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scores of the groups was statistically significant under a predefined effect. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

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Journal Article in Print

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

Journal Article Electronic Publication Ahead of Print

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

Book

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

Chapter in a book

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

Thesis

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

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

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

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

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Original research

Effect of surface sealant on the surface roughness of different composites and evaluation of their microhardness*

Ozge Gurbuz[1], Aylin Cilingir[2], Benin Dikmen[1], Alev Ozsoy[1], Meltem Mert Eren[3]

Purpose
The aim of this study was to evaluate the effect of a surface sealant on the surface roughness of different composites and compare their microhardness values.

Materials and Methods
Sixty disc-shaped specimens were prepared and assigned to 6 groups (n =10). Groups were prepared as follows; Group 1 (Herculite XRV Ultra), Group 2 (Beautifil Bulk Restorative) and Group 3 (Filtek Bulk Fill Posterior Restorative). Groups 4, 5, and 6 were prepared by applying a surface sealant (BisCover LV) on the specimens in groups 1, 2 and 3. Surface hardness of the discs in group 1, 2, and 3 and surface roughness of the discs in all groups were measured using the Vickers hardness test and a profilometer, respectively. One-way ANOVA was used to test for differences among the groups.

Results
No significant differences were observed in the microhardness and roughness between the experimental and control groups for each restorative materials. Group 3 showed the highest surface hardness and group 4 showed the lowest surface roughness values.

Conclusion
Using the BisCover LV resin after the polishing step has no significant effect on the surface roughness. The highest hardness values were obtained for the Filtek Bulk Fill Posterior Restorative after the polishing step. The smoothest surfaces were obtained for all experimental groups using the BisCover LV resin after the polishing step, Herculite XRV Ultra showed lower average roughness values than the other materials.

Keywords: *Roughness; microhardness; resin composite; surface sealant; mechanical properties*

Introduction

Dental composite resins are commonly used restorative materials for the replacement of defects in hard dental tissues (1,2). Despite of satisfactory mechanical and esthetic properties, they have some disadvantages too. Polymerization shrinkage causes some problems such as postoperative sensitivity, secondary caries and marginal leakage. To remove these problems, less than 2-mm- thick layering should be done and it is a time-consuming process. Thus, bulk fill composites have been produced that are claimed to have a low shrinkage stress (3).

Regardless of the cavity class, location and type of the composite material, a smooth surface finish is clinically important because it determines the esthetics and longevity of composite resin restorations (4). Proper finishing of restorations is desirable not only for esthetics but also for good oral health by preventing plaque retention (5). Surface roughness of dental materials can cause microtrauma to the oral tissues and enhance the

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ORCID IDs of the authors: Ö.G. 0000-0002-3468-950X; A.Ç. 0000-0002-9848-9136; B.D. 0000-0003-2174-8934; A.O. 0000-0001-9589-3232; M.M.E. 0000-0002-5903-6636

[1]*Istanbul Medipol University, Faculty of Dentistry, Department of Restorative Dentistry, Istanbul, Turkey*

[2]*Trakya University, Faculty of Dentistry, Department of Restorative Dentistry, Edirne, Turkey*

[3]*Altınbas University, Faculty of Dentistry, Department of Restorative Dentistry, Istanbul, Turkey*

Corresponding Author: Aylin Cilingir

E-mail: aylincinar@hotmail.com

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retention of microorganisms, thereby contributing directly or indirectly to tissue injuries and possible oral diseases (6,7). Therefore, a smooth surface finish is important to maintain good oral health by reducing microorganism retention and plaque accumulation as well as for good esthetic appearance and less recurrent caries and gingival irritation (8-11). Moreover, average surface roughness (Ra) above the 0.2 μm threshold has been reported to increase the colonization and adhesion of bacteria on composite resin surfaces (12). The surface roughness of composite resin is usually determined by the size, hardness, and amount of the filler particles, flexibility of the material, and the hardness and grit size of the abrasive (13).

It has been shown that the surface micromorphology of composite resins after finishing and polishing steps can be influenced by the size, hardness and amount of the filler particles (14). During the polishing of hybrid composites, the harder filler particles are left protruding from the surface, whereas the softer resin matrix is preferentially removed. Therefore, the harder filler particles should be packed close together to protect the soft resin matrix from abrasives (15). The combination of reduced particle dimensions and wider size distribution allows the higher levels of filler loading, resulting in reduced polymerization shrinkage and improved mechanical properties (16). To achieve an effective finishing system for composite resins, the abrasive particles should be relatively harder than the filler materials to prevent the preferential removal of the soft resin matrix during polishing, leaving the harder filler particles protruding from the surface (13). According to earlier work, larger filler particles have resulted in greater Ra values (17,18). The composite resins with higher concentrations of small-sized filler particles have become popular in recent years due to the difficulties in producing smooth surfaces similar to the enamel surface using the composite resins that have larger filler particles. Typically, increased amounts of filler particles result in smoother surfaces because of the decreased particle size and better particle distribution within the resin matrix (13).

Surface sealants have been developed to preserve or improve the mechanical properties of direct restorative materials (19,20). Thus, application of the surface sealants after the polishing step has been recommended to increase the longevity of restorations (21, 22). Liquid polishing materials are low-viscosity, light-polymerized resin formulations with a low amount of filler particles that provide a smooth, sealed surface for interim and composite resin restorations (14,23,24). Surface penetrating sealants (SPS) are unfilled low-viscosity resins polymerized onto the composite surfaces to promote the filling of structural microdefects and microfissures by capillary action (25) for maintaining the surface smoothness, improving the wear resistance (25, 26) and marginal sealing (27) of the restoration. Since various surface defects such as microcracks and irregularities are formed due to the removal of some of the surface particles during finishing, application of the liquid resin to the finished material surface has been

recommended to repair the structural microdefects and improve the abrasion resistance of posterior composite resins (28,29).

However, the effectiveness of sealants in improving the smoothness of composite surfaces is still controversial. Although some authors have suggested that sealants might be desirable to improve the surface finishing of composites (21,30), others have reported no significant reduction in the surface roughness of composites after the simulated abrasion test (31) and also in clinical evaluations after one and five years (25, 32).

Substantial surface hardness of the restoration is one of the main requirements in high stress- bearing areas such as posterior restorations (33) Materials which have reduced surface hardness are more susceptible to deformation (34). Microhardness can be influenced by monomer type, filler type, morphology,volume and weight (34-36). Moreover, finishing and polishing of the restoration can affect the hardness of the composite materials (34).

There are some studies that compare microhardness of different bulk fill composite materials and declare various results (37-41). It is stated that bulk fill composites with low filler content showed lower microhardness than with high filler contents (3,42,43). Despite of various results about the microhardness of different bulk fill composites, there has been no previously reported study that compare the microhardness of bulk fill composites chosen in our study each other.

The aim of this study was to evaluate the effect of the BisCover LV resin sealant on the roughness of different composites and compare their microhardness values. Based on this information, the following hypotheses were tested: (1) surface sealant reduces the roughness of composite materials (2) there were no significant difference between the microhardness of the composite materials used in this study.

Materials and Methods

Specimen preparation

Ten disc-shaped specimens with 10 mm in diameter and 2 mm in thickness were prepared in a teflon mold for each study group (Figure 1)(11). Total sixty disc-shaped specimens were prepared for the surface property tests and divided into 6 groups. Different study groups and materials used

Table 1. *Groups and materials used*

n=10	group 1	group 2	group 3	group 4	group 5	group 6
Composite restoration	Herculite XRV Ultra	Beautifil Bulk Restorative	Filtek BulkFill	Herculite XRV Ultra	Beautifil Bulk Restorative	Filtek BulkFill
BisCover application after restoration	-	-	-	+	+	+

Table 2. *Characteristics of materials tested*

	Composition	Manufacturer	Classification	Filler	Filler loading
Herculite XRV Ultra	Ethoxylated Bis-GMA, TEGDMA, BisEMA	Kerr, Orange, CA, USA	Nano-hybrid composite	SiO_2, Barium silicate glass, Prepolymerized filler with barium silicate glass and silica	71 wt% /54 vol%
Beautifil Bulk Restorative	Bis-GMA, UDMA, Bis-MPEPP, TEGDMA	Shofu Inc, Kyoto, Japan	Giomer based bulk fill resin composite	Surface modified prereacted glass (S-PRG) filler based on fluoroboroalumi nosilicate glass, polymerization initiator	87 wt% /74.5 vol %
Filtek BulkFill	Bis-GMA, UDMA, Bis-EMA(6), procrylat resins	3M ESPE, St. Paul, MN, USA	Bulk-fill paste composite with glass microfibres	Zirconia/Silica, ytterbium trifloride	76.5 wt%/58.5 vol%
Biscover LV	Dipentaerythrrit ol pentaacrylate esters and Etanol	Bisco Inc, Schaumburg, IL, USA	Low-viscosity liquid polish		

in this study are outlined in Table 1, and the properties and type of the used materials are presented in Table 2. The composite resins were poured in a Teflon mold covered with a polyester strip and a glass slide (1mm thick) was then placed over the polyester strip to flatten the surfaces according to the composite manufacturer's recommendation. The restorative materials were light-cured (Optilux Demetron, VLC 403, Danbury, CT, USA, 500 mW/cm^2). Herculite XRV Ultra was applied into the mold and light-cured for 20 s. Beautifil Bulk Restorative and Filtek BulkFill were applied into the mold and light-cured for 20 s and 40 s, respectively. Afterwards, the surfaces of the specimens were polished for 30 s from extra- coarse grain size to extra-fine grain size with polishing discs (OptiDisc, Kerr Hawe, Karlsruhe, Germany). A new polishing disc was used for each specimen and then discarded after each use. Specimens in experimental groups 4, 5, and 6 were etched with 32% phosphoric acid (Uni- Etch, Bisco Inc., Schaumburg, IL, USA) for 15 s. Then, the etched specimens were rinsed with water and air dried before directly applying the BisCover LV resin (dipentaerythritol pentaacrylate in ethanol) (BisCover, Bisco Inc., Schaumburg, IL, USA) using a syringe and an applicator tip. After a 15 s wait for ethanol vaporization, specimens were light polymerized for 30 s with Optilux as the manufacturer's instruction. The light curing unit tip was positioned perpendicular to the specimens' surfaces, and the distance between the tip and the specimen was standardised using a glass microscope slide (1 mm in thickness). All samples were stored in distilled water at 37 °C for 24 hours. This research was conducted at Istanbul Medipol University and Istanbul University Laboratory.

