.4%) | 18 (36%) | 30 (25.2%) |
| 14 | 10 (14.5%) | 8 (16%) | 18 (15.1%) |
| 15 | 9 (13%) | 7 (14%) | 16 (13.4%) |
| 16 | 4 (5.8%) | 2 (4%) | 6 (5%) |
| 17 | 2 (2.9%) | 0 (0%) | 2 (1.7%) |
| Total | 69 (100%) | 50 (%100) | 119 (100%) |

**Table 2.** *Evaluation of $IS_X$ and $IS_T$ parameters stratified by age and sex. ($IS_X$: Vertical distance between the interorbital line (IL) and the sinus floor (IS) in edentulous site; $IS_T$: Vertical distance between the interorbital line (IL) and the sinus floor (IS) in dentate site, Mann Whitney U test; SD: standard deviation).*

| Age | Sex | $IS_X$ | $IS_T$ |
|---|---|---|---|
| | | Mean±SD (median) | Mean±SD (median) |
| 11 | Boys | 20.27±1.87 (20) | 18.53±1.55 (18) |
| | Girls | 19.57±0.98 (20) | 17.29±2.14 (18) |
| p | | 0.460 | 0.110 |
| 12 | Boys | 20.41±2.18 (20) | 19.18±1.88 (19) |
| | Girls | 19.88±2.85(19.5) | 19±3.07 (19) |
| p | | 0.358 | 0.781 |
| 13 | Boys | 20.17±1.95 (20) | 19.25±3.6 (18) |
| | Girls | 19.5±1.34 (19.5) | 18.28±1.27 (18.5) |
| p | | 0.597 | 0.842 |
| 14 | Boys | 20.6±2.17 (20) | 18.8±2.9 (18) |
| | Girls | 21±2.33 (21) | 19.13±2.53 (19) |
| p | | 0.547 | 0.361 |
| 15-17 | Boys | 21.73±2.84 (21) | 20.67±3.27 (19) |
| | Girls | 21.22±3.35 (21) | 19.22±3.42 (19) |
| p | | 0.566 | 0.243 |
| Total | Boys | 20.65±2.25 (20) | 19.32±2.7 (19) |
| | Girls | 20.12±2.25 (20) | 18.56±2.39 (19) |
| p | | 0.222 | 0.276 |

**Table 3.** *Maxillary sinus expansion according to time after extraction (Mann Whitney U test *p<0.05, SD: Standard deviation).*

| Time | Sinus Expansion mean±SD (median) |
|---|---|
| 0-6 months | -1.09±2.12 (-0.4) |
| 6-24 months | 0.73±2.53 (0.6) |
| p | 0.001* |

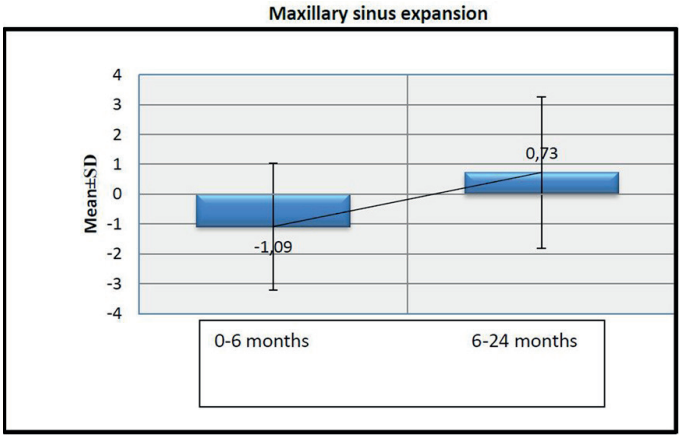


***Figure 2.*** *Maxillary sinus expansion stratified by time factor after extraction.*

was 8.02±1.72 months. The amount of maxillary sinus expansion significantly increased with the time intervals after extractions (p=0.001). Those patients with extractions over six months presented the largest sinus expansions (Figure 2). Evaluation of the correlations between the amount of maxillary sinus expansion and ISX, IST, IZX, IZT measurements is presented in Table 4. A negative correlation was detected between the amount of maxillary sinus expansion and IST (r = -0.438, p=0.001), (Figure 3). There was a positive correlation between the amount of maxillary sinus expansion and IZT (r = 23.8, p=0.009), (Figure 4).

**Table 4.** *The evaluation of the correlations between the amount of maxillary sinus expansion and ISX, IST, IZX, IZT measurements (Sperman's rho *p<0.05, ISX: Vertical distance between the interorbital line (IL) and the sinus floor (IS) in edentulous site IST: Vertical distance between the interorbital line (IL) and the sinus floor (IS) in dentate site IZX : Vertical distance between the interorbital line (IL) and the zygomatic process line (IZ) in edentulous site IZT: Vertical distance between the interorbital line (IL) and the zygomatic process line (IZ) in dentate site).*

| | | Maxillary sinus expansion |
|---|---|---|
| $IS_X$ | r | 0.043 |
| | p | 0.645 |
| $IS_T$ | r | **-0.438** |
| | p | **0.001*** |
| $IZ_X$ | r | -0.057 |
| | p | 0.535 |
| $IZ_T$ | r | **0.238** |
| | p | **0.009*** |

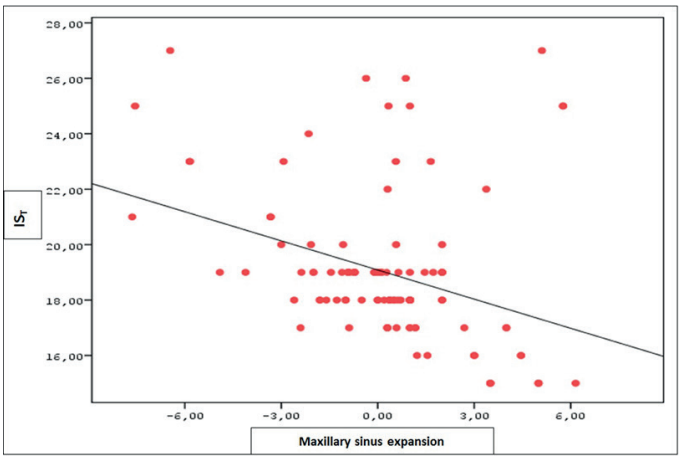


***Figure 3.*** *The negative correlation between the amount of maxillary sinus expansion and IST ($IS_T$: The vertical distance between the interorbital line (IL) and the sinus floor (IS) in dentate site).*

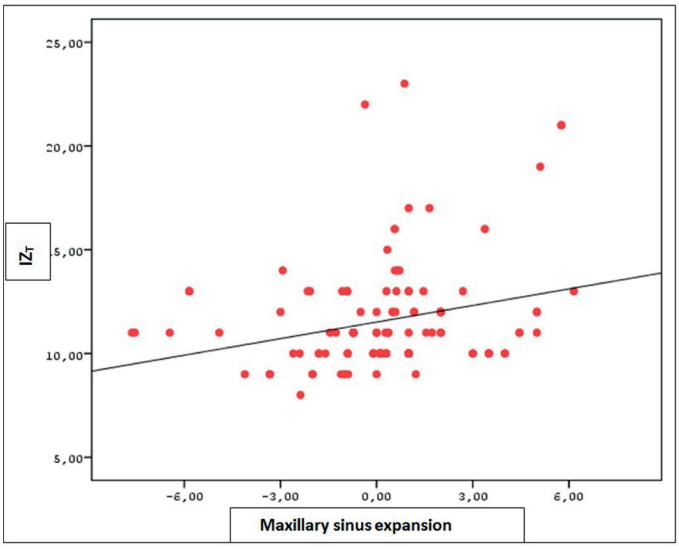


***Figure 4.*** *The positive correlation between the amount of maxillary sinus expansion and $IZ_T$ ($IZ_T$: The vertical distance between the interorbital line (IL) and the zygomatic process line (IZ) in dentate site).*

## Discussion

This study documented the effect of only one upper first molar extraction on the expansion of maxillary sinus in children. Both dentate and edentulous sites of each patient were evaluated. It is important and essential for clinicians to investigate the relation of the upper posterior teeth roots with the maxillary sinus floor for the proper preoperative treatment planning in the maxillary posterior area (2). The ideal time for the loss of the first permanent molars is the age of 8–9 years, before the eruption of the second permanent molar (16). The early eruption of the second molar might establish a good contact relationship with the second premolar (17).

In this study, the amount of time that passed after the loss of the maxillary first permanent molars was during or after the eruption of the second permanent molars. Extractions of first permanent molars in these time intervals may result in some occlusal consequences including incomplete space closure, mesial tilting and distal drifting of the neighboring teeth, over-eruption of the opposing molar, and alveolar bone atrophy (16,17).

The extraction of the maxillary second molars have reported to cause greater sinus expansion compared tothe extraction of maxillary first molars (15). Sharan and Madjar (15) reported that when two or more adjacent posterior teeth are extracted, the sinus volume become larger. The results of this study revealed an lesser expansion of the maxillary sinus floor in the edentulous sites. The negligible amount of sinus expansion could be attributed to there being only one tooth extraction and a transferring of functional forces to the area of the neighboring teeth.

Sinus pneumatization also occurs if mandibular posterior teeth have been missing for an extended time which leads the the opposing maxillary posterior teeth to overerupt (13). Tolstunov et al. (18) reported that the older edentulous patients tend to demonstrate higher rate of bone resorption in the maxilla which causes the enlargement of the sinuses. In the present study, the patients with extractions over six months prior to analysis presented the largest sinus expansions. Sinus also may expand due the presence of large defects in the extraction site and decreased bone resistance because of the extended healing period of the alveolar socket (14).

Several techniques are used to assess the relationship between the roots of the upper posterior teeth and the maxillary sinus (2,4,12,13,15,16). Panoramic radiography allows the visualization of various anatomic structures, is widely available and relatively inexpensive (13). Its disadvantages include overlapping of the anatomical structures, distortion, magnification and inadequate resolution (4,19). CBCT provides multiplanar images in expense to higher doses of radiation, being expensive, and limited availability (20). In this study, the supero-inferior differences of the sinus floor position were measured both in dentate and edentulous sites on the panoramic radiographs.

Atraumatic tooth extraction is important for the preservation of the alveolar bone volume and soft tissues as well (21). Schropp et al. (22) reported a significant reduction in the alveolar ridge width the within the first 3 months after single tooth extractions. Proper mechanics and light forces are recommended to reduce iatrogenic root resorption during orthodontic treatment through the maxillary sinus when the maxillary posterior teeth were extracted and space closure was selected as the treatment approach (13). The treatment plan for edentulous spaces always includes the option of implants in adults, but it is recommended to delay an implant placement until after the cessation of growth in children (23,24).

## Conclusion

This study demonstrated that the extraction of one maxillary first molar resulted in a negligible amount of sinus expansion in children. This can be related to the limited space left after the extraction of only one tooth for the sinus to expand and also to the transfer of the functional loads to the adjacent teeth.

**Ethics Committee Approval:** The Ethics Committee of Istanbul University, Faculty of Medicine has approved the present study protocol (2011/2108-895).

**Informed consent:** Informed consent was provided by the participants' parents.

**Peer review:** Externally peer-reviewed.

**Author contributions:** SK and ES participated in designing the study. SK and MMA participated in generating the data for the study. GA and TLE participated in gathering the data for the study. APE and TLE participated in the analysis of the data. GA wrote the majority of the original draft of the paper. APE participated in writing the paper. All authors approved the final version of this paper.

**Conflict of interest:** The authors have no conflicts of interest to declare.

**Financial disclosure:** The authors declared that this study has received no financial support.

***Türkçe öz:*** *Çocuklarda maksiller birinci büyük azı dişi çekimine bağlı maksiller sinüs genişlemesinin değerlendirilmesi. Amaç: Bu çalışma, maksiller birinci büyük azı dişi çekiminin maksiller sinüsün genişlemesi üzerine etkisini anlamayı amaçlamıştır. Bireyler ve yöntem: Sadece bir maksiller birinci büyük azı dişi çekilmiş olan 119 hasta (yaşları 11-17) çalışma kapsamına alınmıştır. Hem dişli hem de dişsiz bölgelerde sinüs tabanı pozisyonunun superoinferior farkları, panoramik röntgenler üzerinde ölçülmüştür. Maksiller birinci büyük azı dişi çekimi sonrası maksiller sinüsün genişlemesi sabit anatomik yapılarla ilişkili olarak araştırılmıştır. Referans olarak interorbital hat (IL) ve iki zigomatik proses hattı (IZ) kullanılmıştır. IL ile dişsiz bölgelerdeki ($IS_X$) ve dişli bölgelerdeki ($IS_T$) maksiller sinüsün (IS) alt sınırı arasındaki dikey mesafeler ölçülmüştür. Veriler istatistiksel olarak analiz edilmiştir. Bulgular:ISX'de maksiller sinüs genişleme miktarı $IS_T$'ye göre istatistiksel olarak anlamlıydı ($p < 0.001$). En büyük sinüs genişlemeleri, altı aydan daha uzun süre önce çekim yapılan hastalarda tespit edildi ($p < 0.001$). Maksiller sinüs genişlemesi ve $IS_T$ miktarı arasında negatif korelasyon saptandı ($r = -0.438$, $p < 0.001$). Maksiller sinüs genişlemesi ve $IZ_T$ miktarı arasında pozitif bir korelasyon vardı ($r = 23.8$, $p < 0.009$). Sonuç:Bu çalışma, bir maksiller birinci büyük azı dişi çekiminin çocuklarda önemsiz miktarda sinüs genişlemesi ile sonuçlandığını göstermiştir. Sonuçlar sadece bir diş çekimi olması ve komşu dişlerin alanına fonksiyonel kuvvetlerin aktarımı ile açıklanabilir. Anahtar kelimeler: Maksiller sinüs; sinüs genişlemesi; diş çekimi; panoramik röntgen; çocuk*

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Original research

# Evaluation of fracture resistance of roots-filled with various root canal sealers at different time periods

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**Purpose**
The reinforcement effect of 3 various root canal sealers (AH 26, MTA Plus sealer and BioRoot RCS) and gutta-percha at different time periods (1 week and 1 month) were evaluated in the present study.

**Materials and Methods**
Single-rooted, single-canalled, cracks-free 80 mandibular premolars were decoronated to a length of 13mm. Group PC (positive control, n=10): samples were left unprepared and unfilled. Seventy samples were prepared by using the ProTaper Rotary System up to F4. Group NC (negative control, n=10): samples were left unfilled. Remaining 60 samples were assigned into 3 groups; Group 1: AH 26 + F4 gutta-percha (GP); Group 2: MTA Plus sealer + F4 GP and Group 3: BioRoot RCS + F4 GP. Filled samples were divided into subgroups according to storage time: Samples in Groups 1A, 2A, and 3A were stored for 1 week; while Groups 1B, 2B and 3B were stored for 1 month at 100% humidity to allow the complete setting of the sealers (n=10, for each). A universal testing machine at a crosshead speed of 1.0 mm/min was used for fracture testing. For each specimen, the force at the time of fracture was recorded and the data were analyzed statistically.

**Results**
The highest fracture resistance values were obtained in Group PC, while the lowest values were obtained in Group NC. Groups PC and NC were statistically different from each other and from other groups, regardless of time ($p<0.05$). Fracture resistance values of Group AH 26/GP were statistically different from MTA Plus sealer/GP ($p<0.05$) and were statistically similar with BioRoot RCS/GP, irrespective of time. Within group comparisons revealed that there were no statistically differences between samples filled with same sealer at different time periods.

**Conclusion**
Root canal preparation caused decreased fracture resistance. All sealers increased the force values needed to fracture the filled samples compared to unfilled ones. Time factor had no effect on the fracture resistance values.

***Keywords:*** *Calcium silicate sealer; root canal obturation; vertical root fracture; time; endodontics*

## Introduction

One of the major aims of the endodontic treatment is to reinforce the remaining tooth structure with root canal filling materials after chemical and mechanical preparation (1). These filling materials, placed within the root space, might prevent vertical root fractures, which could arise during or following root canal treatment and often lead to extraction of the tooth (2). However, there are conflicting results regarding whether the strength of roots could be enhanced after conventional root canal filling with gutta-percha and sealer, *in vitro* (3-7).

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El-Ma'aita *et al.* (8) reported that mineral trioxide aggregate (MTA) supported the tooth structure and as a result MTA filled roots resisted against vertical root fractures (8). Calcium releasing ability (9) and bioactivity (10) of MTA increase its popularity and this leads to tricalcium silicate-based sealers (TSBSs) production. TSBSs have ability to form hydroxyapatite and a bond between dentin and filling material during the setting process, as a result of this process, mineral infiltration zone is formed (11).

BioRoot RCS (Septodont, Saint-Maur-des-Fosses, France) and MTA Plus sealer (Avalon Biomed Inc, Bradenton, FL) are recently introduced silicate-based sealers to the dental market. BioRoot RCS is a water-based sealer. Powder part of BioRoot RCS is composed of tricalcium silicate, povidone (stickiness agent), and zirconium dioxide (contrast medium); while the liquid part of it is composed of calcium chloride (curing accelerator) with polycarboxylate as an aqueous solution (12,13). BioRoot RCS releases calcium hydroxide after setting (12) and a calcium phosphate phase is formed when it contacts with physiologic solution (13). Micro–computed tomographic analysis revealed that using AH Plus sealer decreased the percentage of voids significantly compared to BioRoot RCS, but no differences were observed regarding fluid flow and microsphere penetration (14). Sealer penetration and interaction with the dentin walls patterns showed diversity between these 2 sealers (14). Powder part of MTA Plus sealer can be mixed with a liquid or a gel. MTA Plus sealer has similar composition to ProRoot MTA however, its particle size is finer than ProRoot MTA (15). In order to obtain root canal sealer, powder could mix with gel, which improves its handling properties and washout resistance (16). DeLong *et al.* (17) reported favorable push-out bond strength values with MTA Plus sealer combined with single gutta-percha cone.

There are several studies evaluating the effects of TSBSs on the fracture resistance of endodontically treated teeth (5-7); so, the aim of this study was to compare the effect of di-/tri-calcium silicate-based root canal sealers on the resistance of endodontically treated roots to vertical root fracture over different time intervals. Epoxy resin sealer (AH 26 Dentsply de Trey, Konstanz, Germany) was employed as reference material for comparison. The null hypotheses of this study were as follows: first, the root canal sealer has no influence on the vertical root fracture resistance of endodontically treated teeth. Second, the resistance to vertical root fracture of endodontically treated teeth filled with different sealers and gutta-percha does not change over time.

## Materials and Methods

*Sample characteristics and preparation*

Eighty extracted caries-free, single rooted, human mandibular premolar teeth were used. Teeth examination was carried out under an operating microscope (Zeiss, Oberkochen, Germany) in order to exclude teeth with microcracks. A digital caliper was used to measure the mesiodistal and buccolingual diameters of the coronal surface. Then, the mean of these dimensions was obtained. If the dimensions of specimens presented a difference of 20% from the mean, they were discarded in order to obtain standardized samples (18). Furthermore, the root canal morphology was determined with buccolingual and mesiodistal radiographs and then, the teeth were randomly assigned into the experimental groups according to root canals shape as round and oval. Until utilization, teeth were stored in deionized water under 4 °C. The specimens were decoronized at the cemento-enamel junction to create length in 13 mm. Randomly selected 10-decoronized samples were represented positive control group (Group PC). The root canals of the remaining 70 teeth were instrumented using the ProTaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) up to F4 with torque controlled endodontic motor (X Smart Plus, Dentsply Maillefer). After each file, 2 mL of 5.25% NaOCl was used to irrigate the root canals. Following last NaOCl irrigation, 2 mL of 17% EDTA was used to remove smear layer for 3 min and finally distilled water was used. Paper points (Dentsply Maillefer) were used to dry the canals. Following instrumentation, 10 teeth were assigned as negative control group without further intervention (Group NC). Remaining 60 teeth were divided into 3 main groups according the root canal sealers were used during root canal filling: Group 1: AH 26 + F4 Gutta-percha (Dentsply Maillefer), Group 2: MTA Plus sealer + F4 Gutta-percha, Group 3: BioRoot RCS + F4 Gutta-percha. Following root filling, excess gutta-percha was removed with heat and an appropriate plugger was used to compact the coronal material. A temporary filling material (Coltosol, Coltene, Whaledent Inc., Altstaetten, Switzerland) was placed over the obturation materials. The 10 teeth from each experimental group (Subgroups 1A, 2A and 3A) were stored at 37 °C at 100% humidity for 1 week to allow the sealers to set, while the remaining 10 (Subgroups 1B, 2B and 3B) were stored under same conditions for 1 month before fracture resistance test.

*Fracture Resistance Test*

Fracture resistance of each sample was tested at the end of the respective time period. However, samples in groups PC and NC were tested at the end of 1st month. Four millimeters of the roots were embedded into the self-cure acrylic resin (Imicryl, Konya, Turkey), that was poured to the cylindrical molds (diameter: 15mm, height: 13 mm), exposing 9 mm of the roots. Similar methodology and set-up were used in

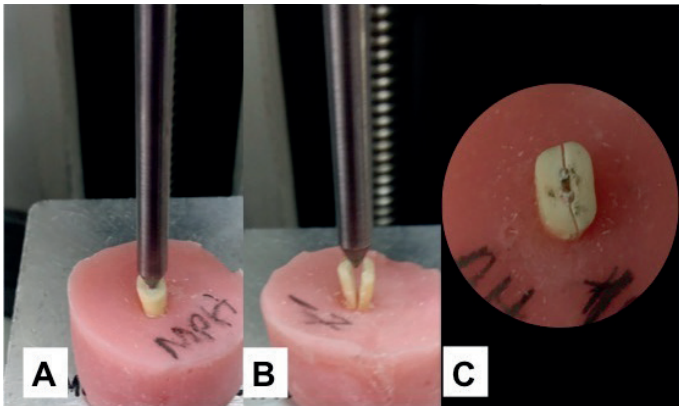


***Figure 1.*** *Representative sample of groups mounted on Universal testing machine (A), vertical root fracture at the end of test (B), example of non-restorable vertical root fracture in buccolingual direction (C).*

**Table 1:** *Experimental groups and fracture resistance values (FRV) of tested samples in Newtons. (*Different superscript letters mean statistical difference between groups p<0.05).*

| Groups | Time periods | | |
|---|---|---|---|
| | 1Week | 1 Month | P Value |
| AH 26/GP | 296.89±38.75 [a] | 334.05±29.31 [a] | 0. 056 |
| MTA Plus/GP | 258.91±37.79 [abd] | 275.34±36.76 [b] | 0.393 |
| BioRoot RCS/GP | 288.85±45.68 [a] | 294.51±52.50 [ab] | 0.768 |
| Group PC | 474.21±62.86 [c] | | |
| Group NC | 205.19±15.28 [d] | | |

previous studies (1-6). Following the removal of temporary filling material, lower plate of the universal testing machine (Instron, Canton, MA, USA) was used for the mounting of the samples (Figure 1A). A vertical compressive loading (rate: 1 mm/min) was applied via spherical steel tip (diameter: 2 mm) to the coronal surfaces of roots until the fracture occurred (Figure 1B-1C). The force required to fracture each specimen was recorded and expressed in Newton.

