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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

Effects of polishing protocols on the surface roughness and color stability of polyetheretherketone (PEEK)

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Purpose
This study aimed to evaluate the effects of different polishing protocols on the surface properties and color stability of the polyetheretherketone (PEEK).

Materials and Methods
A total of 96 disc-shaped specimens were fabricated from PEEK material and divided into 6 different groups: control (CN), "Abraso-Starglanz" polishing paste (A), "Yildiz" polishing paste (Y), "Enhance" polishing system (EN), "Super snap" polishing kit (SS), and silicone polisher (SP). Surface roughness (R_a) were measured with a profilometer and the surface topography was examined under scanning electron microscope. Color differences were measured with a spectrophotometer according to the CIEDE2000 (ΔE_{00}) formulation before and after coffee immersion. Data were statistically analyzed with Kruskall–Wallis and Spearman's correlation analysis ($p<0.05$, $p<0.001$).

Results
A statistically significant difference was observed between the R_a measurements of the polishing protocols ($p<0.001$). R_a measurements except A, Y, and SS groups were found to be higher than the clinical acceptable threshold of surface roughness (0.20 µm). In ΔE_{00} measurements, statistically significant differences were observed between the CN and SP ($p=0.041$), EN ($p=0.001$), and A ($p=0.002$) polishing protocols. No correlation was found between R_a and color stability.

Conclusion
Only in the A, Y and SS polishing protocols, R_a measurements were not found to be risky in terms of acceptable threshold of surface roughness. Polishing protocols have also generally failed to maintain the color stability. Considering the surface roughness and color stability, the "Abraso-Starglanz" paste may be suitable method for PEEK material.

Keywords: *Surface roughness, color stability, polyetheretherketone, PEEK, CIEDE2000*

Introduction

Polyetheretherketone (PEEK) is a synthetic polymeric material that is available in tooth-colored forms for use in dentistry (1, 2). PEEK is biocompatible, has low specific weight, low allergy potential, and low water absorption properties. It also has superior chemical, thermal, and mechanical properties. As a result, PEEK material has started to be used in dentistry as an implant body and superstructure, fixed partial dentures, and infrastructure of removable prosthesis (2-10).

However, like all dental materials, the clinical success and longevity of this material, which is increasingly used as an alternative to traditional restorative materials, highly depend on some parameters (3, 4, 7, 8, 11, 12). One of these essential parameters is the quality of the material surface polishing. This is because the surface roughness of the dental material is a risk factor in the development of bacterial retention, caries, gingivitis,

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periodontitis, peri-implantitis, stomatitis, harmful abrasive effects on natural teeth or restoration, and other problems (1, 7, 8, 12-17). Previous studies have reported that the clinical acceptable threshold for the roughness of a prosthetic restoration surface is 0.2 µm (7, 8, 18). Therefore, it is especially necessary to apply the correct polishing procedures under appropriate conditions to increase the longevity of prosthetic restorations (1, 8).

The polishing quality of the surface can be affected by the material's hardness, wear resistance, and polishing protocols (7, 8, 12). There are many polishing materials and laboratory or chairside polishing protocols that can be used in dentistry (1, 7, 8). However, little is known in the literature about a user guide for these protocols, the effect of polishing on PEEK material surface roughness, and which protocols provide a more successful surface finish of the PEEK material (4, 7, 8, 12-14).

In addition to surface roughness, the color stability of the material is also crucial for the long-term success of the restoration. Many factors affect the color stability in restorative materials, such as the material type and composition, polymerization mode, fabrication process, aging of the material, prolonged exposure to coloring foods, smoking, and oral hygiene habits (3, 18, 19). It has been reported that the polishing quality of the material surface can also affect the color stability (7, 12, 18, 20). However, there is limited knowledge on the effect and long-term performance of polishing protocols and polishing materials on the color stability of PEEK materials (4, 7, 12, 18, 21).

Therefore, this study aimed to evaluate the surface roughness and color stability of PEEK material after applying different polishing protocols. The null hypothesis of the study was that different polishing protocols would not affect the surface roughness and color stability of the PEEK material.

Materials and Methods

Sample size estimation

In this *in vitro* study, the total specimen size was calculated using the G-POWER program with 0.4 effect size, 80% power, and 0.05 sampling error, based on the percentage of the measurement values for the methods to be studied. Based on the calculation, the number of specimens for the ANOVA test was determined by considering 6 independent groups.

Specimen characteristics

A total of 96 disc-shaped specimens of 3 mm thickness and 10 mm diameter were prepared from PEEK material using CAD/CAM production technology (Table 1). Information about the pre-treatment process and polishing protocols were summarized in Figure 1 and Table 2. All polishing materials were used according to the manufacturers' instructions and all polishing procedures were completed by the same researcher (S.C.S.).

Surface roughness

Surface roughness measurements were used to analyze the specimen surface quality of all groups after polishing protocols. In order to prevent any residue on the surface that may affect the roughness results after the polishing protocol, cleaning of the specimens with alcohol and distilled water was repeated (8). Measurements were made with a diamond-tipped contact profilometer (Mahr Perthometer

Table 1. *Information about the test material.*

Brand	Composition	Lot No.	Manufacturer
CopraPeek Light	Polyetheretherketone (≈ 80 %) Titanium dioxide (< 20%) Other additives (< 0.1%)	E10061	Whitepeaks Dental Solutions GmbH & Co. KG, Germany

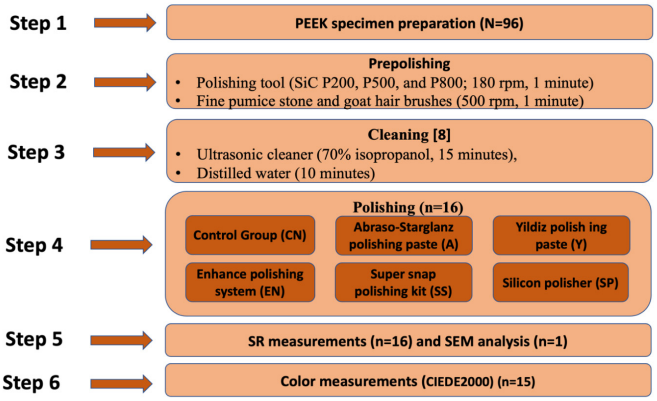

Figure 1. *Step-by-step the study design (SiC: silicon carbid, SR: surface roughness, SEM: scanning electron microscopy).*

Table 2. *Polishing protocols, products and application procedures*

Polishing Protocols	Products	Application Protocols*	Manufacturer
Laboratory	Abraso-Starglanz polishing paste **(A)**	1 minute at 3000 rpm with a polishing buff	bredent GmbH & Co KG, Germany
	Yildiz polishing paste **(Y)**	1 minute at 10 000 rpm with a polishing mop	Yildiz Cila Company, Turkey
	Renfert silicone polisher **(SP)**	1 minute at 12 000 rpm	Renfert GmbH, Germany
Chairside	Enhance Prisma® Gloss™ polishing system **(EN)**	1 minute at 10 000 rpm with polishing cubs and extra-fine composite polishing paste	Dentsply De Trey GmbH, Germany
	Super snap rainbow technique kit **(SS)**	30 second at 12 000 rpm with violet, green and pink discs, respectively	Shofu Dental GmbH, Germany

**Polishing process was repeated by renewing the polishing paste every 30 second. In addition, the buff, cubs, silicone polisher or disk used in the polishing procedure were changed in each specimen.*

M2; Mahr GmbH, Germany) applying a measuring force of 0.7 mN with a trace length of 6 mm. Surface roughness measurements were made at 3 different areas on each specimen by moving the diamond tip of the device along the specimen surface in parallel, and the specimen's average roughness (R_a) were calculated. After each measurement, the profilometer was calibrated with a special calibration block. R_a measurement results of the 5 polished groups were compared with the control group.

A random specimen was chosen from each of the control and polishing groups. The surface topography of these specimens was evaluated in the scanning electron microscope (SEM; Supra40VP, Zeiss, Germany). For topographic examination, specimens' surfaces were coated with 80% gold and 20% palladium using a sputtered device (Q150R ES, Quorum Technologies, UK) to make them conductive. The surfaces were then evaluated using the original ×300, ×600, ×1000, and ×2500 magnifications at 20 kV.

Color measurement

After the polishing protocols and surface roughness measurements were completed, the color parameters (L*, a*, b*) of all groups were measured with a digital spectrophotometer (Vita Easy Shade V, Vita Zahnfabrik, Germany) and recorded according to the Commission International de l'Eclairage (CIE) Lab 3D color system. Color measurements for each specimen were repeated on a white background at 3 different points in the center of the sample at a 90-degree angle to the specimen surface, and the measurement averages were recorded (L_0*, a_0*, b_0*). After each measurement, the device was calibrated.

Staining process

According to the manufacturer's instructions, the staining solution was prepared by dissolving 2 g of coffee (Nescafe Classic, Nestle, Sweden) in 200 mL of boiled distilled water. The specimens were embedded in the staining solution and stored in an incubator (EN055, Nüve, Turkey) at 37 °C for 30 days as static. In order to prevent the decrease in solution efficiency and to prevent coffee particles sedimentation, the staining solution was changed every 2 days (22). After the staining procedure, the specimens were washed with distilled water for 10 minutes and oil-free air-dried. After this procedure, the second color measurements of the specimens were repeated and recorded to compare with the first measurements (L_1*, a_1*, b_1*). All measurements were made by the same practitioner (S.C.S.).

The color change values of the specimens were evaluated with the current CIEDE2000 (ΔE_{00}) color difference formula (18, 23-25):

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

In the formula above, ΔL' represents the difference in lightness, ΔC' represents the difference in chroma, and ΔH' represents the differences in hue. R_T is a correction factor based on chroma and hue differences. The S_L, S_C, S_H concepts describe average factors for lightness, chroma, and hue. K_L, K_C, and K_H are weighed parametric factors expressing the experimental conditions (2, 3, 26). In this study, K_L was set to 2, and K_C and K_H were both set to 1 (27-30).

According to the latest guidance on color measurements, color stability after aging and staining should be assessed based on 50:50% acceptability (ΔE_{00}=1.8) and 50:50% perceptibility (ΔE_{00}=0.8) thresholds (27, 31, 32). In this study, color stability was evaluated with these threshold values. In addition, ΔE_L, ΔE_C, and ΔE_H intermediate components were also calculated and compared.

Statistical analysis

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. The normality distribution of the data was evaluated by the Kolmogorov–Smirnov test. The Kruskall–Wallis test was used to analyze the differences between surface roughness and color stability results according to the polishing protocols. The Mann–Whitney U test was used for pairwise comparisons of groups with significant differences. Spearman's correlation analysis was used to evaluate the correlation between surface roughness and color stability. Significance was evaluated as $p < 0.05$ and $p < 0.001$.

Results

The highest R_a results were found in the CN group, followed by the SP, EN, Y, SS, and A polishing groups (Table 3).

Table 3. *Surface roughness measurements and color stability results of PEEK material after laboratory and chairside polishing protocols.*

Polishing Protocols (n=15)	Surface Roughness (R_a)		p*	ΔE_{00}		p*
	Mean ± SD	Min-Max (Median)		Mean ± SD	Min-Max (Median)	
CN	0.49 ± 0.1	0.37-0.77 (0.47)[a]		4.38 ± 2.03	0.48-7.50 (4.71)[a]	
A	0.11 ± 0.03	0.06-0.21 (0.11)[b]		1.85 ± 1.82	0.21-5.97 (1.3)[b]	
Y	0.16 ± 0.05	0.08-0.25 (0.15)[c]		4.07 ± 2.10	0.76-8.09 (4.46)[a,c]	
SP	0.45 ± 0.08	0.35-0.73 (0.42)[a]	***0.001****	2.87 ± 2.32	0.34-9.11 (2.27)[b,c]	***0.001****
EN	0.29 ± 0.03	0.25-0.38 (0.29)[d]		1.54 ± 1.52	0.28-6.23 (1.20)[b]	
SS	0.12 ± 0.02	0.09-0.18 (0.12)[b,c]		3.63 ± 2.19	0.78-8.4 (3.73)[a,c]	

**Kruskall–Wallis test and Mann–WhitneyU test: $p < 0.05$ and $p < 0.001$. There was no statistically significant difference between the polishing protocols represented by the same letters, but a statistically significant difference was found between the groups with different lettering. SD: Standard deviation.*

A statistically significant difference between CN group and other groups was observed according the R_a measurements (p=0.001; p < 0.001). R_a values for the CN, SP, and EN groups were found to be higher than the acceptable surface roughness threshold level (0.20 µm) for prosthetic restorations. While there was no significant difference between the R_a measurements of the CN and SP groups (p = 0.158), the R_a measurements of all other groups were significantly lower than the CN group (p < 0.001). The SP polishing protocol, which is one of the laboratory-polishing protocol, showed higher R_a values than the A and Y laboratory-polishing protocols (p < 0.001). Of the chairside-polishing protocols, the EN polishing protocol was found to exhibit higher R_a values than the SS protocol (p < 0.001). However, Ra values of the SS polishing protocol were not statistically different from the laboratory-polishing protocols A (p=0.067) and Y (p=0.124) (Table 3).

Images of SEM evaluations for all polishing protocols are presented in Figure 2. According to these images, deep roughness lines were observed along the entire surface in the CN group. Although the surface was smoother in the SP and EN polishing groups than in the CN group, deep roughness areas were found in some parts of the specimens' surfaces, while areas of superficial and fine linear roughness were also detected, especially in groups A and SS.

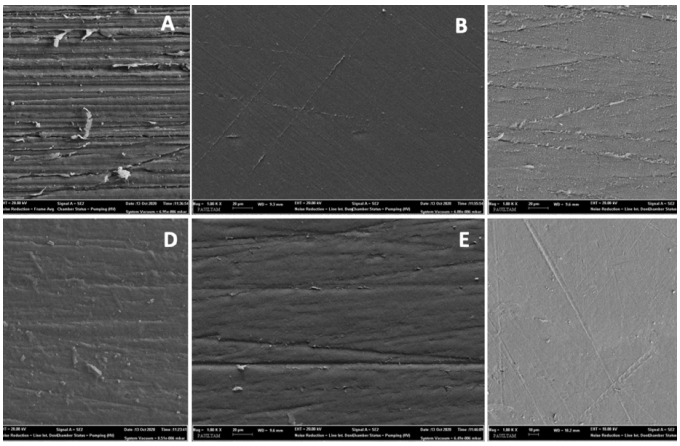

Figure 2. *SEM images (x1000 magnification) of all protocols. A, Control (Group CN). B, Abraso-Starglanz polishing paste (Group A). C, Yildiz polishing paste (Group Y). D, Renfert silicone polisher (Group SP). E, Enhance Prisma® Gloss™ polishing system (Group EN). F, Super snap rainbow technique kit (Group SS).*

The ΔE_{00} measurements of the polishing protocols were also significantly different (p=0.001; p < 0.001). The highest ΔE_{00} measurements were obtained for the CN group, and the lowest for the EN polishing group (Table 3). In ΔE_{00} measurements, statistically significant differences were observed especially between the CN group and SP (p=0.041, p < 0.05), EN (p=0.001, p < 0.001), and A (p=0.002, p < 0.001) polishing protocols. ΔE_{00} measurements for group A was statistically lower than the Y (p=0.007) and SS (p=0.001) groups. However, no significant difference was observed between the Y and SS groups (p > 0.05) (Table 3).

In addition, ΔE_{00} measurements of the A and EN polishing groups were found to be below the acceptability threshold value (ΔE_{00}=1.8). The values obtained for all other polishing protocols were above the threshold limit. In terms of perceptibility, the ΔE_{00} measurements of all groups were found above the threshold limit (ΔE_{00}=0.8) (Table 3). On the other hand, when the relationship between surface roughness and color stability measurements was evaluated, no significant correlation was found (r=0.144, p=0.176).

Table 4 summarizes $\Delta E_L'$ (lightness), $\Delta E_C'$ (chroma) and $\Delta E_H'$ (hue) measurement data of PEEK material after all polishing protocols. According to the statistical analyses, $\Delta E_L'$, $\Delta E_C'$, and $\Delta E_H'$ measurements for all polishing protocols compared were significantly different from each other (p < 0.001). In terms of $\Delta E_L'$ and $\Delta E_C'$, a statistically significant difference was found between the CN group and the A, SP, and EN polishing groups (p < 0.001), while this difference in terms of $\Delta E_H'$ was detected between the CN group and the A, SP, EN, and SS polishing groups (p < 0.001). A detailed comparison of the statistical differences of all polishing protocols is shown in Table 4.

Table 4. *$\Delta E_L'$, $\Delta E_C'$ and $\Delta E_H'$ measurements of PEEK material after all polishing protocols.*

Polishing Protocol (n=15)	$\Delta E_L'$		p	$\Delta E_C'$		p	$\Delta E_H'$		p
	Mean ± SD	Min-Max (Median)		Mean ± SD	Min-Max (Median)		Mean ± SD	Min-Max (Median)	
CN	-2.9 ± 1.64	-5.58 ± -0.13 (-3.06)^{a}		3.09 ± 1.36	0.25 ± 4.72 (3.38)^{a}		-0.98 ± 0.4	-1.78 ± -0.38 (-0.93)^{a}	
A	-1.1 ± 1.33	-4.17 ± 0.13 (-0.7)^{b,c}		1.32 ± 1.4	-0.18 ± 4.09 (1)^{b,c,d}		-0.37 ± 0.3	-1.18 ± -0.1 (-0.31)^{b}	
Y	-2.62 ± 1.61	-5.77 ± -0.35 (-2.55)^{a,d}		2.93 ± 1.33	0.61 ± 5.2 (3.01)^{a,e}		-0.94 ± 0.54	-2.28 ± -0.29 (-0.88)^{a}	
SP	-1.69 ± 1.81	-6.81 ± 0.23 (-1.26)^{b,d}	***0.001***	2.11 ± 1.57	-0.14 ± 5.68 (1.82)^{c,d,e}	***0.001***	-0.61 ± 0.46	-2.05 ± -0.16 (-0.5)^{c}	***0.001***
EN	-0.76 ± 1.18	-4.43 ± 0.43 (-0.54)^{b}		1.03 ± 1.21	-0.39 ± 4.21 (0.98)^{b}		-0.42 ± 0.24	-1.21 ± -0.15 (-0.4)^{b}	
SS	-2.02 ± 1.94	-6.3 ± 1.03 (-2.21)^{a,c,d}		2.43 ± 1.96	-1.22 ± 5.26 (2.98)^{a,d}		-0.57 ± 0.49	-1.77 ± 0.04 (-0.43)^{b,c}	

Kruskall–Wallis test and Mann–Whitney U test: p < 0.05 and p < 0.001. There was no statistically significant difference between the polishing protocols represented by the same letters, but a statistically significant difference was found between the groups with different lettering. SD: Standard deviation.

Discussion

This study initially hypothesized that different polishing protocols would not affect the surface roughness and color stability of PEEK material, but the study results rejected this null hypothesis.

There are only a few studies available that examine the surface properties of PEEK material and how these properties can be enhanced (4, 7, 13, 14). Surface properties and roughness values of restorative materials play a significant role in adhesion, a stage of plaque formation. It is clinically crucial for prosthetic restorative materials to have surface properties that prevent plaque accumulation and adhesion, as well as reduce the risk of caries in surrounding teeth (7).

To ensure that the prosthetic restorative materials exhibit ideal surface properties, it is crucial to select the most effective polishing protocol after performing occlusal adjustments during intraoral trial sessions and production stages of the restorations. The most important factor in choosing a polishing protocol is to achieve a shiny and smooth restoration surface with low Ra values, which prevents bacterial adhesion (7, 13, 14, 16). In the current study, Ra values ranged from 0.06 µm to 0.77 µm. SEM images of the groups with the highest and lowest Ra measurements matched the roughness data. Moreover, only surface roughness measurements taken from two laboratory-polishing protocols (A and Y groups) and one chairside-polishing protocol (SS group) were below the critical surface roughness threshold value for prosthetic restorations (7, 8, 18).

Heimer *et al.* (7) and Hahnel *et al.* (17) consistently compared laboratory and chairside polishing protocols using similar polishing materials and protocols. However, differences in Ra measurements were found between the studies, which may be attributed to changes in application time and speed (7, 17). Furthermore, variations in hardness of the tested PEEK materials may also account for differences in Ra measurements between studies. According to Heimer *et al.* (7), materials with higher hardness may achieve lower Ra values after polishing than softer materials. The PEEK material utilized in the present study contains approximately 20% titanium content and is considerably harder than materials utilized in similar studies. Therefore, it is plausible to obtain lower roughness values in this study, even with similar polishing protocols (8).

The most successful Ra measurements were observed in the A group among the methods tested in this study. This result may be attributed to the use of a liquid-based polishing paste, resulting in finer abrasion and a brighter, slightly reflective surface. The polishing material used in the A polishing protocol may have also contributed to the brighter and slightly reflective surface of the specimen, leading to more successful results in terms of surface roughness (1, 7).

It has been reported that 3-body abrasion techniques, which involve polishing pastes containing aluminum oxide or diamond particles, result in lower surface roughness than 2-body abrasion techniques made by grinding with burs, bonded adhesives, or coated abrasives. Therefore, it is possible that the A polishing protocol, which is a 3-body polishing technique, provided more successful Ra measurements than other polishing protocols and the control group (7, 33). On the other hand, both the EN polishing group and the A polishing group utilized 3-body polishing techniques and liquid-based polishing pastes for similar purposes. However, despite applying the polishing paste at a higher rpm in the EN polishing group, Ra values was higher than those of the A polishing group and the roughness threshold value specified for prosthetic restorations. This discrepancy in Ra values may be due to differences in the grain sizes of the abrasive materials used in these protocols (13).

In this study, coffee was used as the staining agent due to its high staining potential, as reported in previous studies (2, 34, 35). Immersion time is another important factor affecting color stability, and studies have shown that the most significant color change occurs after 30 days (36, 37). To simulate clinical aging, the specimens in this study were immersed for 30 days, which is equivalent to 2.5 years of in vivo use (35, 36, 38). Other factors, such as surface roughness and surface-free energy, have also been reported to affect color stability (3, 20, 37), likely due to the coloring solution being in contact with a larger surface area (3, 20). However, in the present study, no significant correlation was found between surface roughness measurements and color stability ($p > 0.05$). When the color stability of the groups with the smoothest surfaces (A, Y, and SS groups) was examined, the A group showed an acceptable color change ($\Delta E00=1.3$), while the other groups showed higher color changes ($\Delta E00>1.8$). The EN group, which had a surface roughness value above the threshold value (0.29 µm), exhibited the lowest acceptable color change value ($\Delta E00=1.2$) among all groups.

he discoloration of restoration surfaces is affected by electrostatic forces (van der Waals forces), hydrophobic properties, and the absorption or adsorption capacity of the materials (3). In the present study, the main reason for the color changes may be attributed to the PEEK material's lower absorption or adsorption capacity of the coffee staining agent, rather than the surface roughness (34, 35, 37). The positive color stability results achieved in the A and EN polishing procedures may be due to the lower absorption or adsorption possibility of the coloring agent, which is relevant to the surface properties obtained. More detailed evaluations are required in current studies (1, 3, 7).

Few studies in the literature compare the color stability results of PEEK material. The ability to compare the study outcomes has been negatively affected by the fact that different color formulas have been evaluated, different polishing protocols have been used, and there are differences in staining solution and immersion time (2, 3). In most of the studies, the ΔEab formula was used to estimate the color differences, but a newer formula, $\Delta E00$, has been proposed to calculate color differences. Some studies have found a high correlation between the color change data calculated with both formulas. This up-to-date formula has started to be recommended, especially for materials with high chroma, because of its success in detecting small color differences and the visual color difference perception (2, 25, 28, 29, 39). Nevertheless, very few studies have used this new formula for PEEK material or have investigated perceptibility-acceptability threshold values (2, 3, 18). The different formulations used in previous PEEK material studies and the different references used as the basis for the perceptibility-acceptability threshold values mean that the current study results are not comparable

with the results of similar studies. In addition, the difference in the data set values of the "K" parameter in the ΔE00 formula, which affects the color change results, negatively affected the comparability of the studies (2, 18, 30). The K parameters in the present study were selected as 2, 1, and 1 by using data from the literature (27, 28). However, studies in which this value was determined include the results from porcelain materials or human teeth structures (27-29). Research on acceptability thresholds, especially for polymer-based materials, is insufficient and much needed (2).

In this study, only one staining agent was tested to evaluate its effectiveness on color stability. To improve this one, various staining agents, including distilled water, coffee, fruit juice, and their combinations, could be tested. However, it is important to note that other factors, such as the nutritional habits, oral hygiene practices, smoking status, and salivary microflora of patients, may also influence both surface roughness and color stability. Hence, more in vivo and in vitro studies are required to examine the long-term surface and optical properties of PEEK materials and assess the efficacy of polishing protocols on these properties as well as bacterial adhesion.

Conclusion

Many of the laboratory and chairside polishing protocols exhibited risky surface properties for clinical acceptable threshold for the roughness of a prosthetic restoration. The color stability of PEEK material was found to be insufficient in most of the polishing protocols groups. Considering the surface roughness and color change values, the laboratory-based "Abraso-Starglanz" polishing paste protocol may be suitable method for PEEK material.

Türkçe özet: *Parlatma protokollerinin polietereterketonun (PEEK) yüzey pürüzlülüğü ve renk stabilitesi üzerindeki etkisi. Amaç: Bu çalışmada farklı polisaj protokollerinin polietereterketonun (PEEK) yüzey özellikleri ve renk stabilitesi üzerindeki etkilerinin değerlendirilmesi amaçlanmıştır. Gereç ve yöntem: PEEK malzemesinden toplam 96 adet disk şeklinde numune üretildi ve kontrol (CN), "Abraso-Starglanz" cila pastası (A), "Yıldız" cila pastası (Y), "Enhance" polisaj sistemi (EN), "Super snap" polisaj kiti (SS) ve silikon parlatıcı (SP) uygulamalarını içerecek şekilde 6 farklı gruba ayrıldı. Yüzey pürüzlülüğü (Ra) profilometre yardımıyla ölçüldü ve yüzey topografisi taramalı elektron mikroskobu altında incelendi. Renk farklılıkları, örnekler kahveye daldırmadan önce ve sonra CIEDE2000 (ΔE00) formülasyonuna göre spektrofotometre aracılığıyla hesaplandı. Elde edilen veriler Kruskall-Wallis ve Spearman's korelasyon analizi ile istatistiksel olarak analiz edildi ($p < 0.05$, $p < 0.001$). Bulgular: Parlatma protokolleri sonrasında örneklerin Ra ölçümleri arasında istatistiksel olarak anlamlı bir fark gözlemlendi ($p < 0.001$). A, Y ve SS grupları dışındaki Ra ölçümleri, klinik olarak kabul edilebilir yüzey pürüzlülüğü eşiğinden (0.20 µm) daha yüksek değerlerde bulundu. ΔE00 ölçümlerinde CN ve SP ($p=0.041$), EN ($p=0.001$) ve A ($p=0.002$) polisaj protokolleri arasında istatistiksel olarak anlamlı farklılıklar gözlendi. Ra ile renk stabilitesi arasında ise bir korelasyon saptanmadı. Sonuç: A, Y ve SS polisaj protokollerinde elde edilen Ra değerlerinin kabul edilebilir yüzey pürüzlülüğü eşiği açısından riskli olmadığı tespit edilmiştir. Test edilen polisaj protokolleri çoğunlukla renk stabilitesini korumakta başarısız olmuştur. Yüzey pürüzlülüğü ve renk stabilitesi göz önüne alındığında, "Abraso-Starglanz" polisaj pastasının PEEK malzemesi ile kullanıma daha uygun bir yöntem olabileceği tespit edilmiştir. Anahtar Kelimeler: Yüzey pürüzlülüğü, renk stabilitesi, polietereterketon, PEEK, CIEDE2000*

Ethics Committee Approval: The *in vitro* study was approved by the Medical Ethics Committee of Pamukkale University (Approval number: 60116787-020/59518).