Figure 1. *Teflon mold for composites specimens.*

Microhardness measurements

Surface hardness of different composite resins was measured using the Vicker's hardness test because of its ease-of-use and reliability of the measurements (44). The microhardness values for the samples in groups 1, 2, and 3 were obtained using an Innovatest Nexus 4503 hardness testing machine (Innovatest Europe, Maastricht, The Netherlands) for loads of 2.5 – 10 kgf (24.51 – 98.07 N) (Figure 2). The surface hardness measurements were performed using a microscope at 20x magnification under a load of 300 g for 15 s. The applied load and the hold time were kept constant for all samples throughout the study. The measurement was carried out three times in each sample at random locations and a mean value was calculated.

Surface roughness measurements

Surface roughness of different composite resins was determined using the roughness average (Ra) parameter, which represents the arithmetic average of absolute values

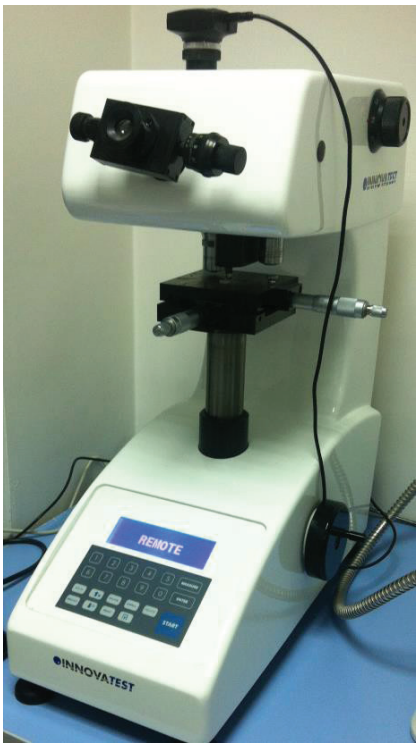

Figure 2. *Hardness testing machine.*

of the profile height deviations from the mean line recorded within the evaluation length (45). A profilometer (Taylor Hobson Surtronic 25, UK) was used for measuring the Ra values of groups 1, 2, 3, 4, 5, 6 with a cut-off value of 0.8 mm, a transverse length of 0.8 mm, and a stylus speed of 0.1 mm/seconds. For surface roughness test, readings were taken at three random locations on each surface and the average roughness value (Ra, μm) was obtained by using the arithmetic mean of these three readings.

Statistical analysis

Statistical analysis of the data was performed with Statistical Package for Social Sciences (SPSS) statistical software (SPSS PC, Vers.15.0; SPSS Inc.; Chicago, IL, USA). Descriptive statistics for continuous variables were calculated and reported in a mean ± standard deviation format. To detect differences among Ra and microhardness values for different groups, a one- way analysis of variance (ANOVA) method was used at the 0.05 level of significance.

Results

Mean microhardness and roughness values for different groups are presented in Table 3 and Table 4. There were no significant differences in microhardness and roughness values between the experimental and control groups for each restorative material ($p>0.05$). Based on the test results, group 3 showed the highest surface hardness and group 4 showed the lowest surface roughness values.

Discussion

The clinical significance of surface roughness and hardness is related to the esthetic restorations (discoloration and wear), the medical consequences of periodontal disease, and the development of secondary caries due to increased plaque accumulation. Wear and microleakege are the main limitations of the composite resins in mainly posterior restorations (46). Several research groups have studied the surface characteristics of different restorative materials (11,31,47-49) using contact profilometers, which detect surface irregularities using a stylus moving vertically across the surface. In addition, clinical studies have shown that the rough surfaces

Table 3. *Mean microhardness values and differences within groups*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		p
					Lower bound	UpperBound	
1	10	61,1370	10,10009	3,19393	53,9118	68,3622	0.105
2	10	58,4860	8,32322	2,63203	52,5319	64,4401	
3	10	66,6520	6,43766	2,03577	62,0468	71,2572	
Total	**30**	**62,0917**	**8,83112**	**1,61233**	**58,7941**	**65,3893**	

Table 4. *Mean Roughness (Ra) values and differences within groups*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		p
					Lower Bound	Upper Bound	
1	10	0,8540	0,23153	0,07322	0,6884	1,0196	0.370
2	10	0,7730	0,24459	0,07735	0,5980	0,9480	
3	10	0,7190	0,14579	0,04610	0,6147	0,8233	
4	10	0,7030	0,25347	0,08015	0,5217	0,8843	
5	10	0,7110	0,25723	0,08134	0,5270	0,8950	
6	10	0,8720	0,19921	0,06300	0,7295	1,0145	
Total	60	0,7720	0,22636	0,02922	0,7135	0,8305	

can promote plaque formation and reduce the efficiency of teeth cleaning procedures (50). Bollen et al. reported that a Ra of 0.2 μm or more could result in accumulation of bacterial plaque, thereby promoting the periodontal diseases and carious lesions (12). However, results from this study showed that the mean Ra and microhardness values obtained at the baseline for the experimental and control groups did not differ statistically from each other.

Effective surface sealants should have good surface wettability, a low contact angle, a low viscosity, and good penetration capability. Microgaps may occur between tooth/restoration interface depending on the polymerization shrinkage during restoring the tooth with resin composites. Surface sealants minimize the wear rates of the resins by filling the microdefects on the restorations (46). Therefore, the presence of low molecular weight monomers was found to be essential in dental sealants (47). It was assumed that a surface sealant containing Bis- GMA combined with low molecular weight monomers (TEGDMA and THFMA) would control its desirable characteristics such as viscosity and surface wettability (25). Also fillers were added to some selants to increase their mechanical properties (46).

The wear resistance of composite resins can be enhanced with a surface sealant, as long as it is annually applied. In an in vivo study, the researchers found that the wear values of the sealed restorations after one year were approximately half of those found in the non-sealed restorations

(25). In addition, these low viscosity resins can increase the wear resistance of the tooth/restoration interface in luting indirect restorations (26).

Although relatively smoother surfaces were obtained with the polyester strips, the use of a glazing material after the polishing step resulted in significantly lower Ra values compared to that obtained with the use of polyester strips alone. The glazing material appeared to fill the structural microdefects, thereby providing a more uniform and smooth surface (30). However, some initial investigations demonstrated the degradation of the glazing materials over time, in spite of their resistance to toothbrushing and staining (31,46,51). Therefore, the limitations of this in vitro study have to be developed and improved in terms of aging.

The effectiveness of sealants in improving the smoothness of composite surfaces is still controversial. Although some authors have suggested that sealants might improve the surface finish (30), others have reported no significant reduction in the surface roughness of composites after the simulated toothbrushing abrasion test and also in clinical evaluations after one and five years (25). The complex structure of a surface cannot be fully characterized by the use of only surface roughness measurements (15).

According to Shintani and others, there were no noticeable differences in plaque accumulation among the surfaces polished using different methods, which resulted in Ra values within the range of 0.7-1 μm (52). Chung reported that restorations with less than 1 μm surface roughness appeared to be optically smooth (14).

The inherent surface roughness of composite resins should be equal or lower than the surface roughness of enamel-to-enamel occlusal contact areas (Ra = 0.64 μm). When comparing the roughness values of optimally polished surfaces, mostly the surface roughness values produced by pressing the restorative materials against transparent matrices such as Mylar strips (53).

Thus, very smooth polished surfaces representative of the clinical situation can be obtained using clear matrices. Although the surface obtained with Mylar strip is perfectly smooth, it is rich in resin organic binder (53). Therefore polishing discs were used to mimic the clinic conditions before applying the surface sealant in this study.

It has been reported that a noticeable decrease in mean surface roughness could be achieved within first five seconds of polishing for practically all restorative materials, but a further decrease of the same magnitude could not be obtained with longer polishing times or the application of additional components (54). Thus, one-step polishing systems offer time saving benefit along with reduced roughness when polishing the composite restorations. Based on this fact one-step surface sealant was used when evaluating the resin surfaces in this study.

The first tested hypothesis was rejected because the surface sealant material decreased the surface roughness of composite resins, but there were no significant differences in the roughness values between the experimental and control groups for each restorative material. Different results may be obtained with different polishing techniques and composite resin materials.

Several factors related to the composite resin compositions were shown to affect the surface hardness of the composite restorative materials (54). It was observed that the mass fraction (55,56), size, and distribution of filler particles have significant effects on certain physical and mechanical properties, including surface hardness of the composite resins (57,58). Moreover, other parameters such as filler particle shape and density, monomer type and ratio, degree of crosslinking, and photoinitiators have also shown significant influence on the surface hardness of restorative materials (55,59).