*Statistical analysis*

Statistical analysis was performed using SPSS package program (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). Univariate analysis of variance (two-way ANOVA) with Bonferroni post-hoc test was used to compare the obtained statistical data ($p<0.05$). Fractures modes were also recorded as favorable (root fractures at the cervical third were classified as restorable) and catastrophic (fractures at the middle and apical thirds were classified as non-restorable) (19). Confidence interval was set to 95% and $p<0.05$ was considered statistically significant.

## Results

The results are shown in Table 1. The highest fracture resistance values were obtained in Group PC, while the lowest values were obtained in Group NC. Groups NC and PC were statistically different from each other and from other groups, regardless of time ($p<0.05$) (Table 2). Fracture resistance values (FRV) of samples filled with AH 26/GP were the highest at the end of each time period, except group PC. FRV of AH 26/GP filled samples were statistically similar with FRV of BioRoot RCS /GP filled samples, while they were statistically different from FRV of MTA Plus sealer/GP filled samples ($p<0.05$), irrespective of time (Table 2).

At the end of 1st week, FRV of AH 26/GP and BioRoot RCS/GP filled samples were statistically different than Group NC and Group PC ($p<0.05$). FRV of MTA Plus sealer/GP filled samples were statistically different from Group PC ($p<0.05$). At the end of 1st month FRV of AH 26/GP filled samples were statistically different from Groups NC, PC and MTA Plus Sealer/GP ($p<0.05$). FRV of BioRoot RCS/GP and MTA Plus sealer/GP filled samples were statistically different both from Groups NC and PC ($p<0.05$). Within group comparisons revealed that there was no statistically significant differences between FRV of samples filled with same sealer at different time periods (Table 1). The most frequent fracture mode was non-restorable fractures in buccolingual direction in all groups (Table 3).

**Table 2.** *Fracture resistance values (FRV) of samples in Newtons, regardless of time. (*Different superscript letters mean statistical difference between groups p<0.05.)*

| Groups | FRV * Mean±SD. (N) |
|---|---|
| AH 26/GP | 315.47±38.50 [a] |
| MTA Plus/GP | 267.12±37.25 [b] |
| BioRoot RCS/GP | 291.68±47.99 [ab] |
| Group PC | 474.21±62.86 [c] |
| Group NC | 205.19±15.28 [d] |

**Table 3.** *Fracture modes of experimental groups (BL: buccoolingual; MD:mesiodistal).*

| Groups | Time periods | | | |
|---|---|---|---|---|
| | 1Week | | 1 Month | |
| | Fracture Modes | | | |
| | Restorable | Non-restorable | Restorable | Non-restorable |
| AH 26/GP | 2 (BL) | 8 (7 BL, 1 MD) | 1 (BL) | 9 (BL) |
| MTA Plus/GP | - | 10 (BL) | - | 10 (BL) |
| BioRoot RCS/GP | 1 (BL) | 9 (BL) | - | 10 (9 BL, 1 MD) |
| Group PC | 10 (BL) Non-restorable | | | |
| Group NC | 1 (BL) restorable and 9 (BL) Non-restorable | | | |

## Discussion

Fracture resistance of endodontically treated teeth has been evaluated in many *in vitro* studies (1-7). Fracture test, performed with the universal testing machine, is the basic method in which progressively increasing vertical loads are applied to teeth until fracture occurs and force at fracture is recorded as Newtons. All parameters except root canal sealers were tried to be standardized in the present study: such as root canal preparation technique, root canal obturation technique; irrigants used during preparation; formation of sealers as liquid and powder; buccolingual and mesiodistal dimensions of selected teeth; storage conditions of teeth. However, this is a difficult task and the potential differences among the groups might be considered as one of the limitations of the present study. Under these limitations, first hypothesis of this study is accepted; however, second one is rejected. Root canal fillings with different sealers and gutta-percha were statistically reinforced the samples compared to group NC.

Different steps of endodontic treatment such as mechanical preparation, obturation and irrigation may weaken the root structure and create susceptible roots to fracture (2,20). Different filling materials are used in order to reinforce the endodontically treated teeth against the root fractures (1,3-7). Root canal sealers, as a complementary part of the obturation, are used to fill the gaps between gutta-percha cones and root canal walls as well as the voids between individual gutta-percha cones (21). Several studies reported that filled samples with gutta-percha and different sealers showed significantly superior fracture resistance than the group NC, as in present study (5,6). On the other hand, there were also studies that reported no clear benefits with the use of root canal sealers in reinforcing endodontically treated teeth in the literature (3,4).

Root canal filling has to be done three dimensionally without voids to prevent bacterial microleakage and to reinforce the tooth structure. Moinzadeh *et al.* (22) reported that cold lateral compaction of gutta-percha resulted in significantly more voids compared with single gutta-percha cone obturation combined with a TSBS, particularly coronally. Considering this information, single-cone technique was used in the present study.

The reinforcement effect of calcium hydroxide, epoxy resin, glass ionomer, methacrylate resins, MTA, polyketone, silicone- or zinc oxide-eugenol (ZnOE) based root canal sealers were evaluated on the endodontically treated roots (1,3-7). Biocompatibility and bioactivity of tricalcium silicate cements are high (10) and they are used to repair lateral root perforations or to fill root-ends initially (23). BioRoot RCS and MTA Plus sealer are recently introduced calcium silicate-based sealers to the dental market. BioRoot RCS was not as cytotoxic as Pulp Canal Sealer and it preserves mouse pulpal stem cells' osteo-odontogenic intrinsic properties (24). Lower flow and higher film thickness values were obtained with BioRoot RCS than that specified for sealers in ISO 6876 (25). These properties could contribute to the resistance of filled roots against vertical fractures. Guneser *et al.* (7) reported that BioRoot RCS might have the potential to increase fracture resistance values of prepared teeth against vertical forces as in the present study.

MTA Plus sealer, which has particle size 50% smaller than ProRoot MTA (15), is marketed as a calcium silicate-based material that can be used during root canal filling, root-end filling, and pulp capping procedures (26). Physicochemical properties of MTA Plus sealer such as calcium ion release, hydration reaction and pH did not change when powder was mixed either with water or gel. However, mixing powder with gel improved the compression, porosity liquid absorption and setting time of MTA Plus sealer compared to distilled water mixture (27). In the present study, gel was used to prepare the root canal sealer. FRV of samples that were filled with MTA Plus sealer/GP were lower in both test periods compared to other samples either filled with BioRoot RCS/GP or AH 26/GP. It has been reported that the chemical composition of MTA Plus sealer was similar to ProRoot MTA (15); however, its porosity, water solubility, and water sorption were higher compared to ProRoot MTA (25). This could be one reason of lower fracture resistance values obtained with MTA Plus sealer.

The highest fracture resistances were recorded in AH 26 group in all evaluated periods among tested sealers. Although Viapiana *et al.* (14) reported that there were more voids in root canals filled with BioRoot RCS/GP compared to an epoxy-resin based sealer (AH Plus)/GP, there were no significant differences between AH 26 and BioRoot RCS regarding fracture resistance. According to the results of present study, higher values were obtained at the end of first month compared to first week, however these differences were statistically insignificant. Hoppe *et al.* (28) reported that push-out bond strength of AH Plus combined with lateral compaction of gutta-percha increased insignificantly at the end of 6 months compared to 24h. El Maita *et al.* (8) showed that vertical root fracture resistance did not change with AH Plus sealer/GP obturation over a period of 6 months. On the other hand, authors reported a significant increase for MTA (8). Turker and Uzunoğlu (29) reported that the highest bond strength for white MTA was seen at the end of 28-day period. Current results were consistent with previous results evaluating epoxy-resin based sealer (AH 26). On the other hand, increase in forces to fracture samples that were filled with TSBSs was insignificant in the current study. Regarding the direction of fractures observed in the present study, most of them occurred in the buccolingual direction as previous studies in which force applied vertically to the samples (3,6,7).

## Conclusion

Under the limitations of this study, it could be concluded that the root canal preparation lowered the fracture resistance values. All sealers increased the force values needed to fracture the filled samples compared to unfilled ones but the time factor had no effect on the reinforcement effect of root canal sealers.

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**Ethics committee approval:** Not provided.

**Informed consent:** Not provided.

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**Author contributions:** EUÖ and SAT participated in designing the study. EUÖ and SAT participated in generating the data for the study. EUÖ gathered and analyzed the data, wrote the majority of the original draft. EUÖ and SAT participated in writing the paper. All authors approved the final version of this paper.

**Conflict of interest:** The authors have no conflicts of interest to declare.

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***Türkçe öz:*** *Çeşitli kök kanal dolgu patlarıyla doldurulan dişlerin farklı zaman dilimlerindeki kırılma dayanımlarının değerlendirilmesi. Amaç: Üç farklı kök kanal dolgu patı (AH 26, MTA Plus sealer ve BioRoot RCS) ve gütaperka ile doldurulan örneklerin 1. hafta ve 1. ayın sonundaki kırılma dayanımı değerlerini incelemektir. Gereç ve Yöntem: Seksen adet tek köklü, tek kanallı, çatlaksız alt çene küçük azı dişi kök boyu 13 mm olacak şekilde dekoronize edilmiştir. Grup PK (pozitif kontrol, n=10): örnekler şekillendirilmemiş ve doldurulmamıştır. Geri kalan 70 örnek en son kullanılan eğe F4 olmak üzere ProTaper döner eğe sistemi ile şekillendirilmiştir. Grup NK (negatif kontrol, n=10): şekillendirilen örnekler doldurulmamıştır. Geri kalan 60 örnek 3 gruba ayrılmıştır: Grup 1: AH 26 + F4 gütaperka konu (GP), Grup 2: MTA Plus Sealer + F4 GP, Grup 3: BioRoot RCS + F4 GP. Doldurulan örnekler saklama süresine göre alt gruplara ayrılmıştır. Grup 1A, 2A ve 3A'daki örnekler 1 hafta; Grup 1B, 2B ve 3B'deki örnekler 1 ay süreyle %100 nemli ortamda bekletilmiştir (n=10/grup başına). Tüm örnekler daha sonra evrensel test cihazında kırılma dayanımı testine maruz bırakılmıştır. Her bir örnek için, kırılma anındaki kuvvetler kaydedilerek uygun istatistiksel analizler yapılmıştır. Bulgular: En yüksek kırılma dayanımı değerleri (KDD) Grup PK'da gözlenirken, en düşük KDD Grup NK'da gözlenmiştir. Grup PK ve NK zamandan bağımsız olarak istatistiksel açıdan hem birbirinden hem de diğer gruplardan farklı bulunmuştur (p<0.05). Zamandan bağımsız olarak yapılan analizde, AH 26 patı/GP ile doldurulan örneklerin KDD'sinin BioRoot RCS/GP ile doldurulan örnekler ile istatistiksel olarak benzerlik gösterdiği (p>0,05), fakat MTA Plus sealer/GP ile doldurulan örneklerden daha yüksek olduğu bulunmuştur (p<0,05). Grup içi yapılan karşılaştırmalar aynı pat ve GP ile doldurulan örneklerin bir hafta ve bir ay sonunda elde edilen KDD arasında anlamlı bir farklılık olmadığını göstermiştir (p>0,05). Sonuçlar: Kök kanal şekillendirmesi anlamlı bir şekilde KDD'nin düşük çıkmasına neden olmuştur. Tüm patlar NK grubuna kıyasla KDD'de artışa yol açmışlardır. Zamanın KDD üzerinde istatistiksel olarak anlamlı bir etkisi bulunamamıştır. Anahtar kelimeler: Kalsiyum silikat kök kanal dolgu patı; kök kanal dolgusu; vertikal kök kırığı; zaman; endodonti.*

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Original research

# Evaluation of the knowledge, attitudes and behaviors of pre-school teachers on oral and dental health in the city center of Trabzon

**Ezgi Baltacı[1], Özgül Baygın[2], Tamer Tüzüner[2], Fatih Mehmet Korkmaz[3]**

**Purpose**
Educators can also contribute to the prevention of early childhood caries (ECC) by educating children about oral diseases and hygiene practices. The aim of this study was to assess the knowledge, attitudes, and behaviors of pre-school teachers concerning oral and dental health in Trabzon.

**Subjects and methods**
The study was carried out with 226 pre-school teachers in the city center using a self-administered questionnaire to evaluate their knowledge, attitudes, and behaviors concerning the oral and dental health of children.

**Results**
Only 29.8% of the respondents had a prior dental education. Although 83% of the teachers said that regular visits to the dentist were effective for caries prevention, only 13.2% said that they made regular visits to a dentist. Approximately 65.8% of the teachers agreed that fluoride strengthens tooth enamel and 35.1% of the teachers said that there were oral health activities in their schools. Additionally, 74.6% of teachers said that they would participate in dental education about children's oral health in the future.

**Conclusion**
Preventive dental health program could be implemented including the proper behaviors related to the importance and treatability of primary teeth, first tooth cleaning, dental visits, the use of fluoride toothpaste, and oral hygiene into the pre-school teachers' undergraduate education programs in the future. This program should be repeated at certain intervals for the prevention of ECC.

***Keywords:*** *Dental education; oral health; preschool; early childhood caries; teacher*

## Introduction

Starting in the prenatal period, it is important to raise parental awareness for early childhood caries (ECC), which includes clarifying incomplete or incorrect knowledge and reshaping the attitudes and behaviors of the parents; these are essential factors for the prevention of ECC. Therefore, the knowledge level of the occupational groups who are in contact with the affected age groups and their parents is important for guiding the parents by providing correct information. One of these occupational groups comprises of teachers. Teachers may be considered alternative staff members in the struggle against dental caries, a preventable disease because they are able to affect a large number of children and parents. The healthy behaviors and lifestyles that are formed in the early years of life are more permanent than those acquired later. The messages required for a healthy life can be repeated every school year (1).

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Schools have an important role in developing programs for the prevention of oral and dental health problems during childhood. There are studies reporting children who are at school for an education are more likely to be positively affected (2). Children need to be given health education to create good oral and dental health, and this education is supposed to cause a beneficial behavioral change in the child. Behavioral changes occur through regular and long-term health education. For this reason, schools are suitable centers for health education because they provide a stable environment and stable conditions. Knowledgeable and communicable teachers are the basic element in the oral and dental health education provided at schools. It is important for the curricula at vocational high schools or the faculties of prospective teachers to incorporate health topics, including health improvement and prevention. Additionally, the content and duration of the curriculum are significant. Moreover, the health education should be made permanent in order to ensure that teachers receive up-to-date health information after graduation (3-6).

One of the important factors in oral health is the management of dental injuries. Since children are involved in sporting activities at school and in situations with close contact or physical activity, eventual injuries may occur due to falls, accidents, etc. Successful management of the process from the time of the incident to the time of the dental visit in these trauma cases increases the chance of successful post-traumatic treatments. Good management of this process depends on the knowledge level of the teacher. The proper orientation of a child and his/her parents can give the dentist a chance for early intervention. It is important for a teacher to know what to do in case of an emergency with regard to the primary and permanent teeth (3-9).

Many studies have shown that the use of teachers in improving the oral health of schoolchildren is successful (10). However, according to some reports, teachers are reluctant to take part in oral health programs requiring supervision (11). It is thought that this may be related to the lack of knowledge of teachers concerning oral health issues (12). The inadequate training of teachers on this subject can cause them to give incomplete or incorrect information to students or not teach the students effectively. In addition, a lack of necessary tools, resources, time, and integrated curriculum of oral health education are some of the limitations of school-based dental education (13).

It is crucial for pre-school and classroom teachers, who have been in contact with children for a long time and have an influence on their education, to receive oral and dental health education. Children spend most of their time in school, especially throughout their formative years. The role of a teacher is critical in these developmental processes. It is internationally recognized that teachers now play a potentially important role in school-based dental education, so the knowledge level of teachers on oral and dental health is very important (14).

The purpose of this study is to identify the knowledge level, attitudes and behaviors of pre-school teachers in the city center of Trabzon concerning oral and dental health, to determine areas of incomplete or incorrect knowledge by conducting a questionnaire, to raise awareness about oral and dental health in pre-school teachers and to ensure that pre-school teachers guide children and their parents with regard to the correct practices.

## Subjects and Methods

*Study design*

The ethics committee approval for the study was received from the Karadeniz Technical University Faculty of Medicine Clinical Research Ethics Committee (2015/103, 04/11/2015), and the necessary permissions were received from Trabzon Governorship Provincial Directorate of National Education. According to the data obtained from the latter governing body, it was determined that there are 564 pre-school teachers in Trabzon, 306 of whom are in the city center. The names and addresses of the schools affiliated with the central district, including the nursery classes, were obtained from the Provincial Directorate of National Education.

In total, 72 schools and 226 teachers received a questionnaire. Teachers were visited at their schools, which were located in the city center of Trabzon, and they were given a questionnaire evaluating their knowledge, attitudes, and behaviors regarding the oral health of children and occupational health. The questionnaire included 40 questions and consisted of three parts. The first part had questions related to demographic information, the second part aimed to measure the level of general knowledge about oral health, and the third part included informational questions about the attitudes and behaviors of the teachers concerning oral health. The types of questions in the questionnaire were open-ended, closed-ended, and multiple choice.

After preparation of the questionnaire, a pilot study was conducted with 10 teachers; the existence of incomprehensible questions was identified, the opinions of the teachers were evaluated, and the necessary corrections were made. Then, the teachers were visited, the purpose of the study was briefly explained, and the questionnaire was given to the voluntary participants. It took an average of 10 minutes for the teachers to complete the questionnaire, and while the teacher was responding to the questionnaire (within working hours), the researcher waited at a certain distance and answered only the questions that the teacher could not understand. Those who were not available or at school on the day of the questionnaire or did not want to participate were excluded from the study. The demographic information section of the questionnaire asked about age, gender, marital status, parental status, number of children, monthly income, duration of professional experience, and whether they received any previous education concerning children's oral health.

The second part, which measured the knowledge of oral health, included information on the consequences of periodontal problems, factors causing decay, effective methods for caries prevention, information about primary teeth and general health, correct and incorrect known facts about fluoride, a child's first dental visit, teeth cleaning, appropriate nutritional options for children, and information on what can be done about permanent and primary teeth dentition trauma.

The third part, which investigated the attitudes and behaviors of teachers concerning their own oral health, included questions about the frequency, duration and time of tooth brushing, preferred tools for interface cleaning, frequency of dental visits, reasons for visiting the dentist,

their views on the attitudes toward the preventive treatments of dentists and the availability of activities related to oral and dental health education in their schools.

*Statistical analysis*

SPSS sotware (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp, USA) was used for the statistical analysis. Descriptive statistics, the comparison of the average scores and related tests were performed. In the questions regarding preventive dentistry, a Chi-square test was used to examine the relationship between correct responses and the status of receiving training, the duration of professional experience, and parental status. Confidence interval was set to 95% and $p<0.05$ was considered statistically significant.

## Results

The results show that, in the survey, 126 of 226 teachers were female, 100 were male, 69.3% had previous education on oral and dental health, 67.5% had children, and 27.2% had more than 10 years of professional experience. The evaluation of teachers' knowledge of tooth decay is given in Table 1. Among the teachers who think that it is possible to have non-carious teeth, there are no statistically significant difference s between the ones who have had education on oral health and those who have not; the ones who have children and those who do not; and the ones whose professional experience was more than 10 years or was less than 10 years ($p>0.05$). Among those who think that the problems in the primary teeth affect permanent teeth and those who think that caries in the primary teeth is treatable, no statistically significant difference was found in terms of previous oral health education, parental status or professional experience ($p>0.05$) (Table 2). As the most effective method for preventing tooth decay, the items regarding tooth brushing (211 people, 92.5%), visiting the dentist regularly (190 people, 83.3%), using dental floss (161 people, 70%), limiting sugary snacks (158 people, 69.3%), using fluoride dental products (89 people, 39%) and chewing sugarless gum (56 people, 24.6%) were chosen. A significant difference exists between the teachers choosing the item "using fluoride dental products" in terms of being educated on oral and dental health or not ($p>0.05$). Accordingly, it is understood that the educated people were of the opinion that consuming fluoride dental products prevented decay. An examination of the other chosen options demonstrated no significant difference in terms of being educated on oral and dental health, parental status or the duration of professional experience ($p>0.05$) (Table 3).

The number of people who think that dental hygiene in children should start at the time of first tooth eruption is 115 (50.4%). Previous education concerning oral and dental health, parental status or the duration of professional experience did not cause any statistically significant result for the teachers who selected this option ($p>0.05$). Among the 40 teachers (17.5%) who reported that the first dental visit should not occur until after a child is 1 year old, there is no statistically significant difference in terms of previous education concerning oral and dental health, parental status or the duration of professional experience ($p>0.05$) (Table 4).

In terms of previous oral and dental health education, parental status, or a professional experience more than 10 years ($p<0.05$), statistically significant results were found among 150 (65.8%) teachers who believe that fluoride strengthens tooth enamel. The number of teachers who stated that fluoride is nutritional for teeth and bones is 100 (36.8%), and among them, a statistically significant difference was identified in terms of being educated on oral and dental health ($p<0.05$), but not in terms of the duration of professional experience or parental status ($p>0.05$). A total of 116 teachers (50.9%) noted that fluoride is an anti-oral bacterial agent and, among them, there was a statistically significant difference in terms of being educated on oral and dental health education ($p<0.05$), but no statistically significant difference was found in terms of parental status or the duration of professional experience ($p>0.05$). Among 69 teachers (30.3%) who noted that the fluoride can treat initial caries, there was a statistically significant difference in terms of being educated on oral and dental health and having more than 10 years of professional experience ($p<0.05$), but being a parent had no significant effect on this option ($p>0.05$). A total of 54 (23.7%) teachers thought that the use of fluoride toothpaste should start in children who were approximately 3 years old, and there was not any statistically significant difference in terms of being educated on oral and dental health, parental status or the duration of professional experience ($p>0.05$) (Table 5).

A total of 161 (70.6%) the teachers indicated that evaluation should be done as soon as possible in the case of mouth injuries/traumas. Among those having oral and dental health education and having children, there was no significant difference ($p>0.05$), but a statistically significant difference existed in the teachers with less than 10 years of professional experience ($p<0.05$). The question, "Which one is best to do for the primary tooth that has been completely removed as a result of injury, falling, etc.?" was answered as "It should never be replaced" by 51 teachers (22.4%) and "I do not know" by 107 teachers (46.9%). No statistically significant difference was found in these respondents in terms of receiving oral and dental health education, parental status or the duration of professional experience ($p>0.05$). The question, "Which one is best to do for the permanent tooth which has completely removed as a result of injury, falling, etc.?" was answered with, "Find the tooth" by 32 teachers, (20.2%), "Rinse it" by 46 teachers (20.2%), "Replace it immediately" by 29 teachers (12.7%), and "I do not know" by 151 teachers (66.2%). Among the respondents who chose these options, there was not any statistically significant difference in terms of receiving oral and dental health education, parental status, or the duration of professional experience ($p> 0.05$) (Table 6).