Informed Consent: Not required.

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Author contributions: SCS, LMS participated in designing the study. SCS participated in generating the data for the study. SCS participated in gathering the data for the study. SCS, LMS participated in the analysis of the data. SCS wrote the majority of the original draft of the paper. LMS participated in writing the paper. SCS, LMS have had access to all of the raw data of the study. SCS, LMS have reviewed the pertinent raw data on which the results and conclusions of this study are based. SCS, LMS have approved the final version of this paper. SCS, LMS guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Original research

Quantitative determination and antibacterial properties of TiO_2 nanoparticle-doped glass ionomer cement: an in vitro study

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Purpose

The aim of the present study is to determine the amount of titanium ions released into the artificial salivary medium by modified glass ionomer cement (GIC) doped with 3% and 5% (w/w) titanium dioxide nanoparticles (TiO_2-NPs), and to evaluate their antibacterial properties.

Materials and Methods

120 cylindrical discs with a diameter of 4 mm and a height of 6 mm were made with 3% and 5% w/w modified GIC containing TiO_2 NPs, divided into two groups of 60, and immersed in a chemically synthesized salivary medium. The samples were quantified over four-time periods: 24 hours, two months, four months, and six months, using inductively coupled plasma mass spectroscopy (ICP-MS), antibacterial properties were evaluated by means of colony forming count (CFU) method.

Results

The amount of titanium ions released from the discs that received 3%(w/w) TiO_2 was highest in the first two months, with no significant release at successive intervals. Also, the second group, which included 5% (w/w) TiO_2, saw a considerable ion release at every interval, with the second month seeing the maximum release. The levels in the 5% (w/w) group were consistently higher when the two concentrations were compared at each of the four time points, indicating a considerable increase in titanium release and antibacterial property with a concentration increase from 3% to 5%.

Conclusion

3% and 5% (w/w) concentrations may be considered safe and exhibit significant antimicrobial effect, titanium ions were discharged at higher rates in 5% (w/w) modified GIC containing TiO2-NPs than in 3% (w/w) modified GIC containing TiO2-NPs.

Keywords: *Antibacterial, Glass ionomer cement, Ion release, saliva, Titanium dioxide.*

Introduction

A common complication in orthodontic patients is the demineralization of enamel surfaces during fixed orthodontic treatment. Plaque accumulation is aided by fixed orthodontic attachments, which provide retentive areas. Streptococcus mutans and Lactobacillus cause a rapid shift in plaque microflora, resulting in an acidogenic environment. These acidic byproducts in plaque are responsible for subsequent enamel demineralization and the formation of white spot lesions (1, 2).

For many years, the orthodontic bonding material glass ionomer cement (GIC) has been used to bond and band orthodontic equipment to tooth structures. By including glass fiber, metals, resin, and zirconia particles, GIC's mechanical qualities have been enhanced (3). Silver, zinc, titanium, and silica nanoparticles are employed to enhance the antibacterial characteristics of bonding agents (4). Dental materials with silver nanoparticles offer great antibacterial characteristics with-

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out altering mechanical properties. Still, they also have drawbacks, including decolorizing the resin matrix, which makes them seem unattractive and makes them poisonous (5,6). Most recently, titanium dioxide-based metal nanoparticles received a lot of interest because of their attractive look, photocatalytic performance, and low toxicity (7).

In a recent study, titanium dioxide nanoparticles (TiO_2-NPs) were added to conventional GIC powder in various concentrations (3%, 5%, and 7% w/w), and it was shown that the addition of 3% and 5% w/w TiO_2-NPs enhanced the mechanical characteristics compared to traditional GIC. On the other hand, 7% weight-weight TiO_2 NP addition caused a loss of mechanical characteristics. The decrease in bond strength could be due to agglomeration of particles, which creates defect sites and disrupts the curing process (8,9). The investigation concluded that a potential bonding agent is 3% and 5% w/w TiO_2-NPs added to a typical GIC powder. However, prior research concentrated mostly on nanoparticles' antibacterial effects and mechanical capabilities, with relatively few studies in the orthodontic literature reporting on the ion release from the modified composite resins (10,11).

The amount of metal ions released by orthodontic equipment and adhesives must thus be taken into consideration in light of the aforementioned. This atomic absorption spectroscopy can be measured using inductively coupled plasma atomic emission spectrometry (ICP-AES) or inductively coupled plasma mass spectrometry (ICP-MS) atomic absorption spectroscopy (AAS). ICP-MS can now accurately complete routine multi-element determinations in biological samples and other matrices because of its enhanced sensitivity and resilience (12, 13).

Evaluation of the amount of titanium dioxide ions released into the synthetic salivary medium from GIC powder doped with 3% and 5% (w/w) titanium dioxide nanoparticles was unprecedented in the current study. The null hypothesis of the study is that there is no significant difference in the rate of titanium release and antibacterial properties between TiO2 NPs impregnated in GIC at 3% and 5% concentrations.

Materials and Methods

Study design

This in vitro study was performed to evaluate the antibacterial activity and quantification of TiO2 ions in modified GIC.

Sample size calculation

Using the G Power 3.1 software, sample size analysis was carried out. The power of this investigation was 80% at a 5% level of significance and an error of = 0.05. 120 observations spread across four distinct time intervals were needed to achieve the predicted effect size of 0.9. Two groups were created, one with GIC and 3% TiO_2 NPs (Group A, n=60), and the other with GIC and 5% TiO_2 NPs (Group B, n=60) of discs. Additionally, each group was split into four separate n=15 subgroups for each of four distinct time periods (i.e., 24 hours, 2 months, 4 months, and 6 months) (6).

Materials used in the study

A commercially available conventional GIC (GC Fuji II, Pyrax Polymers, Roorkee, India) and Titanium dioxide nanoparticles (TiO_2-NPs Anatase, Dry Powder form, Average particle size: 20-30nm, Purity: 99.9%, Nano Research Lab, Jamshedpur, Jharkhand, India) were used in the study.

Equipments used in the study

Vortex shaker, Generic, Elecoptomaufacturer, India (120-240 V, 50/60 Hz), speed range 0- 2000 rpm was used for mixing the GIC with TiO_2 nanoparticles. A scanning electron microscope (JEOL JSM-661 OLV, Tokyo, Japan) was used to assess the uniform distribution of nanoparticles in GIC at Advanced Analytical Laboratory, Andhra University, Visakhapatnam, Andhra Pradesh, India (Figure 1). Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Andhra University, Visakhapatnam was used for quantification of ions. Mueller Hinton agar plate, (HI-Media lab pvt.ltd., Mumbai, India) was used to evaluate the anti-bacterial activity for S. mutans.

Artificial saliva formulation

Artificial saliva composition included 0.381g NaCl, 0.213 g CaCl2.2H2O, 1.114 g KCl, 0.738g KH2PO4, and 2.2g mucin in 1000 ml distilled water and pH 7.

Preparation of 3 % and 5% GIC modification with TiO_2-NP's

To produce 3% modified glass ionomer powder, 9 mg of GIC powder and 0.3 mg of TiO2 NPs should be mixed in a high-speed vortex shaker at 1200 rpm. Similarly, to create 5% modified nano glass ionomer powder, 9 mg of GIC powder and 0.5 mg of titanium powder were mixed in a high-speed vortex shaker at 1200 rpm (14). Scanning electron microscopy (SEM) was also used to analyze the homogenous dispersion of nanoparticles within the glass ionomer powder at 200X magnification (Figure 1).

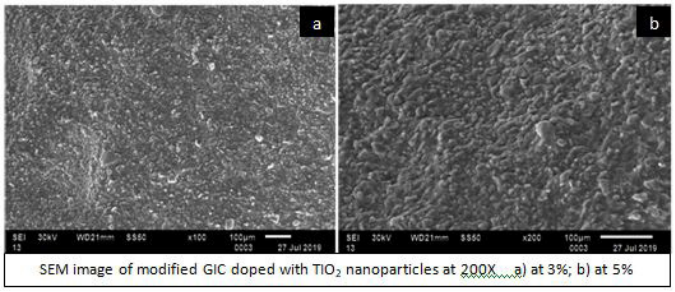


Figure 1. *SEM image of modified GIC doped with TiO_2 nanoparticles at 200x 3% and 5%.*

Disc preparation of 3 % and 5% modified GIC with TiO_2-NP's

According to the manufacturer's instructions, glass ionomer cement was blended in a 1:1 powder-to-liquid ratio, and the modified GIC mix was then shaped into discs that were 4 mm in diameter and 6 mm high. Samples were submerged in 10 ml of artificial saliva at a temperature of 37^0

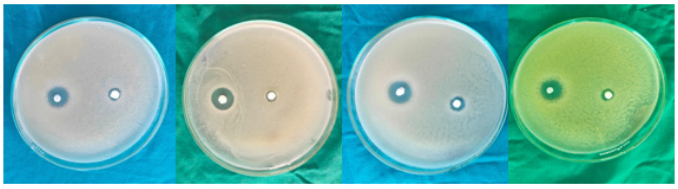
C for four intervals of time after that. Utilizing inductively coupled plasma mass spectrometry, TiO_2 was quantified (ICP-MS)

Quantification of TiO_2 nano particles

Samples with 3% w/w and 5% w/w concentrations of modified GIC discs were isolated from the artificial saliva at 24 hours, 2 months, 4 months, and 6 months. Transfer the salivary medium into separate tubes. The samples were centrifuged at 3200rpm for 15 minutes at -5^{0}C using a centrifuge (REMI C23 PLUS) to achieve impurity-free samples. Samples are diluted with distilled water from 10000 ppm (3% wt/wt) to 50000 ppm (5% wt/wt) to 100 ppb and 50 ppb, respectively, after centrifugation. The PPB range for ICP-MS quantification is limited 0-100. To remove organic materials and eliminate interference from other elements by the detector, the generated 100 PPB and 50 PPB through the dilution process were stabilizedwith supra pure HCL in ICP-MS.

Assessment of TiO_2antibacterial activity

A sterile inoculated loop was used to spread the material onto a blood agar plate with aseptic techniques to prevent growth contamination. To assess the antibacterial activity, the blood agar plates were streaked and then incubated for 24 hours at 37 degrees Celsius in an incubator. After 48 hours, colony-forming units (CFUs) of |S. mutans were counted in both the groups. (Figure 2).



Colony forming units (CFU's) at 24 hours, 2 months, 4 months and 6 months of 3% (right side) and 5% (left side) modified GIC doped with TiO_2

Figure 2. *Antibacterial activity of GIC doped with TiO_2 nanoparticles on colony forming units (CFU's).*

Statistical analysis

Statistical Package for Social Sciences SPSS (ver.26.0, IBM, Armonk, NY, USA) was used to analyze the data and repeated measures of analysis of variance (ANOVA) for multiple groups and independent sample T-test were used for pairwise comparisons. The confidence interval was set to 95% and p values less than 0.05 were considered significant.

Results

A total of 120 discs with GIC were impregnated with 3% (w/w) and 5% (w/w) titanium dioxide nanoparticles, and each sample was placed in a 10 ml artificial salivary medium. The time frames were 24 hours, 2 months, 4 months, and 6 months, and the ion release from the modified GIC discs was calibrated using ICP-MS, and antibacterial properties were evaluated using a colony counting machine. The mean titanium dioxide release from 3% w/w TiO_2 nanoparticles containing GIC (Group -A) was highest between the first and second intervals (0.33 ±0.54), which was statistically significant (p=0.05*). Nonetheless, there was no statistically significant release between the third and fourth intervals. (p=0.28) (Table 1). At the second interval (0.59 ± 0.02, p=0.001), the mean titanium dioxide release from 5% w/w nanoparticles containing GIC (Group-B) was significantly higher. However, at the third and fourth intervals (p=0.001*), the mean scores decreased significantly (Table 2). At all periods, the mean titanium dioxide release in Group B was significantly higher than in Group A. The second interval (p=0.001*) seemed to have the highest mean difference (0.59) (Table 3). The antibacterial test showed a statistically significant difference in CFU/ml of S. mutans in the experimental group between the four periods. There was a decrease in CFUs from first to second interval slight increase in colony counts from second to third interval and further increase from third to fourth interval. Pair-wise intergroup comparison amongst four intervals showed statistically significant p < 0.05 (Table 4) (Figure 3).

Table 1. *Comparison of TiO_2 release from 3% w/w nanoparticles containing GIC*

Time Frame	N	Mean(PPB)	SD	P value
24 hours	15	0.069	0.006	**0.001***
2 months	15	0.333	0.544	
4 months	15	0.274	0.001	
6 months	15	0.187	0.199	
* statistically significant				

Table 2. *Comparison of TiO_2 release from 5% w/w nanoparticles containing GIC*

Time Frame	N	Mean(PPB)	SD	P value
24 hours	15	0.157	0.013	0.001*
2 months	15	0.595	0.022	
4 months	15	0.469	0.016	
6 months	15	0.337	0.019	
Repeated measures ANOVA, * statistically significant, PPB: Parts Per Billion				

Table 3. *Comparison of means between 3% w/w TiO_2 nanoparticles and 5% w/w TiO_2 nanoparticles containing GIC*

Time Frame	N	1% w/w TiO_2 nanoparticles	5% w/w TiO_2 nanoparticles	P value
24 hours	30	0.069	0.157	0.03*
2 months	30	0.333	0.595	0.001*
4 months	30	0.274	0.469	0.02*
6 months	30	0.187	0.337	0.03*
Independent sample T test, * statistically significant.				

Table 4. *Paired comparison of colony counts for 3% w/w and 5% w/w modified GIC*

Time interval	Concentration	Mean±SD	SD Error	value
24 hours	3% w/w	7.42±0.73	0.16	0.001*
	5% w/w	10.71± 0.48	0.28	
2 months	3% w/w	3.23±0.64	0.14	
	5% w/w	4.83± 0.56	0.32	
4 months	3% w/w	4.56±0.69	0.19	
	5% w/w	6.79±0.61	0.38	
6 months	3% w/w	5.36±0.87	0.22	
	5% w/w	8.19±0.75	0.41	

*sign*significant difference.ificant difference.

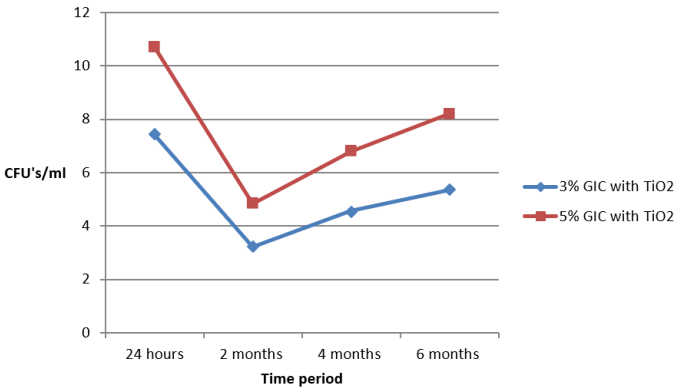


Figure 3. *Mean comparison of CFU's at four time intervals of 3% and 5% modified GIC with TiO_2.*

Discussion

The null hypothesis of the study was rejected, as there was a significant difference in the rate of titanium release and antibacterial properties between GIC impregnated with TiO2 NPs at 3% and 5% concentrations. During orthodontic treatment with fixed appliances, white spot lesions often develop close to the bands and brackets. Bacteria can adhere more effectively, survive longer, and cause more damage thanks to the scaffolding provided by the extracellular polymeric matrix in which they reside. Streptococcus mutans glucosyltransferases (GTFs) can facilitate biofilm development by producing extracellular glucans (15,16). Antimicrobial adhesives that reduce the expression of these GTF genes may help to prevent tooth decay. To prevent the interaction between these factors, it is essential to maintain good dental hygiene by physically removing plaque and applying fluoride or antimicrobial substances that enhance enamel and dentin resistance while limiting bacterial metabolism (17,18). Since patient participation is a challenge (19,20), many practitioners prefer techniques that rely on professional application rather than patient compliance.

Orthodontic bonding materials now have better mechanical and bactericidal properties due to nanotechnology, which involves changing matter atom by atom. They can be included in orthodontic adhesives/cements or coated onto the surfaces of orthodontic appliances to reduce microbial adhesion or enamel demineralization during orthodontic therapy (21). Titanium dioxide nanoparticles (TiO2 NPs) are the most commonly used nanoparticles in dentistry because of their low toxicity and antibacterial qualities (22). TiO2 nanoparticles break down organic molecules when exposed to non-lethal UV radiation by creating hydroxyl radicals and superoxide ions (23,24). The physical and chemical characteristics of standard orthodontic adhesives and equipment should not be affected when nanoparticles are introduced. However, safety must be ensured for a medically appropriate amount of time (21).

Although studies on metal ion release from orthodontic appliances into biological fluids ruled out the possibility of toxic concentrations, even nontoxic levels may cause biological changes in the oral mucosa, such as altered cellular functions, decreased DNA synthesis, and enzyme inhibition. Additionally, orthodontic adhesives must be biocompatible for clinical use in contact with gingival and oral tissues (25,26).

According to Baranowska *et al.* (13), TiO2 nanoparticles have been found to accumulate in the blood (1042 mg/kg bw/day), liver, heart (62.5, 125, and 250 mg/kg bw over 30 days), and spleen (0.324, 648, 972, 1296, 1944, 2592 mg/kg for 24 h, 48 h, 7, 14 days) (22). Some TiO2 nanoparticles have been shown to have inflammatory, oxidative, and genotoxic effects, and as a result, TiO2 has been designated as potentially carcinogenic to humans by the International Agency for Research on Cancer (IARC) (13).

After the materials had fully set, the amounts of ions released into the storage solution were monitored at predetermined intervals using inductively coupled plasma (ICP) analysis (12). The multi-element capacity of inductively coupled plasma mass spectrometry (ICP-MS) enables the simultaneous measurement of many elements in a single analysis, making it a preferable option for tracking element concentrations in solution with less sample preparation and a quicker analysis time (27). In this study, the quantity of titanium released from GIC with two distinct titanium dioxide concentrations (3% w/w and 5% w/w) was monitored over time. Both concentrations had significant antimicrobial activity and adequate shear bond strength, as shown in previous research. Therefore, the analysis was performed on the titanium released from these concentrations with proven qualities, considering both the allowable amounts of titanium and its antibacterial capabilities. Previous studies have demonstrated that the body may react negatively to titanium at levels between 10 and 50 mg/kg (11, 12, 28).

The quantity of titanium that leached out of adhesives at both dosages in the current investigation was substantially lower (about 0.0001-0.0003 mg/kg) than the amount required to cause the aforementioned negative effects discovered in prior studies (22,29). This was observed in both groups at all intervals. Although studies comparing the exact quantities of released titanium in saliva exist, the levels observed in our experiment using 3% and 5% TiO2 concentrations were safe and within allowed limits. The experimental group's antibacterial properties exhibited an increase in antibacterial activity from 24 hours to 2 months. However, there was a decrease in ion release, and the effect on antibacterial properties began to decline from the third and fourth intervals, i.e., 4 months and 6 months, respectively, after impregnation of nanoparticles in GIC. This is because the nanoparticles tend to clump together within the bonding agents. The findings are consistent with the research conducted by Nasalapur *et al.* (30).

Conclusion

Based on the study's results, the release of ions was found to be higher at the second interval (second month) than the first, gradually decreasing at the third and fourth intervals (fourth and sixth months). Additionally, the release of titanium was higher in modified GIC with 5% weight TiO2-NPs compared to 3%. This in vitro study concluded that 1% and 5% concentrations of TiO2-NPs can be considered safe and exhibit strong antibacterial effects.

Türkçe özet: *Titanyum dioksit nanopartikül ilave edilmiş cam iyonomer çimentosunun in vitro olarak tükürük salınım miktarının nicel olarak belirlenmesi ve antibakteriyel özelliklerinin incelenmesi. Amaç: Bu çalışmanın amacı, %3 ve %5 (w/w) titanyum dioksit nanoparçacıkları (TiO2-NP) ile modifiye edilmiş cam iyonomer simanların (GIC) yapay tükürük ortamına saldıkları titanyum iyon miktarını belirlemek ve antibakteriyel özelliklerini değerlendirmektir. Gereç ve yöntem: 4 mm çapında ve 6 mm yüksekliğinde, TiO2 NPs içeren %3 ve %5 w/w modifiye GIC ile yapılmış 120 silindirik disk, 60'lık iki gruba ayrıldı ve kimyasal sentezlenmiş bir tükürük ortamında batırıldı. Örnekler, plazma kütle spektroskopisi (ICP-MS) kullanılarak 24 saat, iki ay, dört ay ve altı ay boyunca dört zaman aralığında ölçüldü. Antibakteriyel özellikler, koloni oluşturma sayısı (CFU) yöntemi kullanılarak değerlendirildi. Sonuç: %3 (w/w) TiO2 içeren disklerden salınan titanyum iyon miktarı, ilk iki ayda en yüksek düzeydeydi ve ardışık aralıklarda önemli bir salınım olmadı. Ayrıca, %5 (w/w) TiO2 içeren ikinci grupta, her zaman aralığında önemli bir iyon salınımı görüldü ve ikinci ay en yüksek salınımı gösterdi. Her dört zaman noktasında her iki konsantrasyon karşılaştırıldığında, %5 (w/w) gruptaki düzeyler, 3% ile karşılaştırıldığında tutarlı bir şekilde daha yüksekti, bu da konsantrasyon artışıyla titanyum salınımında ve antibakteriyel özelliklerde önemli bir artış olduğunu göstermektedir. Sonuç: %3 ve %5 (w/w) konsantrasyonlar güvenli olarak kabul edilebilir ve belirgin bir antimikrobiyal etki gösterir. TiO2-NP içeren %5 (w/w) modifiye GIC'de titanyum iyonları, %3 (w/w) modifiye GIC'den daha yüksek miktarda salınmıştır. Anahtar kelimeler: Antibakteriyel, Cam iyonomer siman, İyon salınımı, Tükürük, Titanyum dioksit.*

Ethics Committee Approval: The study was approved by the institutional ethical committee with reference number SSDC & RI/IRB/IEC/2019-2020/419/9/1.

Informed Consent: Not required.

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Author contributions: TVDM, TSR, KSVR, PS, VSK, VM participated in designing the study. TVDM, TSR, PS, VSK, VM participated in generating the data for the study. TVDM, TSR, KSVR, VSK, VM participated in gathering the data for the study. TVDM, TSR, PS, VM participated in the analysis of the data. TVDM, KSVR, PS, VSK, VM wrote the majority of the original draft of the paper. TVDM, TSR, KSVR, PS, VM participated in writing the paper. TVDM, TSR, PS, VSK have had access to all of the raw data of the study. TVDM, TSR, KSVR, PS, VSK, VM have reviewed the pertinent raw data on which the results and conclusions of this study are based. TVDM, TSR, KSVR, PS, VSK, VM have approved the final version of this paper. TVDM, TSR, KSVR, VSK guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Original research

The effects of different repolishing procedures on the color change of bulk-fill resin composites

Muhammet Fidan[1]

Purpose
The purpose of the study was to investigate the impact of repolishing procedures on the color change of bulk-fill resin composites after being exposed to coffee.

Materials and Methods
Four bulk-fill resin composites (Filtek One bulk-fill, Tetric EvoCeram bulk-fill, Admira Fusion x-tra bulk-fill, Grandio SO x-tra bulk-fill) were tested. Sixty samples were prepared with each resin composite and were randomly divided into two groups: first one received the one-step polishing system (Optragloss) and the other group received the two-step polishing system (Nova Twist) (n=30). After being kept in coffee for 12 days, the samples were divided into three subgroups according to repolishing: one-step repolishing group, two-step repolishing group, and non-repolishing group (n=10 for each). Color measurements of the resin composite samples were determined with a spectrophotometer. The difference in color change was calculated using the CIEDE 2000 color formula. The data were analyzed using three-way ANOVA and Tukey test.

Results
Among composite materials, Filtek One bulk-fill (1.84 ± 0.98) less color change was observed compared to others ($p<0.001$). In terms of polishing systems, Optragloss (2.96 ± 1.51) showed significantly greater color change than Nova Twist (2.21 ± 1.07) ($p<0.001$). The non-repolishing group (3.78 ± 1.25) presented significantly greater color change than the Nova Twist sytem (1.49 ± 0.61) and Optragloss system (2.50 ± 1.01) ($p<0.001$).

Conclusion
The repolishing process reduced discoloration. A two-step repolishing system results in less color change compared to a one-step repolishing application. In polishing systems containing diamond particles, increasing the number of steps can contribute to color stability.

Keywords: *Bulk-fill, color stability, discoloration, repolishing, resin composite*

Introduction

The most popular materials for restorative dental applications are composite resins, which produce excellent outcomes in terms of both aesthetics and functionality. The physical and mechanical characteristics of these materials are continually improving as a result of technological advancements and academic research (1). In recent years, bulk-fill composite resins have been used in posterior teeth, allowing layering up to 4 or 5 mm thick in one step, accelerating the restoration process (2). In the restoration of teeth, composite resins with different sized particles created using nanotechnology are now frequently used (3). Thanks to the improvements made in the structures of composite resins, their clinical life is extended, and the color mismatch between the restored tooth and the material is reduced. The color change of resin materials has gained importance in the choice of restorative materials.

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Discoloration in composites can be due to internal and external factors. Internal factors include self-coloring of the resin material and oxidation or hydrolysis in the resin matrix (4). External factors such as discoloration resulting from exposure to coloring agents and their absorption by the material, as well as the degree of staining varying with oral use, can cause changes in resin-based materials. Beverages such as coffee, tea, cola, and red wine can cause varying degrees of staining on the resin materials (5). Coffee can act as a coloring agent that has the ability to penetrate the organic phase of composite resins and release low-polarity yellow pigments, leading to discoloration (6).

Finishing and polishing processes are important steps after restorations are completed. Optimal finishing and polishing increase both the aesthetics and longevity of the restoration. The presence of irregular areas in the restoration can cause plaque retention, surface discoloration, and the formation of secondary caries (7). Therefore, ensuring the smoothness of the surface of composite resins is a key factor affecting clinical success (1). The color change of materials is affected by external factors, the properties of the particles, polishing systems, and composition (3). The contents of polishing materials include diamond or carbide burs, polishing discs, diamond-embedded rubber spirals, silicon carbide brushes, and polishing pastes. These polishers, which are used in one or more steps in the finishing and polishing process, vary greatly in compositions, type, and hardness of the abrasive (8).

The surface texture of a tooth-colored restoration can have a significant impact on plaque formation, discoloration, wear, and the overall esthetic appearance of the restoration. Therefore, proper finishing and polishing are critical procedures that can increase both the aesthetics and lifespan of restored teeth (4). Recent studies have shown that resin composites, which are commonly used for tooth restoration, exhibit less discoloration after finishing and repolishing (3,4). Studies examining the color stability of the resin materials after repolishing procedures are limited. Although new, simplified polishing systems are less time consuming, there is not enough information about the color change of these finishing and polishing systems with different repolishing procedures. Therefore, the purpose of this study is to assess the impact of various repolishing procedures on the color change of bulk-fill resin composites after exposure to a coffee solution. The null hypothesis of this study is that the repolishing procedures would not affect the color change of bulk-fill resin composites after exposure to a coffee solution

Material and Methods

Ethical approval

The ethical approval for the study was obtained from the Usak University, Faculty of Dentistry, Non-Invasive Clinical Research Ethics Committee (43-43-18).