A microhardness test gives information as to the mechanical properties of the material. A positive correlation has been determined between the hardness and inorganic filler content of composites. Increased organic filler levels result in increased hardness values (60,61).

In a study on filler particle size effect, significant high differences were noticed in the VHN (Vickers hardness number) mean values among bulk-fill and incrementally-fill composite resins, either for top or bottom surfaces. The highest VHN value was obtained for the incremental-fill nanohybrid composite (Grandio) compared to that of the two bulk-fill microhybrid composites (X-tra fil and QuiXfil) (62). According to Moszner et al. (16) and Thome et al. (63), the microhybrid composite resins exhibited higher microhardness values than that of the nanohybrid composite resin.

The second tested hypothesis that, there were no significant differences between the microhardness of the composite materials used was accepted. Despite there were no significant differences between the groups tested, group 3 had the highest microhardness values. This could be attributed to the filler particles of (glass microfibres, zirconia, silica and ytterbium trifloride) the Filtek Bulk-fill. Also it can be speculated that bulk-fill resin composites allow more light to penetrate deep inside and which can results in more polymerized monomers. Our finding's in agreement with a previous study (64).

Therefore, further investigation is necessary to evaluate the surface roughness and microhardness values for different composite resins and polishing techniques.

Conclusion

In this study, the highest hardness values were obtained using Filtek Bulk Fill Posterior Restorative (silane treated ceramic, 3M-ESPE, Germany) after the polishing step. The smoothest surfaces were obtained using a surface sealant after the polishing step, Herculite

XRV Ultra showed lower Ra values compared with those of the other restorative materials. No significant differences were found in the surface roughness of selected composite resins sealed with BisCover LV. Similarly, the microhardness values showed no significant differences among different composite resin materials. Hardness value obtained for group 3 is higher but not significantly different compared to that of the groups 1 and 2. As a result, the glazing material showed a negligible effect on the surface roughness values of different polished composite resins. The current generation of composite resins focused on the filler particle size (nano-fill and bulk- fill) have improved the surface properties such as hardness and roughness of restorative materials. Therefore, the use of sealants to improve the smoothness and hardness of these composite restorations is questionable. Longitudinal clinical trials are necessary to validate this hypothesis and provide further insights into the design of composite resins for clinical use.

Türkçe Öz: *Yüzey örtücü materyalin farklı kompozitlerin yüzey pürüzlülüğü ve mikrosertliği üzerine etkisinin değerlendirilmesi. Amaç: Bu çalışmanın amacı, bir yüzey örtücü materyalin, farklı kompozitlerin yüzey pürüzlülüğü üzerindeki etkisini değerlendirmek ve mikro sertlik değerlerini karşılaştırmaktır. Gereç ve yöntem: Altmış adet disk şeklindeki test numunesi hazırlanmış ve 6 gruba ayrılmıştır (n = 10). Gruplar aşağıdaki gibi hazırlanmıştır; Grup 1 (Herculite XRV Ultra), Grup 2 (Beautifil Bulk Restorative) ve Grup 3 (Filtek Bulk Fill Posterior Restorative). Grup 4, 5 ve 6 da sırasıyla grup 1, 2 ve 3'te kullanılan restoratif materyallere ek olarak bir yüzey örtücü materyal (BisCover LV) kullanılmıştır. Daha sonra Grup 1, 2 ve 3'teki disklerin yüzey sertliği ve tüm gruplardaki disklerin yüzey pürüzlülüğü sırasıyla Vickers sertlik testi ve bir profilometre kullanılarak ölçülmüştür. Gruplar arasındaki farklılıkları test etmek için tek yönlü ANOVA kullanılmıştır. Bulgular: Her bir restoratif materyal için deney ve kontrol grupları arasında mikro sertlik ve pürüzlülük açısından istatiksel olarak anlamlı bir fark gözlenmemiştir. Grup 3 en yüksek yüzey sertliğini, grup 4 ise en düşük yüzey pürüzlülüğünü göstermiştir. Sonuç: Cila aşamasından sonra bir yüzey örtücü (BisCover LV) kullanılması farklı kompozitlerin yüzey pürüzlülüğü üzerinde istatistiksel olarak anlamlı bir etkiye sahip değildir. En yüksek sertlik değerleri, cila aşamasından sonra Filtek Bulk Fill Posterior Restorative için elde edilmiştir. Tüm deney grupları için cila aşamasından sonra yüzey örtücü (BisCover LV) kullanılarak en pürüzsüz yüzeyler elde edilmiştir. Herculite XRV Ultra diğer materyallere göre daha düşük bir ortalama pürüzlülük değeri vermiştir. Anahtar kelimeler: Pürüzlülük; mikro sertlik; rezin kompozit; yüzey örtücü materyal, mekanik özellikler.*

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Author contributions: OG, AC and BD designed the study. OG, AC, BD and MME participated in generating the data for the study. AO and MME participated in gathering the data for the study. OG, AC and AO participated in the analysis of the data. OG and BD wrote the majority of the original draft of the paper. OG, AC and AO participated in writing the paper. All authors approved the final version of this paper.

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Original research

The association between denture care and oral hygiene habits, oral hygiene knowledge and periodontal status of geriatric patients wearing removable partial dentures

Zeynep Turgut Cankaya[1], Aysegul Yurdakos[2], Pelin Gokalp Kalabay[3]

Purpose
This study aims to evaluate the association of the denture care with oral hygiene habits, knowledge and periodontal status geriatric patients wearing removable partial denture (RPD) and to compare the relationship between denture cleaning and tooth brushing frequency.

Patients and Methods
In total, 553 participants wearing RPD for at least 6 months and aged 65–86 years were asked to complete a questionnaire following the clinical examination. Chi-square analysis and Fisher's exact test utilized to analyze the data.

Results
A significant relationship was found between the frequency of smoking, denture age, overnight denture wearing, denture stomatitis and denture cleaning ($p<0.05$). 54.10% of the participants reporting that they did not have information about denture care had poor denture cleaning. Among patients brushing once per day, 26.34% had a good level of denture cleaning; whereas, this was 39.40% for those brushing three times in a day.

Conclusion
The type of advice (verbal or written) and oral hygiene habits demonstrated a more significant impact on the cleaning level of dentures than the socioeconomic level, smoking, overnight use, denture age. The success of denture maintenance might depend on the patients' knowledge of denture care and hygiene habits and geriatric patients' motivation.

Keywords: *Geriatrics; knowledge; oral hygiene; removable dentures; toothbrushing*

Introduction

Edentulousness is a multi-factorial phenomenon that has an impact on the quality of life of individuals (1-4). The maintenance of the continuity of the mastication system and elimination of aesthetic and phonetic problems require a successful restoration and rehabilitation. A removable partial denture (RPD) is intended for partially edentulous patients who cannot have a bridge or an implant due to lack of required teeth to serve as bridge support, high cost and so forth and who want to have replacement teeth for function. Out of various treatment strategies for partial edentulism such as implant- and tooth-supported fixed prostheses, a well-designed partial removable dental prosthesis (PRDP) is still one of the mostly performed treatment modalities (5,6) fulfilling the needs of millions of individuals worldwide (7-9) or applied for aesthetic reasons. Regular oral and denture hygiene habits play important role in maintenance of oral health and long- term use of removable prostheses (3-10).

ORCID IDs of the authors: Z.T.C. 0000-0002-9960-5290; A.Y. 0000-0002-8706-6445; P.G.K. 0000-0002-1263-6183

[1]University of Gazi, Faculty of Dentistry, Department of Periodontology, Ankara, Turkey

[2]Okmeydanı Oral and Dental Health Hospital, İstanbul, Turkey

[3]Private Periodontist, Istanbul, Turkey

Corresponding Author: Zeynep Turgut Cankaya

E-mail: zeynepturgut@gazi.edu.tr

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The awareness and motivation of RPD wearers to maintain a high level of hygiene of residual teeth are extremely important to maintain the health and integrity of teeth and periodontal tissues (11).

A great number of geriatric patients need dental care including RPD. Researches have shown that older people do not clean their dentures properly and do not acquire adequate oral hygiene habits (12-16) due to a number of factors such as social status, age, education, systemic diseases, and smoking (12-15). Also lack of information about the maintenance of oral health and periodic recalls play an essential role in above mentioned situation.

Previous studies have shown a correlation between RPDs and increased risk of periodontal diseases (17, 18). Therefore, proper denture use and care constitutes important component not only for functional and aesthetic reasons, but also for the health of the supporting periodontal tissues and appropriate maintenance of the denture itself for RPD wearers (19). Akaltan et al. concluded that adequate oral hygiene and regular systemic controls can improve periodontal health of patients with RPD (20). The purpose of this study is to determine the relationship between smoking, denture age, denture stomatitis, overnight denture wearing habits, knowledge of denture hygiene maintenance, and oral hygiene habits with denture cleaning among the geriatric population using RPDs. This study also aims to evaluate education and socioeconomic levels of geriatric patients wearing RPD.

Materials and Methods

In total, 553 partial denture wearers aged 65–86 years (mean age 73 ± 11) and using prostheses for at least 6 months completed a questionnaire following clinical examination at the Okmeydanı Oral and Dental Health Hospital, Istanbul, Turkey (Figure 1). The protocol of this study was approved by the Okmeydanı Training and Research Hospital Ethics Committee **(No.838).** This study was performed in compliance with the Declaration of Helsinki.

This study was conducted among geriatric patients applied to the departments of the prosthodontics and periodontology, who already have RPDs. They were informed about the research project and gave their consent in writing.