An evaluation of the personal oral hygiene behaviors of the teachers showed that 73.2% of the teachers brushed their teeth twice a day, but tooth-brushing time was less than 2 minutes; 51.3% of the teachers changed their toothbrushes every 3 months, and only 46.1% used dental floss. Although regular dental visits were one of the most frequently chosen options for the prevention of tooth decay, only 13.2% of the teachers went to the dentist at regular intervals, 42.1% of the teachers visited the dentist only when they had a toothache, 58.8% of the teachers had the most recent visit to the dentist for tooth decay/fillings, and 33.3% of the teachers did not pay enough attention to the preventive treatments by dentists.

**Table 1.** *Evaluation of the knowledge of teachers about tooth decay.*

| | Number | Percentage |
|---|---|---|
| **What are the causes of tooth decay?** | | |
| Inadequate brushing. | 208 | %91,2 |
| Sweet and acidic foods. | 189 | %82,9 |
| Lack of regular dental check. | 147 | %64,5 |
| Dental plaque. | 91 | %39,8 |
| Oral and dental health affects general health. | 216 | %94,7 |
| It is possible to have non-carious teeth. | 171 | %75 |
| Problems in primary teeth affect permanent teeth. | 141 | %61,8 |
| Caries in primary teeth are treatable. | 158 | %69,3 |

**Table 2.** *The relationship between the knowledge of dental caries and education, parental status, and professional experience.*

| | Those who have received education | Those who have not received education | *p* value | Those with children | Those without children | *p* value | <10 years professional experience | >10 years professional experience | *p* value |
|---|---|---|---|---|---|---|---|---|---|
| **It is possible to have non-carious teeth** | n=55<br>%77,9 | n=116<br>%73,4 | 0,582 | n=111<br>%72,1 | n=60<br>%81,1 | 0,191 | n=125<br>%42,8 | n=46<br>%64,5 | 1,000 |
| **Problems in primary teeth affect permanent teeth** | n=43<br>%70,5 | n=98<br>%71 | 1,000 | n=94<br>%67,1 | n=47<br>%77 | 0,214 | n=104<br>%70,7 | n=37<br>%31 | 0,895 |
| **Caries in primary teeth are treatable.** | n=54<br>%84,4 | n=102<br>%75 | 0,190 | n=105<br>%76,6 | n=53<br>%81,5 | 0,545 | n=105<br>%74,5 | n=53<br>%86,9 | 0,076 |

**Table 3.** *The relationship between the knowledge of the prevention of decay and parental status and professional experience (*p<0.05 was considered statistically significant).*

| **Which method is more effective for you to prevent tooth decay?** | **Those who have received education** | **Those who have not received education** | ***p* value** | **Those with children** | **Those without children** | ***p* value** | **<10 years professional experience** | **>10 years professional experience** | ***p* value** |
|---|---|---|---|---|---|---|---|---|---|
| Limiting sugary snacks. | n=53<br>%77,9 | n=103<br>%65,2 | 0,081 | n=105<br>%68,2 | n=53<br>%71,6 | 0,708 | n=113<br>%68,1 | n=45<br>%72,6 | 0,620 |
| Using fluoride dental products. | n=40<br>%58 | n=47<br>%29,7 | 0,000* | n=55<br>%35,7 | n=34<br>%45,9 | 0,138 | n=63<br>%38 | n=26<br>%41,9 | 0,583 |
| Chewing sugarless gum. | n=20<br>%29,4 | n=36<br>%22,8 | 0,373 | n=36<br>%23,4 | n=20<br>%27 | 0,663 | n=39<br>%23,5 | n=17<br>%27,4 | 0,660 |
| Brushing teeth. | n=60<br>%88,2 | n=149<br>%94,3 | 0,190 | n=139<br>%90,3 | n=72<br>%97,3 | 0,104 | n=154<br>%92,8 | n=57<br>%91,9 | 0,783 |
| Using dental floss. | n=44<br>%64,7 | n=115<br>%72,8 | 0,289 | n=104<br>%67,5 | n=57<br>%77 | 0.187 | n=114<br>%68,7 | n=47<br>%75,8 | 0,374 |
| Regular dental visits. | n=51<br>%75 | n=137<br>%86,7 | 0,049 | n=126<br>%81,8 | n=64<br>%86,5 | 0,487 | n=138<br>%83,1 | n=52<br>%83,9 | 1,000 |

**Table 4.** *The relationship between the knowledge of the first dental hygiene and dental visits of children and education, parental status and professional experience.*

| | Those who have received education | Those who have not received education | p value | Those with children | Those without children | p value | <10 years professional experience | >10 years professional experience | p value |
|---|---|---|---|---|---|---|---|---|---|
| Parents should start teeth cleaning at the time of first tooth eruption. | n=37 %54,4 | n=78 %49,4 | 0,487 | n=79 %51,3 | n=36 %48,6 | 0,708 | n=84 %50,6 | n=31 %50 | |
| The first dental visits of children should not be later than 1 year old. | n=13 %19,1 | n=27 %17,1 | 0,860 | n=22 %14,3 | n=18 %24,3 | 0,093 | n=28 %16,9 | n=12 %19,4 | 0,807 |

**Table 5.** *The relationship between the knowledge of fluoride and education, parental status and professional experience (*p<0.05 was considered statistically significant).*

| | Those who have received education | Those who have not education | *p* value | Those with children | Those without children | *p* value | <10 years professional experience | >10 years professional experience | *p* value |
|---|---|---|---|---|---|---|---|---|---|
| Fluoride strengthens the tooth mine. | n=61 %89,7 | n=87 %55,1 | 0,000* | n=94 %61 | n=56 %75 | 0,042* | n=102 %61,4 | n=48 %77,4 | 0,035* |
| Fluoride is nutritional for teeth and bones. | n=35 %51,5 | n=47 %29,7 | 0,002* | n=56 %36,4 | n=28 %37,8 | 0,829 | n=61 %36,7 | n=23 %37,1 | 1,000 |
| Fluoride is an anti-oral bacterial agent. | n=45 %66,2 | n=69 %56,3 | 0,003* | n=77 %50 | n=39 %52,7 | 0,702 | n=81 %48,8 | n=35 %56,5 | 0,303 |
| Fluoride can treat initial caries. | n=34 %50 | n=33 %20,9 | 0,000* | n=48 %31,2 | n=21 %28,4 | 0,783 | n=43 %25,9 | n=26 %41,9 | 0,019* |
| Fluoride toothpaste in children should start around 3 years old. | n=11 %16,2 | n=43 %27,2 | 0,106 | n=41 %26,6 | n=13 %17,6 | 0,180 | n=42 %25,3 | n=12 %19,4 | 0,444 |

An evaluation of the teachers' attitudes toward oral and dental health showed that 64.9% of them did not have any activities concerning oral and dental health in their school, 55.7% knew that they had oral and dental health education in their school curriculum, 71.5% did not have any course on oral health care during their undergraduate education, and 74.6% of them would participate in a course on oral and dental health care in the future.

## Discussion

Among childhood diseases, early childhood caries (ECC) continues to be a highly prevalent disease. ECC afflicts children's quality of life and their learning abilities (15-17). At the same time, other factors that should not be undervalued, including the fact that parents' quality of life is also affected (17), the financial burden that is encountered in coping

**Table 6.** *The relationship between the knowledge of oral injuries and education, parental status and professional experience (*p<0.05 was considered statistically significant).*

| Oral injuries/ trauma | Those who have received education | Those who have not education | *p* value | Those with children | Those without children | *p* value | <10 years professional experience | >10 years professional experience | *p* value |
|---|---|---|---|---|---|---|---|---|---|
| Evaluation should be done as soon as possible in case of oral injuries. | n=43 %63,2 | n=116 %73,4 | 0,124 | n=109 %70,8 | n=52 %70,3 | 1,000 | n=126 %75,9 | n=35 %56,5 | 0,004* |
| A primary tooth which has removed should never be replaced. | n=19 %27,9 | n=32 %20,3 | 0,274 | n=31 %20,1 | n=20 %27 | 0,317 | n=41 %24,7 | n=10 %16,1 | 0,229 |
| Which one is best to do for the permanent tooth, which has completely removed due to an injury? | | | | | | | | | |
| Find the tooth. | n=10 %14,7 | n=22 %13,9 | 1,000 | n=15 %9,7 | n=17 %23 | 0,013 | n=22 %13,3 | n=10 16,1 | 0,732 |
| Rinse the tooth. | n=18 %26,5 | n=28 %17,7 | 0,187 | n=29 %18,8 | n=17 %23 | 0,580 | n=31 %18,7 | n=15 %24,2 | 0,460 |
| Replace the tooth immediately. | n=12 %17,6 | n=17 %10,8 | 0,229 | n=16 %10,4 | n=13 %17,6 | 0,190 | n=17 %10,2 | n=12 %19,2 | 0,106 |
| I do not know | n=42 %61,8 | n=107 %67,7 | 0,386 | n=107 %69,5 | n=44 %59,5 | 0,134 | n=112 %67,5 | n=39 %62,9 | 0,623 |

with ECC (16), and the life-long consequences of the risk of caries in primary dentition that could influence permanent dentition (18).

ECC is a preventable disease. It is a problem that arises due to social, behavioral, medical, and political reasons, and socioeconomic factors, family structure, and social dynamics need to be understood for its solution. ECC is often seen among disadvantaged individuals in the community. For a solution, health politics, social goals, and values need to be reviewed. ECC depends on behavioral elements because it is a result of the daily routines of the individuals around a child, such as parents, caregivers, grandparents. It is possible to create appropriate oral hygiene behaviors and to inform individuals about oral health and the effects of oral health on general health through community-based practices and education (1,4,15).

Since there is no scientific evidence of the superiority of one method over another, the prevention of early childhood caries should not be based on a single approach (19). Therefore, the prevention of ECC requires disseminating information about ECC through approaches that concern the whole society and address its existing etiological reasons. The real challenge in the fight against ECC is to be able to attract the attention of mothers and children before problems arise. Information about ECC should be provided through the media, community education programs, courses for pre- and post-natal parents, women's health clinics, and schools. Education and motivation are not enough to make long-term changes. The behavior of individuals is usually shaped in early childhood and is rooted in the environment surrounding the child and the individual (20-22). To form a behavioral change in a child, it is necessary for the child to be informed by different sources of information and socially supported by his/her parents, peers, and other individuals in their daily lives (23). Teachers, as the people who spend the most time with children throughout their formative years after their parents, may play an important role in informing children about oral health and developing appropriate hygiene behaviors.

Within the scope of this cross-sectional study, primary and secondary schools in the center of Trabzon, including nursery classes, were visited, and 226 teachers were given a questionnaire on their knowledge, attitudes, and behaviors concerning oral and dental health. Our aim was to set the

groundwork for the work that is needed to make school-based dental education a social practice by identifying the issues that teachers had incomplete or incorrect knowledge of. In addition, the teachers' correct answers were investigated in terms of receiving a previous education, parental status and the duration of their professional experience.

In total, 95.6% of the teachers in the study were female, and 94.7% of the teachers thought that oral and dental health affected general health. The proportion of teachers who had this opinion was 84.8% (26) and 74% (24) in the studies in Indian cities. In our study, 75% of teachers stated that it was possible to have non-carious teeth, 61.8% thought that the problems in the primary teeth affected permanent teeth, and 69.3% thought that tooth decay in a primary tooth was treatable. In a Nigerian study28, 89.4% of the teachers reported that tooth decay was not normal, 75% took dental problems seriously, and 76.9% responded that tooth loss can be avoided if it is treated.

Those who believed that using fluoride dental products was effective in preventing tooth decay was 39% in our study, while the proportion was 6.2% (26), 89% (28) and 84% (25) in other studies. The proportion of the teachers who thought that consuming fluoride tooth products were effective in preventing tooth decay was found to be significant in those who had received previous education concerning oral and dental health ($p<0.05$). Compared with the other studies, the knowledge level of the teachers about fluoride tooth products seemed to be inadequate.

The American Academy of Pediatric Dentistry indicates that the cleaning of teeth should start when the first primary tooth is seen in the mouth and that parents must brush their children's teeth twice a day with a toothbrush that is suitable for the child's age. For children under 3 years old, 'a thin smear' or a 'rice size' portion of fluoride toothpaste should be used and, for those between 3-6 years, a 'pea size' portion of fluoride toothpaste should be used. In addition, within 6 months after the first tooth erupts, but before the baby is 1 year old, a dental visit is recommended for a caries risk assessment, parental education concerning oral hygiene and preventive methods, and guidance in oral diseases (29).

In our study, 50.4% of the teachers believed that children should start dental hygiene when their first tooth erupts, whereas 17.5% said that the first dentist visit should not be later than 1 year of age. It is determined that the teachers had incomplete knowledge about the guidance that can be effective in preventing early childhood decay. In similar studies with parents, 28.8% (30) and 52.5% (31) of the parents thought that the first dentist visits should be earlier than 1 year. We believe that the timing of the first dental visit and initial dental cleaning should be emphasized in the social awareness practices that are conducted for the prevention of ECC.

In our investigation of the knowledge level of fluoride, 65.8% of the teachers reported that fluoride toothpaste strengthens tooth enamel, and 30.3% indicated that fluoride toothpaste can treat initial tooth caries. Both outcomes are statistically significant in the teachers who received prior education and had more than 10 years of professional experience. In a similar study (14), 86.3%, and 13.3% of the teachers thought that fluoride toothpaste strengthens tooth enamel and that it could treat initial caries, respectively. Accordingly, we believe that more comprehensive information on fluoride would be beneficial for raising social awareness and reducing the rising prejudice against fluoride.

For the trauma issue, our study showed that 70.6% of the teachers believed that interventions should be performed as soon as possible after the trauma. This rate was reported as 74.7% in another study (32). Unfortunately, in the case of avulsion-type injuries in primary and permanent teeth, the proportion of the teachers who believed that the tooth should be replaced immediately in permanent teeth avulsion was 12.7%. This rate was 43% in a similar study in Singapore (33) and 16.2% in Hong Kong (32). Early interventions in traumatized teeth are described as extremely important for the long-term success of treatment in trauma cases (34,35). In our study, 22.4% of the teachers thought that the primary tooth should never be replaced after primary dental avulsion. This rate was found to be 74.6% in teachers in Hong Kong (32). The fact that the teachers had inadequate knowledge complies with the current literature (36-38). The lack of information on trauma may be due to the lack of trauma experience of the teachers. Teachers' guidance is important for the prevention of trauma and for the proper orientation of parents after trauma. We suggest that the lack of information on this issue should be emphasized.

The curricula in our country's Pre-school and Classroom Teaching departments were reviewed in terms of their oral and dental health education. This examination revealed that there is a compulsory course in the pre-school program called "Mother-Child Health and Diseases" that is taught for 3 hours a week in the 3rd semester. A topic regarding the oral and dental health of children is available in this course. Moreover, in the elective course, "Mother and Child Nutrition," the relationship between nutrition and oral and dental health is mentioned. Only 28.5% of the teachers reported that they had a lesson on oral health during their undergraduate studies. The proportion of the teachers whose schools had oral and dental health activities was 35.1%, and 55.7% of the teachers noted that oral and dental health education for children existed in their school curriculum. In our study, 74.6% said that they would join an educational program about oral and dental health in children if it were given in the future. According to this result, we believe that the curriculum of pre-school teachers' faculties should have more oral hygiene topics. The underlying reasons why an oral health education was not be provided in some schools, even though the school curriculum included it, should be investigated and a solution can be provided. Dental education should be systematized and applied within the scope of health policies.

## Conclusion

School-based dental education is one of the major social steps in fighting tooth decay. While teachers have knowledge of the causes of tooth decay and methods for preventing tooth decay in general, they do not have knowledge of protective practices, fluoride, first dental visits in children, first tooth cleaning and trauma, according to the results from our study. These deficiencies need to be addressed so that teachers can take an active role in school-based dental education, help parents, and positively influence children to help create healthy generations. If teachers have knowledge

about the issue, they can educate children and parents more effectively. For the teachers to reach the desired level of knowledge, the importance of oral and dental health and their education should be emphasized, starting in the undergraduate programs, and continuing awareness about this issue should be made permanent.

**Ethics committee approval:** The ethics committee approval for the study was received from the Karadeniz Technical University Faculty of Medicine Clinical Research Ethics Committee (2015/103, 04/11/2015), and the necessary permissions were received from Trabzon Governorship Provincial Directorate of National Education.

**Informed consent:** Informed consent was provided by the participants' parents.

**Author contributions:** EB and ÖB participated in designing the study. EB participated in generating the data for the study. EB and ÖB participated in gathering the data for the study. TT participated in the analysis of the data. TT and FMK wrote the majority of original draft of the paper. ÖB, TT and FMK participated in writing the paper. All authors approved the final version of the paper.

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***Türkçe öz:*** *Trabzon merkezindeki okullarda, okul öncesi öğretmenlerinin ağız ve diş sağlığı hakkındaki bilgi düzeylerinin değerlendirilmesi. Amaç: Okul öncesi eğitimciler; ağız ve diş sağlığı hakkında yeterli bilgi ve davranışlara sahip olurlarsa, okul temelli dental eğitimde rol alarak, aynı anda çok sayıda çocuk ve ebeveyne ulaşabilirler ve onları, ağız hastalıkları ve hijyen alışkanlıkları konusunda eğiterek erken çocukluk çağı çürüklerinin (EÇÇ) engellenmesine katkı sağlayabilirler. Bu çalışmanın amacı Trabzon merkezindeki okul öncesi öğretmenlerinin ağız ve diş sağlığı hakkındaki bilgi düzeylerini değerlendirerek eksik veya yanlış bilinen konuları saptamaktır. Bireyler ve yöntem: Çalışmada Trabzon merkezine bağlı ana sınıfı ve anaokullarına gidilerek toplamda 226 okul öncesi öğretmenine çocukların ağız ve iş sağlığı hakkında bilgi, tutum ve davranışlarını değerlendiren anket uygulaması yapıldı. Bulgular: Öğretmenlerin sadece %29,8'inin daha öce ağız ve diş sağlığı ile ilgili eğitim aldığı tespit edildi. Çalıştıkları okullarda ağız ve diş sağlığı ile ilgili çalışmalar olan öğretmenlerin oranı %35,1 olarak belirlendi. Öğretmenlerin %74,6'sı ileride çocuklarda ağız ve diş sağlığı ile ilgili eğitim verildiğinde katılmak isteyeceğini belirtti. Koruyucu uygulamalar, ağız yaralanmaları ve ağız hijyeni alışkanlıkları konusunda bilgi eksiklikleri tespit edildi. Sonuç: Süt dişlerinin önemi, tedavi edilebilirliği, çocuklarda ilk diş temizliği ve diş hekimi ziyaretleri, çocuklarda fluorlu diş macunu kullanımı konusunda, ağız hijyeni ile doğru davranışlar ile ilgili uygun bir dental eğitim programı hazırlanarak okul öncesi öğretmenlerine lisans düzeyinden başlayarak belirli aralıklarla tekrarlanmasının EÇÇ'nin engellenmesi konusunda faydalı olacağı kanısındayız. Anahtar kelimeler: Ağız sağlığı; okul öncesi; bilgi; erken çocukluk çağı çürükleri; öğretmen*

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Original research

# Comparison of newly formed microcracks after instrumentation using Protaper Next, Reciproc and Self-Adjusting File systems

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**Purpose**
The purpose of the present study is to assess the rate of newly formed microcracks comparatively during root canal instrumentation by using ProTaper Next, Reciproc, and Self-Adjusting File systems using micro-computed tomography.

**Subjects and Methods**
Thirty mesial roots of mandibular molars were randomly assigned to 3 experimental groups (n = 10) as follows, ProTaper Next, Reciproc and Self-Adjusting File system. Preoperative and postoperative scans were obtained at the isotropic resolution of 13.68 µm. Cross-section images were examined to identify the presence of newly formed dentinal microcracks.

**Results**
The Self-Adjusting File and Reciproc systems caused a higher rate of new microcrack formation than the ProTaper Next group ($p < 0.001$). There were also significant differences in the new microcrack formation between Reciproc and Self-Adjusting File groups ($p < 0.001$).

**Conclusion**
Reciproc and Self-Adjusting File systems caused a higher rate of newly formed microcraks compared to ProTaper Next system.

***Keywords:*** *Dentinal microcracks; micro–computed tomography; Protaper Next; Reciproc; Self-Adjusting File*

## Introduction

The microcracks that occur during root canal shaping procedures can propagate repetitious occlusal forces, be exposed to stresses caused by root canal obturation and retreatment, and result in vertical root fracture (1,2). Numerous studies reported that the use of rotary instrumentation causes microcracks in root dentin (3,4).

In the ProTaper NEXT System (PTN) (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA), which is made by M-wire technology, conventional continuous rotation motion is used. This system has an off centre rectangular cross section with regressive and progressive percentage tapers on a single file. Off-centred rectangular structure provides the file a snake-like, swaggering motion, minimizes the contact between the dentin and file, thus reduces the screw effect (5).

In Reciproc (VDW, Munich, Germany) system, a single file is used with a reciprocating motion. According to the manufacturer, in this system there are 1500 counter clockwise and 300 clockwise movements in the reciprocating motion. Thereby, following three reciprocating motions it enables 3600 preparation after three reciprocating motions. Special

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clockwise and counter clockwise movements of this motion diminish the stress on the instrument (4).

The self-adjusting file (SAF) (ReDent Nova, Ra'anana, Israel) system is a NiTi file and has a hollow cylindrical instrument designed as a thin nickel-titanium lattice with compressible walls. When root canal is prepared, the compressible walls inserted into a narrow root canal allow the instrument to adapt to the root canal shape both along the cross section and longitudinally. Consequently, the SAF instrument three-dimensionally conforms to the root canal with circular and oval cross sections getting maintenance in the original canal shape. In addition, the lattice design of the SAF allows the irrigation solution to flow continuously through the hollow file (6,7).

Recently, micro computed tomography (micro-CT) has been used in assessing dentinal microcracks (8,9). Micro-CT provides reproducible data in all three dimensions and compares the data on each tooth before and after the root canal preparation. However, there is conflicting results between the studies in which the newly microcrack formation was evaluated. De-Deus et al. (8) and De-Deus et al. (9) reported that mechanical enlargement processes can not be related to the formation of new microcracks. However, in another micro-CT study by Ceyhanli et al. (10) the evaluated instrumentation systems increased the number of microcracks when compared to preoperative images. Thus, the aim of this study is to assess newly formed microcracks comparatively during root canal instrumentation by using ProTaper Next, Reciproc, and self-adjusting file systems using micro-CT.