Sample size estimation

The sample size was determined using G*Power 3.1. Considering the parameters examined in a previous study, a medium effect size, 95% confidence level (1-α), and 80% test power (1-β), 10 samples for each group were deemed sufficient (3).

Study materials

Table 1 shows the materials used for this study. CIEDE 2000 formula (Figure 1) and flow chart (Figure 2) are indicated..

Table 1. *Composition of the resin composites*

	Manufacturer	Type	Composition content	Filler weight %	Lot
Filtek One Bulk-Fill Restorative	3M-ESPE, St. MN Paul, USA	Bulk-Fill	UDMA, DDDMA, Zirconia/silica (4-20 nm) cluster filler, ytterbium fluoride (100 nm) AUDMA, AFM, and 1, 12-dodecane-DMA, camphorquinone	76.5 % w	NC6052
Tetric EvoCeram Bulk-Fill	Ivoclar Vivadent, Schaan, Liechtenstein	Bulk-Fill	Bis-GMA, Bis-EMA, UDMA,barium glass, ytterbium trifluoride, mixed oxide, silica nanohybrid;(17% pre-polymers), Lucirin, Ivocerin, camphorquinone	78-81 % w	Z0032W
Admira Fusion x-tra	Voco GmbH, Cuxhaven, Germany	Ormocer bulk-fill	Ormocer, glass ceramics, silica nanoparticles, pigments	84 % w	1918494
Grandio SO x-tra	Voco GmbH, Cuxhaven, Germany	Esthetic bulk-fill	Bis-GMA, Bis-EMA, aliphatic dimethacrylate, Inorganic filler, organically modified silica	86 % w	1910205
Polishing systems					
Optragloss	Ivoclar Vivadent, Schaan, Liechtenstein		Spiral wheel system for composite polishing (diamond-embedded)		ZL09LD
Nova Twist	Nova Twist, Presidental, Munih, Germany		Spiral wheel system for composite polishing (diamond-embedded)		479154

Bis-GMA: bisphenol A glycol dimethacrylate; Bis-EMA: bisphenol A ethoxylated dimethacrylate; TEGDMA: triethylene glycol dimethacrylate, UDMA: urethane dimethacrylate AUDMA: Aromatic urethane dimethacrylate; AFM: Addition-fragmentation monomer; DDDMA: 1, 12-Dodecanediol dimethacrylate

In the current study, four bulk-fill resin composites (Filtek One Bulk-Fill Restorative, 3M-ESPE, MN, USA; Tetric EvoCeram Bulk-Fill, Ivoclar Vivadent, Schaan, Liechtenstein; Admira Fusion x-tra, Voco GmbH, Cuxhaven, Germany; Grandio SO x-tra, Voco GmbH, Cuxhaven, Germany) shade tone equivalent A2 were tested.

Sample preparation

Resin composite specimens were prepared using disk-shaped Teflon molds with a diameter of 8 mm and a depth of 4 mm. The resin composites were placed in holes (bulk layer), and a mylar strip (Hawe Transparent Strip, Kerr Hawe, Bioggio, Switzerland) was placed over the top surface of samples. The Mylar strip was gently pressed with a glass plate at the top of each mold's surface, extruding the excess material. After removing the glass plate, it was polymerized at a power density of approximately 1200 mW/cm^2 for 20 seconds (Elipar S10; 3M ESPE; St. Paul, MN, USA). For surface standardization, a single surface of the samples in each group was finished using 1200-grit silicon carbide abrasive paper with water before application of the polishing systems.

A total of 240 disc-shaped samples were prepared (60 samples in each of the resin composite groups). The resin composite groups were randomly divided into two subgroups (n=30). One group was treated with a one-step polishing system (Optragloss, Ivoclar Vivadent, Schaan, Liechtenstein) using a diamond-embedded spiral wheel for 30 s in dry conditions. The other group was treated with a two-step polishing system (Nova Twist; President Dental, Munih, Germany). Nova Twist polishing system includes prepolishing and highshine polishing diamond-embedded spirals. Each spiral wheel was applied for 15 s in dry conditions. The polishing systems were applied using a handpiece at a speed of 10,000 rpm.

All specimens were rinsed for 10 s and then stored in distilled water in a Nuve Incubator (EN 055, Ankara, Turkey) at 37°C for 24 h. Initial color values of the resin composite samples were determined with a spectrophotometer (Lovibond RT Series, Tintometer Group, UK). Three measurements were made from each sample, the average of these measurements was taken, and the L, a, b values were recorded. The samples were immersed in a 300 ml solution of boiling water containing 3.6 g of coffee (Nescafe Classic; Nestle, Switzerland) and kept in the solution for 12 days (9). The samples kept in the solution at 37°C. The solution refreshed daily. To remove any excess staining agent, the samples were washed with distilled water for 1 minute and then dried following the staining procedure.

Repolishing procedures was applied to the resin composite samples: one-step repolishing (Group RO), two-step repolishing (Group RT), and non-repolishing group (Group RN) (n=10) (Figure 2). For the repolishing procedure, the same polishing protocol was applied to the materials previously described. Following the repolishing procedure, the color values of the samples were remeasured, and the color change values were determined using the CIEDE 2000 color formula (10,11) (Figure 1).

For each pair of samples, $\Delta L'$, $\Delta C'$, and $\Delta H'$ indicate the differences in lightness, chroma, and hue, respectively, using the CIEDE 2000 metric. To adjust for the location of color differences in L', a', and b' values, the weighting functions S_L, S_C, and S_H were used. The parametric factors K_L, K_C, and K_H acted as correction terms for the experimental conditions. Furthermore, R_T was used as a rotation function that takes into consideration how chroma and hue variations interact in the blue region (10,11). In this study, parametric factor values of 1 were accepted based on a previous study (7). Color change threshold values were based on a previous study (11).

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{k_C S_C}\right)\left(\frac{\Delta H'}{k_H S_H}\right)}$$

Figure 1. *CIEDE 2000 color formula used in the present study for color changes.*

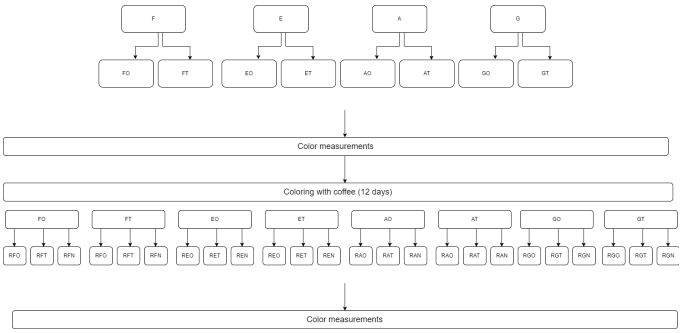

Figure 2. *Flow chart of study plan.*
F; Filtek One bulk-fill, E; Tetric EvoCeram bulk-fill, A; Admira Fusion x-tra bulk-fill, G; Grandio SO x-tra bulk-fill, O; one-step polishing, T; two-step polishing, N; non-polishing, R; repolishing

Statistical analysis

Statistical analysis of the data was performed using SPSS Statistics, Version 25 (IBM Corp., Armonk, NY, USA). For bulk-fill composites, the ΔE_{00} data (normality checked with Kolmogorov–Smirnov and skewness-kurtosis tests) were analyzed using a three-way analysis of variance (main effects and interactions). Tukey's test was used for multiple comparisons ($p<0.05$).

Results

Three-way ANOVA results, significant factors and interaction between the factors showed in Table 2. The means and standard deviations of the color change (ΔE_{00}) values were indicated for the resin composite groups in Table 3. The ΔE_{00} values of the resin materials ranged between (0.96 ± 0.28) and (5.43 ± 1.57). Composite, the polishing sytem, repolishing group were considered main effects for color stability ($p<0.001$, $p<0.001$, $p<0.001$ respectively). Moreover, repolishing effect ($\eta^2=0.675$) was found a more effective factor than composite ($\eta^2=0.315$). In terms of composite materials, Filtek One bulk-fill (1.84 ± 0.98) presented significantly a lower of color change than the other composites ($p<0.001$). There was no significant differences between Tetric-EvoCeram bulk-fill, Admira x-tra bulk-fill, Grandio SO x-tra bulk-fill ($p >0.05$). In terms of polishing sytems, Optragloss (2.96 ± 1.51) presented a significantly greater color change than Nova Twist system (2.21 ± 1.07) ($p<0.001$) (Table 3). The combination of Optragloss with Admira x-tra bulk-fill indicated

Table 2. *Three-way ANOVA results for color change of main effects and interactions between factors*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	341.016[a]	23	14.827	31.607	<0.001	0.771
Intercept	1607.943	1	1607.943	3427.723	<0.001	0.941
f1	46.660	3	15.553	33.156	<0.001	0.315
f2	33.794	1	33.794	72.040	<0.001	0.250
f3	210.220	2	105.110	224.067	<0.001	0.675
f1*f2	22.570	3	7.523	16.038	<0.001	0.182
f1*f3	10.677	6	1.780	3.794	0.001	0.095
f2*f3	12.435	2	6.218	13.255	<0.001	0.109
f1*f2*f3	4.660	6	0.777	1.656	0.133	0.044

a R Squared = .771 (Adjusted R Squared = .747)
(f1;composite, f2;polishing system, f3;repolishing procedure).

Table 3. *Means and standard deviations for* ΔE_{00}

		Composites				
polishing systems	**Groups**	**Filtek One**	**Tetric EvoCeram**	**Admira x-tra**	**Grandio SO x-tra**	**Total**
one-step	Group RN	3.46±0.77	4.58±0.57	5.43±1.57	4.43±0.54	4.47±1.16[a]
	Group RO	1.52±0.48	2.32±0.41	3.64±1.29	3.37±0.46	2.71±1.12[bc]
	Group RT	1.33±0.47	1.61±0.54	1.94±0.45	1.95±0.77	1.71±0.61[d]
	Total	2.10±1.13[A]	2.83±1.38[A]	3.67±1.86[B]	3.25±1.19[C]	2.96±1.51[X]
two-step	Group RN	2.41±0.56	3.79±0.93	2.57±0.34	3.54±0.76	3.08±0.89[c]
	Group RO	1.36±0.26	2.84±0.70	2.14±0.62	2.83±0.69	2.29±0.84[b]
	Group RT	0.96±0.28	1.39±0.54	0.96±0.13	1.76±0.58	1.27±0.53[d]
	Total	1.58±0.73[A]	2.68±1.23[A]	1.89±0.80[D]	2.71±0.99[A]	2.21±1.07[Y]
Total	Group RN	2.93±0.85[ae]	4.18±0.85[c]	4.00±1.84[ce]	3.99±0.79[cf]	3.78±1.25*
	Group RO	1.44±0.38[bd]	2.58±0.62[ag]	2.89±1.25[fg]	3.10±0.63[a]	2.50±1.01**
	Group RT	1.15±0.42[b]	1.50±0.54[d]	1.45±0.59[d]	1.86±0.67[dg]	1.49±0.61***
	Total	1.84±0.98[x]	2.75±1.3[y]	2.78±1.68[y]	2.98±1.12[y]	2.59±1.36

Goup RO; Repolishing one-step, Group RT; Repolishing two-step, Group RN; Non-repolishing. There is no difference between the same lower letter (x-y; between the composites) in the row. There is no difference between the same lower letter (a-g; interaction composite*repolishing) in the table. There is no difference between the same symbol ((*); between the repolishing) in the column. There is no difference between the same capital letter (A-D; interaction composite*polishing) in the table. There is no difference between the same lower letter (a-d; interaction polishing*repolishing) in the column. There is no difference between the same capital letter (X-Y; between the polishing systems) in the column.

the highest color change (3.67 ± 1.86), whereas the combination of Nova Twist with Filtek One bulk-fill indicated the lowest color change (1.58 ± 0.73) (Table 3). In terms of repolishing groups, non-repolishing groups (3.78 ± 1.25) presented a significantly greater color change than Nova Twist system (1.49 ± 0.61) and Optragloss system (2.50 ± 1.01) ($p<0.001$, $p<0.001$ respectively) (Table 3).

Discussion

The surface properties of tooth-colored restorations can affect plaque, discoloration, and the esthetic appearance of the restorations (12). Thus, to maintain the optimal clinical performance and aesthetics of tooth-colored restorations, it is essential to prioritize proper finishing and polishing procedures during the restorative process (13). In this study, the effect of repolishing procedures on the color change of resin composite samples colored with coffee and then polished using different polishing systems was investigated. As a result of the study, it was found that repolishing procedures affected the color change. Therefore, the null hypothesis of the study was rejected.

Coffee which was selected as the coloring solution in our study, is a frequently preferred beverage in daily life. According to the manufacturers, a cup of coffee is consumed in an average of 15 minutes. Assuming that an individual who drinks coffee consumes 3.2 cups of coffee a day, keeping the samples in coffee for 48 hours would correspond to 2 months of coffee consumption (9). Considering these rates, composite resin materials were kept in coffee for 12 days, corresponding to 1 year of coffee consumption. Since composite resin materials can absorb water, liquids containing pigments cause discoloration in the resin composite (14). Coffee contains substances that form a yellow color at low polarity (15). In our study, color change values of polished resin composite samples immersed in coffee for 12 days were observed in the range of (1.84 ± 0.98) and (2.98 ± 1.12). This result is consistent with other studies indicating that coffee

causes color change in resin composites (14,16,17). Water absorption is mostly due to absorption in the resin matrix. The water absorption rate is related to the resin contents of the materials and the bonding of the resin–filler interface. Water absorption causes the resin to expand and become plastic. This creates an environment for micro-cracks that cause stain penetration and color change in the resin composite or the formation of interfacial gaps between the filler and the matrix (18). The Filtek One bulk-fill showed less color change than the others. The color variation and coloring of resin materials are linked to the size, type, and amount of fillers particle (19,20). In the previous study, it was stated that the color change of the Filtek One bulk-fill was lower than the Tetric EvoCeram bulk-fill. The lowest color change of the Filtek One bulk-fill was attributed to the hydrophilic property of the resin material (21). The UDMA matrices can obtain higher hardness values than Bis-GMA based matrices. This could be explained as the UDMA has low viscosity which increase its degree of conversion and form denser polymer network (22,23). Consequently, plasticization effect on the polymer structure of resin composite, leads to the chemical instability and hydrolytic deterioration of resin-filler interface which accordingly reduce polishing retention and color stability over time (19).

Ormocer is a molecule formed by hydrolysis and polycondensation processes with a long chain inorganic silica backbone and lateral organic chain (24). The incorporation of Ormocer in composites results in a more cross-linked polymer network, leading to a higher degree of conversion, reduced polymerization shrinkage, improved surface hardness, increased toughness, and better color stability. Furthermore, when the number of chemical interactions between methacrylate groups increases, the amount of free unreacted monomers in the polymer network decreases (25). Color stability can be due to a mixture of matrix breakdown by acid, colorant penetration/absorption into the material, and colorant adhesion/adsorption to the surface (26). This may indicate that lower color stability and higher solubility are associated with monomer structures, for which colorant affects the chemical structures. In a previous study, microhybrid composites were found to be more stain resistant than nanocomposites and microfilled composites (27). According to Tagtekin *et al.,* Ormocer has a greater surface roughness than standard hybrid resin composites. The color deterioration in the Admira x-tra bulk-fill composite may be because the filler particles in the employed Ormocer are tougher than the matrix (28).

Composite resins exhibit color instability, which can be attributed to both intrinsic and extrinsic factors. External factors that affect the performance of dental composites include the duration and intensity of light curing, as well as exposure to environmental elements such as water, heat, and food coloring agents. Intrinsic factors that can affect color change in composite resin materials include the degree of conversion, the presence of impurities or contaminants, and the type of pigments or dyes used in the materials (29). The color change of materials results from the combination of axis movements. The literature offers several explanations for the color change of resins, including camphorquinone residues, vinyl group changes, polymerization of composite resins, and the breaking of chemical linkages (30). Color stability in restorations may result from a lack of polymerization, which contributes to the absorption of coloring chemicals. Factors may lead to color instability in restorations due to inadequate polymerization, resulting in the absorption of coloring agents (31). Smaller filler particle is less prone to discoloration due to water aging than larger filler particle. However, it is crucial to note that the number of filler particles is partly to blame for staining, since the resin composite can absorb more water (32). A portion of the light is lost due to variables impacting the optical characteristics, such as intrinsic absorbance, porosity, and roughness (19). Light transmittance falls exponentially as absorbance increases, altering reflectance and, as a result, color values (33). However, further inherent factors, such as the purity of the monomers, initiators, inhibitors, activator (type and concentration) and filler loading, have affect the color stability of resin materials (34).

Previous study stated the effect of polishing materials on the color change of resin materials and highlighted that polishing improves the color stability of restorative materials (13). After finishing/polishing, the surface micromorphology of composite materials is affected by the type, amount, size, and hardness of filler particle in composite materials. Previous studies have reported similar constraints, such as the operator variable and type of movement performed during polishing (35,36,37). The final appearance of a tooth-colored restoration can also be influenced by the flexibility of the finishing material, the abrasiveness of the polishing material, the size of the abrasive particles, and the technique used for application (38). Several studies have found that multi-step systems indicate better than one-step systems (7,8). One-step systems can be implemented with a single polishing material, and smooth surfaces are provided in a shorter time (7). The texture of the final surface depends on the technique and material used, but there is no consensus on the materials and techniques that provide the smoothest surfaces for resin composites (39,40).

In this study, the color changes of specimens were analyzed using a spectrophotometer with the CIEDE 2000, which was developed to overcome the limitations of the CIE Lab* (41,42). The evaluation of restorative materials' efficacy and the interpretation of both visual and instrumental data are crucial, perceptibility threshold (PT) and acceptability threshold (AT) are defined as measures of the extent of differences. A color change value that is perceptible by 50% of observers is referred to as 50:50% PT, and the color change value that is clinically acceptable to 50% of observers is referred to as 50:50% AT (43,44). In clinical dentistry, the determination of threshold values for visual perceptibility and acceptability plays a crucial role in the assessment of color differences in dental restorations (45). In our study, color changes of resin composites were considered 50:50% acceptability threshold (AT:$\Delta E_{00} = 1.8$) and 50:50% perceptibility threshold (PT:$\Delta E_{00} = 0.8$) (43). Based on the results of the in this study, the clinically unacceptable color change values for composite resin samples were found to be (3.78 ± 1.25) in the non-repolishing group and (2.50 ± 1.01) in the one-step group after the repolishing procedure. In addition, when polishing systems are evaluated, the Nova Twist system (two-step) had lower color change values than the Optragloss system (one-step). Ideal polishing protocols are described as a selective wear proto-

col using a series of abrasive particles ranging from coarse to fine grit (46). Multistep systems use smaller particles at each step to remove scratches created by the previous step (47). For polishing systems containing diamond particles, a greater number of steps can eliminate the irregularities that occurred during the application stage. This study found that the two-step system preserved the color stability of the repolishing groups. Moreover, the repolishing application contributed to the color stability for both systems. According to Heintze *et al.*, spiral wheels may provide a clinical advantage when used on curved dental surfaces (48). It has been reported that in addition to the size and shape of abrasives, the effectiveness of polishing can also be influenced by factors such as their binding to the matrix and the type and flexibility of the matrix (49). In addition, for color change; the partial eta squared for the repolishing variable was $\eta^2 = 0.675$, while the partial eta squared for the composite variable was $\eta^2 = 0.315$. These findings indicate that the repolishing has a higher effect on the color change than the resin material contents. Based on the findings of the study, it highlights the importance of repolishing for composite resins.

There are limitations associated with this study. This study was conducted *in vitro*, which may not be representative of clinical situations. Intraoral conditions may result in discoloration of restorative materials. Additionally, the extrinsic discoloration caused by beverages could be decreased or even negated with oral hygiene routines. It would be appropriate to consider the effect of brushing in discoloration studies. Future studies using *in vitro* and *in vivo* methods should conduct different parameters and materials.

Conclusion

Repolishing of resin composites exposed to coloring decreases the color change of resin composites. Less color change was observed for the two-step repolishing system compared to the one-step repolishing application. The color change of the one-step repolished and non-polished resin composites were above the clinically unacceptable level after 12 days. Two-step polishing systems may be preferred in terms of maintaining color stability. In polishing systems containing diamond particles, increasing the number of steps can contribute to color stability. The content of the resin material is also an important factor in color stability.

Türkçe özet: *Farklı yeniden cilalama prosedürlerinin bulk-fill rezin kompozitlerin renk değişimi üzerine etkisinin incelenmesi. Amaç: Bu çalışmanın amacı, yeniden cilalama prosedürlerinin kahve solüsyonuna maruz kaldıktan sonra bulk-fill rezin kompozitlerde renk değişimi üzerindeki etkisini değerlendirmektir. Gereç ve yöntem: Dört bulk-fill rezin kompozit (Filtek One bulk-fill, Tetric Evoceram bulk-fill, Admira Fusion x-tra bulk-fill, Grandio SO x-tra bulk-fill) test edildi. Rezin kompozit gruplarının her birinde 60'ar örnek hazırlandı ve polisaj sistemine göre rastgele iki gruba ayrıldı: tek aşamalı polisaj sistemi (Optragloss) ve iki aşamalı polisaj sistemi (Nova Twist) (n=30). Örnekler 12 gün kahvede bekletildikten sonra yeniden polisaj uygulanmasına göre üç alt gruba ayrıldı; tek aşamalı yeniden cilalama grubu, iki aşamalı yeniden cilalama grubu, yeniden cilalama yapılmayan grup (n=10). Rezin kompozit örneklerin renk ölçümleri spektrofotometre ile belirlendi. Renk değişimi farkı, CIEDE 2000 renk formülü kullanılarak hesaplandı. Veriler, üç yönlü varyans analizi ve Tukey testi kullanılarak analiz edildi (p<0.05). Bulgular: Kompozit materyaller bakımından, Filtek One (1,84 ± 0,98) diğer kompozitlere göre anlamlı derecede daha düşük renk değişimi gösterdi (p<0.001). Polisaj sistemleri bakımından Optragloss (2,96 ± 1,51), Nova Twist'e (2,21 ± 1,07) göre daha fazla renk değişimi gösterdi (p<0.001). Yeniden cilalama grupları bakımından, yeniden cilalama yapılmayan gruplar (3,78 ± 1,25), Nova Twist sistemine (1,49 ± 0,61) ve Optragloss sistemine (2,50 ± 1,01) göre anlamlı ölçüde daha fazla renk değişimi gösterdi. Sonuç: Yeniden cilalama işlemi renk değişimini azaltmıştır. İki aşamalı polisaj sistemi uygulandığında, tek aşamalı polisaj sistemine göre en az renk değişikliğini oluşturmaktadır. Elmas partikülleri içeren polisaj sistemlerinde polisaj aşama sayısının artması renk stabilitesine katkı sağlayabilir. Anahtar kelimeler: bulk-fill, renk stabilitesi, renk değişikliği, yeniden cilalama, rezin kompozit*

Ethics Committee Approval: The ethical approval for the study was obtained from the Usak University, Faculty of Dentistry, Non-Invasive Clinical Research Ethics Committee (43-43-18).

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Author contributions: MF participated in designing the study. MF participated in generating the data for the study. MF participated in gathering the data for the study. MF participated in the analysis of the data. MF wrote the majority of the original draft of the paper. MF participated in writing the paper. MF has had access to all of the raw data of the study. MF has reviewed the pertinent raw data on which the results and conclusions of this study are based. MF has approved the final version of this paper. MF guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Original research

Biocompatibility of different resin composites after polymerization with two light curing units: an immunohistochemical study

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Purpose
The aim of this study is to compare the biocompatibility of two different resin composites after polymerization under two different light sources in three different time periods.

Materials and Methods
72 polyethylene tubes polymerized with 2 different resin composites and 2 different light sources (Elipar S10 and Valo) [Group 1: Kalore Elipar S10 (KE), Group 2: Kalore Valo (KV), Group 3: Essentia Elipar S10 (EE), Group 4: Essentia Valo (EV)] were implanted in the dorsal connective tissue of 18 rats. 24 empty polyethylene tubes [Group 5: (Control group)] were implanted in the dorsal connective tissue of 6 rats. Then, the rats were sacrificed after 7th, 15th and 30th days in each time intervals (n=8). Biopsy samples were stained with H&E and examined for inflammation, necrosis, macrophage infiltrate, giant cell and fibrous capsule criteria. Immunohistochemical staining was performed to evaluate proinflammatory cytokines (IL-1β, IL-6 and IL-8).

Results
When the composite groups and the control groups were compared; there was difference statistically significant for the criteria of inflammation at 7th and 15th days, there was no statistical difference between the time points in terms of fibrous capsule and necrosis. When the composite groups and control groups were evaluated in terms of proinflammatory cytokines; statistically significant differences were found at 7th, 15th and 30th days.

Conclusion
All CRs used in this study showed acceptable biocompatibility in the subcutaneous tissues of rats after polymerization with different light sources.

Keywords: *Composite resins, light sources, biocompatibility, rat, immunohistochemistry*

Introduction

The composite resins (CRs) are the most preferred materials due to their mechanical and optical properties for direct restorations in dentistry (1). CRs contain various organic monomers (BIS-GMA, UDMA, HEMA, TEGDMA) at different concentrations (2, 3). After the polymerization of these monomers, residual monomers pass into the dentinal tubules, resulting in delayed pulpal healing, irreversible inflammatory reaction in the pulp, and insufficient dentin bridge formation (4, 5). It has been reported that the release of residual monomers, oligomers and reduced products can have a adverse effect on the biocompatibility of these materials. These monomers disrupt cell metabolism and can cause cytotoxic effects, allergic reactions and mutagenicity (6). It has been reported that CRs containing BIS-GMA, TEGDMA and UDMA are cytotoxic at the cellular level,

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and in addition the order of cytotoxicity for resin monomers is BIS-GMA>UDMA>TEGDMA>HEMA (7-9). For this reason, free BIS-GMA CRs have been produced in recent years to reduce the cytotoxicity of CRs.

Different types of light-curing units (LCUs), conventional quartz tungsten halogen (QTH) lights or lasers can also be used for the polymerization of light-curing restorative materials while light-emitting diodes (LEDs) are used more often today (10, 11). The polymerization of CRs is a critical parameter that affects both the optimal physical properties and biocompatibility of the material. Inadequate polymerization results in poor physical properties of the restoration, solubility in the oral environment, secondary caries and pulp irritation and increased microleakage. On the other hand, the amount of residual monomers may vary depending on the light source used for curing (12). As it is known, the degree of monomer polymer conversion is very important for good mechanical properties and biocompatibility. The degree of light-induced conversion of monomers to polymers is affected by the wavelength, intensity, irradiation time of light, concentrations, types and mixtures of photoinitiators, stabilizers and inhibitors, as well as types and proportions of monomers and fillers (13).

Biocompatibility can be defined as the non-toxic and physiologically non-reactive of a material or its compatibility with a living tissue or system (14). Since these materials are in direct contact with periapical tissues, alveolar bone, pulp and body fluids, biocompatibility is one of the basic conditions (15). The degree of conversion mainly determines the biocompatibility of composite resins, since this factor can determine the greater or lesser release of unpolymerized/residual monomers during curing processes (16). It has been shown that a decrease in the degree of monomer-polymer conversion can lead to a decrease in the physical-mechanical properties of the material and increase in the release of monomers into the oral environment (17).

The local response from the effect of the materials consists of an accumulation of inflammatory cells, primarily macrophages and giant cells (18). Macrophages are crucial for their capacity to engulf and process foreign body and are involved in the release of chemokines responsible for inflammatory cells (13, 19).