The subjects were interviewed by experienced single periodontologist using a structured questionnaire which sought to identify gender, educational status, socioeconomic level, smoking habits, frequency of visiting a dentist, denture age, denture stomatitis, overnight denture wearing, denture cleaning frequency, methods and status of ever being informed by dentist about denture hygiene maintenance and oral hygiene habits (PGK).

SECTION 1: *For Patients*

1. Smoking status
   Yes ( ) No ( )
2. How long have you been using this denture?
   1 year or less ( ) 1-5 years ( ) More than 5 years ( )
3. Do you have any prosthesis-related oral mucosal lesions?
   Yes ( ) No ( )
4. Do you remove your dentures overnight?
   Yes ( ) No ( )
5. How many times do you clean your dentures daily?
   Once a day ( ) Twice a day ( ) Three times a day ( ) Over three times a day ( )
6. Have you ever received any instruction from your dentist on how to clean your dentures?
   Yes ( ) No ( )
7. If yes, in what form were the instructions given?
   Verbal ( ) Written ( ) Both verbal and written ( ) Other ( )
8. How many times a day do you brush your teeth?
   Once a day ( ) Twice a day ( ) Three times a day ( ) Never ( )
9. When you brush your teeth, do you have gingival bleeding?
   Yes ( ) No ( )
10. Have you ever received periodontal treatment?
    Yes ( ) No ( )

SECTION 2: *For Clinicians*

1. Age:
2. Gender:
3. Income:
   Poor ( ) Low ( ) Middle ( ) High ( )
4. Educational Level:
   No schooling degree ( ) Lower secondary school ( ) Intermediate secondary School ( ) High school diploma( )
5. Oral hygiene level:
   Poor ( ) Fair ( ) Good ( )
6. Cleaning of dentures:
   Poor ( ) Fair ( ) Good ( )

Figure 1. *Questionnaire*

The level of prosthesis hygiene was assessed using the Budzt-Jorgensen & Bertram method (21) by an another experienced single prosthodontist (AK) and ranked in categories, namely, good (no plaque or calculus), fair (plaque or calculus covering less than one-third of the prosthesis), or poor (plaque and calculus covering one-third or more of the prosthesis). Prosthesis types were evaluated on the basis of the previous study where oral mucosal lesions among denture wearers was examined (22), and classified as partially removable dental prosthesis in the maxilla and/or mandible. The denture age of prosthesis was categorized in three groups as less than one year (<1 year), between one and five years (1–5 years), equal to and greater than five years (≥5 years). The level of education was classified as illiterate, primary school, secondary school and higher levels; whereas, income level was categorized as poor, low-, middle- and high-income patients. In this study, gingival bleeding was evaluated on the basis of existence of bleeding during tooth brushing. The participants were asked whether they had undergone periodontal treatment before the date of this study.

Statistical analysis

The data was analyzed with SPSS 20 statistical package (SSS Inc., USA).

Ki-square analysis was applied to reveal the relationship between groups of nominal variable. In case where sufficient volume could not be obtained as per 2x2 table cells, Fisher's Exact Test was used. Furthermore, RxC tables were verified with Pearson Ki-Square analysis by using Monte Carlo simulation.

The level of significance was identified as 0.05 during interpretation of the results according to which $p<0.05$ shows meaningful relationship and $p>0.05$ indicates that any meaningful relationship does not exist.

Results

In this study, 51.9% of the subjects were females and 48.1% was male (Table 1). In terms of level of education, 14.47% of participants was illiterate, 63.65% completed primary school education, 10.13% had secondary school degree, and 11.75% held higher education degree (Table 1).

Table 1. *Distribution of demographic characteristics of the subjects*

		n	(%)
Gender	Female	287	51.9
	Male	266	48.1
	Total	553	100
Educational level	Illiterate	80	14.47
	Primary school	352	63.65
	Secondary school	56	10.13
Income	Higher	65	11.75
	Total	553	100
	Poor	103	18.63
	Low	223	40.33
	Moderate	173	31.28
	High	54	9.76
	Total	553	100

While 40.33% of the participants stated that they had low income level, 31.28% and 9.76% reported they had moderate and high individual income levels respectively (Table 1).

Significant relationship was found between the frequency of smoking habits and denture cleaning ($p<0.05$). 50% of the smoking participants and 31.39% of the non-smokers had poor denture cleaning (Table 2).

Furthermore, it was found that denture cleaning was directly associated with the period of time participants used their RPDs, overnight use of RPDs and denture stomatitis ($p<0.05$). Among the participants who reported they had been wearing RPDs less than 1 year, 30.15% had a good level of denture cleaning; whereas, this rate was found as 20% among those wearing RPDs more than 5 years. As for the participants removing their RPDs before sleep and continuing to wear RPDs overnight, 34.26% and 41.24% had poor denture cleaning respectively. While 41.25% of patients with denture stomatitis had poor denture cleaning, only 15% of them had a good level of denture cleaning (Table 2).

In addition, significant relationship was found between the information provided to the wearers about denture care and denture cleaning. Of the participants reported that they did not have information about denture care, 54.10% had poor denture cleaning and 16.39% had good denture cleaning (Table 2).

17.65% of the participants who stated they only received written instruction on denture care and 27.73% whom verbal instruction was given had good level of denture cleaning; whereas, 28.24% of those provided with both verbal and written instruction exhibited good level of denture cleaning (Table 2).

There is also significant relationship between the denture cleaning and the frequency of brushing remaining teeth. 26.34% of the participants brushing once in a day and 36.90% of those brushing three times per day had good level of denture cleaning. Among patients with good level of prosthesis hygiene, 97.08% revealed good level of oral hygiene. On contrary, 92.51% of the patients with poor level of prosthesis hygiene had poor level of oral hygiene (Table 2).

Discussion

Hygiene habits, behaviors and knowledge about the denture care of geriatric RPD wearers have been the subject of researches over the years (23-29). Several factors that may be related to the level of denture cleaning were approached by the questionnaire, within the scope of which gender, education, socioeconomic level, smoking habits, denture age, denture stomatitis, overnight denture wearing, frequency of visiting dentist, knowledge about denture hygiene maintenance and oral hygiene habits were examined (29). The present study was undertaken to determine knowledge about the use and care of partial denture among geriatric patients.

In general, the education and socio-economic levels of geriatric patients are associated with their oral health condition. A previous study has demonstrated that the education level and socio-economic stability is positively associated with interest in oral health (30). The present study mainly

Table 2. *Relationship of prosthesis hygiene level with oral hygiene knowledge, habits and periodontal status of subjects*

		Prosthesis hygiene level								Chi square test	
		Good		Fair		Poor		Total			
		n	(%)	n	(%)	n	(%)	n	(%)	Chi square	p
Smoking status	Yes	28	17.72	51	32.28	79	50.00	158	100	17.991	0.001
	No	116	29.37	155	39.24	124	31.39	395	100		
	Total	144	26.04	206	37.25	203	36.71	553	100		
Denture age	1 year or less	61	33.15	52	28.26	71	38.59	184	100	16.92	0.002
	1-5 years	33	27.73	53	44.54	33	27.73	119	100		
	More than 5 years	50	20.00	101	40.40	99	39.60	25	100		
	Total	144	26.04	206	37.25	203	36.71	553	100		
Denture stomatitis (Prosthesis-related oral mucosal lesions)	Yes	24	15.00	70	43.75	66	41.25	160	100	14.355	0.001
	No	120	30.53	136	34.61	137	34.86	393	100		
	Total	144	26.04	206	37.25	203	36.71	553	100		
Overnight denture wearing	Yes	106	29.53	130	36.21	123	34.26	359	100	6.744	0.034
	No	38	19.59	76	39.18	80	41.24	194	100		
	Total	144	26.04	206	37.25	203	36.71	553	100		
Frequency of denture cleaning	Once a day	63	31.37	73	36.87	63	31.82	198	100	9.42	0.151
	Twice a day	34	30.28	46	42.20	30	27.52	109	100		
	Three times a day	34	25.58	40	31.01	56	43.41	129	100		
	More than three times	13	21.43	22	39.29	22	39.29	56	100		
	Total	144	28.46	181	36.79	171	34.76	553	100		
Received instructions	Yes	134	27.24	188	38.21	170	34.55	553	100	9.202	0.01
	No	10	16.39	18	29.51	33	54.10	61	100		
	Total	144	26.04	206	37.25	203	36.71	553	100		
If yes, in what form?	Written	3	17.65	9	52.94	5	29.41	17	100	*	0.003
	Verbal	33	27.73	43	36.13	43	36.13	119	100		
	Both written and verbal	98	28.24	136	39.19	113	32.56	347	100		
	Other	0	.00	0	.00	9	100.00	9	100		
	Total	134	27.24	188	38.21	170	34.55	553	100		
Frequency of brushing remaining teeth	Once a day	59	26.34	98	43.75	67	29.91	224	100	36.531	0.001
	Twice a day	42	28.00	65	43.33	43	28.67	150	100		
	Three times a day	31	36.90	25	29.76	28	33.33	84	100		
	Never	0	.00	0	.00	14	100.00	14	100		
	Total	132	27.97	188	39.83	152	32.20	472	100		
Gingival bleeding	Yes	56	23.14	86	35.54	100	41.32	242	100	4.223	0.121
	No	88	28.30	120	38.59	103	33.12	311	100		
	Total	144	26.04	206	37.25	203	36.71	553	100		
Periodontal treatment	Yes	24	24.24	37	37.37	38	38.38	99	100	0.242	0.886
	No	120	26.43	169	37.22	165	36.34	454	100		
	Total	144	26.04	206	37.25	203	36.71	553	100		
Oral hygiene level	Poor	0	.00	14	7.49	173	92.51	187	100	809.022	0.001
	Fair	11	4.80	188	82.10	30	13.10	229	100		
	Good	133	97.08	4	2.92	0	.00	137	100		
	Total	144	26.04	206	37.25	203	36.71	553	100		

* Chi square analysis was performed with the help of Monte Carlo Simulation.

includes the population with low socioeconomic and low education levels. The positive correlation between the prosthesis and oral hygiene indicates that denture cleaning is relevant with the level of knowledge of periodontal care. According to these findings, the level of denture cleaning of participants' RPDs was not sufficiently qualified.