## Subjects and Methods

*Study sample*

After the approval by the ethics committee of University of Ordu (17.04.2014-2014/02), human mandibular first and second molars were collected. The reason for extraction of the teeth was unrelated to this study. The residues were removed by cleaning the outer surfaces of the teeth, and they were stored in purified filtered water until they were used. The roots were investigated by a stereomicroscopy under 25× magnification to exclude roots having cracks or craze lines. The selected teeth having similar lengths were decoronated, and the distal roots were separated with a diamond-coated bur using water cooling. The mesial roots were left at approximately 11 mm to obtain a standardized length. Canal patencies of both mesiobuccal and mesiolingual canals were established with a #15 K-file (Mani, Inc., Tochigi, Japan). The exclusion criteria were obstructed in root canals, having two or more apical foramen, curvature angle under 10º and upper 20º, pre-operative canal size greater than ISO size 15 and pre-existing craze lines or cracks.

As a result, 30 mesial roots were included the study. Digital radiographs were taken buccolingually for evaluating canal curvatures and radii. The roots were randomly assigned to three groups (n=10). One-way ANOVA, revealed no significant difference among the groups in terms of the canal curvatures and radii ($p > 0.05$).

In order to simulate the periodontal ligament, the cement surfaces of roots were coated with a silicone impression material. The roots were then embedded in acrylic blocs as described by Liu et al. (11).

*Preoperative Micro-CT Analysis*

The test specimens were scanned with a commercially available high-resolution micro-CT system (SkyScan 1172; Bruker micro-CT, Kontich, Belgium). The X-ray tube was operated at 100 µA and 100 kV with a 0.5 mm Al filter, which has a resolution of 13.68 µm pixels. The scanning process was carried out by a 180° rotation around the vertical axis with a camera exposure time of 1550 ms, a frame average of 3, a rotation step of 0.4°, and a random movement of 20. The samples were scanned for approximately 60 minutes. The axial cross sections were reconstructed using the resulting two-dimensional images (8-bit TIFF). NRecon v.1.6.3 (Bruker micro-CT, Kontich, Belgium) was used to reconstruct the axial cross sections of the inner structure of the specimen with a smoothing of 2, a beam hardening correction of 50%, and an attenuation co-efficient range of 0-0.73.

*Canal instrumentation*

With PTN, Root canals were prepared with a K-file until #20 at the working length (WL). Then the PTN instruments were implemented using an electric motor at 300 rpm at 2 N/cm torque (X-Smart; Dentsply Tulsa Dental, Tulsa, OK). The X1 file (17.04) was used with a slow in-and-out pecking motions until WL was reached. Root canals were then rinsed using 10 ml 5.25% sodium hypochlorite (NaOCl) (Wizard, Istanbul, Turkey), and a #15 K-file was used to confirm patency. Same operations were accomplished with the X2, X3, and X4 files. When using Reciproc, Root canals were prepared with a K-file until #20 at the WL. The RECIPROC instrument was then operated using the "RECIPROC all" mode of the electric motor (VDW Gold, Munich, Germany). The R40 Reciproc file (40/0.06) was used with three slow in-and-out pecking motions and a reciprocating movement until WL was obtained. Root canals were prepared with SAF by using a K-file until #20 at the WL. Then, the SAF 2.0-mm file was applied using an in-and-out vibrating hand piece head (RDT3; ReDent Nova, Ra'anana, Israel) at at 5,000 vibrations per minute and at amplitude of 0.4 mm, as described by Metzger Z et al. [7]. The instrument was used at the WL in each canal with a packing motion for 4 minutes. During the preparation, the canal was irrigated with a pump (Vatea ReDent Nova, Ra'anana, Israel) and 5 ml 5.25% NaOCl per minute.

For all groups, the root canals were rinsed with a totally 40 ml 5.25% NaOCl, and a #15 K-file was used to confirm patency during instrumentation. Then, each canal was dried with sterile paper points. Both canals in the same root were prepared using the same instrument and a new instrument was used for another mesial root.

*Dentinal Microcrack Evaluation*

The images of the specimens before and after the canal preparation were superimposed using Data Viewer software (Bruker micro-CT). The cross-section images of the roots from the apex to the top of the root were examined to detect any dentinal microcracks (n = 7320).

*Statistical analysis*

The images were analyzed by two precalibrated examiners

to identify the presence of dentinal microcracks. In the case of divergence, the images were examined together until reaching an agreement. The formation of the new microcrack was recorded for groups and the data were then statistically analyzed by using chi square test. Data was therefore expressed as frequency. All calculations were performed with SPSS 23 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp, USA) statistical software. Confidence interval was set to 95% and $p<0.05$ was considered statistically significant.

## Results

A total of 21960 cross-sections were examined. Figure 1 shows the number of slices in each group with new formation of microcracks (Figure 2). The SAF and Reciproc systems caused a higher rate of new microcrack formation than that found in the PTN ($p < 0.001$). There were significant differences in the new microcrack formation between Reciproc and SAF groups ($p < 0.001$).

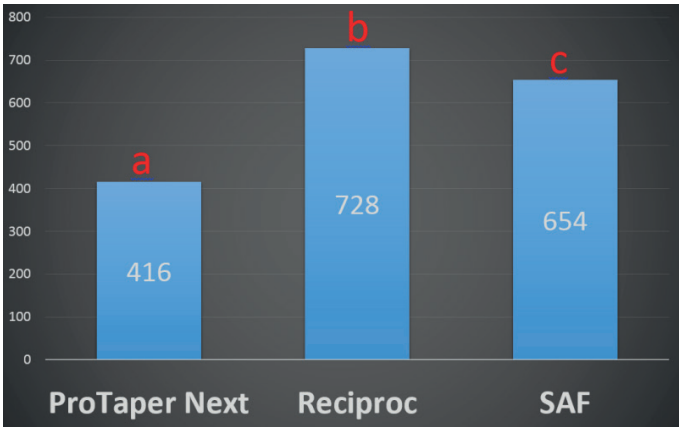


***Figure 1.*** *The number of slices in each group with new formation of microcracks.*

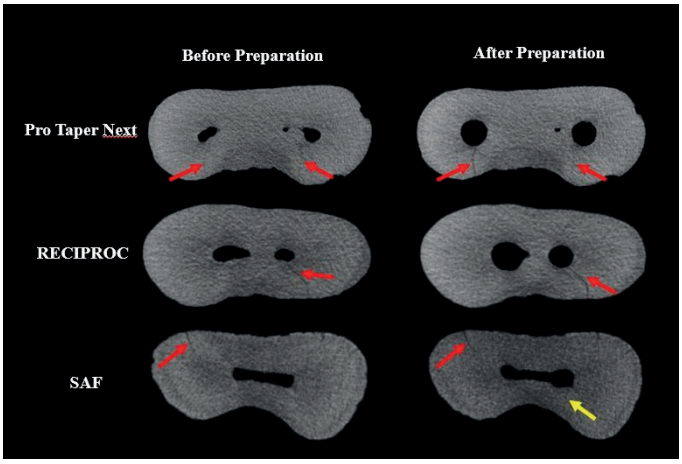


***Figure 2.*** *Representative cross-sectional images of 3 from each experimental group. Red arrows point at microcraks already present before preparation, yellow arrow point at new microcrack.*

## Discussion

In the root sectioning studies, the percentage of dentinal defects created by PTN ranges from 11.6% to 33.3% (12,13); the Reciproc ranges from 3% to 40% (1,4,11,14,15). These researches demonstrated a clear association between root canal preparation and created / propagation of dentinal microcracks. Conversely, the studies that used micro-CT conducted by De-Deus et al. (8,9) reported that mechanical enlargement processes cannot be related to the formation of new microcracks. However, in another micro-CT study by Ceyhanli et al. (10), the evaluated instrumentation systems increased the number of microcracks when compared to preoperative images. Similarly, in the current study, the root canal preparation with SAF, Reciproc, and PTN systems caused new formation of microcracks.

In the root sectioning studies, dentinal microcrack formation was not recorded after the root canal preparation with SAF 1.5 mm (3,11). However, Hin et al. (16) reported that the percentage amount of dentinal damage was 10% by SAF 2.0 mm. In the root sectioning studies, preoperative and postoperative findings cannot be compared and there is no research using micro-CT about microcracks formation by SAF in literature. In the present study, microcrack formation was recorded after canal shaping procedures with SAF 2.0 mm. The rate of newly formed microcrack caused by the SAF system was found statistically higher than that of PTN ($p < 0.001$). These conflicting findings may be due to the different instrumentation sizes (1.5 mm and 2.0 mm) and the differences between the evaluation methods.

According to root sectioning studies, the microcrack formation may be caused by rotational forces that were applied to the root canal walls, and this may be related to instrumentation kinematics, such as tip design, cross-section geometry, taper pitch design, and fluted form (4,15). In a study, Burklein et al. (4) reported that reciprocating files produced significantly more incomplete dentinal microcracks than full-sequence rotary systems. However, Liu et al. (11) and Ashwinkumar et al. (14) informed that the Reciproc instrument caused fewer microcracks than full-sequence rotary instruments. In the present study, Reciproc system caused the highest rate of newly formed microcracks.

In the literature, most of the studies reported that root canal treatment procedures, such as root canal shaping, obturation, and retreatment, cause microcracks in root dentin (4,12). In recent years, many destructive studies examining dentinal microcracks using stereomicroscope have been conducted (3,15). In these studies, samples are obtained by conventional root sectioning method, which causes the loss of a significant amount of dentin. Only a few slices per tooth can be analysed, and root sectioning can cause microcracks in the root dentin (9). In addition, the most important disadvantage of this method is, that because of the impossibility of investigating preoperative root dentin, it is not decided that the present microcracks occur due to the root canal preparation, are already present before, or occur during the root sectioning. Recently, micro-CT has been used in assessing dentinal microcracks. Micro-CT supplies a three-dimensional, highly accurate establishment of the precise location of preoperative microcracks and previously existing dentinal defects throughout the root dentin (8,9). contrast with traditional root sectioning methods, it is possible to evaluate hundreds of slices for each tooth in root using micro-CT imaging. In addition, micro-CT imaging is a non-destructive method that averts the necessity for cutting specimens. This approach arranges further experiments on the

same samples, such as investigating the effect of obturation retreatment post placement and removal procedures on developing dentinal defects. Thus, in the present study a non-destructive method (micro-CT) imaging system was used to evaluate newly formed microcracks.

## Conclusion

Within the limitation of this experimental study, it can be stated that the Reciproc and SAF are more likely to cause higher rate of newly formed microcraks than the ProTaper Next system.

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**Ethics committee approval:** The ethics committee of Ordu University Faculty of Dentistry has approved the study protocol (17.04.2014-2014/02).

**Informed consent:** Informed consent was obtained from all participants.

**Peer review:** Externally peer-reviewed.

**Author contributions:** FÇ, EBÇ, BS and AK participated in designing the study. FÇ, EBÇ, BS and AK participated in generating the data for the study. FÇ, EBÇ and FFK participated in gathering the data for the study. FÇ, EBÇ and BS participated in the analysis of the data. FÇ and EBÇ wrote the majority of the original draft of the paper. FÇ and EBÇ participated in writing the paper. All authors approved the final version of this paper.

**Conflict of interest:** The authors have no conflicts of interest to declare.

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***Türkçe öz:*** *ProTaper Next, Reciproc ve Self-Adjusting File sistemleri kullanılarak yapılan instrumantasyondan sonra yeni oluşan mikroçatlakların karşılaştırılması. Amaç: Bu çalışmanın amacı, ProTaper Next, Reciproc ve Self-Adjusting File (SAF) sistemleriyle yapılan kök kanal instrumantasyonundan sonra yeni oluşan mikroçatlakların mikro-bilgisayarlı tomografi kullanılarak karşılaştırmalı olarak değerlendirilmesidir. Gereç ve Yöntem: 30 adet mandibular molar dişin mesial kökü ProTaper Next, Reciproc ve SAF sistemleriyle hazırlanmak üzere rastgele 3 deneysel guruba ayrıldı (n=10). İşlem öncesi ve işlem sonrası, 13.68 µm izotropik çözünürlükte taramalar yapıldı. Elde edilen kesit görüntüleri üzerinde, yeni dentinal mikroçatlak oluşumunun varlığı incelendi. Bulgular: SAF ve Reciproc sistemler, ProTaper Next sistemine göre daha yüksek oranda yeni mikroçatlak oluşumuna sebep oldu ($p < 0.001$). Reciproc ve SAF gurupları arasında da yeni mikroçatlak oluşumu açısından anlamlı bir tespit edildi ($p < 0.001$). Sonuç: Reciproc ve SAF sistemler, ProTaper Next sistemiyle karşılaştırıldığında daha yüksek oranda yeni mikroçatlak oluşumuna sebep olmuştur. Anahtar kelimeler: Dentinal mikroçatlak; mikro-bilgisayarlı tomografi görüntüleme; Protaper Next; Reciproc; Self-Adjusting File.*

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Original research

# In-vitro evaluation of the effects of insertion and sterilization procedures on the mechanical and surface characteristics of mini screws

**Civan Aved Hergel[1], Yasemin Bahar Acar[2], Mustafa Ateş[1], Nazan Küçükkeleş[1]**

**Purpose**
The aim of the present study was to investigate the effects of insertion and sterilization on primary stability and to examine the mechanical and surface characteristics of mini screws.

**Materials and methods**
140 miniscrews (70 Dual-Top; 70 Ortho-Easy) were divided into 3 groups. Group 1: control group, 10 miniscrews of each brand, evaluated without any primary procedure. Group 2: 30 miniscrews of each brand, each inserted into the sawbone once, then sterilized and tested. Group 3: 30 miniscrews of each brand, each inserted into the sawbone twice, sterilized after each insertion and then tested. The miniscrews were evaluated for changes in primary stability, mechanical and surface characteristics with scanning electron microscopy (SEM) analysis, torsion tests, maximum insertion-removal torques and vertical-horizontal pull out strength tests.

**Results**
The maximum insertion torque values of the unused miniscrews (Group 1) were found to be significantly higher than those of the reused (Groups 2, 3) mini screws ($p<0.05$). Removal torque, vertical-horizontal pull-out strength and torsional strength value changes were found to be statistically insignificant. In SEM analysis, wear and atrophy were seen on the threads of used miniscrews especially in the apical region and the oxide layer was seen to have disappeared from some regions of the coated miniscrews.

**Conclusion**
Although wear and atrophy were detected in SEM analysis of used miniscrews, the overall primary stability and fracture torque resistance tests did not show any significant changes after the first and second insertion and sterilization procedures.

***Keywords:*** *Miniscrew; orthodontic mini-implant; primary stability; scanning electron microscope; sterilization*

## Introduction

Anchorage is defined as the resistance to unwanted tooth movement and anchorage control is the key factor in successful orthodontic treatment (1). Before skeletal anchorage devices, clinicians tried many kinds of mechanics to obtain better anchorage via intraoral and extra oral devices. However, the success of these treatments is greatly affected by patient discomfort, the need for patient cooperation and side effects (2,3). The idea of skeletal anchorage for orthodontic purposes arose from the need to eliminate anchorage losses and patient compliance problems. Gainsforth and Higley (4) performed the first successful application of skeletal anchorage in the mid-1940s with vitalium implants. In 1997, Kanomi (5) introduced first

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mini-implants designed for orthodontic anchorage. With developments in design and material, the miniscrews have become one of the most common skeletal anchorage devices, owing to simple placement, patient comfort and immediate loading capabilities. However, one important issue with miniscrews is that they can become loose due to their non-osseointegrated structure and immediate loading capability. Therefore, the primary stability, the stability immediately after insertion in bone, is crucial for the success of mini screws (5).

Miniscrews can be applied to almost every region of the mouth and can be used for many kinds of mechanics (6,7). As the application process is very easy and well accepted by patients, clinicians can move miniscrews to different regions of the mouth according to anchorage needs (8). Sterilization and recycling of orthodontic materials such as stainless steel braces or nickel titanium archwires have been documented in orthodontic practice. Buckthal and Kusy reported that 52% of clinicians recycle nickel-titanium wires to reduce the costs of orthodontic treatment (9). Some studies have shown that sterilization and recycling have no effect on the mechanical properties of orthodontic wires or braces, while others have shown mechanical or surface alterations (10-12). Like wires and braces, the recycling of miniscrews, although not well documented, is an issue in orthodontic practice.

In a survey carried out in Turkey in 2014, 41% of participants replied that they were using miniscrews more than once (13). Although the recycling and reuse of invasive materials may create an ethical concern and should not be encouraged, re-insertion of a miniscrew can be considered in the same patient (especially in cases of early failure, they can be re-inserted in a different location), if sterilization and previous insertion processes do not create any alteration in mechanical properties. Reports on this subject have shown conflicting results. Mattos et al. (8) found that in used miniscrews, the resistance to torsional forces was decreased and therefore the reuse of miniscrews was not recommended. In contrast, Noorollahian et al. (14). investigated insertion, removal and fracture torque differences between used and non-used miniscrews and found that insertion into bone and processing with 37% phosphoric acid for 10 minutes and 5.25% sodium hypochlorite for 30 minutes and re-sterilization with autoclave, had no adverse effects on insertion, removal, and fracture torque values. Estelita et al. (15) also demonstrated no change in torsional strengths of screws that had undergone recycling protocols

The aim of this study was to investigate the effects of insertion and sterilization on primary stability, mechanical and surface characteristics of miniscrews via SEM analysis, torsion tests, maximum insertion-removal torques and vertical-horizontal pull out strength tests, and thus, to evaluate the viability of reusing miniscrews.

## Materials and methods

140 miniscrews from two different brands were used for the study (70 DualTop G2, Jeil Medical, Seul, South Corea; 70 OrthoEasy, Forestadent, Pforzheim, Germany). Both miniscrews were cylindrical in shape (DualTop miniscrews:1.6 x 8 mm; OrthoEasy miniscrews 1.7 x 8 mm). The miniscrews

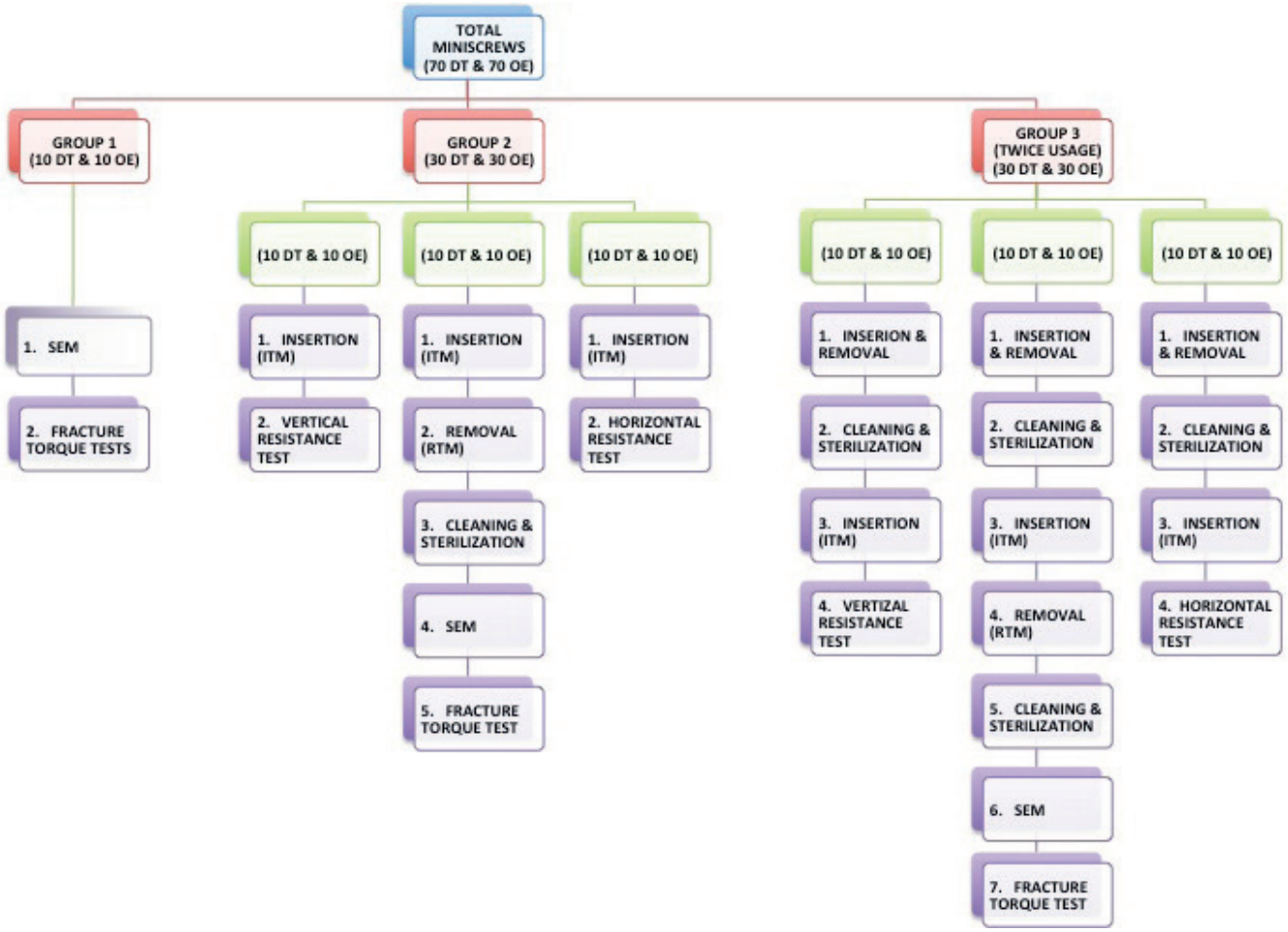


***Figure 1.*** *Contents and procedures of groups.*

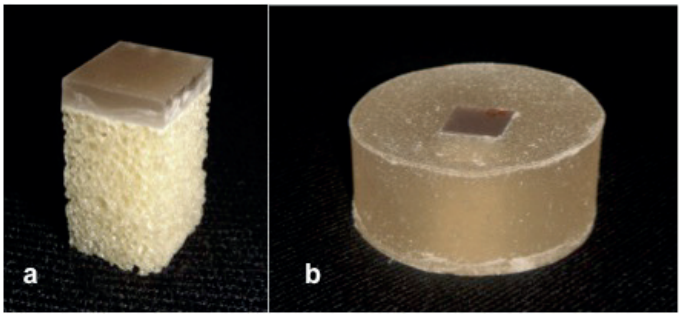


***Figure 2.*** *a) Sawbone artificial bone material; b) Sawbone embedded in acrylic resin.*

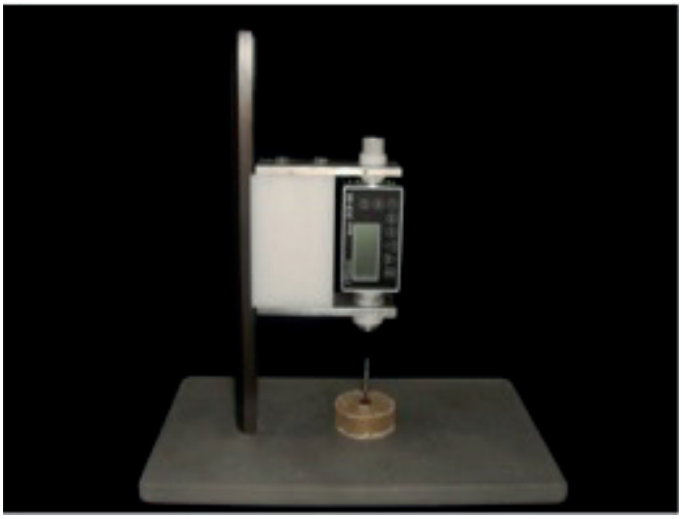

***Figure 3.*** *Cedar DID-05 digital torque-screwdriver.*

were divided into 3 groups. Group 1 was the control group and consisted of 10 miniscrews of each type. They were evaluated as they were received, without any primary procedure and without insertion into the sawbone. Groups 2 and 3 were experimental groups, consisting of 30 miniscrews of each type. Group 2 was inserted into the sawbone once, then sterilized and tested. Group 3 was inserted into the sawbone twice, sterilized after each insertion and then tested. The same researcher repeated all the procedures 1 month later. The contents and procedures of the groups are schematically shown in Figure 1. Sawbone artificial bone material was used for experiments (Sawbone Europe AB, Malmö, Sweden). A short-fiber filled epoxy sheet (2mm thickness; representing cortical bone) was attached to a 20-pcf (0.32 g/cm$^3$) cellular rigid polyurethane foam block (10mm thickness; representing cancellous bone) with 3M acrylate bond (Figure 2). For the specimens to be compatible with the Instron machine, the artificial bone material was divided into 1x1x2.2 cm sized pieces and were embedded into acrylic resin under water-cooling. The uniformity of the blocks was checked with a water gauge and each sample was given a number. Retention grooves were drilled on the epoxy sheet to preserve material integrity during the tests.