Cytokines are a broad category of relatively small proteins that are produced and released for the cell signaling purpose. At the beginning of the acute inflammatory process, monocytes reach the damaged tissue following neutrophils. Irritants in the environment cause the production of proinflammatory cytokines such as interleukin 1 beta (IL-1β), interleukin 6 (IL-6) and interleukin 8 (IL-8), and the release of histamines, prostaglandins and metalloproteinases. As a result of the decrease in the proinflammatory activities of macrophages, anti-inflammatory activity and tissue regeneration begin. Evaluation of the inflammatory reaction in biocompatibility tests is performed by histological methods, which give certain results of tissue response, as they are most commonly associated with immunohistochemical methods (19, 20).

In the light of this information, this study aims to evaluate the biocompatibility of BIS-GMA on rats on the 7th, 15th and 30th days after polymerization with different light sources. The null hypothesis of the this study could be stated as the composites free BIS-GMA would not differ in terms of biocompatibility at different time periods after polymerization with different light sources.

Materials and methods

Ethical approval

This study was carried out with the approval of Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (2020/288).

Sample size estimation

Power analysis was performed to determine the sample size before the research. When α=0.05, β=0.20 (1-β)=0.80 was taken, it was decided to take 24 rat for a sample size and the power of the test was found to be p= 0.80837.

Experimental design

CRs and light sources used in this study are shown in the Table 1. In this study, 24 male Wistar albino rats weighing 200-220 g were used. 18 rats for the composite group and 6 rats for the control group were used since a maximum of 4 incisions can be made on the dorsal part of each rat. In this study, a total of 96 polyethylene tubes (PTs) (inner diameter 1.5 mm, 10 mm long) were kept in 96% alcohol for 20 minutes to ensure aseptic conditions. 24 empty PTs were determined as the control group. After filling 36 PTs with ESSENTIA composites using the incremental technique, 18 PTs were polymerized with the Elipar (EE) light source and 18 PTs with the Valo (EV) light source. Within the KALORE composite group, 36 PTs were filled with this expreimental group using the incremental technique, and 18 PTs were polymerized with the Elipar (KE) light source and 18 PTs with the Valo (KV)

Table 1: *Composite resins and light sources used in our study*

Material (Lot number)	Type	Manufacturer	Composition	
Kalore ™ 1906121	Dental Composite	GC, Corporation. Tokyo, Japan	Fluoro-aluminum-silicate glass, Prepolymerized filler, Silicon dioxide, UDMA, BIS-EMA	
Essentia ™ 1906131	Dental Composite	GC, Corporation. Tokyo, Japan	UDMA, BIS-EMA, BIS-GMA, TEGDMA, Barium glass, Prepolymerized filler, Silica	
			Light Intensity	**Wavelength**
Elipar S10	Light Sources	3M Espe	1200 mw/cm^2	430-480 nm
Valo	Light Sources	Ultradent	1600 mw/cm^2	385-515 nm

light source. All composite groups were polymerized from all surfaces for 20 seconds.

Surgical procedure

The mixture of 0.008 mL/100 g ketamine and 0.004 mL/100 g 2% xylazine hydrochloride (Rompun) was administered intramuscularly to the rats to provide anesthesia. The areas where the PT was planned to be placed before the incision were shaved and disinfected using 5% iodine solution. For the composite groups, 4 different incisions were made in the dorsal part of each rat, 2 on the shoulder and 2 on the waist, on both sides of the midline and at a distance of at least 20 mm from each other. After 1 cm incision was made in the upper left side, KE was placed in the upper left pocket, and 1 cm incision was made in the upper right pocket, and the KV was placed. Then, 1 cm incision was made at a distance of at least 20 mm below the left upper pocket, EE was placed in the lower left pocket, and in the lower right pocket, at a distance of at least 20 mm from the upper right pocket, and the EV was placed in the lower right pocket.

For the control group, 6 rats were used to equalize the sample size with the composite groups, and 3 incisions were made on the dorsal part of the rats. 3 incisions were made in the dorsal part of each rat, 2 mm in the shoulder and one waist region. Following implantation of PTs after blunt dissection for both the Composite and Control groups, the incisions were sutured with 3.0 silk sutures. During the 7th, 15th and 30th days of the experiment, 6 rats from the composite group and 2 rats from the control group were sacrificed with overdose anesthesia at each time interval. The dorsal part of the sacrificed rats were shaved and the locations of the PTs were determined by palpation and removed with intact connective tissue.

Light microscopy and immunohistochemical staining protocols

Biopsy samples were kept in 10% formalin solution for 24-48 hours. After fixation, the tissues were embedded in paraffin blocks and 5 µm sections were taken with a microtome (Leica Corp, Germany). Some of these sections were taken from normal slides for hematoxyleneosin (H&E) staining, and some of them were taken as positively charged slides for immunohistochemistry (IHC) staining. After the deparaffinization process, the preparations taken on normal slides were stained with H&E, and the preparations on positively charged slides were stained with DAB detection kit. Entellan was dripped onto the stained preparations and the closure was performed. The groups were evaluated under light microscope (Olympus Bx50) at 100, 200 and 400 magnifications. Histological criteria and scores used in the study are shown in Table 2.

Statistical analysis

The data obtained by the examination were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 22.0 (IBM SPSS Inc., Armonk, NY, USA). Shapiro - Wilk test was used for the normal distribution test. Accordingly, Kruskall Wallis test was used to compare independent groups, and Mann Whitney U test was used for pairwise comparisons. The Friedman test was used to compare dependent groups. Wilcoxon test was used for pairwise comparisons. The confidence interval was set to 95% and p values less than 0.05 were considered significant.

Table 2: *Histological and immunohistochemical criteria and scores.*

Criteria	Scores			
	0	**1**	**2**	**3**
Inflammation	No detected inflammatory cells	Less than 25 cells (mild)	Between 25 and 125 cells (moderate)	125 or more cells (severe)
Fibrous Capsule	Absent	Thin ≤150 µm	Thick ≥150 µm	
Macrophage Infiltrate	<10 cells	≥10- 20 cells	≥ 20 – 30 cells	>30 cells
Necrosis	Absent	Present		
Giant cell	Absent	Present		
IL-1β	Absent	Mild (<%10)	Moderate (%10-50)	Severe (>%50)
IL-6				
IL-8				

Results

Histological and immunohistochemistry findings

Data for different time periods are presented in Table 3 and Table 4.

Inflammatory cell response

When the comparison between the groups was made on the 7th, 15th and 30th days, the difference was statistically significant on the 7th and 15th days, but the difference was not significant on the 30th day. The highest inflammation value was observed on the 7th day in the EV group, while the lowest inflammation value was observed on the 30th day in the Control group (Figure 1). When the groups were evaluated statistically in three different time periods; the difference was statistically significant in all groups except the control and KE groups ($p<0.05$).

Fibrous capsule, giant cell, macrophage and necrosis

Fibrous capsule thickness was thin in all groups on day 7th, but an increase in capsule thickness was observed on days 15th and 30th. Although giant cell and macrophage infiltration was seen in all groups on the 7th day, it decreased on the 15th and 30th days. Necrosis was observed only on day 7th in all groups (Figure. 1). For fibrous capsule thickness; The difference was not statistically significant in all groups, when the groups were compared on the 7th, 15th and 30th days. When the groups are evaluated statistically in themselves in three different time periods; The difference was statistically significant in all groups except the EE group ($p<0.05$). In terms of giant cell; three was only a statistical difference was found on the 30th day, hen the comparison between the groups

Table 3: *Evaluation of the H&E criteria results for all groups on day 7th, 15th and 30th. In the horizontal column, the same capital letters indicate the difference between the groups, and in the vertical column, the same lowercase letters indicate the difference between the groups (*p<0.05)*

		KE		KV		EE		EV		CONTROL		
Parameters	**Time**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**p**
Inflammation	**7**	$1.16^{A,D,E,F}$	1-2	$1.83^{B,D,G,a}$	1-2	$2.16^{C,E,a,b}$	2-3	$3.00^{F,G,H,a,b}$	3-3	$0.83^{A,B,C,H}$	0-1	**0.001***
	15	1.00^{B}	0-2	1.33	1-2	1.33^{a}	1-2	$2.00^{A,B,a}$	1-3	0.66^{A}	0-1	**0.017***
	30	0.54	0-1	0.83^{a}	0-1	0.83^{b}	0-1	1.16^{b}	1-2	0.50	0-1	**0.129**
	p	**0.057**		**0.034***		**0.023***		**0.020***		**0.157**		
Fibrous Capsule	**7**	1.16^{a}	1-2	$1.00^{a,b}$	1-1	1.50	1-2	$1.16^{a,b}$	1-2	1.16^{a}	1-2	**0.305**
	15	1.66	1-2	2.00^{a}	2-2	1.66	1-2	2.00^{a}	2-2	1.5	1-2	**0.166**
	30	2.00^{a}	2-2	2.00^{b}	2-2	2.00	2-2	2.00^{b}	2-2	2.00^{a}	2-2	**1.00**
	p	**0.025***		**0.014***		**0.83**		**0.025***		**0.025***		
Necrosis	**7**	0.16	0-1	0.33	0-1	0.33	0-1	0.5	0-1	0.16	0-1	**0.709**
	15	0	0	0	0	0	0	0.16	0	0	0	**1.00**
	30	0	0	0	0	0	0	0	0	0	0	**1.00**
	p	**0.317**		**0.157**		**0.157**		**0.083**		**0.317**		
Giant Cell	**7**	1.00^{a}	1-1	1.00^{a}	1-1	1.00	1-1	1.00	1-1	1.00^{a}	1-1	**1.00**
	15	0.66	0-1	0.66	0-1	0.83	0-1	1.00	0-1	0.66	0-1	**0.578**
	30	$0.16^{B,a}$	0-1	$0.16^{A,a}$	0-1	0.50	0-1	$1.00^{A,B,C}$	0-1	$0.33^{C,a}$	0-1	**0.024***
	p	**0.025***		**0.025***		**0.157**				**0.046***		
Macrophage Infiltrate	**7**	$1.83^{D,a}$	1-2	$2.1^{A,a}$	2-3	$2.33^{B,a}$	2-3	$2.66^{C,D,a,b}$	2-3	$1.16^{A,B,C}$	1-2	**0.02***
	15	$1.16^{D,E}$	1-2	1.5^{A}	1-2	$2.00^{B,D,b}$	2-2	$2.00^{C,E,a}$	2-2	$1.00^{A,B,C}$	1-1	**0.001***
	30	$1.00^{A,D,a}$	1-1	1.16^{a}	1-2	$1.00^{B,a,b}$	1-1	$1.5^{A,B,C,D,b}$	1-2	1.00^{C}	1-1	**0.048***
	p	**0.034***		**0.014***		**0.023***		**0.046***				

Table 4: *Evaluation of the IHC criteria results for all groups on the 7th, 15th and 30th days. In the horizontal column, the same capital letters indicate the difference between the groups, and in the vertical column, the same lowercase letters indicate the difference between the groups (*p<0.05)*

		KE		KV		EE		EV		CONTROL		
Parameters	**Time**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**Mean**	**Min-max.**	**p**
IL-1β	**7**	1.00^{A}	1-1	$2.14^{A,B,a}$	1-3	1.28	1-2	1.28	1-2	1.14^{B}	1-2	**0.005***
	15	0.85^{A}	0-1	$1.71^{A,a,b}$	1-2	1.00	1-1	1.00	1-1	1.00	1-1	**0.001***
	30	0.71	0-1	0.57^{b}	0-1	0.85	0-1	0.71	0-1	0.71	0-1	**0.851**
	p	**0.157**		**0.015***		**0.180**		**0.157**		**0.083**		
IL 6	**7**	2.00^{a}	2-2	$1.57^{A,a,b}$	2-3	2.00^{a}	2-2	$2.71^{a,b}$	2-3	$1.28^{A,B,a}$	1-2	**0.001***
	15	1.71	1-2	$2.00^{A,a,c}$	2-2	1.57	1-2	1.85^{a}	1-2	$1.00^{A,B}$	1-1	**0.002***
	30	1.14^{a}	1-2	$1.42^{b,c}$	1-2	0.85^{a}	0-1	1.57^{b}	0-2	$0.57^{A,a}$	0-1	**0.017***
	p	**0.014***		**0.038***		**0.011***		**0.011***		**0.025***		
IL-8	**7**	1.71^{a}	1-2	$1.57^{A,a}$	1-2	2.14^{a}	2-3	$2.14^{B,a,b}$	2-3	$1.28^{A,B,a}$	1-2	**0.010***
	15	1.28	1-2	$1.28^{A,b}$	1-2	2.14	2-3	1.28^{a}	1-2	1.14^{A}	1-2	**0.007***
	30	0.85^{a}	0-1	$0.71^{a,b}$	0-1	1.28^{a}	1-2	0.57^{b}	0-1	0.71^{a}	0-1	**0.113**
	p	**0.014***		**0.014***		**0.034***		**0.014***		**0.046***		

was made on the 7th, 15th and 30th days. When the groups are evaluated statistically in themselves in three different time periods; The difference was statistically significant in all groups except EE and EV groups (p<0.05). For macrophage infiltration; the difference was statistically significant in all time periods (p>0.05), when the comparison was made between the groups on the 7th, 15th and 30th days. The highest macrophage infiltration was seen in the EV group on the day 7th. The lowest macrophage infiltration was observed on the day 30th in KE and Control groups (Figure 1). The difference

in the necrosis variable was not statistically significant in all time periods, When the comparison between the groups was made on the 7th, 15th and 30th days. When the groups are evaluated statistically in themselves in three different time periods; the difference was not statistically significant in all groups.

Interleukins

The difference in IL-1β, was statistically significant on the 7th and 15th days, but the difference was not significant on the 30th day, when the comparison was made between the groups on the 7th, 15th and 30th days. The highest IL-1β was seen on day 7th in the KV group, while the lowest IL-1β was on day 30th in the Control group. When the groups were evaluated statistically within themselves in three different time periods; only the difference in the KV group was statistically significant ($p<0.05$) (Figure 2). For IL-6, the difference was statistically significant in all time periods when the comparison between the groups was made on the 7th, 15th and 30th days. The highest IL-1β was seen on the 7th day in the EV group. The lowest IL-6 was observed on the 30th day in the Control group. When the groups were evaluated statistically within themselves in three different time periods, the difference was statistically significant in all groups ($p<0.05$) (Figure 2). For IL-8, when the comparison was made between the groups on the 7th, 15th and 30th days, the difference was statistically significant on the 7th and 15th days, but there was no difference on the 30th day. The highest IL-8 was seen on the 7th day in the KV and EV groups. When the groups were evaluated statistically within themselves in three different time periods; the difference was statistically significant only in the Control group ($p<0.05$) (Figure 2).

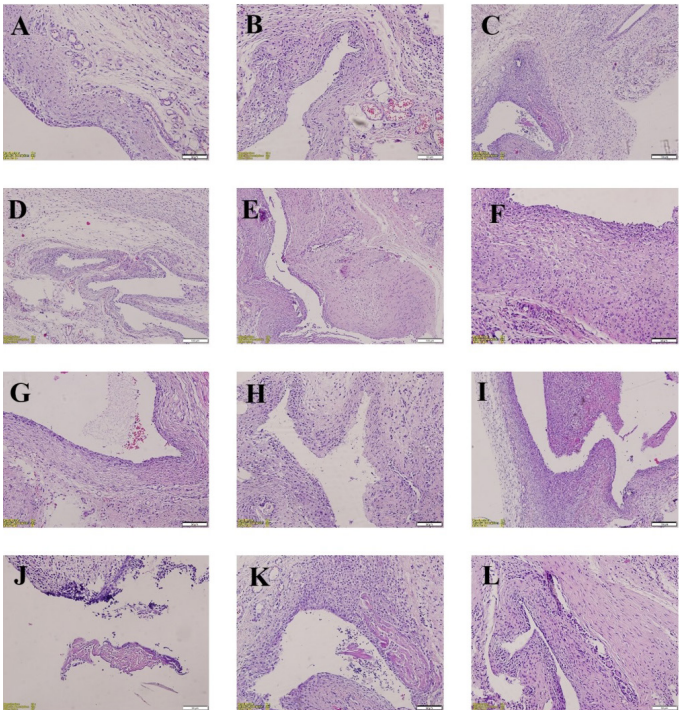

Figure 1. *Histopathological section samples belonging to all groups. A. Mild inflammation, on day 30th Control Group, B. Moderate inflammation, on day 15th KV Group, C. Severe inflammation, on day 7th EV Group, D. Thin capsule, on day 7th KV Group, E and F. Thick capsule, on day 15th and 30th EV Group, G. Mild macrophage infiltration, on day 30th Control Group, H. Moderate macrophage infiltration, on day 15th EE Group, I. Severe macrophage infiltration, on day 7th EV Group, J and K. Necrosis, on day 7th KV Group , L. Giant cells, on day 7th EE Group*

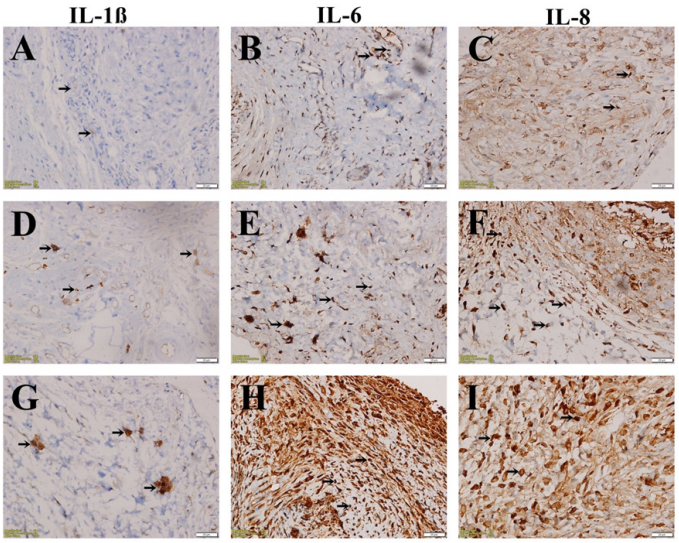

Figure 2. *Immunohistochemical staining images of IL-1β, IL-6, IL-8. A. Mild IL-1β on day 30th Control group, B. Mild IL-6 on day 30th KE group, C. Mild IL-8 on day 30th EE group, D. Moderate IL-1β on day 15th KV group, E. Moderate IL-6 on day 15th EV group, F. Moderate IL-8 on day 15th KE group, G. Severe IL-1β on day 7th KV group, H. Severe IL-6 on day 7th EV group, I. Severe IL-8 on day 7th EE group*

Discussion

Cytotoxicity tests, genotoxicity tests, bone implants and subcutaneous implantation tests are used to determine the effects of dental materials on living tissues. Although cytotoxicity tests are faster and easier, their results can be insufficient for clinical applications (21, 22). It has been stated that local inflammation and toxicities can be determined by implantation of dental materials into subcutaneous connective tissue in experimental animals. Since the inflammatory tissue response after subcutaneous implantation tests is similar to pulp and connective tissue, it is considered a reliable method to evaluate the biocompatibility of dental materials (23). Since PTs are inert in subcutaneous implantation tests, materials are placed in these tubes and their biocompatibility is evaluated (24). For these reasons, different CRs used in this study were filled in PTs and implanted in the subcutaneous connective tissue of the rats.

The standard staining technique with H&E staining is used for histological examination of tissues. Although this simple and cheap staining technique is capable of revealing important cellular details, it identifies only limited protein, enzyme and tissue structure (25, 26). Since IHC involves a specific antigen-antibody reaction, it has a significant advantage over these conventionally used staining techniques, which identifies only limited protein, enzyme and tissue structure. In basic research, this technique is also used to determine the location and distribution of biomarkers within tissues (27). Since more specific results are obtained, IHC staining method was used together with H&E staining in our study and

proinflammatory cytokines (IL-1β, IL-6 and IL-8) were evaluated. CRs are restorative materials used quite frequently in dentistry (28, 29). CRs contain various organic monomers (BIS-GMA, UDMA, HEMA, TEGDMA) at different concentrations (2, 3). It has been reported that these monomers may cause toxic effects due to incomplete polymerization and residual monomer formation (29, 30). BIS-GMA is the monomer with the lowest monomer polymer conversion degree (DC) according to the literature. DC is BIS-GMA < BIS-EMA < UDMA < TEGDMA respectively (31). In this study, Essentia CR containing BIS-GMA and TEDGMA and Kalore CR free BIS-GMA were used.

Mesquita *et al.* (32), evaluated the biocompatibility of three different resin-based cements and reported that resin cements containing BIS-GMA had the highest cytotoxicity and CD68 levels compared to other cements. It has been reported that resin-based dental materials with a high BIS-GMA concentration cause an increase in phagocytic cells such as CD68, overexpression of proinflammatory cytokines, and cause a long-term inflammatory process in rats (33, 34). In our study, both inflammation and proinflammatory cytokines (IL-1β, IL-6 and IL-8) were found to be high on the 7th day in the EV and EE groups. In addition on day 15th, both the cytokine and the inflammatory process continued, while at day 30th both decreased.

Jun *et al.* (35), evaluated the biological properties of the biomonomer without BIS-GMA, which they developed to avoid the estrogenic and cytotoxic effects of BIS-GMA, reported that the biomonomer they developed had a high viability level of human oral keratinocyte and MCF-7 cells. Shinkai *et al.* (36), evaluated the toxicity of resin-containing adhesives in the pulp reported that dental adhesives containing HEMA and TEDGMA showed more severe inflammation. However, they reported that the permeability of the adhesive monomers to the pulp tissue and the degree of polymerization of the adhesives may also be related to the irritation of the pulp tissue. In present research, low inflammation, proinflammatory cytokines (IL-1β, IL-6 and IL-8) were observed in the KV and KE groups free BIS-GMA and free TEDGMA. This may be caused by the release of lower amounts of residual monomers (37). Castaneda *et al.* (38), evaluated the cytotoxicity of silorane-based resin composite and BIS-GMA-containing resin composite, and reported that BIS-GMA containing composite resin caused higher inflammation. Similarly, in our research, EE and EV groups showed higher inflammation and proinflammatory cytokines (IL-1β, IL-6 and IL-8) compared to KE and KV groups on the 7th day. Kamalak *et al.* (39), evaluated the cytotoxicity and biological properties of bulk-fill composites reported that inflammatory cytokines such as IL-6, IL-8 and TNF-α were high in cell culture. Silva et al. (24), evaluated the biological properties of BIS-GMA containing endodontic canal filling paste and silicate-based root canal filling pastes, reported that BIS-GMA-containing canal filling paste had higher IL-6 than other root canal filling pastes. Similar to the results of the study evaluated the effects of resin-containing composites on inflammatory cytokines such as IL-6, IL-8 and TNF-α in our research, IL-1β, IL-6 and IL-8 were found to be high in all resin composite groups on the 7th day (40). Different light sources and curing modes affect the release of resin monomers that have an impact on the biocompatibility and cytotoxicity of dental composites (41-43). Feiz *et al.* (44), evaluated the biocompatibility of resin composites after polymerization with different light sources, and reported that high-intensity light source caused toxic effects on inflammation and fibroblasts.

Munksgaard *et al.* (45), compared the residual monomer amounts of BIS-GMA and TEDGMA containing resin composites polymerized using different light sources, and reported that the high-intensity light source left less residual monomer. However, it has also been reported that a high-intensity light source may cause damage to the pulp and periodontal tissues during polymerization (46). Ergun *et al.* (47), evaluated the cytotoxicity of resin-based luting cements after polymerization at different times, and stated that strong LED light for a long time caused a toxic effect on fibroblasts. Yap *et al.* (48) evaluated the cytotoxicity of resin composites after polymerization with different light sources, and observed that the high-intensity LED light source caused a more cytotoxic effect than the QTH light source. When they compared the LED light sources within themselves, reported that the resin composite polymerized with the high-intensity LED light source was more cytotoxic.

Tunç *et al.* (49) evaluated the cytotoxicity of compomers on pulp fibroblasts after polymerization with different light sources, reported that the LED light source with high light intensity was more toxic on fibroblasts. They stated that the cytotoxicity of hydrophobic monomers such as BIS-GMA and UDMA is greater than that of hydrophilic monomers such as 2-hydroxyethyl methacrylate and TEGDMA (6). In addition, higher light intensity and higher temperature rise is another factor that causes this situation (50).

In our experiment, inflammation and IL-1β, IL-6 and IL-8 proinflammatory cytokines were found to be higher in the EV and KV groups polymerized with a high light intensity VALO light source, compared to the control group, on the 7th day. Mild to moderate levels of inflammation and proinflammatory cytokines were observed in the KV and EV groups on the 15th day. This may be due to the continued release of unreacted monomers. In addition, the response of the immune system of rats to foreign bodies may be delayed (44). In addition, from the 7th to the 30th day, the thickness of the fibrous capsule increased, while the macrophage infiltration and giant cell decreased. It is also stated that the cytotoxic effect increases depending on the dose and changes over time. Inflammation and proinflammatory cytokine levels of all composite groups used in our study decreased over time. Necrosis is defined as the uncontrolled death of the cell and is associated with the resulting increase in non-viable cells and increased release of inflammatory cytokines. In our study, necrosis was observed on the 7th day in all composite groups. On day 15th, necrosis was seen only in the EV group.

Conclusion

Within the limits of this animal experiment, it can be stated that all composites used in the present study demonstrated acceptable biocompatibility in the subcutaneous tissues of rats. However, pulp protective materials should still be considered for deep dentin caries which are close to the pulp tissue, due to the high inflammation rate observed on the 7th day.

Türkçe özet: *Rezin kompozitlerin iki farklı ışık cihazıyla polimerizasyonu sonrası biyouyumluluğu: immünohistokimyasal çalışma. Amaç: Bu çalışmanın amacı, iki farklı rezin kompozitin, iki farklı ışık kaynağı ile üç farklı zaman diliminde polimerizasyon sonrası biyouyumluluğunu karşılaştırmaktır. Gereç ve Yöntem: 2 farklı rezin kompozit ve 2 farklı ışık kaynağı (Elipar S10 ve Valo) ile polimerize edilmiş 72 polietilen tüp [Grup 1: Kalore Elipar S10 (KE), Grup 2: Kalore Valo (KV), Grup 3: Essentia Elipar S10 (EE), Grup 4: Essentia Valo (EV)] 18 ratın dorsal bağ dokusuna implante edildi. 24 adet boş polietilen tüp [Grup 5: (Kontrol grubu)] 6 ratın dorsal bağ dokusuna yerleştirildi. Daha sonra ratlar 7., 15. ve 30. günlerden sonra her zaman aralığında (n=8) sakrifiye edildi. Biyopsi örnekleri H&E ile boyandı ve inflamasyon, nekroz, makrofaj infiltratı, dev hücre ve fibröz kapsül kriterleri açısından incelendi. Proinflamatuar sitokinleri (IL-1β, IL-6 ve IL-8) değerlendirmek için immünohistokimyasal boyama yapıldı. Bulgular: Kompozit grupları ile kontrol grupları karşılaştırıldığında; 7. ve 15. günlerde inflamasyon kriterleri açısından istatistiksel olarak anlamlı fark bulunurken, fibröz kapsül ve nekroz açısından günler arasında istatistiksel fark yoktu. Kompozit grupları ve kontrol grupları proinflamatuar sitokinler açısından değerlendirildiğinde; 7., 15. ve 30. günlerde istatistiksel olarak anlamlı fark bulundu. Sonuç: Bu çalışmada kullandığımız tüm kompozit rezinler, farklı ışık kaynakları ile polimerizasyon sonrası ratların deri altı dokularında iyi biyouyumluluk gösterdi ve böylece klinik restoratif tedavilerde bu materyallerin güvenle kullanılabileceği kanısındayız. Anahtar kelimeler: kompozit rezin, ışık cihazları, biyouyumluluk, rat, immünohistokimya*

Ethics Committee Approval: This study was carried out with the approval of Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (2020/288).