Smoking and its relation with the oral hygiene level have been subjects of many articles. Previous reports in literature support that smoking deteriorates oral hygiene (31). Findings of this study concerning higher percentage of good oral and denture hygiene among non-smokers compared to the smokers are consistent with the outcomes revealed by several previous studies (31, 32).

The behavior of the dentists and dental staff is the primary tool guiding the behavior of the geriatric patients. The attitude, body language and communication skills of the dentist are critical to create positive dental visit experience. This study revealed the correlation between the level of cleaning of participants' RPDs and the manner in which patients were advised concerning the care of their denture. 70.53% of the patients who received both had better denture cleaning in comparison with the patients provided only with verbal instructions. In accordance with the result of previous studies, this result shows the importance of giving detailed information to patients (17, 33-35).

Geiballa et al. showed that the majority of dentists did not pay attention to the post treatment instructions concerning the maintenance of fixed prosthesis (36).

It is important to have regular dental visits and follow patients which will allow monitoring of patient oral health. Regular calls maintain good level of cleaning of participants' RPDs (35) due to the fact that updated verbal and written instructions are provided (28, 37).

In the geriatric population studied, 33.15% of the participants wearing their RPDs less than 1 year had good level and 38.59% had poor level of denture cleaning. 20% of the participants using RPDs more than 5 years had good level and 39.6% had poor level of denture cleaning. This study also presented that denture age had adverse effect on level of cleaning of dentures. It was claimed that patients gave more attention to clean their dentures after they were provided with updated instruction. In compliance with the results of previous studies, these results showed that a large number of patients had to be informed about denture cleaning and care (27, 30).

This study also revealed significant relationship between overnight denture use and the level of cleaning of dentures ($p < 0.05$). The result of this study showed that subjects wearing their RPDs overnight had poor level of denture cleaning. A previous study reported that wearing partial denture continuously resulted in more plaque accumulation compared to those using the partial denture only during the day (38). Some studies revealed that denture stomatitis was associated with failure to remove denture overnight (23-25). According to the previous studies, the rate of denture stomatitis differed between 15 and 71%, and the prevalence of denture stomatitis had strong relationship with denture hygiene and denture plaque amount (39, 40). Furthermore, higher rate of denture stomatitis was found among patients with poor and fair level of denture cleaning compared to the patients with good level of denture cleaning ($p < 0.05$).

RPDs may increase the risk of caries, damage on periodontium and the amount of stress on natural teeth due to poor oral hygiene, increased plaque and calculus accumulation, and transmission of excessive forces from occlusal surfaces of the frame of RPDs to the periodontal structures. Adverse impacts of the removable partial dentures on the periodontium can be eliminated, if good oral hygiene is maintained. Ideally, partial denture wearers should brush their remaining teeth after every meal by which periodontal health of the remaining dentition can be maintained. On the other hand, RPDs of the patients whom professional tooth cleaning was applied regularly have only minor adverse effects on the periodontium (19, 41). Shigeto *et al*. showed that RPS-wearing patients who received periodic maintenance care 4 times/year were effective to maintain good periodontal conditions (42).

Dula et. al. assessed education, motivation and awareness of the patients during the stage of RPD's construction and concluded that planned prosthetic treatment with an appropriate design and good oral hygiene could reduce the possibility of occurrence of periodontal disease of abutment teeth (43).

This study further revealed that good level of cleaning of denture was maintained among geriatric patients, who brushed their remaining teeth three times a day. In this framework, it can be recommended that periodontal disease should be eliminated before construction of partial dentures, and the natural teeth should be prepared to provide stability and support for the denture.

The oral cavity offers ideal bacteria breeding area and those affected with periodontal disease are exposed to increased risk of potentially fatal bacteria that enter the bloodstream via infected oral tissue. According to the present study, periodontal treatment before prosthetic construction had no effect on the level of denture cleaning. The reason is that geriatric patients may not correct oral hygiene habits depending on the inadequacy of hand skills. In this study, overnight denture wearing condition was considered; whereas, in-water storage of RPD, which affects periodontal health and denture cleaning, was not analyzed. Therefore, it is recommended that further studies should question whether patients keep their RPS within the water. As xerostomia and the type of brushing method have also impact on the oral hygiene and periodontal health, further studies should also take these two factors into consideration within the scope of their examinations in relevant field.

Conclusion

It could be concluded that the level of cleaning of dentures is associated with giving detailed information to the geriatric patients about how to use their RPDs, type of advice (verbal or written), regular dental visits, good oral hygiene, smoking habits, socioeconomic level, overnight use and denture age. Furthermore clinicians play an important role on maintenance of patient motivation. It is possible to prevent the periodontal diseases by providing detailed information about the oral hygiene habits to the patients and having them maintain adequate oral and denture hygiene. Consequently the clinicians should advice and motivate the patient in RPD maintenance and oral hygiene procedures.

Further longitudinal studies are needed to better evaluate the effect of periodontal treatment before prosthetic construction in geriatric population.

Türkçe Öz: *Hareketli bölümlü protezi olan geriatrik hastalarda oral hijyen alışkanlıkları, periodontal durum ve oral hijyen bilgisi ile protez bakımı arasındaki ilişkinin değerlendirilmesi. Amaç: Bu çalışmanın amacı hareketli parsiyel protez kullanan geriatrik hastalarda oral hijyen alışkanlıkları ve oral hijyen bilgisi ile protez bakımı arasındaki ilişkinin değerlendirilmesidir. Bu çalışma aynı zamanda protez temizliği ile diş fırçalama sıklığı arasındaki ilişkinin değerlendirilmesini de amaçlamaktadır. Hastalar ve yöntem: 65-86 yaşları arasında en az 6 aydır parsiyel protez kullanan toplam 553 hastaya klinik değerlendirmeyi takiben anket yapılmıştır. İstatistiksel değerlendirme için Chi- square analizi ve Fisher's Exact testi kullanılmıştır. Bulgular: Sigara kullanımı sıklığı, protez yaşı, gece protez kullanımı, protez stomatiti ve protez bakımı arasında önemli bir ilişki bulunmuştur (p<0.05). Protez bakımı ile ilgili bilgisi olmadığını belirten hastaların %54.10'unun protez bakım seviyesi zayıf düzeyde bulunmuştur. Günde 1 defa dişlerini fırçaladıklarını belirten hastaların %26.34'ünün protez bakım düzeyinin iyi seviyede olduğu bulunurken dişlerini günde 3 defa fırçaladığını belirten hastaların %39.4'ünün de protez bakım düzeyinin iyi seviyede olduğu bulunmuştur. Sonuç: Hastalara verilen bilgilendirmenin ne şekilde olduğunun (yazılı veya sözlü) ve hastaların ağız hijyeni alışkanlıklarının protez bakım düzeyleri üzerine etkisinin sosyoekonomik seviye, sigara kullanımı, gece protez kullanımı, protez yaşı gibi faktörlerden daha önemli olduğu gösterilmiştir. Anahtar Kelimeler: ağız hijyeni; bilgilendirme; geriatri; hareketli bölümlü protez; diş fırçalama.*

Ethics Committee Approval: The protocol of this study was approved by the Okmeydanı Training and Research Hospital Ethics Committee **(No.838).** This study was performed in compliance with the Declaration of Helsinki.

Informed Consent: The informed consents were provided by the participants.

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Original research

The effects of cavity disinfection on the nanoleakage of compomer restorations: an in vitro study*

Ipek Arslan[1], Ozgul Baygin[2], Tamer Tuzuner[2], Fatih Erdemir[3], Aykut Canakcı[3], Fatih Mehmet Korkmaz[4]

Purpose
Cavity disinfection, in addition to routine caries removal methods, is recommended to eliminate the microorganisms. The aim of this study was to compare the effect of various systems Er,Cr:YSGG lasers, diode lasers and FotoSan and agents Corsodyl; Cervitec and Cervitec Plus and Fluor Protector—on the nanoleakage of compomer restorations when used for cavity disinfection.

Materials and Methods
A total of 40 intact human deciduous molar teeth containing Black V cavities (3×2×1.5 mm) on the buccal and lingual surfaces parallel to the cementoenamel junction were randomly divided into 8 groups according to the cavity disinfection methods. The antibacterial agents and systems were applied according to the manufacturer's instructions. Restorations were completed using a compomer. The restored teeth were then subjected to thermocycling for 500 cycles in a water bath at 5°C and 55°C with a dwell time of 30 seconds. After the thermocycling procedures, 1-mm sticks were obtained from the center of each cavity to prepare for the nanoleakage test. After the teeth were sectioned, they were immersed in 50 wt % ammoniacal silver nitrate solution for 24 hours and dipped in photo-developing solutions for 8 hours with fluorescent light irradiation. The samples were examined under a scanning electron microscope (SEM). The non-parametric Kruskal-Wallis and Mann-Whitney U test ($p<0.05$) were applied.