*Maximum insertion and removal torque measurements*

Insertions of mini screws were performed using a Cedar DID-05 digital torque screwdriver (Checkline Europe GmbH&Co.KG, Gronau, Germany). To place the miniscrews perpendicular to the artificial bone, a special apparatus was used (Figure 3). With this apparatus, the angulation of the torque-meter was fixed and could not be changed. The torque-meter could only move vertically and rotate around its axis. The miniscrews were inserted with the digital torque-meter until a 1.2mm gap was left between the bone and the head of the miniscrews and the peak values of torques were recorded. Removal of 20 miniscrews from each group was applied with the same screwdriver and peak values were recorded.

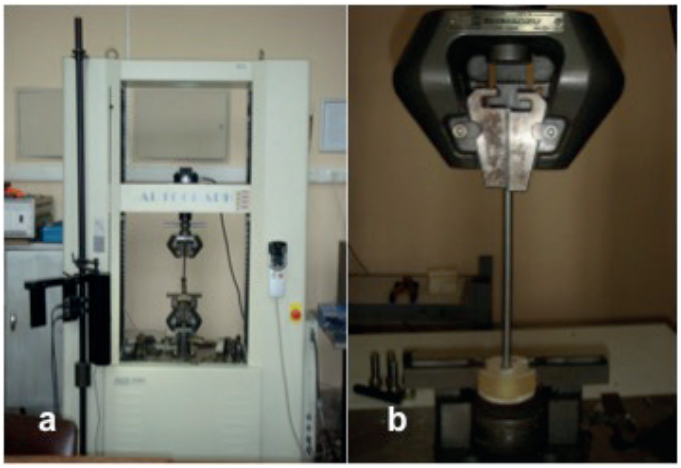


***Figure 4.*** *a) Schimatzu AG-IC Instron machine; b) Vertical resistance test mechanism of DualTop miniscrews.*

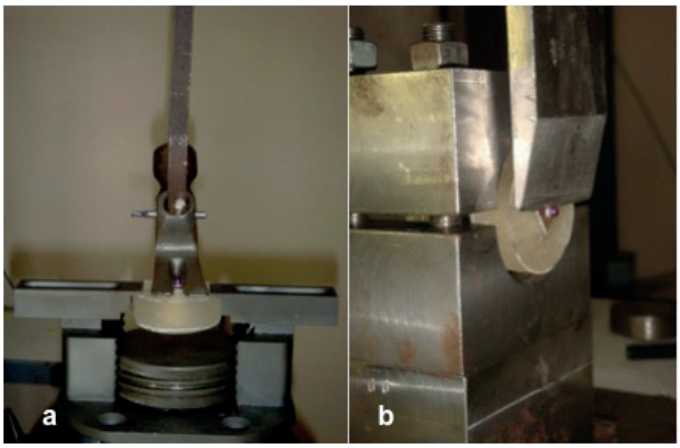


***Figure 5.*** *a) Vertical resistance test mechanism of OrthoEasy miniscrews; b) Horizontal resistance test mechanism.*

*Vertical and Horizontal Resistance Tests*

Horizontal and vertical resistance tests were performed using a Schimatzu AG-IC Instron machine (Schimatzu Co., Tokyo, Japan) (Figure 4a). For vertical resistance tests of DualTop miniscrews, a 0.8mm round wire was passed through the hole of the miniscrew (Figure 4b). For vertical resistance tests of OrthoEasy miniscrews, a notched device was passed under the head of the screw, since OrthoEasy miniscrews do not have holes on the head (Figure 5a). For horizontal resistance tests a knife-like device was applied to the heads of the miniscrews (Figure 5b). The force value of 0.6mm displacement of the head was recorded.

*Cleaning and sterilization*

Miniscrews that were to be examined with SEM after insertion into the sawbone, were first cleaned of debris with a Codyson CD-3800A ultrasonic cleaner (Codyson Electrical Co. Ltd., Shenzhen, China) for 30 minutes and rinsed. Cleaning solution was prepared using 1lt distilled water and 5ml Endozyme (Ruhof Co., Long Island, New York, USA). Then, each miniscrew was packed separately, sterilized at 135°C for 10 min and dried for 55 min with Statim 7000 (SciCan Ltd., Toronto, Canada).

*Scanning Electron Microscopy Examinations*

Scanning electron microscopy examinations were performed with JEOL JSM-5910LV(JEOL Ltd. Tokyo, Japan) and Energy scatter spectrometer INCAx-sight 7274 (OXFORD Industries, England) with 133-eV resolution. Images of 10 miniscrews (5 DT, 5 OE) from each group were taken at 20x, 40x, 100x, 200x, 500x, 1000x, 2000x, 5000x and 10000x magnifications to examine surface modifications.

*Fracture Torque Tests*

To understand torsional strength differences between the non-used and re-used miniscrews, fracture torque tests were applied. A 10mm thick cortical bone layer of bovine femur bone was used for these tests. The miniscrews were inserted in the bones until breaking point. Peak torque value before fracture was recorded.

*Statistical Analysis*

SPSS for Windows 15.0 (SPSS, Chicago, Ill) software was used for statistical analyses. The Kolmogorov-Smirnov test was used to evaluate normal distribution of data. To evaluate intergroup parameters of normally distributed data, the Student's t test and One-way Anova test were used. To evaluate intergroup parameters of non-normally distributed data, the Kruskal Wallis and Mann Whitney U tests were used. The group that caused the difference was identified with the Mann Whitney U test. Pearson Correlation analysis was used to evaluate the relationships of parameters. Data reliability was assessed by intraclass correlation coefficient (ICC). The results were evaluated at a statistical significance level of $p<0.05$ and a 95% confidence interval.

## Results

The insertion and removal torque values of Group 2 and 3 are shown in Table 1. The insertion torque values of Group 2 were significantly higher than those of Group 3. A decrease was determined in the removal torque values of Group 3, but this difference was not statistically significant.

The vertical and horizontal resistance values of Groups 2 and 3 are shown in Table 2. There was no statistically significant difference in the vertical and horizontal resistance values between Groups 2 and 3 in the DualTop miniscrews. The vertical resistance value changes of Groups 2 and 3 in the OrthoEasy miniscrews were also statistically insignificant, but the horizontal resistance of Group 3 in the OrthoEasy miniscrews was significantly higher than that of Group 2 OrthoEasy miniscrews.

The fracture torque values of all the groups are shown in Table 3. There was no difference between the groups in respect of fracture torque values.

SEM examinations showed noticeable wear in the used miniscrews. (Figure 6,7) Wear was increased in Group 3. The same operator repeated all the tests 1 month later. ICC was found to be close to 1.00.

**Table 1:** *Insertion and removal torque values (Ncm) for DualTop and OrthoEasy miniscrews in Group 2 and Group 3*

| | | Group II | | Group III | | p |
|---|---|---|---|---|---|---|
| | | n | Mean±SD | n | Mean±SD | |
| Dual Top | InsertionTorque | 30 | 26.98±0.68 | 30 | 26.57±0.47 | 0.011* |
| | RemovalTorque | 10 | 24.32±0.33 | 10 | 24.18±0.33 | 0.370 |
| OrthoEasy | InsertionTorque | 30 | 47.48±0.37 | 30 | 47.17±0.44 | 0.004** |
| | RemovalTorque | 10 | 32.28±0.33 | 10 | 32.17±0.40 | 0.511 |

**Table 2:** *Vertical and horizontal resistance values (N) for DualTop and OrthoEasy miniscrews in Group 2 and Group 3*

| | | Group II | | Group III | | p |
|---|---|---|---|---|---|---|
| | | n | Mean±SD | n | Mean±SD | |
| Dual Top | Horizontal Resistance | 10 | 518.05±67.15 | 9 | 529.90±26.07 | 0.626 |
| | Vertical Resistance | 9 | 174.60±43.87 | 10 | 203.27±14.58 | 0.092 |
| OrthoEasy | Horizontal Resistance | 9 | 591.32±99.55 | 10 | 589.06±47.28 | 0.952 |
| | Vertical Resistance | 10 | 270.21±65.47 | 10 | 319.76±22.97 | 0.045* |

**Table 3:** *Fracture torque values for DualTop and OrthoEasy miniscrews according to the groups*

| FractureTorque | Dual Top | | OrthoEasy | |
|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD |
| Group I | 10 | 33.67±1.44 | 10 | 29.04±0.39 |
| Group II | 10 | 33.73±1.11 | 10 | 29.11±0.41 |
| Group III | 10 | 33.60±1.44 | 10 | 28.98±0.57 |
| p | | 0.976 | | 0.817 |

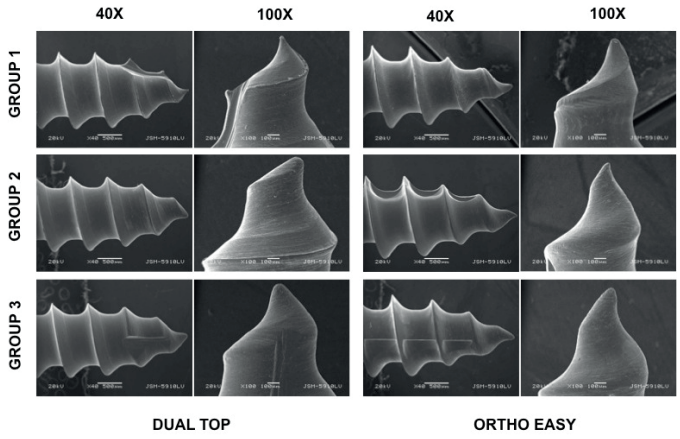


***Figure 6.*** *SEM images (40x and 100x magnification).*

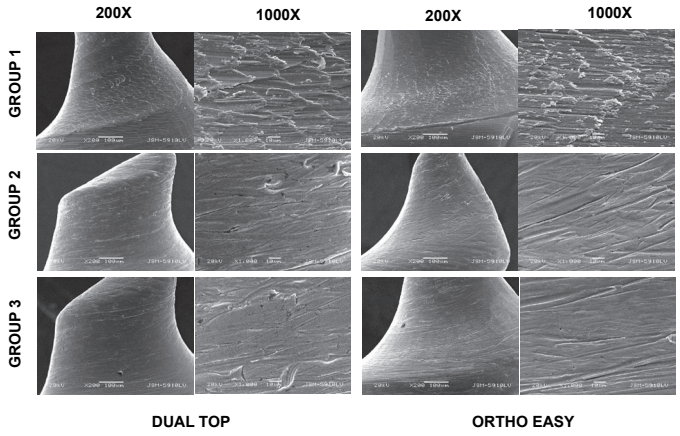


***Figure 7.*** *SEM images (200x and 1000x magnification).*

## Discussion

Widespread usage of miniscrews has led to better treatment results in critical anchorage cases. In some cases, changing the miniscrew position may be necessary. The possibility of recycling miniscrews would decrease treatment costs and improve treatment results (14). However, there are ethical considerations about the reuse of invasive medical materials among different patients. Nevertheless, especially in cases of early failure, re-insertion of the miniscrew in the same patient may be an option if the materials do not have any structural and functional changes (8). However, expected changes in tip and threads may change the insertion performance of miniscrews during re-insertion. The aim of this study was to assess the impact of insertion and sterilization processes on primary stability, mechanical and surface characteristics of miniscrews.

Primary stability is affected mainly by miniscrew design, bone quality and insertion angle (16-18). To assess primary stability, periotest, resonance frequency analysis, insertion and removal torque measurements and pull-out strength tests have been used in previous research (5,19,20). Periotest and resonance frequency analysis often have low accuracy and values for different screw types may not be comparable (21). Insertion and removal torques and pull out strength tests are the most common methods for assessment of primary stability (18,22,23).

For in-vitro evaluation of primary stability of miniscrews, various bone specimens, such as femurs or ribs of animals, jaws of cadavers or artificial bone materials, have been used previously (5,24-26). Artificial bone materials provide uniform bone thickness and density. They have been used in various studies that have aimed to understand the effects of miniscrew design on primary stability, in order to eliminate bone specimen differences (19,27). In the present study, artificial bone material was preferred to standardize the variables related to bone materials. Having a uniform cortical bone thickness, bone density and a fixed vertical load and direction were advantages in this study design.

Researchers have used several methods for the cleaning process of miniscrews. Mattos et al. used ultrasonic cleaner with an enzymatic detergent (Endozyme), Noorollahian et al. used 37% phosphoric acid and sodium hypochlorite solution and Estelita et al. used sandblasting with 90 µm $Al_2O_3$ particles prior to ultrasonic cleaning (8,14,15). In the present study, there were no tissue remnants attached to the miniscrews because artificial bone was used. However, in order to simulate the cleaning process of used miniscrews, ultrasonic cleaning with Endozyme was performed.

Many researchers investigating the effects of screw diameter have reported that an increase in screw diameter increased primary stability (5,28). In addition, Iijima et al. showed that miniscrews of smaller diameter were less resistant to torsional forces (29). In the present study, miniscrews of similar diameters were used. Surprisingly, the fracture torque values were found to be lower in the OrthoEasy miniscrews. This could have been due to the thinner inner diameter of the OrthoEasy miniscrews and it was observed that fracture occurred at the thinnest part of the middle third of the threaded region.

Many studies have compared the primary stability of different kinds of miniscrews, but few have evaluated the primary stability of reused miniscrews. The insertion and removal torque values of the OrthoEasy miniscrews were significantly higher than those of DualTop miniscrews. Maximum insertion torque values are created at the neck region of miniscrews. The torque values of the OrthoEasy miniscrews were higher because the neck region of OrthoEasy miniscrews are thicker. The insertion torque values of the reused miniscrews were lower than those of the new miniscrews. This finding could be due to abrasion and smoothening of the surface that was observed in SEM analysis. The mean removal torque values of reused miniscrews were also decreased but the difference was not statistically significant. No statistically significant difference was determined in the resistance test results. When all the parameters of primary stability were compared together, it was concluded that the difference between the twice-used and new miniscrews in respect of primary stability was not significant.

One of the most dangerous scenarios of miniscrew failure is breakage of miniscrew during insertion or removal. Mattos et al. found a statistically significant decrease in the fracture torque values of the reused miniscrews and it was concluded that reuse of miniscrews should not be recommended, although fracture torque values were higher than the insertion torque values suggested by Motoyoshi et al. (8,17). In contrast, Noorollahian et al. and Estelita et al. found no difference between new and reused miniscrews (14,15). The fracture torque values of the present study were consistent with the findings of the studies by Noorollahian et al. and Estelita et al.

Microscopic evaluation of reused miniscrews is crucial for assessment of structural changes. Chatzigianni et al. and Mattos et al. showed that there were no alterations in material structure, such as corrosion, cracks or defects on reused miniscrews (5,8). However, Mattos et al. observed smoothening of threads, atrophy of the tip region and scratches on reused miniscrews (8). In the present study, the same alterations were observed in reused miniscrews. It can be thought that alterations in the tip and threads would change insertion performance of miniscrews during re-insertion but these alterations made no significant difference in the second insertion of the used miniscrews. However, the decrease in insertion torque value was significant after the second insertion.

## Conclusion

Although wear and atrophy were seen in used miniscrews, the overall primary stability and fracture torque resistance did not show a significant change after the second insertion. It should be kept in mind that there are ethical considerations about the re-use of invasive medical materials among different patients and this study evaluated in-vitro changes in miniscrews up to the second re-insertion procedure. Within the limits of this study, it can be concluded that miniscrews can be re-inserted, especially in early failure cases, for a second time without a significant change in their mechanical and structural properties, but only if cleaning and sterilization processes are applied methodically.

**Ethics committee approval:** Not required

**Informed consent:** Not required.

**Peer review:** Externally peer-reviewed.

**Author contributions:** CAH and NK participated in designing the study. CAH and MA participated in generating the data for the study. CAH participated in gathering the data for the study. MA participated in the analysis of the data. CAH and YBA wrote the majority of the original draft of the paper. YB participated in writing the paper. All authors approved the final version of this paper.

**Conflict of interest:** The authors have no conflicts of interest to declare.

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***Türkçe öz:*** *Yerleştirme ve sterilizasyon işlemlerinin minividaların mekanik ve yüzey özellikleri üzerindeki etkilerinin in-vitro olarak incelenmesi. Amaç: Çalışmanın amacı, yerleştirme ve sterilizasyon işlemlerinin minividaların mekanik ve yüzey özellikleri üzerindeki etkilerinin incelenmesidir.Gereç ve yöntem: 140 minivida (70 Dual-Top; 70 Ortho-Easy) 3 gruba ayrılmıştır. Grup 1: kontrol grubu; her iki markadan 10'ar adet minivida herhangi bir işlem uygulanmadan incelenmiştir. Grup 2 ve Grup 3: çalışma grupları; her iki markadan 30'ar minivida. Grup 2'de minividalar yapay kemik bloğuna bir kez yerleştirilip sterilize edilerek test edilmiştir. Grup 3'de minividalar yapay kemik bloğuna iki kez yerleştirilip çıkarılmış; her yerleştirilme sonrası sterilizasyon işlemleri yinelendikten sonra testler uygulanmıştır. Minividalar primer stabilite, mekanik özellikler ve yüzey özellikleri açısından Taramalı Elektron Mikroskobu analizi, burulma testi, maksimum yerleştirme ve çıkarma torku, yatay ve dikey çekme kuvveti testleri ile değerlendirilmiştir. Bulgular: Grup 1'in maksimum yerleştirme tork değerleri Grup 2 ve 3'e göre anlamlı şekilde yüksek bulunmuştur ($p<0.05$). Ancak çıkarma torku, yatay ve dikey çekme kuvveti ve burulma direnci değerlerindeki değişim istatistiksel olarak anlamlı bulunmamıştır. Taramalı elektron mikroskobu analizinde, Grup 2 ve 3'te minividaların özellikle apikal bölgelerinde aşınma olduğu ve kaplamalı minividaların oksit tabakasının kısmen kaybolduğu gözlemlenmiştir. Sonuç: Çalışma grubu minividalarının taramalı elektron mikroskobu analizinde aşınma ve atrofi gözlenmesine rağmen mekanik testlerde kontrol grubu ile arasında anlamlı bir fark bulunmamıştır. Anahtar kelimeler: minivida; ortodontik mini-implant; primer stabilite; taramalı elektron mikroskobu; sterilizasyon.*

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Original research

# In vitro antibacterial activity of self-etch bio-active dental adhesives after artificial aging

**Purpose**
The aims to evaluate the antibacterial effect of different bioactive component containing dental adhesives before and after artificial aging.

**Materials and Methods**
Two bio-active adhesives; Clearfil Protect Bond and FL Bond II, two non-bioactive adhesives, Clearfil SE Bond and Clearfil S3 Bond were used for this study. Antibacterial activities of the fresh and aged samples against *Streptococcus mutans* were investigated with Direct Contact Test. Data were analyzed with Kruskal Wallis and Mann Whitney U multiple comparison tests.

**Results**
For fresh samples FL Bond II and Clearfil Protect Bond exhibit similar antibacterial effect but Clearfil Protect Bond showed significantly higher antibacterial effect after aging the samples ($p < 0.05$).

**Conclusions**
The incorporation of bio-active antibacterial components into adhesive systems may be considered as a fundamental component in inhibiting residual *Streptococcus mutans* when considering the antibacterial effect of fresh samples of bio-active adhesives.

***Keywords:*** *Bio-active adhesives; antibacterial activity; S.mutans; Direct Contact Test; artificial aging*

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## Introduction

Today in the treatment of dental caries, resin-based composites are widely preferred. Nevertheless, microleakage and the tendency for plaque accumulation is a downside of the resin composite material, which occurs due to polymerization shrinkage and is generally followed by secondary caries (1). Although the continuous improvement in composite restorative materials and dentin adhesive systems, it is not yet possible to completely prevent microleakage (2-4).

Therefore Ultimate novelty in adhesive materials should focus on special features rather than altering present technologies. With this being accomplished, bio-active adhesive materials could promote prognosis of restorative treatments (5).

In order to gain bio-active properties such as antibacterial, matrix metalloproteinase inhibitor, remineralization and anti-plaque effects; quaternary ammonium compounds based resin monomers, silver and calcium phosphate nanoparticles, ion-releasing glass fillers and growth factors such as 4-META/MMA (4-methacryloxyethyl trimellitate anhydride/methacrylate) are added into the resin materials (5,6).

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12-methacryloyloxydodecylpyridinium bromide (MDPB) is a polymerizable and bactericide, quaternary ammonium compound. When the MDPB incorporated resin is cured the antibacterial component is immobilized and not released from the material after polymerization. These component is damaged in contact with bacteria (7).