Informed Consent: Not required.

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Original research

Evaluation of gap formation for different adhesive agents in primary teeth with optical coherence tomography

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Purpose
This study aimed to evaluate gap formation between the tooth surface and restorative material in terms of microleakage by using optical coherence tomography (OCT) for self-etch and selective-etch applications of two different universal and one self-etch adhesives.

Materials and Methods
Sixty non-caries, primary molar teeth were divided into six groups; self-etch and selective-etch application ways of two different universal and one self-etch adhesive systems (n:10). After Class-V cavities were prepared, every tooth was distributed randomly in groups to apply adhesion procedure and then, all cavities were restored with polyacid-modified composite resin. Microleakage was evaluated by measuring the gap between the tooth surface and restoration by a blind researcher with Image J Software from OCT images. During statistical analysis, the significance level was accepted as $p<0.05$.

Results
According to the statistical analysis of the measurements obtained by Image J Software, selective-etch groups showed less gap formation than self-etch groups for each tested adhesive ($p<0.05$), and self-etch adhesive without etching showed significantly highest gap formation among all groups ($p<0.05$).

Conclusion
Universal adhesives with a selective-etching step might be preferred over self-etch adhesives for long-lasting polyacid-modified composite resin restorations in primary teeth. However, obtained results should be considered with another prospective clinical study for long-term prognosis.

Keywords: *Adhesion, Gap formation, microleakage, optical coherence tomography, polyacid-modified composite resin*

Introduction

In today's dentistry, Black principles, which means 'expand to protect', have been replaced by minimally invasive treatment principles. In minimally invasive treatment procedure, only caries is removed and remained dental tissue is restored with using adhesives (1). Adhesive systems used today are fourth, sixth and seventh generation systems (2-4). Among these, fourth generation adhesive systems are 'etch & rinse' systems that remove the smear layer with 34-37% orthophosphoric acid applied to both enamel and dentin surfaces. Because of the acid etching step for both enamel and dentin surfaces, fourth generation adhesive systems are also called 'total-etch' systems (5). Sixth generation adhesive systems are self-etch adhesive systems that apply in two-steps and do not require etching step (2-4). These adhesives can be applied in one or two steps depending on whether primer and adhesive are in same or separate bottle (6). Seventh generation adhesive systems are called 'universal' or 'multimode' adhesives, which are

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produced as a single bottle and can be used as both self-etch and total-etch (4). While these systems incorporate features of total-etch and self-etch systems together, their bond strength can be increased due to the 'selective etching' step (7,8). Selective etching means applying shorter time acid-etch to only enamel surface such as 15 seconds of 34-37% orthophosphoric acid to increase enamel bond strength (7,8).

The main bonding mechanism to enamel and dentin in adhesive systems is to provide micromechanical locking and prevent microleakage problem (2,3). Various methods can be used to evaluate the presence of the microleakage between restoration and tooth surface (9). These methods are dye penetration tests (liquid penetrate inspection), radioisotopes, chemical agents, bacterial methods, air pressure method, neutron activation analysis, electrochemical studies, microscopic examination methods and optical coherence tomography (9-12). Optical coherence tomography (OCT) is a non-interventional imaging method that was introduced in the early 1990s and recent years, preferred in dentistry. OCT provides a micron-level cross-sectional view of biological tissues so that it has a wide range of uses (10,11). The imaging technique of OCT is a measurement based on the low coherence-centromere logic and correlation of the reflected light from the sample with a reference light (10-12).

This in-vitro research study aimed to evaluate gap formation between the tooth surface and restorative material in terms of microleakage by using OCT to compare self-etch and selective-etch application ways of two different universal and one self-etch adhesive agents in polyacid-modified resin composite restorations applied in primary molar teeth. The tested null hypotheses were the selective-etch application would show the similar gap formation as the self-etch application regarding that the type of adhesive would not be a contributory factor in gap formation.

Materials and Methods

Ethical approval

Extracted non-carious primary molar teeth due to the spontaneous eruption of permanent teeth from healthy patients with no systemic disorders were collected following ethical protocol no: D-KA 18/13 approved by Başkent University Institutional Review Board and included in the study within 6 months after receiving their informed consents.

Study design

Sample size of each group was calculated for binary primary outcome measures as the evaluation of microleakage for primary teeth. So, 10 samples per group with a total number of 60 samples were required to detect a significant difference for a two-sided type I error at 0.05, 0.40 effect size and 90% power. However, after obtaining OCT images, according to the post-hoc analysis, it was decided to evaluate a total of 210 images and 30 images per each group with Image J Software to detect a significant difference at 0.05 error level, 0.40 effect size and 95% power. Besides the power analysis, the study was conducted as double-blind so, the researcher who had done OCT imaging and Image J Software was unaware of group distribution.

Extracted non-carious primary molar teeth due to the spontaneous eruption of permanent teeth from healthy patients with no systemic disorders were collected following ethical protocol no: D-KA 18/13 approved by Başkent University Institutional Review Board and included in the study within 6 months after receiving their informed consents. Also, before preparation, primary molar teeth with any hypomineralized areas on enamel surfaces or restorations on any surfaces were excluded.

Sample preparation

Extracted 60 primary molar teeth were disinfected in 0.5% chloramine and stored in sterile distilled water until all samples were embedded in clear acrylic resin blocks. After the auto polymerization, standard Class-V cavities were prepared on the buccal surfaces of the primary molar teeth. Class-V cavity preparations that do not extend onto root surface were performed on the buccal surface of each tooth using a round diamond instrument, ISO size number 009 (Komet Dental Gebr. Brasseler GmbH & Co. KG, Lemgo, Germany) at high speed and air-water spray cooling. Class-V cavities were also standardized by using a periodontal probe as depth 2mm, width 4mm, height 2mm.

All prepared teeth were randomly distributed to the groups with different adhesive agents with self-etch and selective-etch application ways. Distribution of adhesive agents and application ways amongst the groups were given in Table 1. In the self-etch group, there is not any acid-etching step before adhesive application and adhesive agent applied according to the manufacturer's instructions. However, in selective-etch groups, 37% orthophosphoric acid was applied to the only enamel surface for 15 seconds, then washed out for 10 seconds and dried with

Table 1. *Groups distribution for the adhesive materials used in this study.*

Material	Application Procedure
Scotchbond™ Universal Adhesive, 3M, USA	**Group1:** Self-etch application (n=10) Universal adhesive application without acid-etching
Scotchbond™ Universal Adhesive, 3M, USA	**Group 2:** Selective acid-etch application (n=10) 15 seconds acid-etching + Universal adhesive application
All-Bond Universal™, Bisco, USA	**Group 3:** Self-etch application (n=10) Universal adhesive application without acid-etching
All-Bond Universal™, Bisco, USA	**Group 4:** Selective acid-etch application (n=10) 15 seconds acid-etching + Universal adhesive application
Prime&Bond NT, Dentsplay, USA	**Group 5:** Self-etch application (n=10) Adhesive application without acid-etching
Prime&Bond NT, Dentsplay, USA	**Group 6:** Selective acid-etch application (n=10) 15 seconds acid-etching + Adhesive application

air for 10 seconds. After etching or without etching, bonding agents were applied to all surfaces of the cavities for 10 seconds by a separate microbrush for each tooth then, dried with 10 seconds and polymerized 20 seconds with LED (Elipar S10, 3M ESPE, Seefeld, Germany) according to the manufacturers' recommendations. All light-curing procedures were performed with the same LED-curing unit operating in a continuous mode while emitting a light-intensity of 1200 mW/cm^2 with a polimerization distance of 1mm standardized by a curing disc. The output of the LED-curing unit was verified after every three measurements by using a radiometer (Bluephase Meter II; Ivoclar Vivadent, Amherst, New York).

After the adhesive application step, polyacid-modified resin composite was condensed to the cavities and restorations were polymerized for 40 seconds with the same LED device according to the manufacturers' recommendations. After polymerization, all restorations were polished with a pear-shaped finishing bur to finish irregular areas at restoration borders and give final contour to the restoration. Then, abrasive disks were used for final polishing. After restorative procedure, thermal cycle procedure (Thermocycler THE 1100/1200, SD Mechatronik, Westerham, Germany) was carried out for all teeth samples before the microleakage evaluation. Thermal cycles were applied to the samples at 5-55±20C, with a waiting time of 15 seconds and a transfer time of 10 seconds.

After thermal cycle, all teeth samples were evaluated with Optical Coherence Tomography to take images from all borders of the restorations by a second researcher to be blind of group distribution. A set-up was designed for study samples to stand still in front of the OCT device to take appropriate images. In Figure 1 and Figure 2, adjustments made to capture appropriate images can be seen in pseudocolors (Figure 1A and Figure 2A) and grey scale (Figure 1B and Figure 2B), respectively. All obtained images were evaluated with Image J Software Program to take quantitative data to compare different adhesive groups and different application ways of these groups by the same second blind researcher who was unaware of group distribution of all images.

Evaluation with optical coherence tomography

During the OCT (Zeiss Cirrus HD-OCT, Carl Zeiss Meditec, Jena Germany) procedure, restored tooth embedded in acrylic resin blocks were placed in front of the objective at the top of the device. Then, the sample was moved to left-right and up-down by the arm of the device and light beam was orthogonally scanned to the tooth surface and restoration interface in such a way that infrared beam traversed over the tooth surface, the air which was the gap between tooth surface and restorative material and restoration regions, respectively. The scanning probe was positioned at distance of 3cm from the restoration.

The entire tooth-restoration borders were scanned, which means from one approximal surface to the other by taking cavosurface margins guidance. So, after the infrared beam was scanned over tooth-air-restoration surfaces, each scan provided images at per 200µm, which enabled us to determine marginal gaps between tooth surface and restorative material. According to the working principle of OCT, gap areas reflect in high viscosity colors than tooth and restorative material. These adjustments in the OCT device which had done and the features of the OCT images in pseudocolors and grey scale due to the different viscosities of the tooth and restorative material can be seen in Figure 1 and Figure 2.

Measurement with Image J Software

After images were obtained with OCT, approximately 90 images were taken from every tooth sample and 30 images were randomly chosen from these to take quantitative results with Image J Software (Imaging Processing and Analysis in Java, National Institute of Health, Bethesda, MD). Image J Software was used to measure the gap between tooth surface and restorative material by drawing 'paintbrush tool' with the guidance of different color reflections of the tooth, restorative material, and air between them. All OCT images were taken in pseudocolors to differentiate the borders of the restoration (Figure 1A and Figure 2A), tooth and gap area whereas during the Image J measurements, colors of all images were converted to grey scale to draw the circumference of the gap areas (Figure 1B and Figure 2B). Also, each measurement was repeated three times to prevent or reduce the number of faulty measurements for each image. These quantitative values which were pixel values were saved as excel tables and compared between all the groups.

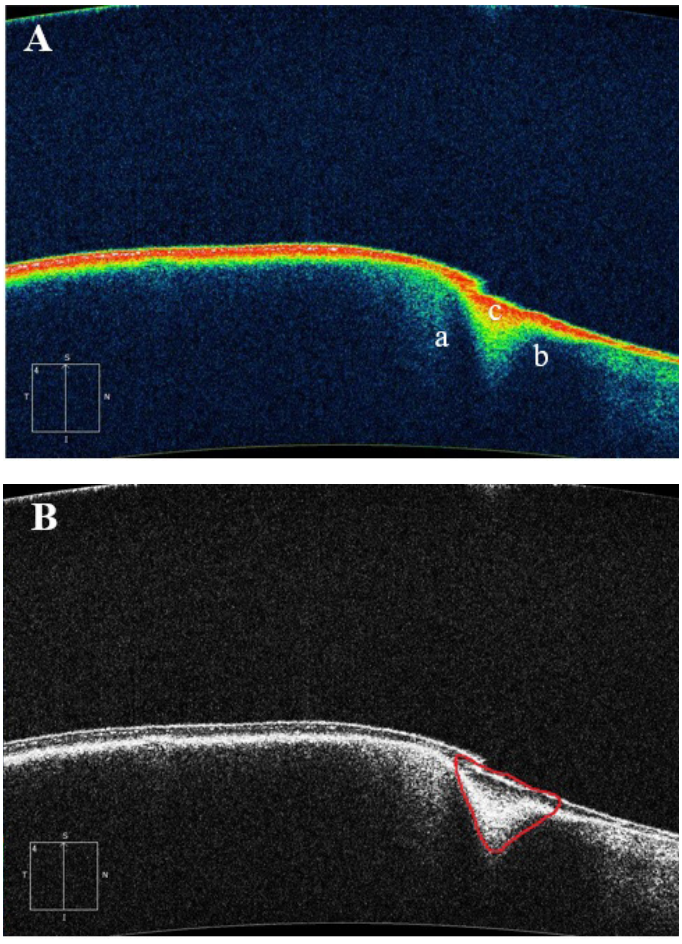

Figure 1. *OCT image of one of the teeth samples in self-etch group (Group 5) (A) showing regions in pseudocolors; a: Restoration; b: Tooth; c: Gap area; (B) drawing of the gap area with Image J of the same OCT image in grey scale.*

Statistical analysis

SPSS V. 20.0 (Statistical Package for Social Sciences, IBM SPSS, Armonk, NY, USA) software for Windows was used for statistical analysis. Kruskall Wallis test and Mann-Whitney U tests were used, respectively, for multiple and pairwise comparisons. The confidence interval was set to 95% and p values less than 0.05 were considered significant.

Results

According to the obtained values from Image J Software, analyzed results showed statistically significant differences between groups in terms of gap measurements between the tooth surface and restorative materials ($p<0.05$). Table 2 shows the post-hoc analysis of Image J results of the OCT images of the enamel/restoration interface, providing means and standard deviation values of the gap formation and statistical differences between groups. According to binary comparisons, Group 5 showed significantly higher gap measurements than all other groups ($p<0.05$). Group 6 had shown significantly higher gap measurements than Group 1, 2, 3 and 4 ($p<0.05$). Also, Group 1 showed higher gap measurements than Group 2, 3 and 4 ($p<0.05$). However, there was no statistically significant difference between Group 2, 3 and 4 ($p\geq0.05$).

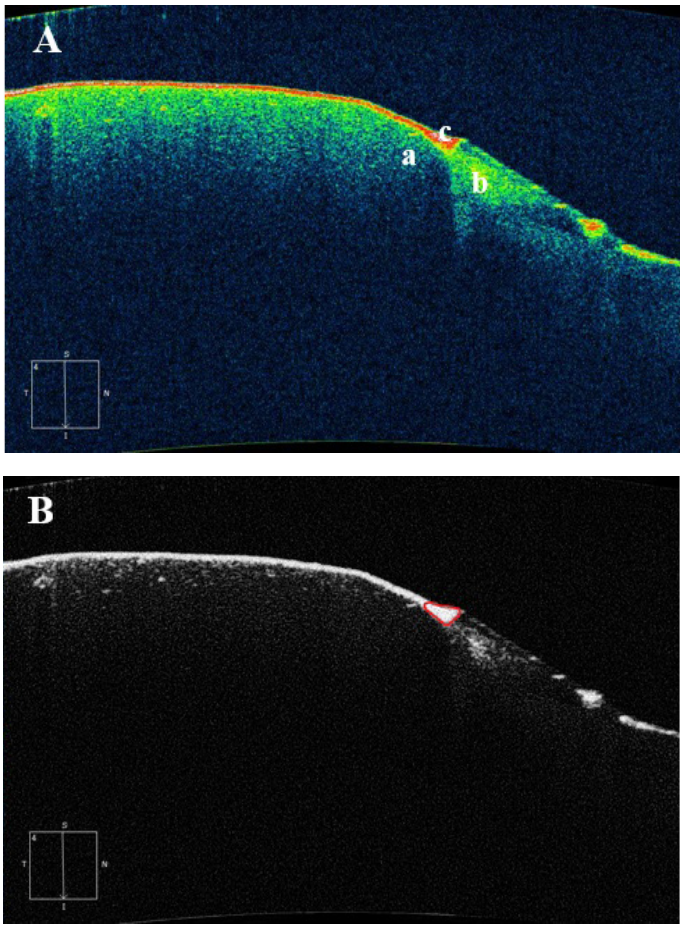

Figure 2. *OCT image of one of the teeth samples in Scotchbond Universal Adhesive with selective-etching group (Group 2) (A) showing regions in pseudocolors; a: Restoration; b: Tooth; c: Gap area; (B) drawing of the gap area with Image J of the same OCT image in grey scale.*

Discussion

Adhesive systems have important technical requirements, particularly in pediatric restorative dentistry (1,13). The most important technical problems during restorative procedures are cavity preparation, saliva isolation, and material adaptation (13). Therefore, developments in adhesive dentistry have primarily aimed to eliminate these issues. According to studies, fourth-generation systems provide higher bond strength to both enamel and dentin than other systems (2-5), and long-term follow-up studies have demonstrated successful results in terms of retention, marginal adaptation, and secondary caries development (2,4,5). As a result, fourth-generation adhesives are currently considered the gold standard when compared to other systems (2-5).

However, longer etching times, especially in primary teeth, can cause more technical problems and decrease the clinical success of restorations. The most significant clinical failure is microleakage between the tooth surface and restorative material due to insufficient acid-etch or isolation problems after the etching step (7,13). Therefore, in recent years, self-etch adhesives have been preferred in pediatric restorative dentistry to decrease chair time and increase the clinical success of restorations by eliminating these technical problems during restorative procedures (14,15).

Studies have shown that there are advantages and disadvantages of self-etch adhesives compared to total-etch adhesives (2-4,7). Self-etch adhesives require less technical precision due to fewer application steps than other generations and can be applied in a shorter chair time (14). While their clinical sensitivity is lower, their bond strength is also lower than total-etch agents (14,16). Additionally, enamel bond strength is not as sufficient as dentin bond strength in self-etch agents, since enamel is more resistant to acids than dentin (2,4,6). To address this issue, recent advancements in adhesive systems have recommended applying a selective-etching step before these adhesives to improve adhesion, increase enamel bonding, decrease marginal microleakage, and increase clinical success. For this purpose, universal adhesives may be an alternative that can be used with or without an acid-etching step in a one-step application.

Table 2. *Evaluation of gap measurements formed between enamel and restoration interface amongst all groups.*

Measurements	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	P-value
Mean (SD)	0.63(0.12) *G5, G6	0.50(0.10) *G1, G5, G6	0.48(0.08) *G1, G5, G6	0.41(0.10) *G1, G5, G6	1.67(0.46)	1.10(0.33) *G5	**0.001**
Min-Max Median	0.44-0.97 0.62	0.32-0.76 0.49	0.36-0.68 0.46	0.26-0.56 0.41	0.85-2.44 1.78	0.58-1.78 1.04	

Kruskall Wallis Test; Significancy level at $p<0.05$; sd: Standard deviation; Min: Minimum value; Max: Maximum value; * refers to statistically significant difference between the stated groups in each column according to post-hoc analysis with pairwise comparisons.

However, to our knowledge, there is no study comparing both universal and self-etch adhesives, with or without a selective-etch step, in primary teeth. Therefore, we aimed to compare these adhesive materials and their different application methods before condensing polyacid-modified resin composites in terms of marginal gap formation as another way to determine the risk of microleakage formation. Class-V cavities, also known as cervical cavities, were preferred due to the difficulties in isolation, caries access, and proximity to the gingival margin. In addition, the design of the cavity has a high configuration factor (C-factor), which leads to a high risk for microleakage (17,18). Currently, adhesive systems can provide a favorable marginal seal and decrease marginal microleakage, especially at the cervical margins of the cavity (16,19). In an in-vitro study evaluating microleakage with dye penetration of total-etch and universal adhesives in Class-V restorations, Cirligeriu *et al.* (20) recommended that selective-etch application should be used to improve marginal sealing, especially in cervical restorations and cavities with high C-factors. Therefore, in this study, Class-V cavities were prepared on the buccal surfaces of all teeth and standardized using a periodontal probe to a depth of 2mm, width of 4mm, and height of 2mm.

Polyacid-modified resin composites, which share some similarities with both composite resins and glass-ionomers, have been used for primary teeth restorations for a long time (1,13). Due to their lower polymerization shrinkage and easy condensation compared to composite resins, and similar physical properties with primary teeth, such as erosive tooth wear, pediatric dentists prefer to restore with polyacid-modified resin composites (13). While the material can be used with both self-etch and total-etch adhesives, the literature suggests that application with total-etch adhesives leads to higher clinical success in terms of lower marginal microleakage due to the acid-etching step of the enamel surface (14,16,17).

Long-term microleakage in the oral environment can result in consequences such as discoloration of the tooth or restoration surface, sensitivity, and secondary caries development (18-20). Therefore, in this in-vitro study, all teeth samples were put through thermal cycling to simulate the oral environment and ensure marginal microleakage development before OCT imaging. However, according to Marchesi *et al.* (3), microleakage results for self-etch and total-etch systems did not differ at immediate evaluation or after one year of storage in artificial saliva.

OCT is a method used for diagnosing carious lesions and periodontal diseases, and has also been used to evaluate microleakage localization, continuity, and gap width between composite resin restoration and tooth surface using different adhesives (9). OCT provides quantitative values and does not require additional processes, making it a preferred method for measuring the gap between the tooth surface and restorative material (10-12). Previous studies have shown that OCT evaluation can yield significantly logical results in adhesion studies (11,21-25), and can safely be used to evaluate microleakage in different adhesive techniques (13,26-29). However, OCT has a measurement depth limit of around 2-3mm in many tissues, which might affect results in adhesion studies, although the image resolution is 10-100 times better than ultrasound imaging methods (26-29).

In this study, all cavities were prepared at a depth of 2mm, width of 4mm, and height of 2mm, and the restorative material was polymerized for 40 seconds at one time. However, despite the cavity depth being 2mm, the base of the cavities could not be seen in the images taken with OCT due to the properties of dental tissues and their light transmittance or reflection, which is different from that of soft tissues like the eye.

The study conducted by Haak *et al.* (26) evaluated the selective-etch and self-etch application methods of Scotchbond Universal Adhesive with composite resin restorations in permanent teeth using OCT assessment. The authors reported that the self-etch application method of Scotchbond Universal Adhesive showed significantly higher gap measurements, but there was no significant difference between total-etch and selective-etch applications. Furthermore, the authors concluded that OCT is a sensitive method to evaluate microleakage which cannot be evaluated clinically, and requires lesser time to obtain data and provide bidirectional perspective compared with other evaluation methods (9-12). The authors also noted that aging due to storage time and immediate evaluations did not have any statistical difference in microleakage development.

Another study by Rosa *et al.* (5) reported different bond strength results for total-etch and self-etch application methods of different universal adhesives in permanent teeth. While there was no statistically significant difference in the Scotchbond Universal adhesive groups, there was a statistical difference in the All-Bond Universal adhesive groups. In the present study, the self-etch group without selective-etch application (Group 5) showed significantly higher gap measurement results than all other groups, which suggests a higher microleakage level. This result is supported by the findings of the studies reported by Rosa *et al.* (5) and Haak *et al.* (26). It also supports the general acceptance that selective-etch application before adhesive application during restorative procedures in primary teeth improves enamel bonding and decreases microleakage in terms of marginal gap formation, similar to what is observed in permanent teeth.

In the present study, binary comparisons revealed a significant difference between Group 5 and 6, both of which used the same self-etch adhesive agent, but with and without acid-etch. The lower gap measurements in Group 6 indicated that the acid-etching step effectively decreased gap formation. Furthermore, Group 1, which used Scotchbond Universal Adhesive without selective acid-etch, had statistically higher gap measurements than other universal adhesive groups (Groups 2, 3, and 4). This result supports the findings of Rosa *et al.* (5) and the recommendations of manufacturers that universal adhesive agents can be used with or without selective-etch. However, dentists should opt for adding the selective-etch step to their restorative treatment procedures instead of self-etch to achieve long-term successful prognosis for restorations.

Therefore, selective-etch application could be a better way to increase enamel bonding and reduce marginal microleakage with respect to the adhesive agent. As dentin bonding is easier to handle than enamel bonding, selective etch should be preferred to increase enamel bonding and reduce marginal microleakage, according to the authors' and the present study's results. The first hypothesis was rejected

due to the lower gap formation in the selective acid-etch groups than in the groups without acid-etching, which was verified for both self-etch adhesives and universal adhesives. The second hypothesis was also rejected because the self-etch group, with or without acid-etching, showed statistically significant higher gap measurements than the universal groups. However, it is essential to consider prospective clinical studies to eliminate the technical features of in-vitro studies and evaluate long-term prognosis.

Conclusion

Preferably, selective-etch and universal adhesive applications should be used to restore primary teeth with poly-acid-modified resin composites, as opposed to self-etch adhesives, especially without selective-etch applications. Additionally, OCT may be preferred as a sensitive and minimally invasive evaluation method in adhesive dentistry studies.

Türkçe özet: *Süt dişlerinde farklı adeziv ajanlar için mikrosızıntı oluşumunun optik koherens tomografi ile değerlendirilmesi. Amaç: Bu çalışmada, iki farklı üniversal ve bir self-etch adezivin self-etch ve selektif-etch uygulamaları için optik koherens tomografi (OCT) kullanılarak diş yüzeyi ile restoratif materyal arasındaki boşluk oluşumunun mikrosızıntı açısından değerlendirilmesi amaçlanmıştır. Gereç ve Yöntem: Altmış çürüksüz süt azı dişi, iki farklı üniversal ve bir self-etch adeziv sistemin self-etch ve selektif-etch uygulama yolları (n:10) şeklinde altı gruba ayrıldı. Sınıf-V kaviteler hazırlandıktan sonra her diş rastgele gruplara dağıtılarak adezyon işlemi uygulandı ve ardından tüm kaviteler poliasit modifiye kompozit rezin ile restore edildi. Mikrosızıntı, kör bir araştırmacı tarafından Image J Yazılımı ile OCT görüntülerinden diş yüzeyi ile restorasyon arasındaki boşluk ölçülerek değerlendirildi. İstatistiksel analiz sırasında anlamlılık düzeyi p<0,05 olarak kabul edildi. Bulgular: Image J Software tarafından elde edilen ölçümlerin istatistiksel analizine göre, test edilen her adeziv için selektif-etch grupları self-etch gruplarına göre daha az boşluk oluşumu gösterdi (p<0,05) ve asitleme yapılmadan uygulanan self-etch adeziv, tüm gruplar arasında önemli ölçüde en yüksek boşluk oluşumunu gösterdi (p<0,05). Sonuç: Süt dişlerinde uzun ömürlü poliasit modifiye kompozit rezin restorasyonları için self-etch adezivlere göre selektif asitlemenin ardından uygulanan üniversal adezivler tercih edilebilir. Ancak, elde edilen sonuçlar uzun vadeli prognoz için prospektif klinik çalışmalar ile değerlendirilmelidir.* Anahtar kelimeler: *Adezyon, Boşluk Oluşumu, Mikrosızıntı, Optik Koherens Tomografi, Poliasit Modifiye Kompozit Rezin*

Ethics Committee Approval: The present study protocol has been reviewed and approved by the Başkent University Institutional Review Board (project no:D-KA 18/13).

Informed Consent: Participants provided informed constent.

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Author contributions: DSU, LA, RET participated in designing the study. DSU, LA, RET participated in generating the data for the study. DSU, LA, RET participated in gathering the data for the study. DSU, RET participated in the analysis of the data. DSU wrote the majority of the original draft of the paper. DSU, LA, RET participated in writing the paper. DSU, LA, RET have had access to all of the raw data of the study. DSU has reviewed the pertinent raw data on which the results and conclusions of this study are based. DSU, LA, RET have approved the final version of this paper. DSU guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Original research

Prophylactic and therapeutic effects of (6)–shogaol on alveolar bone loss in experimental periodontitis*

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Purpose
(6)-Shogaol is the most prevalent bioactive compound in ginger. The aim of this study was to examine both the prophylactic and therapeutic effects of (6)-shogaol in an experimental periodontitis model.