Results
The Er,Cr:YSGG laser group showed significantly less nanoleakage than all of the tested groups ($p<0.01$). The diode laser, Fluor protector and FotoSan groups showed similar nanoleakage to that of the control group ($p>0.05$). The Corsodyl ($p<0.01$) and Cervitec ($p<0.001$) groups showed significantly higher nanoleakage than the control group.

Conclusion
Er,Cr:YSGG laser irradiation which showed lower nanoleakage scores from either control or tested groups can be recommended for cavity disinfection Additionally, a diode laser and FotoSan, which have antibacterial effects and no negative effect on leakage, can be used for cavity disinfection.

Keywords: *Er,Cr:YSGG laser; Diode laser; FotoSan; Antibacterial agents; Nanoleakage*

Introduction

Traditional restorative dentistry aims to remove all infected tooth structures and obturate the area with biocompatible filling materials (1). However, none of the currently used caries removal methods eliminate all of the microorganisms in the cavities consistently (2). Several studies have shown the existence of bacteria in dentin even after using caries detector dyes (3,4). Researchers have proved that fermentative microorganisms remained viable under non- antiseptic restorations for as long as 139 days (5).

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ORCID IDs of the authors: I.A. 0000-0002-8648-3554; O.B. 0000-0003-2220-7654; T.T. 0000-0001- 5817-5928; F.E. 0000 0002 0449 9801; A.C. 0000 0001 5244 6467; F.M.K. 0000-0001-9324-2014

[1]*Recep Tayyip Erdoğan University, Faculty of Dentistry, Department of Pediatric Dentistry, Rize, Turkey*

[2]*Karadeniz Technical University, Faculty of Dentistry, Department of Pediatric Dentistry, Trabzon, Turkey*

[3]*Karadeniz Technical University, Faculty of Engineering, Department of Metallurgical and Materials Engineering, Trabzon, Turkey*

[4]*Karadeniz Technical University, Faculty of Dentistry, Department of Prosthodontic, Trabzon, Turkey*

Corresponding Author: Ipek Arslan

E-mail: sareipekarslan@gmail.com

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Thus, cavity disinfection, in addition to routine caries removal methods, is recommended to eliminate the microorganisms and reduce potential secondary caries, pulp sensitivity and pulp inflammation before restoring the cavities (6, 7).

The treatments of carious lesions has been changing as the knowledge about the caries process has increased (8). Some authors have advised that new approaches should remove only infected dentin and provide an opportunity for the affected dentin to undergo remineralization (8,9). The main problem with this approach is the remaining cariogenic bacteria. Thus, cavity disinfection has gained importance with new approaches (8,10).

In the literature regarding cavity disinfection, various systems and agents are suggested. Understanding the disinfection mechanisms and their effects on the sealing ability of restorative material is essential in the selection of disinfection methods (11).

Chlorhexidine (CHX) is a commonly used cavity disinfection agent in dental procedures. Its disinfection effect occurs upon its binding to the amino acids in microorganisms, and its effects can continue for several hours. Due to these properties, CHX is an excellent antibacterial agent (12,13). However, in the literature, there is disagreement concerning the effects of chlorhexidine on the sealing ability of resin restorative materials. Some researchers have argued that chlorhexidine has no adverse effects on bond strength and leakage (14,15). Conversely, some studies have reported that chlorhexidine increases leakage scores (16,17).

Currently, combinations of chlorhexidine with other antibacterial agents—e.g., fluoride or thymol—are commercially available. In the present study, Corsodyl gel containing 1% CHX digluconate, Cervitec gel (combination of 0.2% CHX digluconate and sodium fluoride gel) and Cervitec Plus Varnish (combination of 1% CHX diacetate and 1% Thymol) were used. Many studies have reported that these combinations showed less antibacterial activity than CHX (18,19). Wallman *et al.* (19) reported that CHX gel was more effective in reducing MS in saliva compared with Cervitec. The other study showed that CHX-containing dentifrice was more effective in reducing MS in saliva compared with Cervitec (19).

Fluoride is the most popular anticaries agent in dentistry. Its antibacterial activity has been demonstrated many times (20-22). Fluoride is not designed specifically for cavity disinfection, but some properties of fluoride such as its ability to inhibit active growth of cariogenic bacteria, remineralize the affected dentin, and increase the microhardness of dentin (8, 23) were thought to make it usable as a cavity disinfectant. Some recent studies have tested it for cavity disinfection (8, 24).

Photoactivated disinfection (PAD), also known as antimicrobial photodynamic therapy, is a disinfection method that can be used in both restorative and endodontic treatment to eliminate microorganisms. Its disinfection principle is based on a photosensitiser, which is irradiated by a specific wavelength of light (25). After irradiation, singlet oxygen is produced that causes bacterial cell wall rupture and faster antibacterial effects (26). Previous studies have demonstrated the reduction by 95- 99.9% of the viable cell count with PAD (27,28).

Laser therapy is a disinfection system that is effective against oral bacteria, associated or not with a photosensitiser. The antibacterial action of a laser is related to its thermal effects and photodisruption (29). Despite its well-known antibacterial action, studies concerning the use of lasers for cavity disinfection are limited. One previous study proved its effectiveness against caries-related bacteria (30).

The ideal cavity disinfectant should provide both strong antimicrobial action and not interfere with the sealing ability of restorative materials (31). When the sealing ability is disrupted, marginal leakage may occur. The occurrence of leakage between restorative material and teeth may decrease the longevity of restoration (32). Nanoleakage is described as the diffusion of nanoscale ions or molecules in the hybrid layer of the restoration (33). Silver nitrate (AgNO3), which is detectable by both SEM and TEM, is used to evaluate nanoleakage (34).

The aim of this study was to compare the effect of various systems—Er,Cr:YSGG lasers, diode lasers and FotoSan, which is a PAD system—and agents—Corsodyl; Cervitec and Cervitec Plus, which contains CHX in their combination; and Fluor Protector—on the nanoleakage of compomer restorations when used for cavity disinfection. The null hypotheses tested were as follows: 1) The systems and agents that were used in the study would have no effect on nanoleakage; 2) nanoleakage would not differ between the systems or agents.

Materials and Methods

Specimen preparation

Ethical approval of the present study was obtained from the Ethics Committee of Karadeniz Technical University, Faculty of Medicine (Protocol # 2015/149). A total of 40 intact human deciduous molar teeth extracted for exfoliation or orthodontic reasons were collected and cleaned with pumice. The teeth were stored in 0.5% Chloramine T aqueous solution following the extraction. Standardized class V cavities (3×2×1.5 mm) were prepared on the facial and lingual/palatinal surfaces of each tooth with a diamond bur (Diatech Swiss Dental Instruments, Switzerland; 881-012-8 ml), parallel to the cementoenamel junction. Next, the teeth were randomly divided into eight experimental groups of 10 teeth, each according to cavity disinfection method. The antibacterial agents and systems were applied according to the manufacturer's instructions (Table 1).

Restoration

After disinfection, all samples were restored with a compomer (Dyract eXtra, Dentsply, Germany) according to the manufacturer's instructions. Prime&Bond NT (Dentsply, Germany) were used as bonding agent. Finishing was achieved by using flexible polishing discs. The restored teeth were then subjected to thermocycling for 500 cycles in a water bath at 5°C and 55°C with a dwell time of 30 seconds. After the thermocycling procedures, 1-mm sticks were obtained from the center of each cavity to prepare for the nanoleakage test.

Preparation for nanoleakage test

Two layers of nail varnishes were applied to sticks up to 1 mm from the restoration margins. The specimens were then

Table 1. *Agents and systems used for cavity disinfection and application forms*

Agents-Systems	Application Forms
Group 1: Control	• No disinfection process applied.
Group 2: Corsodyl (GlaxoSmithKline USA)	• 1% CHX Digluconate gel was applied to the dentin for 1 minute • Excess gel was removed from the cavity with a clean cotton pellet
Group 3: Cervitec Gel (Ivoclar, Schaan, Liechtenstein Germany)	• A combination of 0.2% CHX Digluconate and Sodium Fluoride gel was applied to the dentin for 2 minutes.
Group 4: Cervitec Plus (Ivoclar, Schaan, Liechtenstein)	• A combination of 1% CHX Diacetate and 1% thymol varnish (Ivoclar Vivadent) was applied to the dentin for 2 minutes.
Group 5: Fluor Protector (Ivoclar, Schaan, Liechtenstein)	• A 1% difluorosilane varnish was applied to dentin for 1 minute.
Group 6: PAD (FotoSan, CMS Dental, Denmark)	• A fotosensitiser containing 0.01% toluidine blue was applied to the dentin. • The teeth were irradiated with red light (660 nm wavelength and 100 mW
Group 7: Diode laser (Biolase, San Clemente, CA)	• The dentin surfaces were irradiated with a diode laser with a wavelengths of 940 nm, 1- W power output, and 20-Hz frequency. • A sapphire tip, 600 µm in diameter and 6 mm in length was used to deliver the laser light.
Group 8: Er:Cr;YSGG laser (Waterlase MD; Biolase, San Clemente, CA)	• The dentin surfaces were irradiated with an Er,Cr:YSGG laser with a wavelength of 2780 nm, 1-W power output, and 20-Hz frequency. • A sapphire tip, 600 µm in diameter and 6 mm in length, was used to deliver

immersed in 50 wt% AgNO3 solution in the dark chamber according to Tay *et al.* (35) for 24 hours and then were rinsed with running water for 5 minutes, dipped in photodeveloping solutions for 8 hours with fluorescent light irradiation to reduce the silver or diamine silver ions to metallic silver (36) and again washed with running water for 5 minutes.