Another method for generating antibacterial bio-active adhesive was combined silver (Ag) and amorphous calcium phosphate (NACP) nanoparticles to dental adhesives (5). In vitro investigations revealed that Ag and NACP nanoparticles containing adhesives have remineralization and antibacterial features. These effects might be of great benefit to improve bond strength and prevent to secondary caries (8,9).

In recent years, a great number of surface pre-reacted glass fillers (S-PRG) containing dental materials were introduced by Shofu Inc. (5). These fillers are established by initiating an acid-base reaction between fluoroboroaluminosilicate glass and aqueous polyacrylic acid. Researchers reported that these bio-active fillers induce remineralization and show antibacterial effects (10,11) through the release of ions such as fluoride, strontium, sodium, boron, aluminum and silicon (12,13).

While presently marketed dental adhesives have satisfactory clinical performance, it is suggested that adhesive materials containing bio-active components could contribute to better outcomes. Antibacterial bio-active components are still in the experimental stage and only MDPB and S-PRG are available on the market.

The aim in this study is to investigate the antibacterial effect of different bio-active component containing dental adhesives.

## Materials and methods

*Adhesives*

Two bio-active and two non-bio-active adhesive systems were used in this study: Protect Bond (MDPB containing adhesive resin), FL Bond II (S-PRG containing adhesive resin), Clearfil SE Bond and Clearfil S3 Bond. Adhesive systems were evaluated in this study are shown in Table 1.

*Direct Contact Test (DTC)*

*Streptococcus mutans* (ATCC 25175) was used in this study. The DCT is based on the bacteria counting method in 24-well microliter plates (24-well, flat-bottom Cellstar, Copenhagen, Denmark). Each tested adhesive resins were 20µL adsorbed to sterile paper disks (Oxoid, Hants, UK), adapted to the sidewall of the plate and cured with LED light curing unit (SDI Radii Plus, SDI Limited, Australia), according to manufacturer's instructions.

For group 1, 20 µL bacterial suspension ($1{,}5x10^8$ CFU/ml) was placed on each sample and placed on plates incubated at 37°C, in a vertical position, for one hour. Wall of uncoated wells were contaminated with bacterial suspensions and used as control. During incubation, evaporation of suspension liquid ensured direct contact between bacteria and the tested material. Then, 3ml of tryptic soy broth (TSB) broth was added to each of the wells and gently mixed for two minutes. The bacterial suspension of each well was transferred and serially diluted in TSB. Culturing aliquots 0,1/l onto tryptic soy agar

**Table 1:** *Adhesive composition and application procedure. (Abbreviations: MDP: 10-Methacryloyloxydecyl dihydrogen phosphate, MDPB: 12-Methacryloyloxydodecyl pyridinium bromide, Bis-GMA: bisphenol A glycidyl methacrylate, HEMA: Hydroxyethylmethacrylate, S-PRG: surface pre-reacted glass ionomer, UDMA: urethane dimethacrylate, TEGDMA: Triethylene glycol dimethacrylate).*

| Adhesive System | Material Type | Composition | | Manufacturer |
|---|---|---|---|---|
| Clearfil Protect Bond, Bioactive dental adhesive | Two component self-etch adhesive | Primer | MDP, MDPB, HEMA,Hydrophilic dimethacrylate, Water | Kuraray Medical Inc., Okuyama, Japan |
| | | Bond | MDP, Bis-GMA, HEMA,Hydrophobic dimethacrylate,dl-Camphorquinone,N,N-Diethanol-p-toluidine,Silanated colloidal silica, Surface treated sodium fluoride | |
| FL Bond II, Bioactive dental adhesive | Two component self-etch adhesive | Primer | Water, Ethanol,Carboxylic acid monomer, Phosphoric acid monomer and initiator | Shofu Inc., Kyoto, Japan |
| | | Bond | S-PRG filler based on fluoroboroalimoslicte glass, UDMA, TEGDMA, 2-HEMA, initiator | |
| Clearfil SE Bond | Two component self-etch adhesive | Primer | MDP, HEMA, Hydrophilic dimethacrylate, Water | Kuraray Medical Inc., Okuyama, Japan |
| | | Bond | MDP, Bis-GMA, HEMA,Hydrophobic dimethacrylate,dl-Camphorquinone,N,N-Diethanol-p-toluidine,Silanated colloidal silica | |
| Clearfil S3 Bond | One component self-etch adhesive | | MDP, HEMA, Bis-GMA, Hydrophilic aliphatic dimethacrylate, Hydrophobic aliphatic methacrylate, Colloidal silica, dl-Camphorquinone, Accelerators, Initiators, Water | Kuraray Medical Inc., Okuyama, Japan |

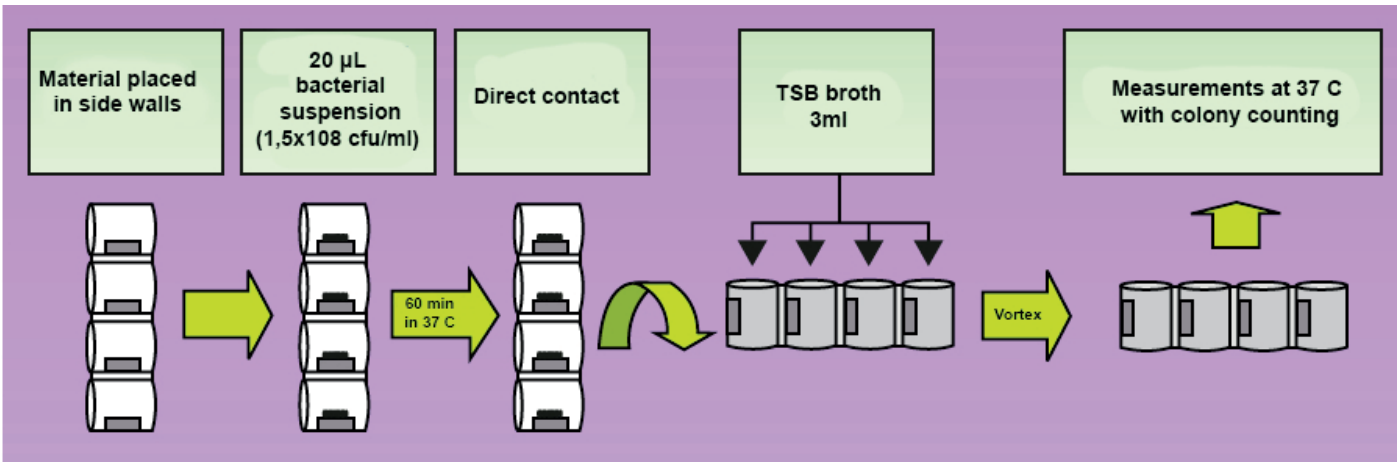


***Figure 1.*** *Schematic drawing of direct contact test performed.*

after serially diluted range from $10^{-1}$ to $10^{-3}$ detected surviving bacteria. After 24 hours incubation at 37°C, colonies were counted, and Cfu/ml was calculated (Figure 1).

For group 2, after the polymerization of the adhesives, 3ml of sterile distillated water was added to each of the wells. At the end of the aging period (7 days) samples were removed from the distillated water. The antibacterial activity of fresh and aged samples was tested at the same time as described above. All experiments were performed in triplicate.

*Statistical analysis*

The collected data from all groups were imported to Statistical Package for Social Sciences 16.0 for Windows software, (SPSS Inc., Chicago, IL, USA). The nonparametric Kruskal-Wallis one way analysis of variance by ranks and Mann-Whitney U tests were used for the multiple and pairwise comparisons, respectively. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

## Results

The results of the antibacterial effects of the bio-active dental adhesives from DCT are presented in Figure 2. For fresh samples Clearfil Protect Bond and FL Bond II shows exhibited the most effective antibacterial activity against *S.mutans*. The difference between these two and the other groups including the control were significant ($p < 0.5$). When compared with fresh and aged samples, only Clearfil Protect Bond showed significant antibacterial effect after aging. Although fresh samples of FL Bond II exhibit similar antibacterial effects to Clearfil Protect Bond , when aged, antibacterial effects are similar to adhesive groups without bio-active component . There is no difference between the aged and fresh samples among the adhesive systems without the bio-active component.

## Discussion

Over the last decade, composite resin materials have become highly preferable in restorative dentistry (14). Because of their superior aesthetic properties than dental amalgams and ability to be used with minimally invasive preparation techniques, moreover the potential for bonding

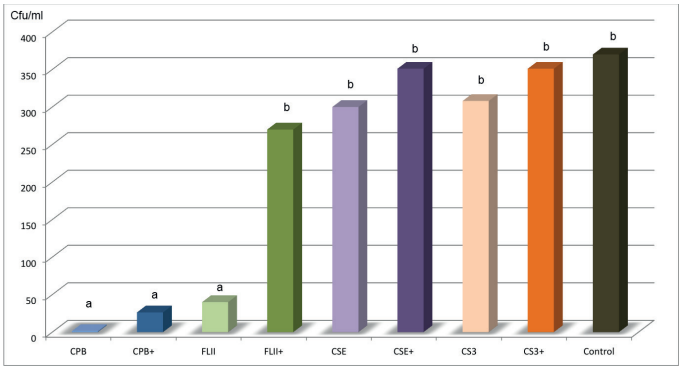


***Figure 2.*** *Bacterial growth rate after direct contact with tested materials (Ab abbreviated 103). Identical letters indicate that mean values were not significantly different (p=0.05). (Abbreviations: PB: Clearfil Protect Bond, PB+: Clearfil Protect Bond aged, FLII: FL II Bond, FLII+: FL II Bond aged, CSE: Clearfil SE Bond, CSE+: Clearfil SE Bond aged, CS3: Clearfil S3 Bond, CS3+:Clearfil S3 Bond aged) .*

to dental hard tissues, composite materials are often used to restore decayed or traumatized teeth, and for the aesthetic restoration of discolored and malpositioned teeth, as a direct and indirect restorative material (15). Nevertheless, some studies have revealed shorter longevity and higher failure rates for amalgam compared to composite restorations (16-20). One of the major reasons for failure of composite restorations is secondary caries (15-17,19-22).

The etiology of the seconder caries is an infectious disease of bacterial origin, similar to primary caries (23), and consist mainly of *S. mutans*, *Lactobacilli* and *Actino-myces naeslundii* (24). The demineralization mechanism is the same as the primary caries, but the presence of restorative material creates some differences. The cariogenic attack in secondary occurs also from the tooth-restoration interface (15). Furthermore, other studies reported that the amount of plaque and caryogenesis was directly related to restorative material used (15,17-20, 24).

In general, composite resin materials are differed from amalgam and glass-ionomers, because they do not exhibit antibacterial properties (25). Studies have been reported that they show antibacterial properties due to metal ions in amalgam and fluorid-releasing capacity of the glass ionomer (26-28).

A number of experimental composites have been developed by combining antibacterial bio-active components to the resin or filler content of dental composites (29-33). Recent studies report that the mechanical properties of some of these materials are much the same to traditional composites (32,34), but lack information about a large part of their mechanical and aesthetic characteristics, furthermore antibacterial bio-active component containing composites are still in the experimental phase and other than fluoride-releasing composites are commercially unavailable.

At the present time, no suitable method or material has been developed to provide antibacterial activity to composite materials, on the other hand much has been done about the antibacterial activity of adhesive systems (5). If dentin adhesive systems can exhibit antibacterial effects during placement of the restoration, this may provide inactivation of residual bacteria. In addition, after the restoration was completed, the antibacterial activity of the adhesive systems may be effective to inactivated bacteria in the dental plaque.

Many attempts have been made to produce dental adhesive systems that have bio-active component which could contribute to better prognosis of restorative treatments (5,9,35,36). The current study evaluated the inhibition of bacterial growth by DTC of two bio-active self-etch adhesive systems against *S. mutans*. Conventional Agar Diffusion Test (ADT) (37,38), tooth cavity model technique (39,40) dentin discs method (41) and bacterial penetration on the histological sections of extracted teeth (42,43) have been used in previous studies to investigate the antibacterial effect of dental adhesives. The DCT is a quantitative and reproducible technique that simulates the contact of the test microorganism with dental adhesives similar to microleakage. The technique also provides for better control of potential confounding factors than ADT (44-46). Tooth cavity model technique and dentin discs method involves a dentin substrate in methodology and according to the researchers they provides more reliable results about antibacterial activity of dental adhesives (39-41). However, these methods may not be suitable for investigating the antibacterial activity of dentin adhesives after aging process (42,43). The bacterial penetration test (42,43) provides information about the ability of the tested adhesives to prevent bacterial microleakege but does not indicate the ability to inhibit residual bacteria on the cavity. In this study, to evaluate the antibacterial activity of fresh and aged adhesive samples formed by direct contact with *S. mutans*, DTC method was used.

*S. mutans* was selected as the test microorganism because it is the primary pathogen responsible for the initiation of caries and the development of secondary caries (24). As in this study *S.mutas* has been used in many studies to examine the antibacterial effect of dental materials (10,39,40,47).

Among the fresh samples tested, Clearfil Protect Bond and FL Bond II exhibited antibacterial activity compared to the other self-etch adhesives and control ($p<0.05$). Furthermore, only the Clearfil Protect Bond's antibacterial activity persisted within the aged samples. The prolonged antibacterial effect of Clearfil Protect Bond is related to the antibacterial MDPB molecule. After curing, MDPB-containing resins inhibit the growth of bacteria in contact with the material, thereby act as a "contact inhibitor" (5). Studies have shown significant reduction of *S. mutans* number, when incubated in contact with the cured primer/adhesive surface containing MDPB (7,48).

In this study, fresh samples of FL Bond II also exhibited antibacterial activity compared to the other self-etch adhesives ($p < 0.05$) and this antibacterial effect is similar to the Clearfil Protect Bond ($p > 0.05$). However this antibacterial activity did not persist when it was aged.

Studies have shown that fluoride released by fluoride-releasing dental materials are intense during the first week of immersion in water; but reduce later on (49,50). Considering these studies, the reason for the decrease in antibacterial activity in aged FL Bond II samples might be related to loss of most of the fluoride concentration during the aging process.

## Conclusion

This study highlights the incorporation of bio-active antibacterial component into adhesive systems which may become an essential factor in inhibiting residual *S.mutans* in the cavity.

**Ethics committee approval:** Not provided.

**Informed consent:** Not provided.

**Peer review:** Externally peer-reviewed.

**Author contributions:** GD, ME and GG participated in designing the study. GD, ME and HŞ participated in generating the data for the study. GD, ME and HŞ participated in gathering the data for the study. GD, ME and HŞ participated in the analysis of the data. GD wrote the majority of the original draft of the paper. GD, ME, HŞ and GG Participated in writing the paper. All authors approved the final version of this paper.

**Conflict of interest:** The authors have no conflicts of interest to declare.

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***Türkçe öz:*** *Self-etch bio-aktif dental adezivlerin yapay yaşlandirma sonrasi antibakteriyel etkilerinin in-vitro olarak incelenmesi. Amaç: Bu çalışmanın amacı; farklı bioaktif içeriklere sahip dental adezivlerin yapay yaşlandırma öncesinde ve sonrasında antibakteriyel etkilerinin değerlendirilmesidir. Gereç ve Yöntem: Çalışmada; iki adet biyo-aktif adeziv; Clearfil Protect Bond, FL Bond II ve iki adet biyo-aktif içeriğe sahip olmayan dental adeziv; Clearfil SE Bond ve Clearfil 3S Bond kullanılmıştır. Adeziv sistemlerin Streptococcus mutans'a karşı antibakteriyel etkinliklerinin değerlendirilmesinde direk temas testi kullanılmıştır. Veriler Kruskal Wallis ve Mann Whitney U çoklu karşılaştırma testleri kullanılarak analiz edilmiştir. Taze hazırlanmış örneklerde FL Bond II ve Clearfil Protect Bond benzer antibakteriyel etki göstermiştir ($p > 0.05$), ancak yaşlandırılmış örneklerde Clearfil Protect Bond'un antibakteriyel etkinliği anlamlı derecede yüksektir ($p < 0.05$). Sonuç: Taze hazırlanan bio-aktif adeziv örneklerinin göstermiş olduğu antibakteriyel etki dikkate alındığında, bio-aktif içeriğe sahip adezivler kavitedeki reziduel S.mutas eliminasyonu amacıyla kullanılabilir. Anahtar kelimeler: Bio-actif adezivler; antibakteriyel aktivite; S.mutans; Direkt Kontak Testi; yapay yaşlandırma*

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Original research

# The effects of autologous platelet concentrate on the healing of intra-bony defects: a randomized clinical trial

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**Purpose**

The present study aimed to evaluate the clinical and radiographic effects of autologous platelet concentrate (APC) on the healing of intra-bony defects filled with β-tricalcium phosphate (β-TCP) and covered with collagen membranes.

**Subjects and methods**

This study included 30 defects of 14 systemically healthy subjects. All of them had, at least, two deep intra-bony, inter-proximal periodontal defects. Minimum probing pocket depth (PPD) was 6 mm. Clinical and imaging examination was performed both at baseline and at 3, 6, and 9 months after surgery.

**Results**

Both the test and control group revealed a significant reduction in all variables when compared with the base line. Mean reduction of the PPD in two groups at each follow-up time point showed no significant difference. Means of the clinical attachment gain of the same groups were significantly different ($p<0.05$). Mean gingival recession at 3 month was not significant. However, the means of gingival recession coverage of two groups were significantly different at 6 and 9 months ($p<0.05$ for both).

**Conclusion**

Sites treated with APC are more likely to demonstrate more clinical attachment gain and recession coverage at the end of 9 month compared to those without APC.

***Keywords:*** *Intra-bony defects; periodontal regeneration; periodontitis; platelet rich plasma; β-tricalcium phosphate*

## Introduction

Periodontitis is a site-specific disease that causes periodontal attachment and bone loss (1). In the general population, periodontitis is a common cause of tooth loss (2). One of the important goals of periodontal treatment is to reduce the pocket depth (3). Non-surgical treatment is indicated in cases with moderate periodontitis. On the other hand, standard treatment protocol should be supported with periodontal surgery in the presence of deep pockets and intrabony defects (4).

According to Melcher's hypothesis (5), the selected cell population in the periodontium can regenerate periodontal tissues provided that the cells could occupy the periodontal wound. Guided tissue regeneration (GTR) is a regenerative technique in which a physical barrier was positioned between the flap and the root surface to prevent the migration of the connective tissue and epithelium. This provides an opportunity for the pluripotent ligament cells to multiply on the root surface (6).

Tricalcium phosphate (TCP) is an alloplastic bone substitute which has been extensively researched since the 1970's. This resorption rate of this

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material has been reported to be similar to that of the new bone apposition (7). Its potential to stimulate the bone formation is higher than hydroxyapatite, but lower than that of the Bioglass (6,8).

SynOss®(Dentacare, Pune, India) is a commercially available pure phase Beta TCP. It meets the purity requirements for bioceramics of the American standards (ASTM F 1088-87). It is biocompatible, carries no risk of disease transmission and shows no immunogenic reactions (9). SynOss® has unique forms of the granules mimicking the bone macroscopic structure (9). It fully degrades within 6-12 months which is close to the native bone apposition rate, depending on the patient's individual regeneration potential (10).

Various types of absorbable and non-absorbable materials have been tested as membranes for GTR (11,12). Perio-Col® GTR is a bio-absorbable Type I collagen membrane manufactured from the air-bladder of edible fresh water fish. Perio-Col® is a non-toxic, non-allergenic and non-immunogenic biocompatible material (13). Especially when combined with platelet rich plasma, Perio-Col® GTR membrane has shown promising results in the treatment of intra-bony defects (14).

Mitogenesis, chemotaxis and cell differentiation during the repair and/or regeneration process are regulated by growth factors (15). Improved osseous defect fill has been reported in patients treated with platelet derived growth factors (PDGF/IGF-I) during its first human clinical trial (16).

PDGF, insulin-like growth factor (IGF) and transforming growth factor- β (TGF-β) enhance wound healing and provoke regeneration(17).PRP's potential in new bone formation was first reported in 1998(18). The aim of the present study is therefore to examine the effect of autologous platelet concentrate on periodontal regeneration when used in combination with an alloplastic bone graft and GTR membrane. The null hypothesis of this research is that no difference can be observed between study and control groups.

## Subjects and Methods

*Study participants*

The study was approved by the ethics review and research board (Approval no. 16/2013, MGV 30134/43246). The study design was a randomized clinical case-controlled trial that follows the guidelines cited by CONSORT. Written, informed consents were obtained from all patients. Thirty defects of 14 systemically healthy patients were included. Male to female ratio was 1:1, their age range was 20 to 55 years and mean age was 34 ± 10.57 years.

*Patient selection*

Having no contraindication for periodontal surgery, good oral hygiene habits, having at least two intra-bony defects, one at each quadrant or contra-lateral sides of the same region, having minimum 6 mm of probing pocket depths (PPD) and having radiographic angular bone defect of at least 4 mm at baseline were the inclusion criterion. Patients with more than 1 mm of mobility, prosthetic restoration and/or endodontic treatment in the affected teeth, those who are allergic to drugs, pregnant and/or lactating, smokers or having any other contraindication for periodontal surgery were excluded.

*Study design*

Thirty selected sites in 14 patients were randomly allocated into either one of the Site A or Site B groups stratified by the treatment plan, each site in different quadrants. Defects in Site A were filled with β-TCP mixed with autologous platelet concentrate (APC) and then covered with absorbable collagen membrane (Perio-Col®-GTR). Defects in site B received also a β-tricalcium β-TCP but no APC and they were again covered with the same collagen membrane. The healing process was recorded by following the baseline scores and those registered after3, 6, and 9 months after surgery. Full mouth plaque index (PI) (19), full mouth papillary bleeding index (PBI) (20), PPD, clinical attachment level (CAL), gingival recession (REC) were determined at each time point. A cast model was obtained from alginate impression and occlusal stents from acrylic resin were fabricated for probe positioning (21).

*Radiographic parameters*

After initial therapy, standardized radiovisiographs (RVG) (Kodak DS, Rochester, NY, USA)were taken with the RINN XCP system (DENTSPLY, Tulsa, Oklahoma, USA), at baseline, and after 3, 6, and 9 months following surgery. The following landmarks were identified on the radiographs: Cemento-enamel junction (CEJ), base of the defect (BD), alveolar crest (AC), cemento-enamel junction to root apex (RA) (Figure 1). Pre- and post-treatment radiographs (RVG) pairs were compared to investigate the presence of crestal bone resorption (ACC), bone defect filling (BF) and the extent of defect resolution which was calculated with the following formula: DR= (BF−ACC).