Materials and Methods
Thirty-five male Wistar albino rats were divided into four groups. In the healthy group (n=5), no intervention was undertaken. In the periodontitis group (n=10), periodontitis was induced by ligature placement for 14 days. In the prophylaxis group (n=10), periodontitis was induced with ligature placement for 14 days, and during this time, 20 mg/kg/day of (6)-shogaol was administered via oral gavage. In the therapeutic group (n=10), periodontitis was induced with ligature placement for 14 days, and following the removal of the ligature, 20 mg/kg/day of (6)-shogaol was administered via oral gavage for 14 days. Alveolar bone loss was histometrically measured, and malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GP), nuclear factor kappa B (NF-κB), receptor activator of nuclear factor kappa B ligand (RANKL), and osteoprotegerin (OPG) were immunohistochemically analyzed.

Results
Alveolar bone loss was significantly lower in the healthy group than in the remaining groups, as well as in the therapeutic group than in the periodontitis group ($p<0.001$). RANKL/OPG was significantly higher in the periodontitis group compared to the remaining groups and in the prophylaxis group compared to the therapeutic group ($p<0.001$). MDA was significantly lower in the healthy group than in the remaining groups ($p<0.001$). SOD was significantly lower in the periodontitis group than in the prophylaxis and therapeutic groups ($p=0.039$ and $p=0.042$, respectively). GP was significantly lower in the healthy group than in the prophylaxis and therapeutic groups ($p=0.031$ and $p=0.002$, respectively).

Conclusion
The administration of (6)-shogaol modulated the RANKL/OPG balance and antioxidant status in rats with ligature-induced periodontitis.

Keywords: *Experimental periodontitis, ginger, oxidative stress, RANKL/OPG, shogaol*

Introduction

Chronic inflammation of the periodontium begins with complex subgingival biofilms containing many periodontal pathogens. In response to these pathogens, the excessive host response causes the release of cytokines, proteinases, and osteoclastogenic factors responsible for soft and hard tissue destruction (1). For the activation of osteoclasts that initiate bone resorption, the signal is delivered by the receptor activator of nuclear factor kappa B (NF-kB) [RANK], its ligand (RANKL), and osteoprotegerin (OPG). RANK is a receptor found on the cell surfaces of osteoclasts and osteoclast precursors that stimulates the proliferation and differentiation of cells to form the osteoclast phenotype. RANK is connected to

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RANKL. On the contrary, OPG is a soluble receptor produced by many cells, such as osteoclasts, and modifies the effects of RANKL by inhibiting the RANKL/RANK interaction. The degree of bone loss depends on the RANKL/OPG ratio. This rate increases dramatically in the area of active periodontal disease and positively correlates with disease severity. RANKL and OPG can be detected in gingival tissue, gingival crevicular fluid, saliva, and serum, providing reliable knowledge concerning periodontal disease activity and alveolar bone loss (2). Reactive oxygen species (ROS) are produced as a result of the normal cellular metabolism of host defense cells against bacterial pathogens. However, when ROS are produced in large amounts, they have destructive effects. It is known that oxidative stress, which expresses an imbalance between free radical formation and the antioxidant defense mechanism, damages cellular macromolecules sensitive to oxidative damage and plays a role in the pathogenesis of many chronic degenerative diseases, such as periodontal disease. Oxidative stress is generally determined by the measurement of malondialdehyde (MDA), which is the final product of lipid peroxidation, and antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GP) (3).

Ginger is a plant that possesses anti-inflammatory, antioxidant, antibacterial, antiarthritic, antiangiogenic, antithrombotic, anticancer, hypolipidemic, and antidiabetic properties. Ginger contains many active compounds involved in various biological activities (4-6). Several studies have reported the effectiveness of compounds found in ginger in improving symptoms of chronic inflammatory diseases. Among these compounds, studies have focused on the antioxidant and anti-inflammatory effects of gingerol and shogaol, which are usually the main components of ginger. In many cases, (6)-shogaol has been reported to exhibit better biological activities than gingerol. Additionally, the anti-inflammatory or antioxidant effects of shogaol have been demonstrated in conditions such as cancer, Alzheimer's disease, Parkinson's disease, cardiovascular disease, diabetes, and allergies (7-13).

In the literature, there are no studies evaluating the effects of the systemic administration of (6)-shogaol on both the prevention and treatment of periodontitis. Therefore, this study aimed to assess both the prophylactic and therapeutic effects of (6)-shogaol on alveolar bone loss in rats with experimentally induced periodontitis. Accordingly, the null hypothesis of the study was established as 'systemic administration of (6)-shogaol would reduce the destruction of periodontal tissues in the ligature-induced periodontitis model.'

Materials and Methods

Animals

Thirty-five male Wistar albino rats (250±25 g) were obtained from a certified commercial laboratory animal facility. The rats were housed in plastic cages at 22±2 °C under a 12-hour light/dark cycle. Commercial rat pellet feed and water were provided ad libitum. All experimental procedures and applications were approved by the Animal Research Ethics Committee of the University (ethics committee report number: 17/21) and the study was conducted following the ARRIVE guidelines (14).

Experimental design

The rats were randomly assigned to one of the following four groups: Healthy group (n=5) (no ligature placement; only standard laboratory diet/water were provided). Periodontitis group (n=10) (ligature placement for 14 days; standard laboratory diet/water were provided). Prophylaxis group (n=10) [ligature placement and administration of 20 mg/kg/day (6)-shogaol for 14 days). Therapeutic group (n=10) (ligature placement for 14 days and administration of 20 mg/kg/day (6)-shogaol for 14 days after ligature removal).

Experimental induction of periodontitis and (6)-shogaol admin istration

All ligature-induced periodontitis procedures were performed under general anesthesia induced with ketamine hydrochloride (75 mg/kg) and xylazine (10 mg/kg), and ligatures were placed with 3.0 silk sutures (Ruschmed, İstanbul, Turkey) around the left mandibular first molar. All ligatures were positioned subgingivally, and lost or loose sutures were replaced. All ligature placements were performed by the same operator (D.B.). (6)-shogaol (ChemFace, Wuhan, China), dissolved in 0.1% dimethyl sulfoxide (Sigma-Aldrich D2650, Germany), was orally administered to the rats according to their weight at the same time of day.

Histomorphometric analyses and histopathological evaluation

The rats in the healthy, periodontitis, and prophylaxis groups were sacrificed on day 15, and those in the therapeutic group were sacrificed on day 29, using a CO_2 euthanasia cabinet, and their mandibles were collected. The left mesio-distal segment of each mandible was dissected, fixed in 4% buffered paraformaldehyde for 48 h, and decalcified in 8% formic acid for 14 days. The tissues were trimmed, washed, dehydrated, and embedded in paraffin wax. The paraffin-embedded tissues were sectioned at a thickness of 4–5 µm longitudinally and stained with hematoxylin and eosin. The slides were examined under light microscopy (Olympus BX51 trinocular microscope and Leica DFC450 digital camera, Germany), and digital photomicrographs were taken. The distance between the cemento-enamel junction (CEJ) and the alveolar crest (AC) was measured using digital imaging software (Leica Qwin image analysis software, Germany), and all measurements were performed at six different areas (three buccal and three lingual surfaces), and a mean value for each tooth was calculated. Histopathologically, the severity of inflammatory cell infiltration was scored as 0 if there were no cells, 1 if there were one to five cells, 2 if six to 10 cells, and 3 if more than 10 cells. All analyses were performed at 20x objective magnification.

Immunohistochemical analyses

Immunoperoxidase analyses were performed using a commercial immunoperoxidase kit [UltraVision Polyvalent (Rabbit-Mouse) HRP Kit, Labvision/Thermo, USA] to determine the expression of MDA, SOD, GP, RANKL, OPG, and NF-κB. Briefly, tissue sections were deparaffinized in xylene and hydrated through graded alcohols. To unmask antigens, the tissue sec-

tions were placed in a microwave in citrate buffer for 20 min at the highest potency. Then, endogenous peroxidase activity was inhibited using 0.1% H_2O_2 in methanol for 10 min, and non-specific labeling was blocked by pre-incubation with normal goat serum in 10 min. Thereafter, the sections were incubated with anti-MDA (Ab6463, Abcam, UK), anti-SOD (sc-101523, Santa Cruz Inc., Texas), anti-GP (sc-133160, Santa Cruz Inc., Texas), anti-RANKL (Ab-169966, Abcam, UK), anti-OPG (Ab-203061, Abcam, UK), and anti-NF-κB (sc-8414, Santa Cruz Inc., Texas) antibodies for 60 min in a humidity chamber at room temperature. The sections were then incubated with the biotinylated secondary antibody for 15 min, labeled with horseradish peroxidase for 15 min and, 3-Amino-9-ethylcarbazole (AEC) chromogen substrate solution (TA-125-HA AEC Chromogen Kit, Thermo, USA) for approximately 10 min. They were counterstained with Mayer's hematoxylin for 1 min and suspended in a water-based mounting medium. As the isotype-negative control, normal mouse serum, instead of the primary antibody, was used. The density of positive staining was measured using digital imaging software (Leica Qwin image analysis software, Germany) under a 20 × objective lens. The integrated optical density of all immunopositive staining was measured, and the mean MDA-, SOD-, GP-, RANKL-, OPG-, and NF-κB-positive areas and the total area were calculated. After calculating the proportion (% pixels) of the stained area to the whole field, the mean (in % pixels) staining area for each slide was determined.

Statistical analysis

The normality of the data distribution was examined using the Shapiro-Wilk test. In the comparisons between the groups, the variables that met the normality and variance homogeneity conditions were evaluated using the analysis of variance (ANOVA) test. When a difference was detected, the Bonferroni-corrected independent-samples t-test was used to determine the groups that caused the significant difference. The Kruskal-Wallis test was conducted to determine differences in variables that did not comply with a normal distribution, and the Bonferroni-corrected Mann-Whitney U test was used to determine the group from which the significant difference originated. Comparisons between the groups for normally distributed values that did not meet the homogeneity of variance were undertaken using the Welch-ANOVA test, which is the corrected version of the ANOVA test, and the Games-Howell binary comparison results were reported to determine the differences between the groups where the differences were significant. SPSS v. 22.0 (IBM Corp. released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used for statistical analyses. The statistical significance level was accepted as $p<0.05$.

Results

The study was completed with 35 rats. No complications or any obvious signs of systemic disorders occurred during the study period, and no animals died or were excluded.

Histopathological findings and histomorphometric analyses

Microscopic images of the tissue samples examined are shown in Figure 1. In the healthy group, cement, periodontal ligament, and alveolar bone had a normal appearance, with no inflammatory reaction or bone resorption.

Cement resorption was observed in all samples in the periodontitis group. In addition, osteolysis, characterized by osteoclastic activity at alveolar bone borders, was observed. Intense inflammatory cell infiltrations rich in lymphocytes and neutrophils were found, especially in regions with high osteoclastic activity and cement resorption. Hyperemic blood vessels and newly formed young capillaries were also found in the periodontal ligament.

In the prophylaxis group, cement resorption was observed, albeit at a lower rate than in the periodontitis group. In addition, enlarged blood vessels were observed in the periodontal ligament, similar to the periodontitis group, but there was less vascularization. While a small number of osteoclastic activities were encountered in this group, mild inflammatory cell infiltration was noted in osteoclast circles and around enlarged capillaries. However, osteoblastic activity was found in all samples. In the therapeutic group, much lower vascularization and inflammatory cell infiltration were observed compared to the periodontitis group. Similarly, there was osteoclastic activity in the alveolar bone margin in only two cases, while osteoblastic activity was more common than in the prophylaxis group. During the histopathology analysis, inflammatory cell infiltration was scored semi-quantitatively, and when compared between the groups, the number of rats with a score of 0 was one in

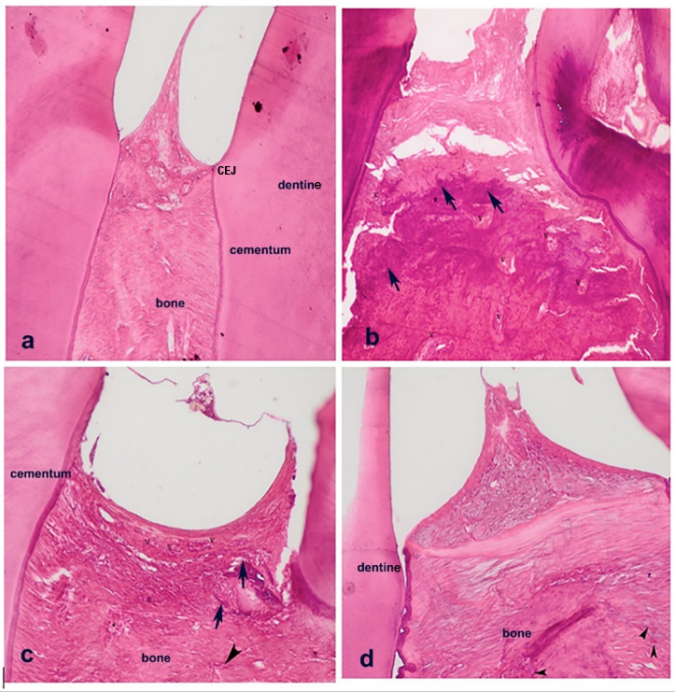

Figure 1. *Microscopic images of the tissue samples of all groups (hematoxylin and eosin staining). (a) Healthy group: Normal periodontal membrane, alveolar bone, and cement. (b) Periodontitis group: Periodontium with intense vascularization (v), hyperemia with osteoclasts (arrow) at the alveolar bone border, and moderate inflammatory cell infiltration (asterisk). (c) Prophylaxis group: Mild vascularization (v) and mild inflammatory cell infiltration (asterisk) in the periodontal ligament, osteoclasts (arrow), and osteoblasts (arrowhead) in alveolar bone. (d) Therapeutic group: Osteoblasts (arrowhead) formed at the border of the alveolar bone.*

each of the prophylaxis and therapeutic groups, while none of the rats in the periodontitis group scored 0.

The CEJ-AC distance (μm) was measured in all sections and found to be statistically significantly lower in the healthy group than in the remaining groups ($p < 0.001$ for all). It was also significantly lower in the therapeutic group than in the periodontitis group (p=0.001) (Table 1).

Immunohistochemical findings

The results of the tissue analyses for SOD, GP, and MDA are shown in Table 2 and Figure 2. The SOD level was statistically significantly lower in the periodontitis group compared to the prophylaxis and therapeutic groups (p=0.039 and p=0.042, respectively); however, there was no statistically significant difference between the periodontitis and the healthy group (p=0.931). The GP level was found to be statistically significantly lower in the healthy group compared to the prophylaxis and therapeutic groups (p=0.031 and p=0.002, respectively), while no statistically significant difference was detected between the periodontitis group and the healthy group. The MDA level was statistically significantly higher in the periodontitis group than in the healthy group (p=0.004), and it was also statistically significantly lower in the healthy group than in the prophylaxis and therapeutic groups (p=0.012 and p=0.017, respectively). The MDA did not statistically significantly differ between the periodontitis group and the prophylaxis or therapeutic group. The results of the tissue analyses for RANKL, OPG, RANKL/OPG, and NF-κB are shown in Table 3 and Figure 2. The RANKL level was observed to be statistically significantly higher in the periodontitis group compared to the healthy and therapeutic groups (p<0.001 and p<0.001, respectively). There was no statistically significant difference in the RANKL level between the periodontitis group and the prophylaxis group. The OPG level was statistically significantly lower in the periodontitis group than in the prophylaxis and therapeutic groups (p=0.002 and p<0.001, respectively). It was statistically significantly lower in the healthy group compared to the therapeutic group (p=0.049). However, no statistically significant difference was found when the periodontitis group was compared to the healthy group. The RANKL/OPG ratio was statistically significantly higher in the periodontitis group than in the healthy, prophylaxis, and therapeutic groups (p=0.005, p=0.006, and p=0.005, respectively), but it was statistically significantly higher in the prophylaxis group than in the therapeutic group (p=0.011). The NF-κB level was statistically significantly higher in the periodontitis group than in the healthy, prophylaxis, and therapeutic groups (p<0.001, p=0.017, and p=0.023, respectively).

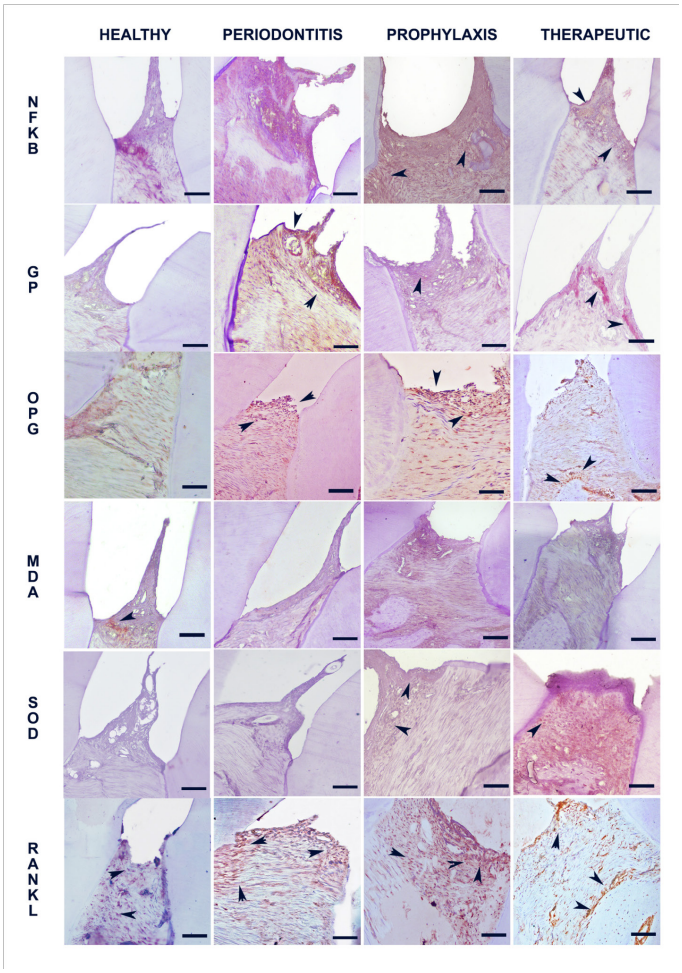

Figure 2. *Immunoperoxidase positive expression (arrow heads) of NFKB, GP, OPG, MDA, SOD, and RANKL antigens in the mesio-distal histological section of each experimental group (healthy, periodontitis, prophylaxis, and therapeutic), avidin-biotin complex immunoperoxidase test results, and Mayer's hematoxylin counterstaining. Bar = 220 μm.*

Table 1: *Comparison of the CEJ-AC distance (μm) between the groups (mean ± standard deviation)*

	Healthy Group	Periodontitis Group	Prophylaxis Group	Therapeutic Group	F	p
CEJ-AC (μm)	28.61±12.21	101.78±11.46[a]	87.23±17.4[a]	76.26±9.34[a,b]	36.586	**<0.001**

CEJ: cemento-enamel junction; AC: alveolar crest; F: ANOVA test statistic; [a]significant difference from the healthy group; [b]significant difference from the periodontitis group

Table 2: *Comparison of the SOD, GP, and MDA levels between the groups (% area)*

	Healthy Group	Periodontitis Group	Prophylaxis Group	Therapeutic Group	F, χ^2	p
SOD Median (min; max)	3.91 (2.40; 4.86)	1.54 (0.88; 4.69)	4.68[b] (2.68; 6.94)	4.44[b] (2.83; 7.92)	9.695[&]	**0.021**
GP Median (min; max)	0.73 (0.42; 2.64)	2.34 (0.99; 3.54)	2.92[a] (2.40; 5.77)	3.4[a] (2.61; 5.62)	15.611[&]	**0.001**
MDA Mean ± SD	1.14±0.54[b]	2.09±0.56	4.35±1.70[a]	5.71±2.46[a]	15.817[*]	**<0.001**

F, *: Welch-ANOVA test statistic; χ2, &: Kruskal-Wallis test statistic; SOD: superoxide dismutase; GP: glutathione peroxidase; MDA: malondialdehyde; SD: standard deviation; [a]significant difference from the healthy group; [b]significant difference from the periodontitis group

Table 3: *Comparison of RANKL, OPG, NF-κB, and RANKL/OPG between the groups (% area)*

	Healthy Group	Periodontitis Group	Prophylaxis Group	Therapeutic Group	F, χ^2	p
RANKL Median (min; max)	1.48[b] (0.82; 2.55)	11.34 (7.36; 14.59)	4.36 (1.96; 7.11)	1.64[b] (1.39; 3.40)	24.657[&]	**<0.001**
OPG Median (min; max)	2.32 (1.64; 3.40)	0.93 (0.56; 3.92)	5.24[b] (3.44; 8.24)	6.39[a,b] (3.11; 13.86)	23.270[&]	**<0.001**
RANKL/OPG Mean ± SD	0.66±0.31[b]	12.43±6.44	0.86±0.31[b]	0.38±0.24[b,c]	12.353[*]	**<0.001**
NF-Kb Median (min; max)	1.59[b] (1.16; 2.11)	6.08 (3.87; 8.72)	3.30[b] (2.46; 4.02)	3.32[b] (2.89; 4.91)	23.806[&]	**<0.001**

F, *: Welch-ANOVA test statistic; χ2, &: Kruskal Wallis test statistic; RANKL: receptor activator of nuclear factor kappa B ligand; OPG: osteoprotegerin; NF-κB: nuclear factor kappa B; SD: standard deviation; [a]significant difference from the healthy group; [b]significant difference from the periodontitis group; [c]significant difference from the prophylaxis group

Discussion

In recent years, many plants with anti-inflammatory and antioxidant properties have been utilized in the treatment of periodontal diseases. Ginger contains active ingredients such as shogaol, gingerol, paradol, and zingerone, each with various biological activities. Among these, shogaol stands out as one of its most active components (15). Studies have primarily focused on the antioxidant and anti-inflammatory effects of gingerol and shogaol, which are typically the main components of ginger. Recent research indicates that (6)-shogaol is more stable than (6)-gingerol and exhibits stronger pharmacological effects, including antioxidant, anti-inflammatory, and anticancer properties, compared to the latter (7, 16). Moreover, shogaol has demonstrated anti-inflammatory or antioxidant effects in conditions such as cancer, Alzheimer's disease, Parkinson's disease, cardiovascular disease, diabetes, and allergies (8-13). Several in vitro and in vivo studies have investigated the antioxidant and anti-inflammatory potential of (6)-shogaol. Pan *et al.* (17) demonstrated that the topical application of (6)-shogaol inhibited the activation of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 in murine macrophages in a skin model. Ahn *et al.* (18) showed that (6)-shogaol down-regulated NF-kB activation and the expression of COX-2, resulting in the inhibition of toll-like receptor-mediated signaling pathways. In another study, (6)-shogaol reduced the release of interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF) in human mast cells (19). In a study employing human primary chondrocytes, (6)-shogaol reduced the activity of cathepsin K (20). Various animal models have reported that (6)-shogaol plays a crucial role in anti-inflammatory action and allergic inflammatory reactions (12, 21). However, to the best of our knowledge, this is the first study to evaluate both the preventative and therapeutic effects of (6)-shogaol administration in an experimental periodontitis model.

According to animal studies, the placement of ligatures around the teeth, allowing for the accumulation and colonization of microorganisms, decreases tissue integrity and creates mechanical trauma in the dentogingival region (22). This leads to the ulceration of the sulcular epithelium after plaque deposition, facilitating the invasion of connective tissue and initiating periodontal tissue loss, similar to the process in humans. This model is widely accepted as the closest to natural plaque formation (23). Male animals are more frequently used in experimental studies because female animals may exhibit significant differences in phenotypes, such as gene expression and changes in the genome, due to their hormone cycles (24). Rats are the most extensively studied rodents in research on the pathogenesis of periodontal diseases and are widely employed in experimental periodontitis models due to their similarity to humans in terms of the periodontal structure of molar tooth regions, low cost, high availability, and easy feeding and breeding. In light of the literature, we used male Wistar albino rats for our experimental model. We induced experimental periodontitis over 14 days by tying ligatures around the mandibular left first molar teeth of the rats.

The RANKL/OPG ratio is an indicator of normal bone resorption and deposition regulation during bone remodeling (25). Bone tissue destruction that occurs during periodontitis is regulated by the balance between the levels of RANKL and OPG, and therefore, their ratio increases in the event of destruction (26, 27). It has been suggested that the RANKL/OPG ratio can be a good indicator of periodontitis (2). Kim *et al.* (28) investigated the preventative use of (6)-shogaol against periodontitis and showed that it had an anti-osteoclastogenic role, inhibiting RANKL-induced mitogen-activated protein kinase activation, Ca2+ signaling, ROS generation, nuclear factor-activated T cells, and cytoplasmic 1 induction in osteoclast precursors. In another study evaluating the anti-metastatic activity of (6)-shogaol, it was revealed that this compound suppressed bone resorption by decreasing RANKL expression in osteoblasts (29). In the current study, we also observed that (6)-shogaol had a significant effect on the RANKL-OPG balance. In both therapeutic and prophylaxis groups, the OPG level was significantly higher than in the periodontitis group. The RANKL level was significantly lower in the therapeutic group than in the periodontitis group. The RANKL/OPG ratio and the NF-kB level were significantly higher in the periodontitis group compared to the remaining groups. The RANKL/OPG ratio was also significantly lower in the therapeutic group than in the prophylaxis group.

In periodontal diseases, bacterial cell components and inflammatory cytokines activate polymorphonuclear leukocytes (PMNLs), which exhibit oxidative activity with ROS production. Furthermore, fibroblasts, osteoclasts, epithelial cells, and neutrophils increase ROS release, causing damage to host tissue (3, 30). Tissue destruction develops through mechanisms such as the activation of pro-inflammatory cytokines due to ROS activity, production of prostaglandin E2 (PGE2) via lipid peroxidation, and NF-κB release (31). It has also been suggested that ROS acts as an intracellular signaling device for osteoclast differentiation, inducing periodontal tissue destruction and being associated with osteoclastic bone resorption (32).

MDA is a low-molecular-weight aldehyde in volatile form resulting from lipid peroxidation, occurring as a product of prostaglandin biosynthesis during oxidative stress or through the destruction of some molecules. Tissue destruction due to ROS is measured by MDA, an indicator of lipid peroxidation (33). Antioxidants are substances that significantly delay or prevent the oxidation of an oxidizable substrate and neutralize the free radical formation that can occur due to oxidative stress (34). GP and SOD are the most important antioxidants that protect cells from oxidative damage caused by free oxygen radicals (3).

(6)-shogaol has been shown to be the strongest scavenger of superoxide and hydroxyl radicals among all ginger constituents (7). It has been demonstrated that (6)-shogaol can significantly reduce cellular oxidative stress and suppress ROS production in various cells, including PMNLs, endothelial cells, and epidermal keratinocytes (7, 11, 35, 36). In a recently published study by Qi and Han (37), an endotoxin-induced experimental periodontitis model was created, and ROS, lysosomal enzymes, lipid peroxide, and acute-phase protein levels were reported to decrease after the administration of (6)-shogaol. Moreover, antioxidant enzymes and non-enzymatic antioxidant systems increased. In 2019, Nonaka *et al.* (38) investigated the effects of (6)-shogaol on the expression of advanced glycation end-product-induced oxidative and anti-oxidative responses, IL-6, and intercellular adhesion molecules in human gingival fibroblasts and demonstrated the efficacy of this compound in the prevention and treatment of diabetes mellitus-associated periodontitis.