SEM/EDX and Elemental Mapping analyses

The sticks were embedded into acrylic resin prior to polishing. The specimens were polished with descending grits of silicone carbide papers (600, 1200 and 2500) and diamond polishing paste then conditioned with 5% phosphoric acid for 5 sec and immersed in ethanol solution (70%) for 10 sec. They were coated with a thin layer of gold (sputtering) and analyzed using SEM in the backscattered mode. Quantitative analyses of AgNO3 uptake into the hybrid layer were performed as a percentage with EDX analyses. Elemental mapping of the samples was performed using SEM-EPMA. The elements in the samples were marked with different colors.

Statistical analysis

Statistical analyses were performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, III, USA). Shapiro-Wilks test was used to evaluate the distribution of the data. The Non-parametric Kruskal-Wallis and Mann-Whitney U tests ($p<0.05$) were applied. The group that caused the difference was identified with the Mann Whitney U test.

Results

AgNO3 accumulations in all samples were seen in SEM images, EDX and elemental mapping analyses. In some SEM images, cracks were visible in the materials but this was not

Table 2. *The effect of the agents and systems used in the study on the nanoleakage values of compomer restorations*

	Ag (%) Mean Value	Standard Deviatio	Ag (%) Max Value	Ag (%) Min Value	Comparison with control
Group 1	42.7[b,c]	5.75	53	35	------
Group 2	73.4[a,c]	11.06	80	45	0,000
Group 3	58.1[a,b,c,d]	10.35	67	42	0,001
Group 4	49.5[b,c]	14.21	67	29	0,126
Group 5	48.0[b,c]	15.01	67	22	0,286
Group 6	37.9[b,c]	12.14	54	21	0,692
Group 7	43.0[b,c]	4.05	53	39	0,378
Group 8	20.5[a,b]	5.19	26	15	0,000

Kruskal Wallis and Mann-Whitney U multiple comparison test were used with the significance level of 0.05.
[a]Groups that are statistically different from the Group 1 ($p<0.01$), [b]Groups that are statistically different from the Group 2 ($p<0.01$),
[c]Groups that are statistically different from the Group 8 ($p<0.01$), [d]Groups that are statistically different from the Group 6 ($p=0,001$)

taken into consideration as an important result because the samples were subjected to vacuum conditions. SEM images and elemental mapping showed that AgNO3 uptake were generally noted at the base of the hybrid layer.

The resin-dentin interfaces in the eight groups were analysed with SEM-EDX; AgNO3 deposition was observed. Eight groups were analysed using line scanning: Si, C, and Ca element peaks were detected. The means, minimum and maximum uptake values (%) of AgNO3, standard deviation and p values compared with the control group are shown in Table 2. The Corsodyl ($p<0.01$) and Cervitec ($p<0.001$) groups showed significantly higher nanoleakage than the control group. The Er,Cr:YSGG laser group showed significantly less nanoleakage than the control group ($p<0.001$).

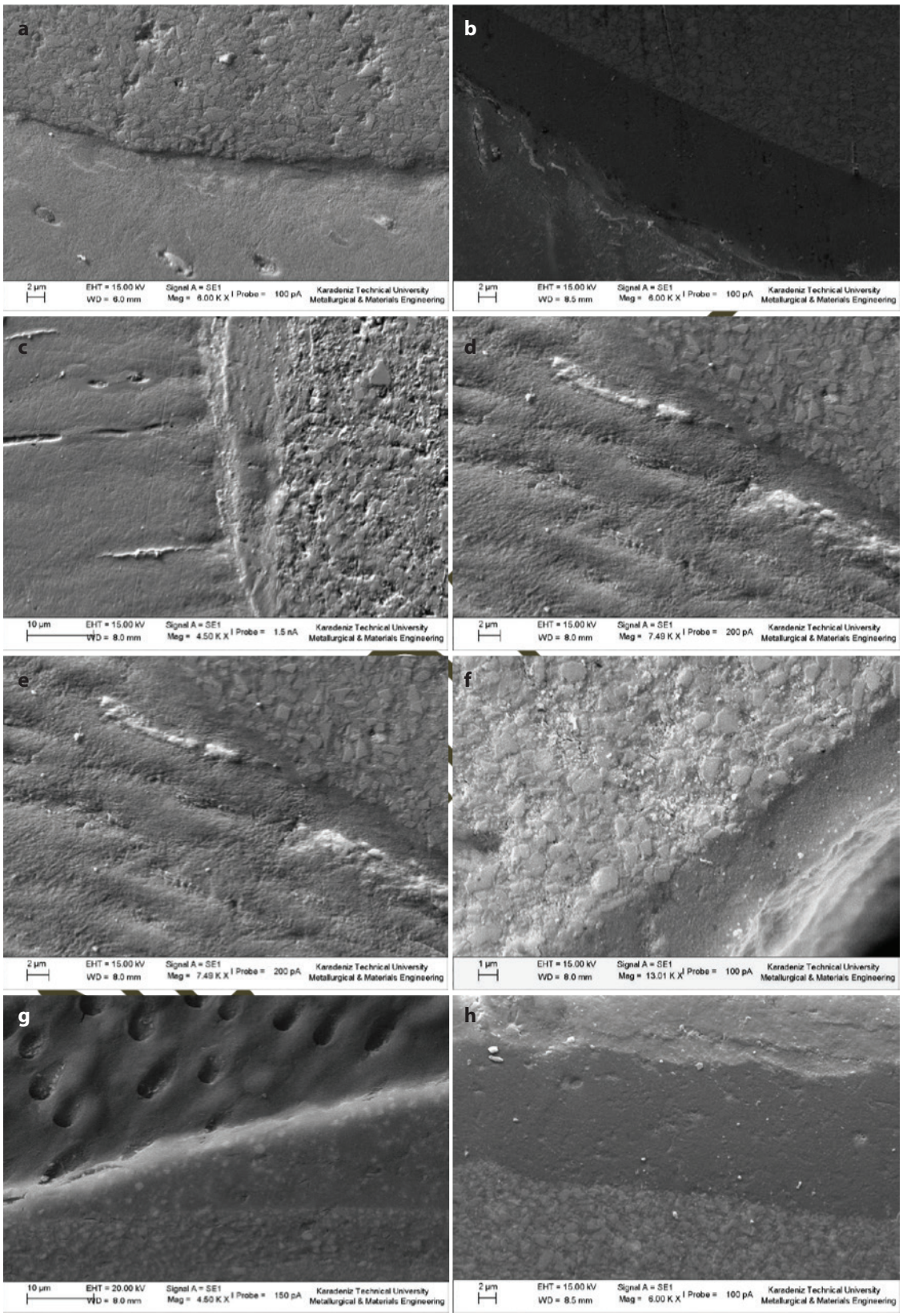

Figure 1. *Representative backscattered SEM images of groups. a: Control group, b: Corsodyl group, c: Cervitec group, d: Cervitec Plus group, e: Fluor Protector Group, f: FotoSan group, g: Diode Laser group, h: Er, Cr:YSGG Laser group.*

The Cervitec Plus, Flour Protector, FotoSan and diode laser groups showed similar nanoleakage results to the control group (p>0,1). The Corsodyl group showed higher nanoleakage than all the tested groups (p<0.01). The Er,Cr:YSGG laser group showed significantly less nanoleakage than all the tested groups (p<0.01). The SEM images, as well as the findings on elemental analysis and SEM-EDX analysis of the groups, are given in Figures 1-3.

Discussion

The presence of bacteria in the smear layer of the restored tooth is the major cause of secondary caries and failure of restoration (37,38). None of the currently used caries removal methods eliminate all the microorganisms in the cavities (2). Thus, cavity disinfection procedures are recommended to eliminate these residual bacteria (6,7). One of the main problems with cavity disinfection is increased leakage between dentin and resin restorative material by interfering