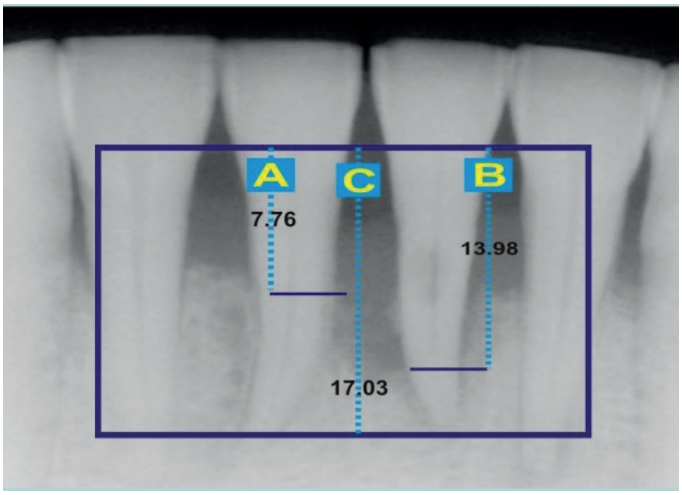


***Figure 1.*** *Radiographic planning of the treatment (A: cemento-enamel junction to alveolar crest, B: cemento-enamel junction to base of the defect, C: root).*

*Surgical procedure*

An hour prior to surgery, venous blood was collected and stored in a 10 ml tube that contains 3.2% sodium citrate as anticoagulant. Tubes were then placed in a manual centrifugation device (e-tek, Remi laboratory, India). The first step was completed in 13 minutes at 1000 rpm and the second one in 10 minutes at 2000 rpm. APC was collected from the bottom layer of the test tube (Figure 2). 10% calcium chloride

***Figure 2.*** *Autologous platelet concentrate.*

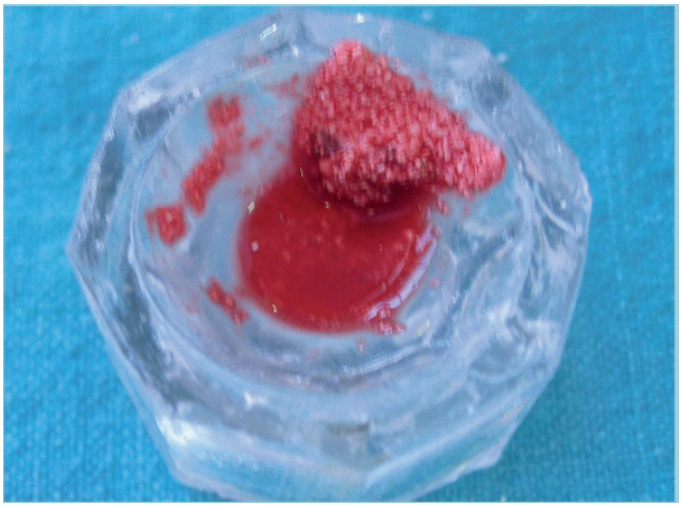

***Figure 3.*** *SynOss mixed with autologous platelet concentrate and patients' blood.*

***Figure 4.*** *Pre-suturing of the membrane and graft placed in the defect.*

was added to activate APC, and then the mixture was left undisturbed for 15-20 minutes to ensure a proper gel formation.

The surgical procedure was performed under local anesthesia, β-tricalcium phosphate granules (SynOss®) of 300µ - 600µ particle size, were mixed with 2-3 drops of fresh blood from the same defect and then mixed with the coagulated APC (Figure 3). Bio-absorbable membrane (Perio-Col®) was used for guided tissue regeneration. The membrane was placed and sutured over the defect with absorbable suture material (Vicryl® 4-0 reverse cutting, Ethicon, Johnson and Johnson, India). β-tricalcium phosphate granules mixed with APC was packed in the defect up to the level of the surrounding bony walls (Figure 4). Mucoperiosteal flaps were then approximated and sutured at their initial position using 3-0 silk. In the control sites, the same procedure was carried out, but β-tricalcium phosphate granules were mixed with normal sterile saline instead of APC, and the root surface was not coated with APC. Recall appointments were made after 1, 3, 6, and 9 months post-surgery for clinical evaluation. Post-operative patients' evaluation was done clinically and radiographically at 3, 6, and 9 months (Figure 5 and Figure 6).

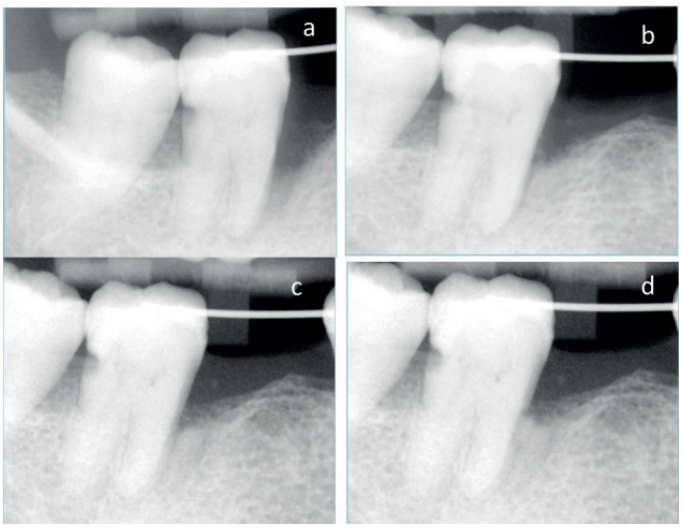


***Figure 5.*** *Control site radiographic evaluation at baseline (a), three months (b), six months (c) and 9 months (d).*

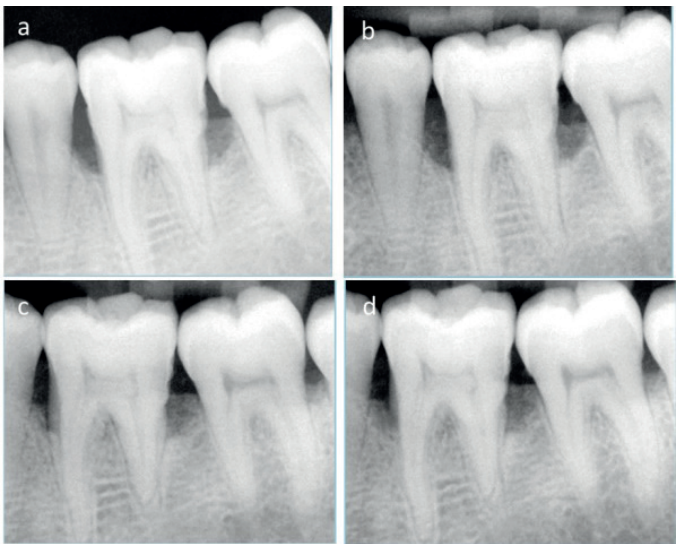


***Figure 6.*** *Test site radiographic evaluation at baseline (a), three months (b), six months (c) and 9 months (d).*

*Statistical analysis*

All the clinical and radiographic parameters recorded were transferred to Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), and subjected to the following statistical analysis: the paired-t test was performed to compare intra-group variation, Intergroup variation between the two groups was compared by using a non-parametric, rank test (Mann-Whitney U test). Confidence level was set to 95% and p<0.05 was considered significant.

## Results

Of the 14 patients who have completed the study, seven were male and seven were female. The mean baseline PI score was 0.83±0.05 and 0.38±0.17 at 9 months follow up which indicated a significant mean 54% reduction to 0.45±0.19 (p<0.001) (Figure 7). Also, the mean PBI score at baseline (0.83±0.19) dropped down to 0.14±0.14 at 9 months which shows significant 84% reduction (0.69±0.22) (p<0.001) (Figure 8). The reduction in PPD between the baseline and at 9 months was statistically significant in both experimental site A and experimental site B (p<0.05). The CAL between baseline and 9 months was statistically significant in both groups (p<0.05). At 9 month time point, the difference in the mean CAL gain (1.4 mm) was statistically significant (p<0.05) (Table 1). After 9 months from baseline, the mean REC gain was statistically significant in both groups (p<0.05). The mean REC between the two groups at 6 months and 9 months was 0.86 mm and 1.06 mm respectively which was statistically significant (p<0.05) (Table 1).

Both groups showed a statistically significant (P<0.001) gain in bone defect fill compared to the baseline and 1, 3 and 9 months, with the maximum reduction recorded at the end of the 9 month follow-up (Table 1). The mean defect fill was statistically significant in both groups when the baseline was compared to 1, 3 and 9 months, with the maximum defect fill recorded at the 9 month interval (p<0.05 for each). Both groups revealed gradual increase in the defect resolution percentage from baseline to 1, 3 and 9 months. A comparison between the two groups revealed a difference of 1.78±0.37%, 3.57±0.69% and 0.50±0.08% at 3 months, 6 months and 9 months respectively, but this was not statistically significant.

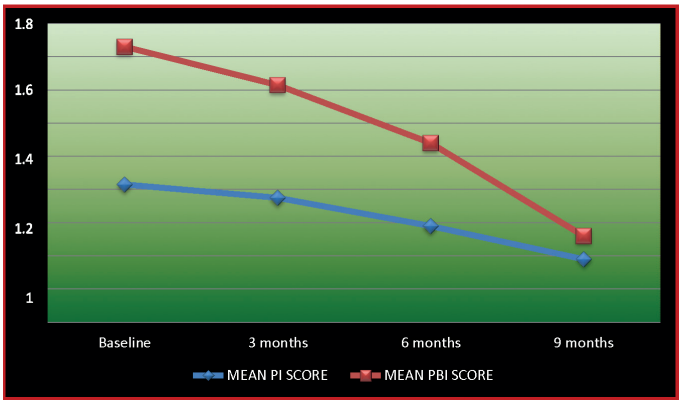


***Figure 7.*** *Changes in the mean plaque (PI) and papillary bleeding index (PBI) score over time.*

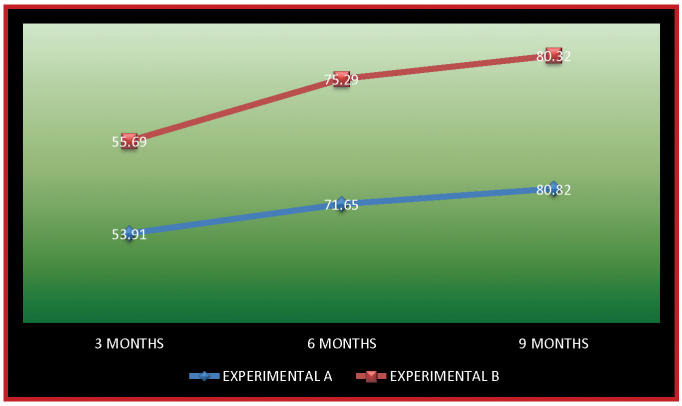


***Figure 8.*** *Comparison of the percentage of mean radiographic defect resolution over time.*

## Discussion

Histologic evidence gathered from experimental studies indicated that the application of growth factors such as PDGF and IGF might be beneficial in the periodontal regenerative therapy when applied in the short term (21,22). Less post-operative membrane exposures and higher rate of bone apposition have been reported following the use of PRP, β-TCP and GTR up to 6 months although no difference was found at 12 months (23). However, the treatment of bone defects with β -TCP, β-TCP + PRP and β-TCP + GTR did not show significant differences among 3 groups (24).

The present study evaluated the effects of growth factors on GTR, by delivering an increased concentration of growth factors directly to the defect site along with the benefit of the barrier action of the collagen membrane and scaffold effect of the β-TCP bone graft. In this study, the platelet density in the resulting APC preparation was higher (573.27%) than the one obtained by Landesberg (18) (205.7%) and Marx (25) (338%). This difference could be due to variations in the preparation

**Table 1.** *Presentation of the mean and standard deviations of the study variables stratified by the time point parameter (PPD: pocket probing depth,CAL clinical attachment level,REC: recession coverage,BD: bone defect fill,ACC: alveolar crest change, RED: radiographic extent of bony defect).*

| | Experimental Site A | | | | | | Experimental site B | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PPD | CAL | REC | BD | ACC | RED | PPD | CAL | REC | BD | ACC | RED |
| Base line | 8.07±1.53 | 9.40±1.80 | 1.60±1.18 | 11.59±3.56 | 4.12±2.34 | 7.47±3.71 | 7.73±1.39 | 8.60±2.03 | 0.87± 1.13 | 9.57± 2.51 | 3.58±1.44 | 5.99±2.07 |
| 3 Months | 3.93±1.28 | 5.53±1.41 | 1.60±1.12 | 8.06±2.91 | 4.39±2.95 | 3.68±3.11 | 4.33±0.90 | 5.53±1.51 | 1.20± 1.21 | 6.75± 3.06 | 3.98±1.86 | 2.77±1.98 |
| 6 Months | 3.27±0.96 | 4.47±1.46 | 1.20±1.08 | 6.76±2.78 | 4.56±2.92 | 2.20±2.48 | 3.40±1.18 | 4.73±1.83 | 1.33± 1.40 | 5.99± 2.99 | 4.26±2.28 | 1.73±1.96 |
| 9 Months | 2.87±0.83 | 3.93±1.33 | 1.07±1.03 | 5.37±2.27 | 4.09±2.98 | 1.28±1.17 | 3.13±1.19 | 4.53±2 | 1.40± 1.40 | 5.42± 2.90 | 3.94±2.10 | 1.48±2 |

steps such as force or time. Before being applied to the test defect, 10% calcium chloride solution in 6:1 ratio was used to activate the platelet concentrate. This ratio is the minimal amount of CaCl2 to neutralize the citrate and induces the platelet concentrate clotting. In contrast to Marx et al.(18), no xenogenous material such as bovine thrombin was added in order to prevent possible infection or contamination. To accelerate the coagulation process, some drops of collected venous blood were added to the β-TCP before mixing it with APC, which is consistent with the work of Appel et al.(26).

Post-operative membrane exposure rate in the control sites of the present study was 13.33% and no membrane exposure was found at the test sites. Growth factors included in the APC might have therefore positively affected the soft tissue closure in test sites. Consistently, PDGF released from platelet's' α-granules has been reported to show mitogenic and chemotactic effects on fibroblasts and proliferative effects on endothelial cells (27).

Both test and control groups showed significant reduction in PPD and higher CAL gain after 3, 6, and 9 months compared to baseline values. The PPD reduction and CAL gain was higher in sites treated with APC at 3, 6 and 9 months than those without APC. However, no significant difference was found between the two, although the test site had significantly gained more CAL at 9 months compared to the control site.

All the sites, except for the two in the control group, showed ≥ 3 mm of CAL gain in both groups at the end of 9 months. Around 53.3% of the control and all of the test sites showed CAL gain of ≥ 4 mm at the end of 9 months. Previous studies in which only the membranes were used, CAL gain of at least 4 mm was found in 51.6%, 38.7%, and 69% of cases (28,29). The lower CAL gain found in the present one could be due its longer duration.

The defect fill variable showed an acceptable outcome. In the test site, the mean defect fill was 30%, 42% and 54% at 3 months, 6 months and 9 months respectively, which was highly significant when compared to baseline values. These findings were consistent with others (30). A similar pattern was observed in the control sites with a mean defect fill of 29%, 37% and 43% at 3 months, 6 months and 9 months respectively. This was also significant when compared to the baseline which is line with previous research (31). The difference between the means of two groups, however, was not significant.

Following periodontal surgery, remodeling of the alveolar bone leads to crestal resorption. In the present study, the APC sites showed supracrestal bone formation as compared to the site where APC was not used. However, the results were not statistically significant. In this variable, our findings were not consistent with previous studies where there was no supracrestal bone formation (32). The results obtained in this study with regards to the gain in alveolar crest height at the end of 9 months could be due to the stickiness of the APC gel as well as the small (300-600μ) particle size of ss-TCP granules. However, the surgical technique used in this study (i.e. pre-suturing of membrane before placement of ss-TCP), could be another reason by preventing the dislodgment of the bone graft due to fresh bleeding because of suturing.

CAL gain found in the present study acted together with significant bone gain in the defects in both groups. The imaging study showed substantial changes in the hard tissue changes compared to those reported in previous research (33,34). However, this finding should be considered cautiously because of the radiopacity of the bone graft granules. Increased bone turnover rate at 6 weeks after the application of rh-PDGF has been shown previously (35). In addition, positive effects of growth factor combinations, such as IGF-I and PDGF-BB or TGF-β1 and FGF, on the bone cells' proliferation and activity have been reported in vitro (36).

## Conclusion

At 9 months, the PPD reduced and CAL gain increased in both groups. However, the test sites' CAL gain was higher. REC was also more pronounced in the test groups. Both groups showed considerable defect fill rates but no significant difference was observed between them. In addition, although not significant, APC sites showed supracrestal bone formation compared to those without APC. The pre-suturing of the membrane and the use of APC improved the handling properties of β-TCP. APC could have been prepared without the use of bovine thrombin, although it would have taken slightly longer to form a sticky gel and release growth factors.

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**Ethics committee approval:** The study was approved by the ethics review and research board (Approval no. 16/2013, MGV 30134/43246).

**Informed consent:** Written informed consents were obtained from all patients.

**Conflict of interest:** The authors have no conflicts of interest to declare.

**Financial disclosure:** The authors declared that this study has received no financial support.

**Peer review:** Externally peer-reviewed.

**Author contributions:** NK, ND and SSA participated in designing the study. NK, NAA and NA participated in generating the data for the study. NK, ND, NAA and AG participated in gathering the data for the study. ND, SSA and NA participated in the analysis of the data. SSA and NAA wrote the majority of the original draft of the paper. NK, SSA and AG participated in writing the paper. All authors approved the final version of this paper.

***Türkçe öz:*** *Otojen trombosit konsantrasyonlarının kemik içi defektlerin iyileşmesi üzerine etkisi: randomize kontrollü çalışma. Amaç: Bu çalışma, otojen trombosit konsantrasyonlarının β-trikalsiyum fosfat (β-TCP) ile doldurulmuş ve kollajen membran ile örtülmüş kemik içi defektlerin klinik ve radyografik olarak iyileşmesini incelemeyi amaçlamaktadır. Gereç ve yöntem: Bu çalışma sistemik olarak sağlıklı 14 bireyin 30 defektini içermektedir. Her birinin, en az iki derin kemik içi, interproksimal periodontal defekti bulunmaktadır. Minimum sondalanabilir cep derinliği (SCD) 6 mmdir. Klinik ve görüntü muayenelerinin her biri başlangıç ve cerrahi sonrası 3., 6. ve 9. aylarda yapılmıştır. Sonuç: Hem test hem de kontrol grubu başlangıç seviyesi ile karşılaştırıldığında tüm sonuçlarda anlamlı bir azalma göstermiştir. Her iki grupta da SCD'nin her muayene zamanındaki ortalama azalışı anlamlı bir farklılık göstermemiştir. Aynı grupların ortalama klinik ataşman kazançları anlamlı farklılık göstermiştir (p<0.05). 3. ayda ortalama dişeti çekilmesi kapanması anlamlı değildir. Fakat, her iki grubun 6. ve 9. aylardaki ortalama dişeti çekilmesi kapanma miktarı anlamlıdır (p<0.05 her*

*ikisi için). Tartışma: OTK ile tedavi edilmiş bölgeler edilmemiş bölgelere oranla 9. ayın sonunda daha fazla klinik ataşman kazancı ve dişeti çekilmesi kapanması göstermeye eğilimlidir. Anahtar kelimeler: Kemik içi defekt; trombositten zengin plazma; β-trikalsiyum fosfat.*

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Original research

# In vitro antimicrobial activity of different electrochemically-activated solutions on enterococcus faecalis

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**Purpose**

The aim of this *in vitro* study was to assess and compare the antimicrobial efficacy of different electrochemically-activated solutions (ECA) and contemporary irrigants, in root canals infected with *Enterococcus faecalis*, used with or without EndoActivator (EA).

**Materials and methods**

A hundred single-rooted human teeth were prepared. Ninety of the root segments were infected with *E. faecalis* for four weeks, and divided into eight test groups (n = 10) (four with and four without EA sonication) and a positive control (n = 10). The irrigants tested were electrochemically-activated solutions produced by the Medilox® (ECA-MX) and Envirolyte® devices (ECA-EN), 2% CHX and 2.5% NaOCl. The root specimens were irrigated with 5 mL of the test solution, with additional sonic agitation applied to the EA groups. The dentine samples that were obtained from the walls were cultured, and the antibacterial efficacy was evaluated by counting the colony-forming units.

**Results**

The ECA-EN, 2.5% NaOCl and 2% CHX were more effective than the ECA-MX ($p < 0.05$) with the addition of EA sonication, showing no statistical difference in the elimination of *E. faecalis*.

**Conclusion**

The ECA-EN shows potential as an endodontic irrigant, while EA usage gives no benefit in reducing bacteria from root canals.

***Keywords:*** *Electrochemically-activated solution; EndoActivator; endodontics; Enterococcus faecalis; root canal irrigants*

## Introduction

Microorganisms and their products play substantial roles in pulpal and periapical diseases (1,2). Therefore, bacterial elimination in the root canal system is the primary measure to prevent major periapical diseases, like apical periodontitis (3). This can be achieved by chemo-mechanical preparation, including root canal irrigation and inter-appointment medication (3). Ideally, effective endodontic irrigants should exhibit antimicrobial activity, and an absence of toxicity toward the periapical tissues (4).

Sodium hypochlorite (NaOCl) has been widely used as a root canal irrigant, due to its strong antimicrobial properties and tissue dissolving ability (4). Other advantages of NaOCl include its low price, easy accessibility and long shelf life (5). However, the cytotoxicity of NaOCl when it comes in contact with periapical or oral mucosal tissues is an undesirable characteristic (6). Chlorhexidine gluconate (CHX) has also been suggested as both an irrigant and intracanal medicament (7,8). CHX exhibits broad spectrum

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antimicrobial activity, as well as substantivity, but lacks tissue dissolving capacities in the root canals (9).

Electro-chemically activated solutions (ECA), denominated super oxidized water, are electrochemically processed aqueous solutions generated from tap water, using low concentration salt solutions. Their usage in endodontic therapy has been investigated in several studies, which evaluated the ECA solutions with regard to their antibacterial activity (10,11), tissue dissolving capacity (12), ability to debride root canals and smear layer removal (6,13). Recently, Medilox® (SOOSAN E&C CO., LTD., Seoul, Korea) and Envirolyte® (Envirolyte Industries International Ltd., Tallinn, Estonia) electrolysis devices have been introduced in medicine to manufacture ECA solutions endowed with a disinfecting capacity. The ECA solution produced by the Medilox® device (ECA-MX) contains about 50-80 ppm of hypochlorous acid (HOCl), with a pH of 5.0-6.5 and an oxidation-reduction potential (ORP) of 800-1000 mv, according to its manufacturer. The ECA solution generated by the Envirolyte® device (ECA-EN) has a greater concentration of HOCl (500-700 ppm), with a pH range of 7.0-7.5 and an ORP of 700-900 mv, according to its manufacturer. To date, no studies have compared the antimicrobial effects of different ECA solutions as root canal irrigants, other than the present study.