It has also been suggested that (6)-shogaol may be a potential agent for the treatment of cardiovascular disease via its inhibition of the production of PGE2 and different pro-inflammatory cytokines, the release of nitric oxide, the expression of iNOS, and the increase of antioxidant enzymes, such as SOD and GP (10, 39). Similarly, in our study, the SOD level was significantly higher in the prophylaxis and therapeutic groups than in the periodontitis group, and the GP level was significantly higher in the prophylaxis and therapeutic groups compared to the healthy group. These results were attributed to the antioxidant properties of (6)-shogaol.

As expected, the MDA level was significantly higher in the periodontitis group than in the healthy group. Interestingly, the MDA level was significantly lower in the healthy group than in the prophylaxis and therapeutic groups. This may be due to the psychological stress to which the rats were exposed every day due to the administration of (6)-shogaol by oral gavage, the rats' immobility during this period, and the possible irritation of their throats during gavage, all factors that can increase oxidative stress. The examination of this marker in serum samples can clarify this situation. Accordingly, the lack of serum samples and radiographic examination of periodontitis can be considered limitations of this study.

Conclusion

In this study, the administration of (6)-shogaol, an active ingredient of ginger, reduced periodontal inflammation, RANKL, and NF-kB expression and increased OPG, SOD, and GP expression in rats with experimentally induced periodontitis. It is possible to conclude that (6)-shogaol reduces alveolar bone loss by affecting the RANKL-OPG balance and antioxidant status. Further studies are needed to optimize the dosage and route of administration of (6)-shogaol and investigate its efficacy based on in vivo models.

Türkçe özet: *Deneysel periodontitiste (6)-shogaol'ün alveoler kemik kaybı üzerindeki profilaktik ve terapötik etkileri Amaç: (6)-shogaol, zencefildeki en yaygın biyoaktif bileşiktir. Bu çalışmanın amacı, deneysel bir periodontitis modelinde (6)-shogaol'ün hem profilaktik hem de terapötik etkilerini incelemektir. Gereç ve Yöntem: Otuz beş adet erkek Wistar albino rat dört gruba ayrıldı. Sağlıklı gruba (n=5) herhangi bir müdahale yapılmadı. Periodontitis grubunda (n=10) 14 gün boyunca ligatür yerleştirilmesi ile periodontitis oluşturuldu. Profilaksi grubunda (n=10) 14 gün ligatür yerleştirilmesi ile periodontitis oluşturuldu ve bu süre içinde oral gavaj ile 20 mg/kg/gün (6)-shogaol verildi. Terapötik grubunda (n=10) 14 gün ligatür yerleştirilmesi ile periodontitis oluşturuldu ve ligatürün çıkarılmasını takiben 14 gün 20 mg/kg/gün (6)-shogaol oral gavaj ile uygulandı. Alveolar kemik kaybı histometrik olarak ölçüldü ve malondialdehit (MDA), süperoksit dismutaz (SOD), glutatyon peroksidaz (GP), nükleer faktör kappa B (NF-κB), nükleer faktör kappa B ligandının reseptör aktivatörü (RANKL) ve osteoprotegerin (OPG) immünohistokimyasal olarak analiz edildi. Bulgular: Alveoler kemik kaybı, sağlıklı grupta diğer gruplara göre ve terapötik grupta ise periodontitis grubuna göre anlamlı olarak daha düşüktü (P<0.001). RANKL/OPG periodontitis grubunda diğer gruplara göre ve profilaksi grubunda terapötik gruba göre anlamlı olarak daha yüksekti (P<0.001). MDA, sağlıklı grupta diğer gruplara göre anlamlı olarak düşüktü (P<0.001). SOD, periodontitis grubunda profilaksi ve terapötik gruplara göre anlamlı olarak daha düşüktü (sırasıyla P = 0.039 ve P = 0.042). GP, sağlıklı grupta profilaksi ve terapötik gruplara göre anlamlı olarak daha düşüktü (sırasıyla P = 0.031 ve P = 0.002). Sonuç: (6)-shogaol uygulaması, ligatür indüklü periodontitisli ratlarda RANKL/OPG dengesini ve antioksidan durumunu modüle etti. Anahtar Kelimeler: Deneysel periodontitis, zencefil, oksidatif stres, RANKL/OPG, shogaol*

Ethics Committee Approval: All experimental procedures and applications were approved by the Animal Research Ethics Committee of the University (number: 17/21)

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: DB, MKH, EO participated in designing the study. DB, OK, TA participated in generating the data for the study. DB, OK, TA participated in gathering the data for the study. DB, MKH, OK, TA participated in the analysis of the data. DB, MKH, OK, TA, EO wrote the majority of the original draft of the paper. All authors participated in writing the paper. DB, OK, TA have had access to all of the raw data of the study. DB, MKH, OK, TA, EO have reviewed the pertinent raw data on which the results and conclusions of this study are based. All authors have approved the final version of this paper. All authors guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original research

Comparative evaluation of digital radiography, electronic apex locator and simultaneous working length determination on postoperative pain after root canal treatment: a randomized clinical trial

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Purpose
The study aimed to compare postoperative pain after root canal preparation using three different methods of working length determination.

Materials and Methods
60 patients diagnosed with symptomatic irreversible pulpitis were randomly divided into three groups based on the method of working length (WL) determination. Group 1: digital radiograph (DRG), Group 2: electronic apex locator (EAL), Group 3: the simultaneous working length control (SLC) method using an endomotor with an integrated apex locator. The root canal treatments were completed in a single visit, and patients were asked to record their pain response using the Visual Analog Scale (VAS) at 6, 24, 48, and 72 hours postoperatively.

Results
Group 1 (DRG) recorded the highest postoperative pain score, while the lowest was recorded by Group 3 (SLC). There was a statistically significant difference in the VAS pain scores between DRG and SLC ($p<0.05$) at 6-, 24- and 48-hour intervals.

Conclusion
Within the limitations of this study, it can be concluded that the SLC can be a helpful working length determination technique to reduce postoperative pain.

Keywords: *Electronic apex locator, postoperative pain, radiograph, root canal therapy, simultaneous working length control, visual analogue scale*

Introduction

The American Association of Endodontics has defined working length (WL) as "the distance from a coronal reference point to the point at which the canal preparation and filling should terminate" (1). Precise determination of WL is essential for the success of endodontic treatment, as both over and under-instrumentation can adversely impact the outcome. A WL calculated beyond the apical foramen can result in the extrusion of debris, irrigants, and root overfilling (2). This can intensify and prolong postoperative discomfort and reduce the odds of treatment success by 62% (3). Conversely, when the WL is short of the minor apical diameter, insufficient canal debridement and underfilling can occur, leading to an increased risk of apical periodontitis and decreased success rates. Short root canal fillings have 3.1% higher odds of being associated with apical periodontitis, and for every uninstrumented millimeter, there is a 12% reduction in the success of treatment (3,4). Conventionally, WL determina-

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tion has relied on radiographic methods. The technological advancement towards digital imaging has overcome several limitations of the traditional X-ray film radiographs. Digital imaging offers faster image processing with enhanced image quality, eliminates chemical processing, minimizes hazardous waste, requires less radiation than films via digital intraoral sensors and therefore reduces radiation exposure to the patient. Currently, there are two primary modes of digital intraoral imaging used in dentistry. The first is computed radiography, which employs photostimulable phosphor (PSP) plates to produce images. In contrast, direct digital radiography uses solid-state detectors, such as charge-coupled devices (CCDs) or complementary metal-oxide semiconductors (CMOS) systems. The digital detectors in essence changes how we acquire, store and display images. However, radiographic methods including digital dental radiography retains several limitations, including image distortion, exposure to ionising radiation, compromised image clarity due to the superimposition of anatomic structures and the incapacity to determine the location of apical constriction/ foramen, which may significantly vary from the radiographic apex (5).

The incorporation of electronic apex locators (EALs) with conventional radiography has significantly improved the precision and accuracy of working length (WL) determination. The number of radiographs required has also decreased as a result of this integration, lowering the radiation exposure to patients. Sunada developed the first apex locator which utilized direct current to determine the length of a root canal. The device was based on the principle that both the mucous membrane and the periodontium have uniform electrical resistance values. The initial generations of EAL had unreliable readings and poor accuracy in the presence of root canal irrigants or tissue fluids. The modern generation of EALs, such as the Propex Pixi apex locator, employs multiple frequency to measure change in impedance as the minor apical foramen is reached (6). These advanced apex locators are equipped with integrated microprocessors that can process using algorithm calculations the continuous change in impedance data as the files are advanced within canal. This enables the apex locators to determine the working length of a tooth with precision. Studies have reported an estimated accuracy in WL determination of 90% with modern electronic apex locator (7,8).

The simultaneous working length determination is a newer development that allows clinicians to clean and shape root canals while monitoring the file's position inside the canal using dynamic feedback from EAL. The Tri Auto ZX2 (J Morita Corp, Tokyo, Japan) is an endodontic motor with a built-in EAL that provides continuous feedback during root canal instrumentation and allows clinicians to make real-time adjustments to the WL. The motor has auto apical reverse and auto apical stop operation, which ensures that as soon as the tip of the file reaches the apical foramen, the motor reverses the file safely or stops rotating, reducing the risk of over- instrumentation (9).

Pain control is a major concern for endodontic patients and dentists. The frequency of postoperative pain occurrence ranges from 3% to 58% (10). Periradicular tissue irritation during root canal therapy triggers an acute inflammatory response, causing the release of chemical mediators and alterations in local adaptation and pressure in the periapical tissue (11). Precise determination of the WL can impact the occurrence of postoperative pain (12).

The present study aimed to compare the postoperative pain levels associated with three different WL measurement techniques: 1) Digital radiographic method (DRG), 2) EAL determination, and 3) Simultaneous length control using an endomotor with integrated Electronic Apex Locator (SLC). The null hypothesis was that there is no significant difference in the levels of postoperative pain associated with the three different WL measurement techniques in endodontic treatment.

Materials and Methods

Ethical approval

The present in-vivo study was conducted in the Department of Conservative Dentistry and Endodontics. It was approved by the ethics committee of the faculty of medicine and registered in the Clinical Trials Registry of India. (Reference id CTRI/2019/07/019960).

Sample size determination

The sample size calculation was done using Gpower software (Franz Faul, University of Kiel, Germany) with an effect size of 0.82, alpha error =0.05 and power of 0.8.

Study design and patient selection

This study was designed as a parallel-group, randomized clinical trial with three arms, each with an equal allocation ratio of 1:1:1 ratio. A total of 60 patients (33 men and 27 women) were enrolled for this in-vivo study after obtaining a written informed consent. The study subjects were recruited from a pool of patients referred to the Department of Endodontics between July 2019 to June 2020. Only single-rooted teeth were taken into consideration for the study. Healthy patients having symptomatic irreversible pulpitis and without any periapical lesion or any systemic disease were included in this study. Patients with systemic diseases, swelling, sinus tract, severe periodontal disease or resorption, history of bruxism clenching or previously initiated or completed root canal treatment requiring retreatment were excluded. An Intraoral periapical radiograph was used to confirm the presence of a single root. The clinical history of lingering thermal, spontaneous or referred pain suggestive of symptomatic irreversible pulpitis was confirmed by pulp sensibility test by cold refrigerant spray (Endo Ice, Hygienic Corporation, Akron, OH) and electric pulp test (Gentle Pulse, Parkell, New York, USA). After the endodontic access, the diagnosis was confirmed by the presence of vital bleeding pulp tissue.

Root canal preparation

The maxillary teeth were anesthetized using a local infiltration technique, whereas the mandibular teeth were anesthetized by inferior alveolar nerve block supplemented with buccal infiltration or mental nerve block. The anes-

thetic solution used was Lidocaine with 1:80000 adrenaline (Lignox 2%, Indoco Remedies, India). A rubber dam was placed, and an access cavity was prepared with the help of Endo-access burs (Dentsply, USA). Size #10K and #15K hand (Mani Inc, Japan) files were passively inserted into coronal two-thirds of a root canal as pathfinding files. Coronal flaring was done using the Protaper Gold shaper files (S1, S2, SX). Working length was estimated as soon as the 15 K hand file appeared to reach and bind at the tentative working length. The tentative working length was established by measuring the radiographic length of the tooth on a diagnostic radiographic digital image acquired by paralleling technique and subtracting a safety factor of 2mm to ensure that instruments would not be extended beyond the apical foramen. Based on the method of working length determination, patients were randomly assigned into three groups. Block randomization with a block size of 6 patients (with each block containing two patients per treatment arm) was done using software (available on www.randomizer.org). Random sequence generation was done by a person not involved in the study and revealed to the clinician at the time of the procedure. The three experimental groups assigned were:

Group 1 Digital Radiographic method (DRG): In this group, the WL of the canal was established by digital radiograph (Sopix^2, Acteon) using Weine's method by subtracting 0.5mm from the distance measured from the radiographic apex. The images were acquired by a long cone paralleling technique using a positioning device (Rinn XCP-ORA, Dentsply Sirona). Digital radiography was done using a CMOS sensor (Sopix2 Acteon) with a 25 pl/mm resolution. The root canal preparation was done by Protaper Gold using F3 as the final file with NSK Endomate DT endomotor(NSK, Japan) as per the manufacturer's instruction.

Group 2 Electronic apex locator method (EAL): In this group, the WL was established by using Propex Pixi (Dentsply, USA) apex locator following the manufacturer's instructions. The lip clip was placed in the mouth, and the file clip was attached to the 15 K file. The file was advanced in the root canal till the 00 reading was obtained in the Propex Pixi apex locator. The rubber stopper was adjusted to a coronal reference point. The file was removed from the canal, and the distance between the rubber stopper and the tip of the file was measured on the endodontic ruler (Dentsply, USA). 0.5mm was subtracted from the value to obtain the final WL. The root canal was prepared with a Protaper Gold system up to size F3 thourough NSK Endomate DT (NSK, Japan) endomotor according to the manufacturer's instructions.

Group 3 Simultaneous length control using endomotor with integrated Electronic Apex Locator (SLC). The preparation in this group was done using Tri Auto ZX2 (J Morita, Japan) endomotor, which has a built-in apex locator. The mode was set as an auto-apical stop to ensure that there was no over-preparation during instrumentation. Preparation was done up to Protaper Gold size F3.

For all the groups, in between each file used, the canals were copiously irrigated with 5.25% sodium hypochlorite (Prime Dental, India). Flutes of the files were cleaned with wet gauze after each instrumentation, signs of distortion or wear of the file were checked, and apical patency of the root canal was maintained with a #10K file. Following completion of the biomechanical preparation, a radiograph was taken after placing a 6% size 30 master cone gutta percha (Meta Biomed, Korea) to the working length. The canals were obturated using epoxy resin sealer (AH Plus, Dentsply Maillefer) and cold lateral compaction of the gutta-percha. The entire treatment was performed by a single operator (post-graduate endodontic resident) as a single visit endodontic procedure. Ibuprofen 400 mg was prescribed to the patients, with instructions to use it as a rescue analgesic only in the event of unbearable pain.

Pain evaluation

Patients were provided with a questionnaire for recording the postoperative pain intensity and analgesic intake at 6, 24, 48 and 72 hours. They were instructed on how to use a VAS (Visual Analog Scale) to rate their pain and document their responses in the questionnaire. Furthermore, before each time interval, patients were reminded via a phone call and an electronic message to submit their response.

Statistical analysis

The data were assessed for homogeneity by the homogeneity of variance and normality by the Shapiro-Wilk test. Data on gender and dental arch and analgesic intake after procedure was evaluated by the $\chi 2$ test, while data on age and preoperative pain was analyzed by the One-Way ANOVA test. The postoperative pain scores were analyzed statistically using the Kruskal Wallis and Mann-Whitney U test, and Wilcoxon signed ranks test at a significance level of $p < 0.05$. All statistical analysis was conducted in a blind manner at a confidence interval of 95% (p=.05) and performed using SPSS 20.0 software (IBM Corp, Armonk, NY, USA).

Results

From the total sample of 60 patients, 33 (55%) were men, and 27(45%) were women. Each patient had only one tooth included in the study, making a total sample of 60 patients with 60 teeth. Out of the total teeth that were treated, 41 (68.3%) of them were maxillary teeth comprising 32 (53.3%) incisors and 9 (15%) premolars. 19 (31.7%) of them were mandibular teeth consisting of 11 (18.3%) incisors and 8 (13.3%) premolars. Two patients (one from the DRG group and one from the SLC group) did not respond and were lost during the follow-up. The mean age of the patients was 24.4 ±8.45, ranging from 16-52 years. The characteristics of the patient and the demographic data are shown in Table 1. The demographic data among the three groups were found to have no statistically significant difference among them.

All the methods of working length evaluation resulted in postoperative pain. The highest VAS pain scores for all three experimental groups were recorded at 6 hours postoperatively, and these scores decreased gradually over the 72-hour observation period (Figure 1). Intra-group comparison by Wilcoxon signed ranked tests showed that statistically significant differences existed within a group at 6, 24, 48 and 72 hours postoperatively for all the groups ($p<0.05$). The mean postoperative pain values evaluated after 6, 24, 48, and 72 hours are shown in Table 2. There was no significant difference in postoperative pain between males and females in any group at 6, 24, 48 and 72 hours.

Table 1: *Demographic data and preoperative pain levels*

Demographic data	Digital Radiograph	Electronic Apex Locator	Simultaneous Length Control	P value
Ageφ	24±8.2	23±10.1	26±7.8	p>0.05
Gender χ				
Male	11	13	12	p>0.05
Female	9	7	8	
Dental Archχ				
Maxillary	15	12	14	p>0.05
Mandibular	5	8	6	
Mean preoperative pain scoreφ	7.8±0.85	7.9±0.97	7.6±0.93	p>0.05

Φ: one way ANOVA test χ: χ2(chi square test)

Table 2: *Mean postoperative Pain at 6, 24, 48 and 72 hours and rescue medications needed in each group*

	Mean Postoperative Pain VAS scale (cm)				No. of patients taking analgesics
	6 hours	24 hours	48 hours	72 hours	
DRG	3.76[a]	2.59[a]	1.86[a]	0.71[a]	8[b]
EAL	3.12[ab]	2.06[ab]	1.71[a]	0.50[a]	3[a]
SLC	2.24[b]	1.51[b]	0.42[b]	0.34[a]	2[a]

The postoperative pain scores were analyzed statistically using the Kruskal Wallis test and Mann-Whitney U and Wilcoxon signed ranks test at a significance level of p <0 .05. Same superscript in a column indicates no significant difference, DRG= Radiographic method , EAL= Electronic Apex Locator , SLC= Simultaneous Length Control

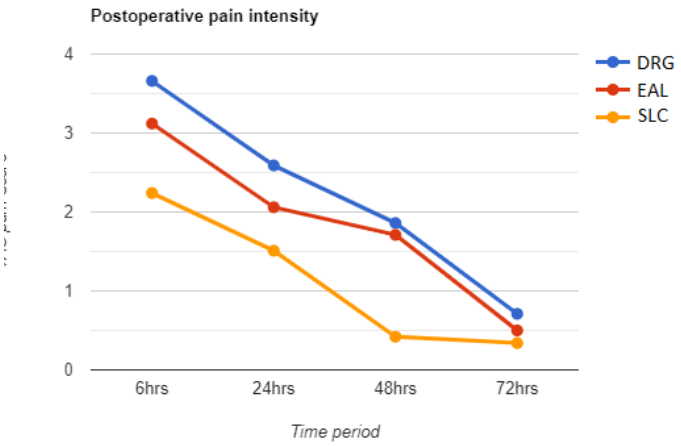

Figure 1. *Line graph illustrating the VAS pain scores over time in the three groups (DRG, EAL, SLC) DRG= Radiographic method, EAL= Electronic Apex Locator, SLC= Simultaneous Length Control.*

In this study, the SLC group (Group 3) experienced the least postoperative pain compared to all other experimental groups. The SLC group recorded the lowest VAS scores at all observed time intervals (6, 24, 48, and 72 hours). The study found a significant difference in VAS pain scores at 6, 24, and 48-hour intervals between Group 1 (DRG) and Group 3 (SLC) (p<0.05). Additionally, a significant difference in VAS pain scores was observed between Group 2 (EAL) and Group 3 (SLC) at the 48-hour interval. No statistically significant difference in postoperative pain levels existed between Groups 1 (DRG), Group 2 (EAL) and Group 3 (SLC) at the 72hour observation interval. 13 patients overall, with eight patients from Group1 (DRG), three patients from Group 2 (EAL) and two patients from Group 3 (SLC) required postoperative analgesics. There was a statistically significant difference in the postoperative analgesic intake of DRG (Group 1) compared to EAL (group 2) and SLC (group 3) (p<0.05).

Discussion

During endodontic treatment, clinicians primarily rely on radiographs and electronic apex locators for determining the working length. A recent innovation is the introduction of endodontic motors with integrated electronic apex locator, which simultaneously prepares and monitors the WL. There are very limited studies assessing the accuracy of the simultaneous working length determination. The present study was therefore conducted to evaluate and compare the effect of Digital radiography, electronic apex locator and dynamic working length measurement on postoperative pain. The present study found a statistically significant difference in the pain levels between the Group 1 (DRG) and Group 3 (SLC) at 6, 24, and 48 hours postoperatively. Thus, the null hypothesis of no difference in the postoperative pain levels between the experimental working length determination groups was rejected.

Radiographs are essential in endodontics during diagnosis, treatment and postoperative evaluation. However, radiographic images are a two-dimensional view of a three-dimensional object. To overcome this limitation, the present study employed a horizontal cone shift technique by taking two radiographic images of the same tooth at different horizontal angles and used the SLOB (same lingual opposite buccal) principle to interpret the images. These methods were utilized to attain an understanding of the tooth's three-dimensional anatomy and allowed for the verification

of the diagnosis of single-rooted teeth that were included in the study. Clinicians frequently rely solely on radiographic images to estimate the working length during root canal treatment. In order to prevent magnification errors that can result from incorrect angulation of the X-ray beam to the sensors, a long cone paralleling technique with a positioning device (such as the Rinn XCP-ORA from Dentsply Sirona) was implemented in this study. Digital radiography using CMOS (Complementary Metal Oxide Semiconductor) sensors was used in the study to acquire the images. Direct digital radiography systems for dental imaging use CMOS and CCD sensors. Compared to CMOS sensors, which are more recently available on the market, CCD sensors are well-established in the industry and have been used for many years. CCD sensors are known for producing images of high quality and low noise. A scintillator layer absorbs light in a CCD sensor before emitting photons that are captured by a photoconductive layer. The photoconductive layer then generates electrical charges that are read out by the CCD chip. On the other hand, CMOS sensors capture and amplify the electrical charges produced by X-ray energy using an array of tiny transistors. Unlike CCD, each pixel in a CMOS sensor has its own amplifier circuit, making the sensor more power-efficient and faster. Comparative studies have reported the quality of images made by the CCD and CMOS intraoral X-ray detectors to be similar (13, 14). However, there is possible cost saving and decreased power requirements associated with the adoption of CMOS technology.

In the present study, among all the experimental groups, radiographic determination of working length was associated with the highest recorded VAS pain scores at all the observed time intervals (6,24,48, and 72 hours). Additionally, a significantly higher number of patients in Group 1 (DRG) required rescue analgesic medications compared to Group 2 (EAL) and Group 3 (SLC). The radiographic method of WL determination used in the present study involves an arbitrary estimation of the apical foramen by subtracting a predetermined length from the radiographic apex. However, the variation in the position of the radiographic apex and the actual apical foramen is a primary cause of inaccuracy in radiographic WL determination, which may explain the higher postoperative pain scores observed in this group of patients (15).

Electronic apex locators use electrical measurements to precisely locate the apical constriction for working length determination, unlike the radiographic method that relies on an arbitrary estimation. Modern apex locators, like Propex Pixi, measure the changes in electrical impedance at two different frequencies, allowing for accurate and reliable measurements even in the presence of blood, pus, or other materials (16). In the present study, it was observed that the group using electronic apex locators had lower median pain scores on the visual analogue scale (VAS) at all the intervals measured (6, 24, 48, and 72 hours) compared to the group that used radiographic working length determination. However, the difference in the postoperative pain scores between the two groups was not statistically significant. One possible reason for potential errors in working length determination using an electronic apex locator is that the process involves manually measuring and transferring the working length to an endodontic file by marking it with a rubber stop. These manual techniques can introduce inaccuracies, as the rubber stop may be incorrectly placed or move during use, and the visual estimation may not be precise. These errors could impact the accuracy of the procedure in clinical practice. The results were consistent with the study by Tuncer and Gerek (17), where they reported no significant difference in the severity of postoperative pain between working length determination by digital radiography and electronic apex locator.

The integrated endomotor Triauto ZX 2 enables simultaneous instrumentation and working length control. These motors are capable of detecting and stopping at the working length, which minimizes the risk of over-instrumentation beyond the apex. This feature can help reduce periapical tissue damage and postoperative pain. This was confirmed in our research as the simultaneous working length determination (Group 3) resulted in the least amount of pain which was significantly lesser than the radiographic group (Group 1) at 6, 24 and 48 hours ($p<0.05$). The findings of this study are consistent with the results obtained by Arslan *et al*. (18), who found that the group that underwent simultaneous length control during root canal preparation had lower postoperative pain levels on day 1 than the control group in which working length and instrumentation were accomplished separately.

The present study found that the highest VAS pain scores were consistently recorded at 6 hours postoperatively, and these scores decreased gradually over the 72-hour observation in all the experimental groups. The results were statistically significant when intragroup comparison across the observed time intervals were made in all the experimental groups. These findings are consistent with the findings of several other studies (17,19,20). The most likely explanation for the higher pain scores observed at the 6-hour interval is that the patient's anesthesia fully wears off by this time, and the patient starts to experience pain. The allodynic and hyperalgesic pain responses are elicited by the nerve endings sensitized by the acute inflammatory mediators. However, the initial acute inflammation gradually decreases over a few days, and as a result, the pain reduces. Most patients in this study experienced little to no pain after 48 and 72 hours of treatment, and there was no significant difference in pain levels between the groups after 72 hours. This aligns with the findings of a prior study, which showed that pain severity tends to decrease over the course of several days, often decreasing by half after just one day (21). Tuncer and Gerek (17) reported minor to no pain within 12-48 hours of treatment, with pain decreasing to 22.9% and 27.3% within 24-48 hours.

Factors such as preoperative diagnosis and instrumentation techniques used during root canal treatment influence post-endodontic pain. In the present study, single rooted teeth with irreversible pulpitis were included to allow consistency in completing the biomechanical preparation and obturation in a single visit. A recently conducted systematic review found that after a root canal treatment was done in a single visit, there is a lower frequency of short-term post-obturation pain than performing a multiple-visit root canal treatment, without any significance in the rate of healing (22). All the procedures were performed by a single operator (post graduate resident) to offset interoperator variations in results obtained. Crown down rotary instrumentation was

utilized for the biomechanical preparation of the root canal system, as research has shown that it results in significantly lower debris extrusion compared to manual step back preparation (23,24). Coronal flaring is crucial in determining the appropriate initial apical size, as demonstrated by Pecora *et al.*, who found that pre-flaring significantly enhanced the precision of Root ZX electronic apex locator measurements, regardless of the file used (25, 26). Therefore, in the present study, coronal flaring was performed prior to determining the working length.

In the present study, visual analog scale (VAS) was used to measure the pain intensity which has been used previously in several studies to measure post-endodontic treatment pain (27,28). The VAS scale was the choice in this study because of the following reasons 1) It has a continuous scale which allows patients to indicate their pain intensity with high sensitivity 2) The quantitative measurements obtained by VAS pain scale is helpful in the statistical analysis and result interpretation 3) It is easy for the patient to understand and use.