Attempts at the complete elimination of bacteria from the root canal system have resulted in the usage of adjunct devices to improve irrigation efficiency. One recent system, the EndoActivator (EA) (Dentsply/Tulsa Dental Specialties, Tulsa, OK), has received substantial attention because of its proposed properties. The EA is a cordless, battery-operated hand piece with a sonic motor, and it has been designed to enhance the cleaning efficacy of the irrigation of the root canal system. The EA system uses non-dentine cutting polymer tips in three different sizes (small/yellow, medium/red, large/blue) and the sonic motor provides 3-speed options including 2.000, 6.000 and 10.000 cpm. The EA has been recommended to activate EDTA and NaOCl solutions (14). For example, Pasqualini et al. (15) revealed increased antibacterial activity with the sonic activation of NaOCl. Considering application time, irrigant activation for 30 seconds during a 60-second period of QMix application has been proposed to enhance debris and smear layer removal potential of the EA (16).

The purposes of this study were to determine and compare the ex vivo susceptibility of *Enterococcus faecalis* to 2.5% NaOCl (Caglayan Kimya, Turkey), 2% CHX (Drogsan, Turkey) and two different ECA solutions (ECA-MX and ECA-EN), and to evaluate whether the addition of EA to the standard irrigation protocol results in a greater elimination of *E. faecalis* from the root canals. The null hypothesis tested were: 1) there are no differences among tested irrigant groups, and 2) EA sonic activation does not improve antibacterial effectiveness of irrigation solutions.

## Materials and methods

*Root dentine specimen preparation*

One-hundred single-rooted intact human teeth, extracted for orthodontic or periodontal reasons, were used in this study. They were stored in 1.3% NaOCl for <3 months to disinfect the surface and to remove organic debris before use. The calculus was removed with periodontal cretuars; then, the crown was cut off and the root were shortened apically, leaving a root segment with a length of 10 mm. The root cementum was not removed.

The root canal instrumentation was performed by using ProTaper files (Dentsply Maillefer, Ballaigues, Switzerland) to size #F3, under irrigation of 1 mL of 2.5% NaOCl (Caglayan Kimya, Turkey) between each file. The removal of the smear layer was carried out in an ultrasonic bath (Bandelin Sonorex, Berlin, Germany) with the sequential use of 17% EDTA (pH 7.3) (Merck KGaA, Darmstadt, Germany) and 5.25% NaOCl (Caglayan Kimya, Turkey), for 5 min each (17). The root specimens were then placed in test tubes containing phosphate buffered saline (PBS) (Sigma-Aldrich, Germany), and autoclaved for 20 min at 121°C. Each sterile test specimen was incubated in 2 mL of tryptic soy broth (bioMerieux, France) for 24 hours at 37°C, to confirm the sterility.

*Infection of root specimens*

A clinical strain of E. faecalis organisms (A197A) was grown on tryptic soy agar (TSA) (BioMerieux, France) for 24 hours, at 37°C (18). A 24-hour-old E. faecalis suspension was adjusted spectrophotometrically to $OD_{600} = 0.6$.

Ninety root segments were infected with the *E. faecalis* strain for 4 weeks at 37°C. During the infection period, the media was changed every second day. The purity of the cultures was controlled once per week, based on the colony morphology on the TSA plates and cellular characteristics. Bacteria penetration into dentinal tubules was checked using scanning electron microscope. Ten samples from the negative control group were used to check the sterility, and submerged in PBS before sampling.

*Irrigation*

The teeth were randomly divided into eight experimental groups (n = 10) (four without the EndoActivator combination and four with) and two control groups (n = 10). The four irrigants tested were ECA-MX, ECA-EN, 2% CHX and 2.5% NaOCl.

Following the contamination period, the apical portions of the root segments were sealed with ethanol sterilized sticky wax to prevent flow of the solution, and then fixed on sterile glass petri dishes. Each root canal in the experimental groups was treated with 5 mL of each test solution, using a 27-gauge syringe. In the EA groups, the test solutions were left in the root canal, and the sonic activation was applied for 60 seconds, by inserting the red 25/04 polymer EA tip into the root canal at the highest speed (10,000 cpm). The infected teeth that served as the positive control were rinsed with 5 mL of PBS solution, while the sterilized teeth of the negative control group were left untreated.

*Sampling procedure*

At the end of the irrigation period, the root canals were immediately dried with sterile paper points, and stored in a freezer for 1 hour at -27°C. The dentine samples were obtained from the canal walls with Gates Glidden burs (# 3, 4 and 5) under aseptic conditions (Figure 1), and the burs never made contact with the outer surfaces of the root segments while sampling. The dentine chips obtained were transferred to glass vials containing PBS/glass beads, and vortexed for 30 seconds (19). The PBS with the re-suspended enterococci was then diluted

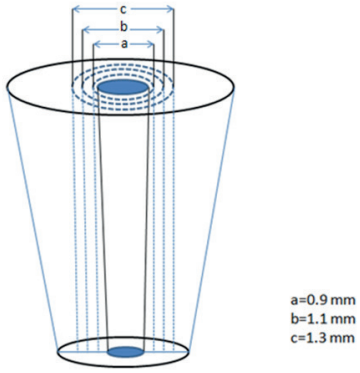


***Figure 1.*** *Schematic view of sampling procedure.*

to 10-fold. Then, 25 µL droplets from each of the four parallel dilutions were inoculated on TSA plates, and incubated at 37°C for 48 h. The visible colonies from the appropriate dilutions yielding 5-50 colonies were counted, and the colony forming unit (CFU) $mL^{-1}$ was calculated and transformed to $log_{10}$.

*Scanning electron microscopy (SEM)*

One extra sample from each test and positive control group was incubated with *E. faecalis* and treated as described above, to illustrate the colonization of the bacteria, and show the efficiency of the disinfection methods with the SEM. The samples were fixed in a 2.5% glutaraldehyde solution, and then evaluated (EVO LS10, Zeiss, Oberkochen, Germany). The SEM micrographs were taken from representative areas at various magnifications (×2000 and ×15000).

*Statistical analysis*

The data was analysed by using the SPSS (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp, USA). The statistical analyses were performed on $log_{10}$ converted data. The data were nonparametric, because of the absence of normal distribution; therefore, the Kruskal-Wallis test and Mann-Whitney test were used to compare the different groups, with the significance level at $p< 0.05$.

## Results

The *E. faecalis* growth after sampling on the TSA plates and the CFUs are listed in Table 1. All of the specimens in the positive control group showed the presence of growth (Figure 2a) in the agar plates, whereas all of the negative control specimens remained free of growth. The ECA-EN, 2.5% NaOCl and 2%

**Table 1.** *E. faecalis growth after sampling and mean values and standard deviations (SD) of colony-forming units (CFU).*

| Groups (n=10) | Samples with CFU-negative | Samples with CFU-positive | Log10 CFU (mean ± SD) |
|---|---|---|---|
| ECA-MX | 0 | 10 | 3.75 ± 0.65 |
| ECA-EN | 10 | 0 | 0 ± 0 |
| 2% CHX | 8 | 2 | 0.47 ± 0.95 |
| 2.5% NaOCl | 10 | 0 | 0 ± 0 |
| ECA-MX+EA | 1 | 9 | 3.27 ± 0.17 |
| ECA-EN+EA | 10 | 0 | 0 ± 0 |
| 2% CHX+EA | 9 | 1 | 0.35 ± 0.70 |
| 2.5% NaOCl+EA | 10 | 0 | 0 ± 0 |
| Co-p | 0 | 10 | 4.34 ± 0.25 |
| Co-n | 10 | 0 | 0 ± 0 |
| Co-p: Positive control, Co-n: Negative control | | | |

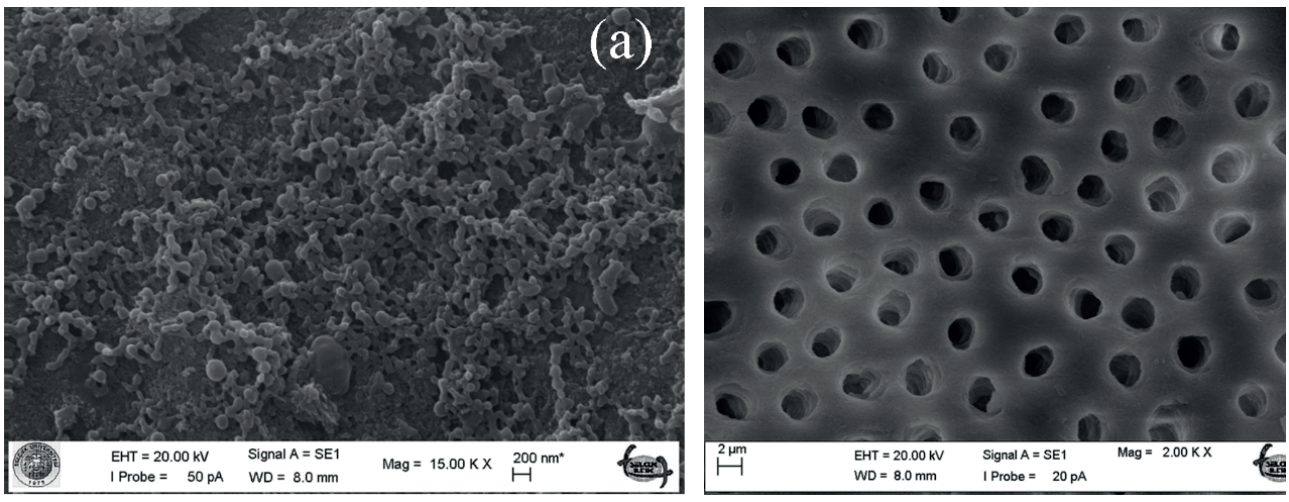


***Figure 2.*** *(a) Scanning electron micrograph showing colonization of the root canal walls by E. faecalis A197A after a four-week infection period (original magnification, ×15.000). (b) Scanning electron micrograph showing open dentinal tubules without enterococcus species.*

**Table 2.** *Statistical comparison between test group pairs in an extracted tooth model*

| Test groups | | Significance (p value) |
|---|---|---|
| A | B | |
| ECA-MX | ECA-EN | 0.000* |
| ECA-MX | 2% CHX | 0.000* |
| ECA-MX | 2.5% NaOCl | 0.000* |
| ECA-EN | 2% CHX | 0.436 |
| ECA-EN | 2.5% NaOCl | 1.000 |
| 2% CHX | 2.5% NaOCl | 0.436 |
| ECA-MX | ECA-MX+EA | 0.077 |
| ECA-EN | ECA-EN+EA | 1.000 |
| 2% CHX | 2% CHX+EA | 0.863 |
| 2.5% NaOCl | 2.5% NaOCl+EA | 1.000 |

*Significant at 0.05 level ($p<0.05$)

CHX showed stronger bactericidal effects, and there were no statistically significant differences among these groups ($p > 0.05$) (Table 2). Both the ECA-EN (Figure 2b) and 2.5% NaOCl groups, and their EA combinations, completely eliminated the *E. faecalis*. Moreover, the statistical analyses indicated significantly lower bacterial reduction ($p<0.05$) in the ECA-MX group, when compared with the other tested irrigation solutions. Overall, there was no significant difference between the irrigant groups and irrigant groups combined with the EA.

## Discussion

The findings of this study revealed that ECA-EN, NaOCl and CHX showed better antibacterial activity against *E. faecalis* than ECA-MX. Therefore, our first hypothesis was rejected. The secondary result of the present study showed no significant difference between irrigant groups and irrigant + EA groups. Consequently, our second hypothesis was accepted.

The infected tooth model used in this study was a modification of the one previously described by Haapasalo and Qrstavik (20), and this method has the advantage of simulating clinical conditions. However, variables such as root canal morphology, density of dentine, degree of calcification in dentine, content of dentinal tubules and amount of dentine chip samples are difficult to standardize in this dentine block model (21). The dentine block model appears to be more appropriate for this study since dentine and dentine components inhibit the antimicrobial activity of various root canal medicaments (22). In the present study, the root canals were contaminated with *E. faecalis* for 4 weeks. *E. faecalis* is a facultative bacteria frequently isolated in endodontic infections, especially in retreated cases (23). Its resistance to antimicrobial agents and ability to invade the dentinal tubules are possible reasons for the presence of *E. faecalis* in the microflora of persistent apical periodontitis (20). In order to provide sufficient time for the *E. faecalis* to penetrate into the dentinal tubules, a 28-day infection period was selected (20). The eradication of this bacterium from the root canals is important; therefore, strong antibacterial agents and effective methods are required for the killing of enterococci in infected root canals and dentine.

Neutralization of the irrigant to decrease possible substantive effects was not performed due to the absence of standard deactivating agent proper for all endodontic irrigants used. Also, neutralizing step does not take part in the clinical use of irrigants. Additionally, some of the components in neutralizing agents may possess antibacterial action on the biofilms therefore may lead to deceptive results (24). Dentine powder analysis was immediately carried out subsequent to irrigation under same experimental conditions to eliminate prolonged contact time of irrigation solutions (25).

In the present study, various ECA solutions demonstrated different effects on *E. faecalis*. The results suggest that ECA-EN, when used alone or combined with EA, has greater antibacterial activity (100% bacterial reduction) against *E. faecalis* than ECA-MX. Different ECA solutions can be produced by a similar electrolysis process, but the product can have a different antimicrobial activity due to the differences in the ORP values and the pH of the solutions (26). These two ECA solutions had similar ORP values, while the ECA-EN had a neutral pH, and the ECA-MX was slightly more acidic. It has been proposed that the neutral pH of the ECA solutions might be responsible for the longer shelf-life and perpetuation of the microbicidal activity (27). Therefore, the more potent bacterial reduction observed in the ECA-EN group could be explained by the neutral pH of this solution.

The main biocidal reagents in ECA solutions are chlorine related substances, such as chlorine ($Cl_2$), hypochlorous acid ions ($ClO^-$) and hypochlorous acid ($HClO^-$). The active component of ECA solutions is predominantly HOCl, which is known to be biocompatible and antimicrobial against a broad spectrum of microorganisms (28). Therefore, when comparing ECA solutions, it is necessary to take into account the difference in the HOCl concentration. ECA-EN has an approximately ten times higher concentration of HOCl than ECA-MX, which might be another explanation for the strong antimicrobial activity of ECA-EN.

The basic materials in ECA solutions are purified water and a small quantity of sodium chloride (NaCl). Unlike ECA-EN, the ECA-MX is generated at the point of use by passing Annexol solution over the electrodes, instead of NaCl. Annexol, which contains hydrochloric acid (HCl), NaCl and water, provides stability to the solution, according to the manufacturer. Although the manufacturer alleges improved antibacterial efficacy by means of Annexol, it appears that the Annexol had no substantial effect on the antimicrobial properties of the ECA-MX. On the other hand, the use of either NaCl or Annexol in the electrochemical procedure is not the only difference between the two ECA solutions. The HOCl concentration may influence the antimicrobial capacity more than the input material used in the electrolytic process.

NaOCl has extensive uses in endodontic treatment, but its potential toxicity on vital tissue has led to further investigations for alternative irrigants. One of the known advantages of the ECA solution is its non-toxicity when in contact with vital biological tissues (29). A novel ECA solution (Aquatine EC; Sterilox, PuriCore, Malvern, PA, USA) has been proposed as a highly biocompatible irrigating solution that permits the pulp stem cells to survive and attach to the root canal dentine, in regenerative endodontic therapy (30). Gomi et al. (31) reported that ECA solutions had mild cytotoxicity

on pulp cells when compared to NaOCl. In addition, Gonzales-Espinoza et al. (32) revealed that a pH neutral super-oxidized solution is less cytotoxic than antiseptic hydrogen peroxide concentrations. ECA-EN could have major advantages over NaOCl with regard to biocompatibility, and further studies are required to evaluate its cytotoxic effects.

Based on the results of the present study, ECA-EN is equally as effective as NaOCl, because both the ECA-EN and 2.5% NaOCl killed 100% of the bacteria in the root canal dentine. Our results corroborate the findings of previous studies that demonstrated the comparable antimicrobial activity of ECA solutions with NaOCl (31,33). In contrast, the findings of the present study are not in accordance with the findings of Marais & Williams (11), who showed no antibacterial activity with ECA solutions having pHs of 7.0 and 9.0, whereas the ORP levels and HOCl concentrations, which are considered to be important parameters for antimicrobial action, were not reported in that research. One recent study showed that although super oxidized water has the ability to prevent the growth of *E. faecalis*, NaOCl exhibited better antimicrobial action (34). The differences amongst the results of these studies and the present study could be attributed to the differences in the chemical properties of the ECA solutions used.

Hypochlorous acid (HOCl) and hypochlorite ($OCl^-$) are chlorine by-products, and in chlorine containing aqueous solutions, hypochlorous acid is the predominant form below a pH of 7.6; above this value, the predominantly active form is hypochlorite (35). The sodium hypochlorite solution that was used in the present study had a pH of 11-12; thus, the entire available chlorine content was in the form of hypochlorite. However, the germicidal activity of hypochlorous acid is superior to that of hypochlorite (36). ECA-EN has been confirmed to have good antimicrobial properties through the prepotency of hypochlorous acid, and based on the results of this study, it may be an attractive alternative to NaOCl.

Chlorhexidine (CHX) is a potent broad-spectrum antimicrobial agent that has substantive characteristics (9); therefore, it has been suggested for a final irrigation due to its residual antimicrobial activity (4). In addition, CHX has been found to be more effective against gram-positive bacteria than gram-negative bacteria (4). CHX effectively killed *E. faecalis* in the present study, which is in accordance with previous studies showing that CHX is an effective antibacterial agent (9,37). In the CHX with EA combination group, only one sample resulted in positive cultures on the plates, and in the CHX group, two samples showed *E. faecalis* growth on the agar plates. In a recent extracted tooth and membrane biofilm study, NaOCl, CHX and super oxidized water were investigated with regard to their antibacterial activities. The NaOCl was shown to be the most effective endodontic irrigant, while the 2% CHX and super oxidized water could not provide adequate disinfection (38). However, no other published study has compared the ECA solutions and CHX under the same experimental conditions as the present study.

The findings of the present study did not reveal a significant difference between the intracanal microbial reduction obtained by standard irrigation alone, and the reduction obtained with standard irrigation and EA sonication. This is consistent with the results of a recent in vivo study that evaluated the effects of EA on bacterial elimination (39) and the report of Ordinola-Zapata et al. (40) who showed that biofilm removal with EA was similar to conventional needle irrigation. This also corroborates the findings of an *in vitro* study which indicated that the sonic activation of EDTA and NaOCl with EA after chemomechanical preparation did not lead to better antibacterial activity (41). However, it was not the same as the results of Bago et al. (42), who reported that EA usage with NaOCl provides better antimicrobial activity when compared with NaOCl irrigation alone. This could be attributed to the differences in the infection period, since they used 7 days instead 28 days, which we used in the present study, and bacteria in young biofilms are more susceptible to endodontic irrigants than bacteria in old biofilms (43). Our results are also contradictory to the results of Shen et al. (44), who stated that sonic agitation improves the effectiveness of chlorhexidine. The previous study used an in vitro biofilm model on sterile hydroxyapatite discs, so the methodological differences may also be responsible for the different results.

In another *in vitro* study, sonic and ultrasonic activation were found to provide superior penetration of sodium hypochlorite at the apical third of the root, compared to traditional needle irrigation alone (45). When comparing these results, it should be considered that the apical 3 mm part of the roots was removed in the present study, in order to prevent deterioration of the results due to anatomical variations, and EA may show its real contribution to the conventional chemo-mechanical preparation on the apical region of the roots. The mechanism of action for EA involves acoustic microstreaming inside of the root canal system, and EA has been proposed to produce cavitation bubbles. A recent study evaluating the cleaning mechanisms of sonic and ultrasonic activated irrigation showed that cavitation was not found during sonic agitation, unlike the cavitation shown to arise during ultrasonic activation (46). One reason for the inefficiency of EA might be the non-occurrence of cavitation. Further *ex vivo* and *in vivo* studies evaluating the contemporary irrigants, together with extended EA sonication times, are needed.

## Conclusion

ECA-EN can be considered to be as effective as 2.5% NaOCl in the elimination of *E. faecalis*. The addition of EA to standard irrigation was not effective in the further reduction of viable bacteria that resides within the root canal system. The use of ECA-EN could be recommended for endodontic irrigation procedures since it showed good antibacterial activity and ECA-EN may be a promising alternative to the relatively more cytotoxic NaOCl.

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***Türkçe öz:*** *Farklı elektrokimyasal olarak aktive edilmiş solüsyonların Enterococcus faecalis'e karşı in vitro antimikrobiyal etkinliği. Amaç: Bu in vitro çalışmanın amacı, EndoActivator (EA) ilavesiyle ya da yalnız başına kullanılan farklı elektrokimyasal olarak aktive edilmiş solüsyonlar ve geleneksel yıkama solüsyonlarının, Enterococcus faecalis ile enfekte edilmiş kök kanalları üzerindeki antimikrobiyal etkinliğini değerlendirmek ve karşılaştırmaktır. Gereç ve yöntem: Bu çalışma için 100 adet tek köklü insan dişi hazırlandı. Doksan adet kök segmenti E. facealis ile 4 hafta boyunca enfekte edildi ve bir pozitif kontrol grubu (n=10) ve 8 deney grubuna ayrıldı (n=10) (4 gruba ilave EA aktivasyonu uygulandı). Test edilen irrigasyon solüsyonları; Medilox® cihazından (ECA-MX) ve Envirolyte® cihazından (ECA-EN) elde edilen elektrokimyasal olarak aktive edilmiş solüsyonlar, %2'lik CHX ve %2.5'luk NaOCl'dir. Kök örnekleri 5ml deney solüsyonuyla yıkandı, EA gruplarında ilave olarak sonik ajitasyon uygulandı. Kök kanal duvarlarından elde edilen dentin örneklerinin kültürü yapıldı, koloni oluşturan birimler sayılarak antibakteriyel etkinlik değerlendirildi. Bulgular: ECA-EN, %2.5'luk NaOCl ve %2'lik CHX, E. faecalis'in eliminasyonunda ECA-MX'ten daha etkili bulundu (p < 0.05). İlave EA sonik aktivasyonu istatistiksel olarak anlamlı antibakteriyel etkinlik göstermedi.Sonuç: ECA-EN, endodontik irrigasyon solüsyonu olarak potansiyel taşırken EA kullanımı kök kanallarından bakteriyi azaltmada ekstra fayda sağlamamıştır. Anahtar kelimeler: Elektrokimyasal olarak aktive edilmiş solüsyon; EndoActivator; Enterococcus faecalis; kök kanal yıkama solüsyonları; endodonti*

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