The visual analog scale used in this study relies on the patient's subjective perception of pain, which can result in variability in scoring. The study participants were limited to single-rooted teeth with irreversible pulpitis, and therefore, the findings may not be applicable to more complex clinical situations, such as multirooted teeth, severe root curvatures, pulp necrosis, or periapical lesions. Future research should explore postoperative pain outcomes in these situations to ensure broader generalizability of the results.

Conclusion

Within the limitations of this study, it can be concluded that the SLC technique resulted in significantly lower postoperative pain after 6, 24 and 48 hours, than the DRG method, and could be a beneficial non-pharmacological method to reduce postoperative pain. The postoperative pain levels in the 72-hour assessment period were reduced to minimal or no discomfort level irrespective of the WL determination technique used.

Türkçe özet: *Kök kanal tedavisinden sonra postoperatif ağrıda dijital radyografi, elektronik apeks bulucu ve eşzamanlı çalışma uzunluğu belirlemenin karşılaştırmalı değerlendirmesi: randomize bir klinik çalışma Amaç: Çalışma, kök kanal preparasyonundan sonra postoperatif ağrıyı üç farklı çalışma uzunluğu belirleme yöntemi kullanarak karşılaştırmayı amaçladı. Gereç ve Yöntem: Semptomatik irreversibl pulpitis tanısı alan 60 hasta, çalışma boyu (WL) belirleme yöntemine göre rastgele üç gruba ayrıldı. Grup 1: dijital radyografi (DRG), Grup 2: elektronik apeks bulucu (EAL), Grup 3: entegre bir apeks bulucu ile bir endomotor kullanan eş zamanlı çalışma uzunluğu kontrolü (SLC) yöntemi. Kök kanal tedavileri tek seansta tamamlandı ve hastalardan postoperatif 6, 24, 48 ve 72. saatlerde Visual Analog Skala (VAS) ile ağrı yanıtlarını kaydetmeleri istendi. Bulgular: Postoperatif ağrı skoru en yüksek Grup 1'de (DRG), en düşük ise Grup 3'te (SLC) kaydedildi. DRG ve SLC arasında 6, 24 ve 48 saatlik aralıklarla VAS ağrı skorlarında istatistiksel olarak anlamlı fark vardı (p<0,05). Sonuç: Bu çalışmanın sınırlamaları dahilinde, SLC'nin postoperatif ağrıyı azaltmak için yararlı bir çalışma uzunluğu belirleme tekniği olabileceği sonucuna varılabilir. Anahtar Kelimeler: elektronik apeks bulucu; ameliyat sonrası ağrı; radyografi; Kök kanal tedavisi; eş-zamanlı çalışma uzunluğu kontrolü; görsel analog Ölçeği*

Ethics Committee Approval: The study protocol was approved by the ethics committee of the faculty of medicine and registered in the Clinical Trials Registry of India. (Reference id CTRI/2019/07/019960).

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: BS, SA, SKM participated in designing the study. BS participated in generating the data for the study. BS, SA, DL participated in gathering the data for the study. BS, SA, DL participated in the analysis of the data. BS, SA wrote the majority of the original draft of the paper. BS, SA participated in writing the paper. DL, SKM has had access to all of the raw data of the study. BS, SA, DL, SKM has reviewed the pertinent raw data on which the results and conclusions of this study are based. BS, SA, DL, SKM have approved the final version of this paper. BS, SA, DL, SKM guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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Original research

The location of mandibular foramen relative to the occlusal plane: a study on anatolian dry mandible

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Purpose
The study aims to evaluate the location of mandibular foramen (MF) with respect to the occlusal plane (OP) and its location on the ramus using Anatolian dry mandibles.

Materials and Methods
A total of 115 dry mandibles with mandibular molars were analyzed. The distance between the MF and the OP was examined with a flat metal plate. Group A was above the OP; group L was at level, and group B was below the OP. The distances between the MF and anterior border (A-MF), sigmoid notch (U-MF), posterior border (P-MF), and lower border (L-MF) were measured. The symmetry between the two sides was examined. Pearson chi-square and Student's t-test were performed for statistical analysis.

Results
According to the analysis, 50.23% of MF was located below the OP ($p<0.05$). The mean distances of Groups A and B were 3.45 and 4.78 mm, respectively. There was no difference between the left and right in groups ($p>0.05$). The distance A-MF was 14.71 mm. There was no statistical difference between the distances A-MF and P-MF or U-MF and L-MF.

Conclusion
Half of the MF (50.23%) was located below the occlusal plane with a mean distance of 4.78 mm. It may be helpful to place the needle 3-4 mm above the OP and 1.5-2 mm back of the anterior border to obtain a successful inferior alveolar nerve block. The MF was located at the center of the medial surface of the ramus.

Keywords: *Anatomy, mandible, mandibular foramen, mandibular nerve, occlusal plane*

Introduction

Modern dentistry denies painful practices. However, it is not possible to deny the failure rate of the inferior alveolar nerve block (IANB), which has been reported to be between 13% and 57% (1,2). More dramatically, 90% of the clinicians reported difficulties with obtaining proper anesthesia in dental practice (1). IANB, which is used in the treatment of mandibular posterior teeth, is one of the local anesthesia techniques that require the most diligent and target-oriented application in dental practice. IANB, in other words, the Halsted technique, or 'mandibular nerve block', which is a misnomer according to Malamed (3), is based on the deposition of an anesthetic solution in the pterygomandibular space, which includes the inferior alveolar nerve and foramen mandible. The inferior alveolar nerve enters the mandibular canal through the mandibular foramen and provides innervation to the mandibular molars and premolars along its course (1,3). Various reasons lead to the failure of the anesthetic technique including anatomic variations, uncommon physiology, and the presence of infection; however, the prom-

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inent reason for the failure is the inability to determine the mandibular foramen location (4). Since the needle penetration depth or location is adjusted according to the surrounding tissues or landmarks, it is substantial to have a fixed reference point that can be observed directly in the mouth while administering anesthesia, instead of hard-to-observe anatomical formations to ensure the proximity of the anesthetic solution to the nerve (5,6).

For IANB, another important point in addition to the failure of anesthesia is the possible complications including hemorrhage, soft or hard tissue necrosis, or nerve damage, that may occur during the application (6). Therefore, meticulous knowledge about the location of the MF and the topography of the ramus is required. The morphological dimensions of the mandible and the topography of the mandibular foramen relative to the occlusal plane (OP) were evaluated using dry mandible (4,7-10) or radiography (11-15) in different populations. Since different methodologies of radiographic measurement may change in determining the location of MF (13,15), dry mandibles provide substantial data as a direct observation method despite having a limited sample size. Altunsoy *et al.* (12) reported the relative location of MF in children using cone-beam computed tomography (CBCT) in the Turkish population. Oğuz and Bozkır (16) analyzed the morphology of the ramus using Turkish dry mandibles but provided no information about the OP and the relative location of the MF. When considering the effects of racial and methodological factors, more studies are needed to obtain well-established data. The objective of this study is to evaluate the relative location of MF to the OP and to analyze the topographic placement of the MF on the ramus using Anatolian dry mandibles. The null hypothesis of the study was that the MF would not be above the OP.

Materials and Methods

Study design

The study was conducted in the anatomy department laboratory of Akdeniz University Faculty of Medicine and Süleyman Demirel University Faculty of Medicine with the obtained permissions. A total of 298 dry mandibles were scanned. Age and gender were unknown. Inclusion criteria were mandibles with intact ramus and with mandibular molars for analyzing the relative location to the OP. Exclusion criteria were ragged or deformed mandibles excessively enough to affect the measurement, mandibular molars in supraocclusion or inclined mandibular molars, and mandibular molars with advanced crown destruction. The supraocclusion was regarded if the distance between the cementoenamel junction and the alveolar bone was more than 1 mm. For the study, 115 dentate dry adult human mandibles were selected.

Cadaveric measurements

We measured the distance between the MF and the most concave point of the anterior border (coronoid notch) (A-MF), posterior border of the ramus (P-MF), sigmoid (mandibular) notch (U-MF), and lower border of the ramus (L-MF), at the left and right sides (Figure 1). The center of the mandibular foramen was taken as a fixed reference point. While determining the occlusal plane, two buccal cups of the second mandibular molar were used according to the basic geometry logic of "a line passes through two points." A rigid flat metal plate of 0.5 mm in thickness was placed on the second molar and stabilized. In the inner surface of the ramus, the point on the line referred by the metal plate and in the MF alignment was marked with a fine-tipped erasable pen, and the distance between the MF and the mark was measured with a digital caliper.

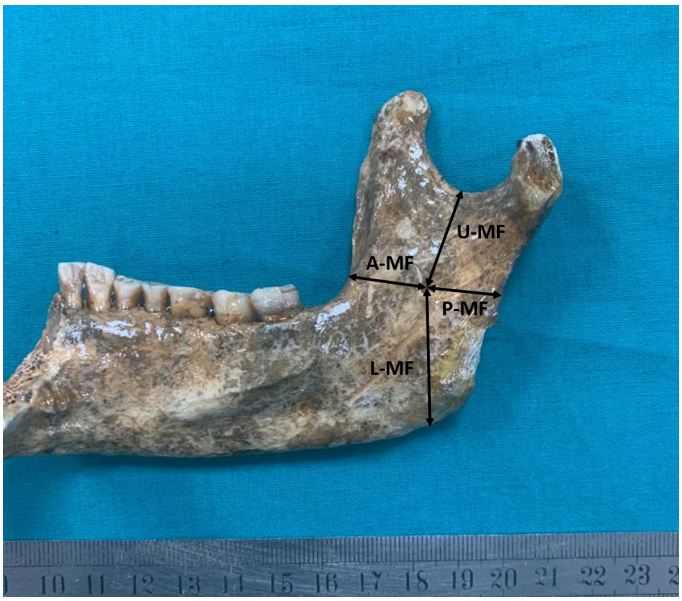

Figure 1. *Morphometric measurements of the ramus (MF: Mandibular foramen. A-MF: the distance between the MF and the most concave point of the anterior border. P-MF: the distance between the MF and posterior border of the ramus. U-MF: the distance between the MF and sigmoid notch. L-MF: the distance between the MF and lower border of the ramus).*

All measurements were performed independently by two observers (DY, a 6-year experienced endodontist, and AMN, a 10-year experienced periodontist) twice to obtain inter-class and intra-class reliability. Prior to measurements, for the calibration of observers, the measurements of 21 dentate mandibles (10% of the total sample size) were performed, and Cohen's kappa values were obtained (ranging from 0.88 to 0.93). The relative location of the MF to the occlusal plane and morphometric measurements was recorded.

Study groups

The relative location of the MF to the OP was allocated to three groups; Group A: MF was above the OP, Group L: MF was at the level of the OP, and Group B: MF was below the OP (Figure 2). Each measurement was performed three times, and the average value was recorded for statistical analysis. All measurements were recorded in millimeters. A digital caliper (SC-6, Mitutoyo Corporation, Tokyo, Japan) was used for the measurements.

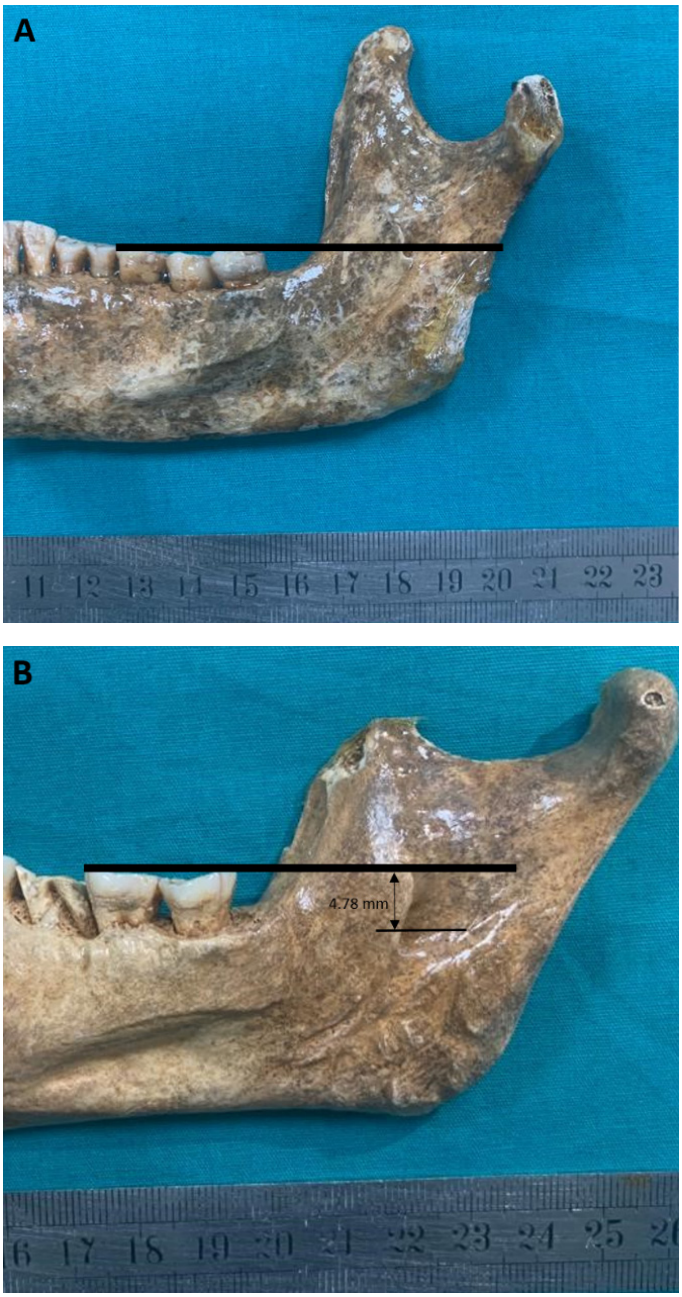

Figure 2. *Representative image of the measurement of the location of the mandibular foramen. A: Group L; at level, B: Group B; below the occlusal plane.*

Statistical analysis

Dataset was analyzed using SPSS software version 26.0 (IBM SPSS Inc, Armonk, NY, USA). The normality distribution of the data was analyzed using Kolmogorov-Smirnov. Levene's test was performed to determine the homogeneity of the data. Pearson chi-square test was applied for the symmetry of the relative location of the MF to the OP. Morphometric measurements were analyzed with Student's t-test. Interclass correlation coefficient (ICC) was used for the reliability of the observers. Statistical difference was considered significant with a 95% confidence interval at $p<0.01$ for the ICC, and $p<0.05$ for other tests.

Results

There was no statistical difference in the intra-observer and inter-observer measurement values. For the morphometric measurements of the MF, ICC values were 0.845 and ICC=0.913. The mean and standard deviations of upper, lower, anterior, and posterior measurements and symmetry of the MF location are presented in Table 1. The distance between the MF and anterior border of the ramus was 14.71 mm. The distance between the MF and posterior border was 12.67 mm. The distance of the MF to the sigmoid notch was 22.61 mm, and the distance to the basis of the ramus was 23.04 mm. According to Student's t-test, no significance was detected between the distances A-MF and P-MF, and similarly, in the distances U-MF and L-MF. Hence, the MF was located approximately at the center of the ramus. There was also no significant difference between the left and right sides in terms of morphometric measurements. The morphometric distances to the OP, and percentages of Group A, L, and B were shown in Table 2. The mean distance between the MF and OP in the group A was 3.45 mm, while group B was 4.78 mm (Figure 2). The MF was located in an area ranging from 11.04 mm superior to 9.89 mm inferior to the occlusal plane. The frequency of groups A, L, and B were 38.43%, 11.34%, and 50.23%, respectively. The most observed (50.23%) location was Group B, which means it was below the OP ($p<0.05$). No statistical difference was detected in the symmetry of the level of the MF according to the OP between the left and right sides.

Table 1: *Morphometric measurements (in mm) and symmetry of the location of the mental foramen*

	Total	Symmetry		P value
		Right	Left	
	Mean (±Std)	Mean (±Std)	Mean (±Std)	
Anterior	14.71 (±2.24)	15.77 (±2.51)	13.65 (±1.69)	0.074
Posterior	12.67 (±2.18)	12.19 (±2.60)	13.15 (±1.98)	0.067
P value	0.19	0.07	0.34	
Upper	22.61 (±4.45)	21.92 (±4.90)	23.03 (±3.87)	0.14
Lower	23.04 (±3.23)	23.41 (±3.83)	22.67 (±2.97)	0.063
P value	0.24	0.16	0.21	

No statistical difference was observed between morphometric data or two sides according to the Student's t-test ($p<0.05$).

Table 2: *Relative location of the mental foramen to the occlusal plane*

	Total		Right		Left		P value
	Mean (±Std)	%	Mean (±Std)	%	Mean (±Std)	%	
Group A	+3.45 (±1.29)	**38.43%**	+3.12 (±1.27)	41.01%	+3.78 (±1.35)	35.85%	0.16
Group L	N/A	**11.34%**	N/A	12.17%	N/A	10.51%	0.27
Group B	-4.78 (±1.28)	**50.23%**	-4.56 (±1.34)	49.25%	-5.01(±1.13)	51.21%	0.08
P value		**0.001**		**0.002**		**0.001**	

Group A: above, Group L: at level, Group B: below the occlusal plane
Bold p values mean statistical difference according to the Pearson chi-square test ($p<0.05$). Minus indicates below the OP and indicates above the OP.

Discussion

Within the limited sample size of the study, the most important result of the study is that 50.23% of the MF was located below the OP. Endodontic treatment, extraction, and most dental treatments require block anesthesia rather than local infiltration that does not supply proper anesthesia due to the thick compact bone of the mandible (15). The clinician has blind access to the MF as it is covered by soft tissue, but the solution must be injected as close to the MF as possible to rule out the possible complications including hemorrhage, and persistent injury to the nerve, and for successful anesthesia (13). Therefore, for proper anesthesia, a clinical reference point is required. Previous studies indicated that MF was positioned below the OP in the range of 80.4% to 19.87% in African, Thai, Zimbabwean, Brazilian, and East Indian populations as described in Table 3 (5,7,9-15,17-26). Different methodologies or ethnicity lead to this wide range of results. One of the methodological differences was the measurement point of the MF. The middle of the superior line of the MF (14), the lingula (7,15), or the center of the MF (4,8) was assumed when analyzing the relative location. In the present study, the center of the MF was accepted as the measurement point. Different reference points in different studies make it difficult to compare in millimeters, and the distance of the area where anesthesia will be applied relative to the occlusal plane should be interpreted carefully. Besides the reference point that is covered with soft tissue cannot be directly observed clinically. Another reason for the differences in MF location may be due to various definitions of the occlusal plane in the studies. The first molar (11,20-22), second molar (7,13), or mandibular molars (17-19), the line passing through the canine and molars (23), or the adjusted occlusal plane (14) were considered for the measurements. According to Russ (20), the first molar is the best reference point for IANB. However, the first molar is the first permanent tooth to erupt, it is the most frequently decayed or lost. In the present study, we considered mandibular molars as a reference line using a metal flat line. Taking a single mandibular molar as a reference will affect the measurement, reducing the accuracy of the investigation when considered that the tooth has occlusal wear, restoration, or decay, or that it was in supraocclusion position or pathologic migration. Taking the occlusal plane as a reference by including molars instead of a singlemolar provides a more precise definition of the occlusal plane.

A previous study performed on dry mandibles analyzed the level of the MF according to the coronoid notch (10). However, the coronoid notch is covered by the moveable mucosa and may not present a stable point like molar tubercles. Thus, since it is a difficult point to estimate, its precision in clinical practice is reduced when considering the quantitative data. The coronoid notch properly guides the anteroposterior position of the needle, but its reference characteristic for the vertical level of the MF should be interpreted with caution.

The most important drawback in the evaluation of the occlusal plane is the irregularity of the mandibular teeth. The irregular mandibular teeth can alter the data, consequently, the precise location of the MF in mm cannot be compared properly in different populations. Likewise, various reference points, definitions of the occlusal plane, differences in measurement methodologies, and several measurement media may cause millimetric changes in the distance of MF from the occlusal plane, as well as racial factors. On the other hand, in the quantitative analysis of a dry skull, the measurements are performed on an amorphous surface, and minor angle differences affect the results. Besides, due to the curve of Spee, the buccal tubercle levels of mandibular molars may be divergent (27). Therefore, instead of specifying the numeric data belonging to the distance of the MF, which is not already a clinically observable point, determining the area where the MF is located frequently, such as above, below, or at the level of the occlusion, may create a clinically easier and more applicable approach. Therefore, although we provided quantitative data in the present study, we emphasized the areas where MF was concentrated. Group B (below the OP) was higher. The relative location of MF was similar on each side. This result was congruent with previous studies (11,12,14,20). Investigation of the dry mandible provides a direct observation. However, for observation on panoramic radiography, a patient's improper head movement or position may create a false asymmetry between the left and right sides.

We found that the location of the MF was at the center of the ramus, which is consistent with previous studies conducted with Turkish people or other populations (7,16). While measuring the distance of the MF to the anterior border on the ramus, the most concave point, the coronoid notch, was accepted, as in most previous studies (10,14). Topographic measurements of MF localized in the ramus are important for IANB (15). However, during injection, since the posterior border, the sigmoid notch, and the medial aspect of the ramus are not seen totally, clinically observable or palpable points are of greater importance in determining the technique of the anesthesia.

Table 3: *Location of mandibular foremen in previous studies*

Study	Ethnicity	Methodology	Sample size	References	Location
Afsar	Canadian	Panoramic	79	First molar	1.9 mm above
Hwang	Chinese	Cephalometric	112	*Undetermined*	4.16 mm above
Feurerstrein	French	CBCT	260	Mandibular teeth	2-3 mm above
Altunsoy	Turkish	CBCT	20	Mandibular molars	2.5-3.6 mm above
Blatcher	American	CBCT	203	Second molar	9 mm above
Al-Shayyab	Jordanian	CBCT	224	First molar	2.5 mm above
Jang		CBCT	125	Second molar	8.85 mm above
Bunyarit	Malay	CBCT	87	*Undetermined*	10 mm above
Kang	Korean	CT	59	First molar	3.8 mm above
Zhou	Korean	CBCT	106	Mandibular molars	12.4% above (2.5mm) 3.3% at level 84% below (4.5mm)
Russa	Tanzania	Cadaveric mandible	44	First molar	10 mm above
Monnazzi	Brazilian	Dry mandible	44	Mandibular crest	0.02 below
Thangevalu	Indian	Dry mandible	102	Mandibular molars	Few mm above
Kositbowornchai	Thai	Dry mandible	23	First molar	10 mm above
Nicholson	East Indian	Dry mandible	80	Second molar	2.5% above 22.5% at level 75% below
Palma	Brazilian	Dry mandible	82	Mandibular molars	15.1% above 0.8% at level 84.1% below
Jansisyonont	Thai	Dry mandible	146	Mandibular molars	80.13% above (4.5 mm) 19.87% below (3.1 mm)
Mbarjorgu	Zimbabwean	Dry mandible	38	Mandibular molars	29.4% above 47.1% at level 23.5% below
Mwaniki	African	Dry mandible	79	Mandibular molars	4.7% above 30.7% at level 64.6% below
The result of the present study	**Anatolian**	**Dry mandible**	**105**	**Mandibular molars**	**38.43% above (4.5 mm) 11.34% at level 50.23% below (3.1 mm)**

CBCT: cone-beam computed tomography

The direct method using skulls or radiographic method was used to determine the relative location of MF with the OP (5,7,9-15,17-26). Although the skull provides direct observation, when the investigation is based on an "imaginary line" without systematic methodology, this approach makes such valuable data as direct observation subjective and unrepeatable. Therefore, the accuracy of the measurements would be skeptical. In the present study, it was used a metal line to determine the OP alignment directly, similar to the study of Palma *et al.* (9) which used plastic to compare the level previously.

The limitation of the study is that it concerns a certain population as it was conducted in dry skulls. In the present study, the effect of age and gender or different skeletal patterns was not examined. Besides, another limitation of the study is that the dry mandibles were obtained from one region of Anatolia and they were limited in number. More clinical and cross-sectional studies on different populations and a wider sample are needed. From a different perspective, future studies should focus on clinical applications of different anesthetic techniques rather than detecting the exact localization of MF that has different variations. The strength of the study is that it is the first study in which the relationship of the occlusal plane with MF was examined in the Anatolian population by direct observation using a dry mandible.

Conclusion

Due to the limitation of this study, it was concluded that the distance of MF to the coronoid notch was 14.71 mm. The mandibular foramen was located at the center of the medial surface of the ramus. The mandibular foramen was located below the occlusal plane, with a rate of 50.23%. The MF located was in an area that was between 4.78 mm below and 3.45 mm above the plane of occlusion. This anatomic data may be useful in the projection of the needle during

IANB. The take-home message of the present study is that the highest rate of success can be achieved during anesthesia when the needle is positioned slightly above the occlusal plane (approximately 3.5 - 4 mm) and 1.5 - 2 cm behind the anterior edge, given that the results that showed 50% of MF was located below the occlusal plane and the 14.71 mm distance to the coronoid notch. Besides, the results can be informative for the location of osteotomies during maxillofacial surgeries in terms of the avoidance of permanent damage to vital structures including nerves. Nevertheless, it is crucial to remark that the data of the study demonstrated a benchmark result. In clinical practice, it is essential to perform analysis on a case-by-case basis.

Türkçe özet: *Mandibular Foramenin Oklüzal Düzleme Göre Konumu: Anadolu Kuru Mandibulaları Üzerinde Yapılan Bir Çalışma. Amaç: Bu çalışma, Anadolu kuru mandibulaları üzerinde mandibular foramenin (MF) oklüzal düzleme (OP) göre konumunu ve ramus üzerindeki yerleşimini değerlendirmeyi amaçlamaktadır. Gereç ve Yöntem: Mandibular azı dişleri olan toplam 115 kuru mandibula analiz edildi. MF ve OP arasındaki mesafe düz bir metal plaka ile ölçüldü. Grup A, OP'nin üzerinde; Grup L PO seviyesinde ve grup B OP'nin altında olacak şekilde gruplandırıldı. MF ile ramus ön sınırı (A-MF), sigmoid çentik (U-MF), ramus arka sınırı (P-MF) ve ramus alt sınırı (L-MF) arasındaki mesafeler ölçüldü. Sağ ve sol arasındaki simetri incelendi. İstatistiksel analiz için Pearson ki-kare ve Student t-testi yapıldı. Bulgular: Analize göre MF'nin %50.23'ü OP'nin altında yer aldı (p<0.05). Grup A ve B'nin ortalama mesafeleri sırasıyla 3.45 ve 4.78 mm olarak tespit edildi. Gruplarda sağ ve sol arasında fark bulunmadı. A-MF mesafesi 14,71 mm'ydi. A-MF ve P-MF veya U-MF ve L-MF mesafeleri arasında istatistiksel fark tespit edilmedi (p>0.05). Sonuç: MF'nin yarısı (%50.23) ortalama 4.78 mm mesafe ile oklüzal düzlemin altında yer alıyordu. MF, ramusun medial yüzeyinin merkezinde konumlandı. Bu sonuçlara göre başarılı bir inferior alveoler sinir bloğu elde etmek için enjektör OP'nin 3-4 mm yukarısına ve ön sınırın 1.5-2 mm arkasına yerleştirmek yararlı olabilir. Anahtar kelimeler: Anatomi, mandibula, mandibular sinir, oklüzyon, lokal anestezi*

Ethics Committee Approval: Necessary permissions were obtained from Akdeniz University Faculty of Medicine and Süleyman Demirel University Faculty of Medicine.

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