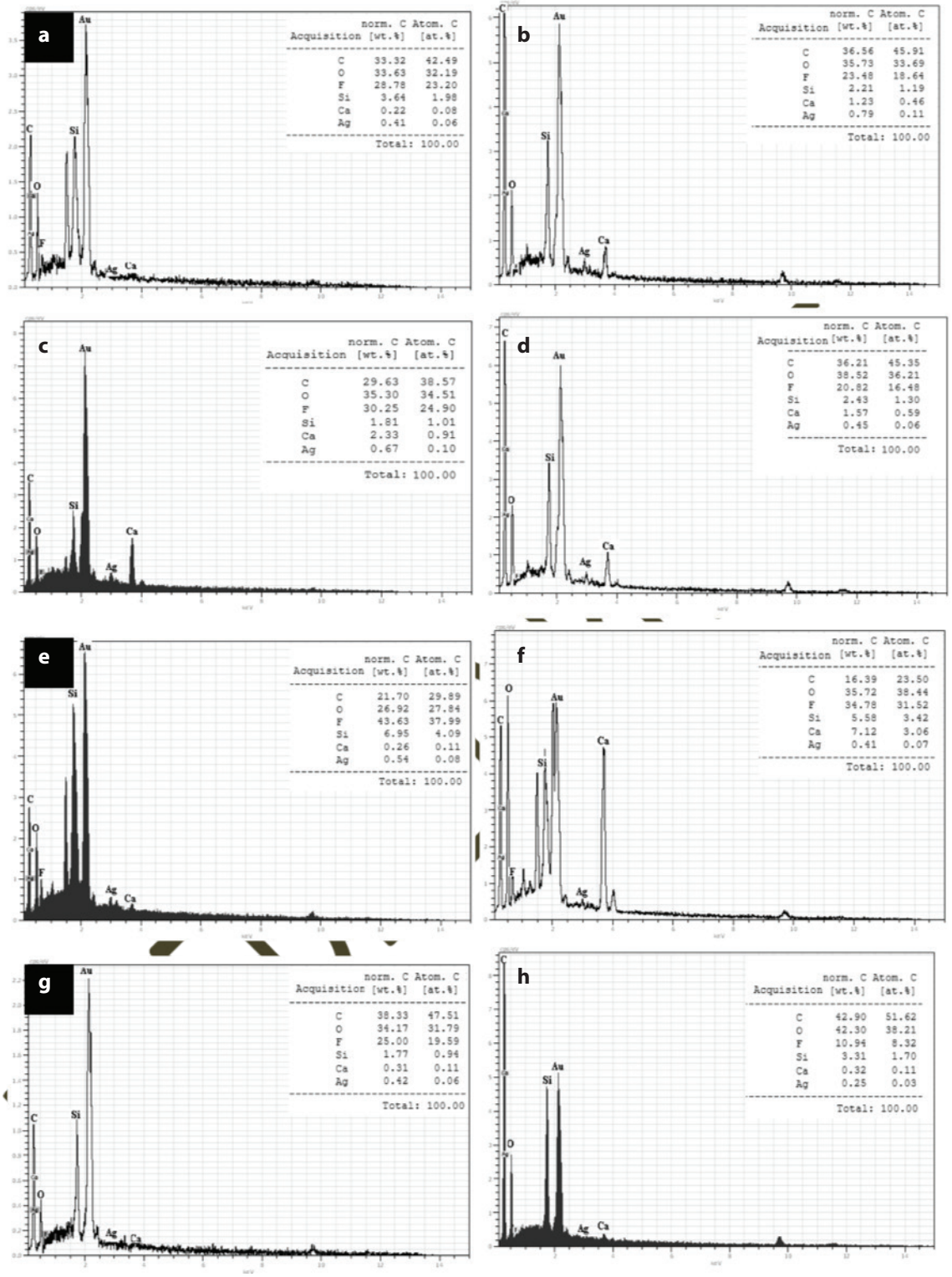

Figure 2. *The Ag ion uptake percentages of groups. a: Control group, b: Corsodyl group, c: Cervitec group, d: Cervitec Plus group, e: Fluor Protector Group, f: FotoSan group, g: Diode Laser group, h: Er, Cr:YSGG Laser group.*

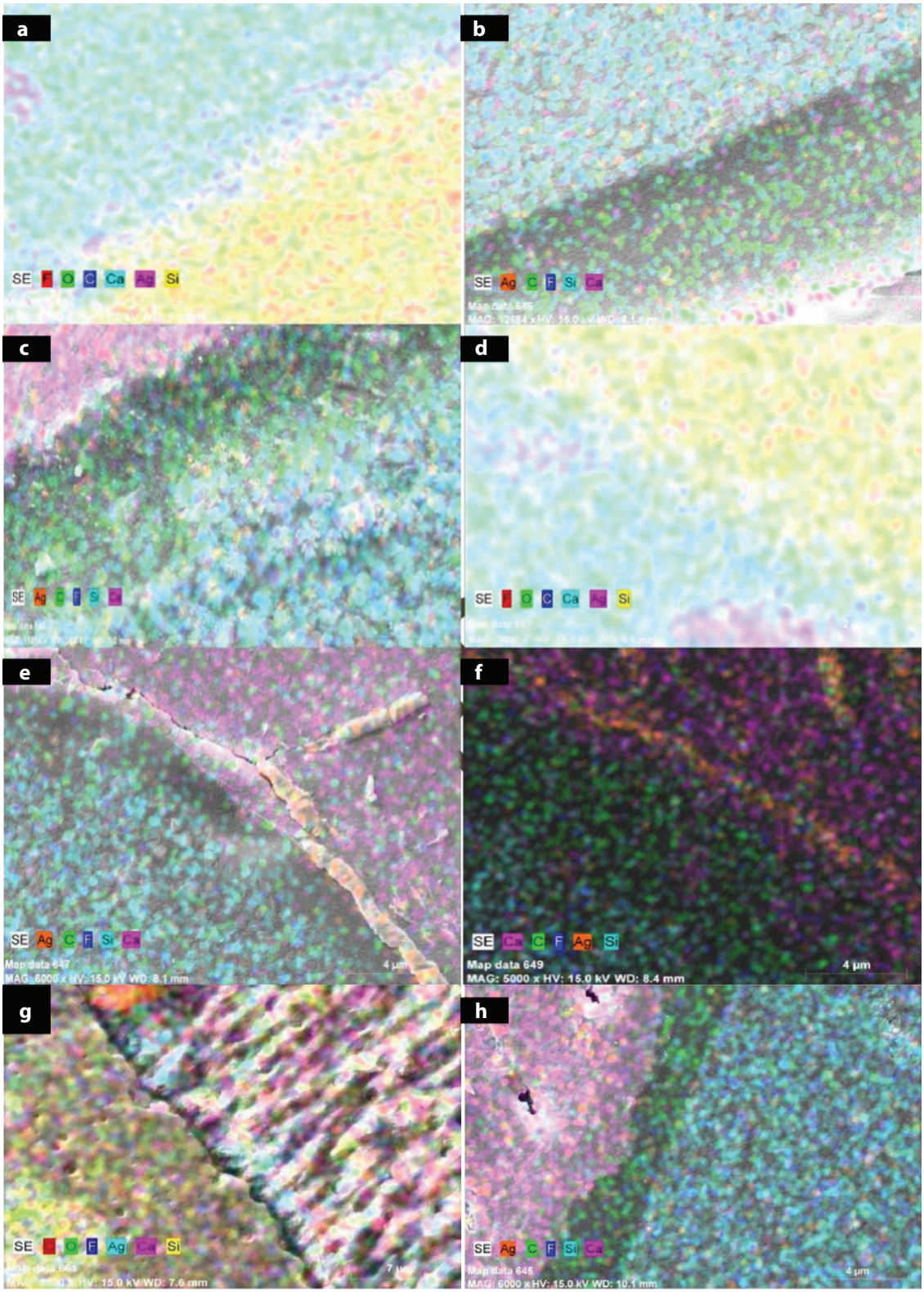

Figure 3. *Distrubition of the ions were shown with mapping. Colours shown at the bottom of the SEM images represent the ions written in. a: Control group, b: Corsodyl group, c: Cervitec group, d: Cervitec Plus group, e: Fluor Protector Group, f: FotoSan group, g: Diode Laser group, h:Er,Cr:YSGG Laser group.*

with the sealing ability (16). Because of these reasons; with the aim of comparing the effect of various systems; Er,Cr:YSGG lasers, diode lasers and FotoSan, which is a PAD system and agents; Corsodyl Cervitec and Cervitec Plus, which contains CHX in their combination; and Fluor Protector on the nanoleakage of compomer restorations when used for cavity disinfection, the present study was designed.

Leakages have been mostly investigated at the micro scale (39). However, the diameter of the stained particles used in microleakage studies was larger than that of the bacteria; thus, the leakage was not detected accurately. So, researchers have searched for new methods (40). Nanoleakage refers to the nanosize leakage that occurs around collagen fibrils in the hybrid layer. In nanoleakage studies, staining is per-

formed using AgNO3 solution. The AgNO3 solution particles are approximately 0.59 nm in size. The sizes of the bacteria that live in the mouth vary between 0.5 and 1 nm. Thus, AgNO3 is considered a suitable staining solution for leakage studies (41). Until now, the effects of cavity disinfection on nanoleakage have not been investigated. All the studies conducted previously were microleakage studies.

Currently, various cavity disinfectants have been used and include chlorhexidine, fluoride gels, sodium hypochloride, benzalkonium-based solutions, propolis and *Aloe vera* (42,43). Technological devices like lasers or PAD systems may be alternative procedures for cavity disinfection (27,30).

Chlorhexidine is the accepted gold standard antibacterial agent that is commonly studied when used as a cavity disinfectant (17). Speculation exists concerning the effects of chlorhexidine on the sealing ability of resin-restorative materials. Some researchers have found that chlorhexidine does not have an adverse effect on the bond strength (14,15). On the other hand, studies have demonstrated controversial results that chlorhexidine increases leakage scores (16,17). The studies that found chlorhexidine increased leakage scores used self-etched adhesive systems. This situation can be attributed to negative interactions between chlorhexidine and the self-etched adhesive systems (17).

In the present study, an increased nanoleakage score was found in the chlorhexidine group. This result also concurs with studies using the self-etched adhesive system (11,17) such as PrimeBond NT, which was used in this study.

The combinations of chlorhexidine with other antibacterial agents, such as fluoride or thymol, are commercially available. In the present study, Cervitec gel (chlorhexidine digluconate and sodium fluoride) and Cervitec Plus varnish (chlorhexidine diacetate and thymol) were used. Although the Cervitec groups showed significantly higher nanoleakage than the control group ($p<0.001$), the Cervitec Plus group showed similar nanoleakage results to the control group ($p>0.1$). This situation could be explained with the concentration differences between the Cervitec and Cervitec Plus groups. Cervitec has a gel form, but Cervitec Plus has a varnish form.

The studies concerning the effect of fluoride on leakage are limited, and in most of the studies, fluoride was used as a desensitiser or demineralising agent (44,45). Selveraj *et al.* (45) used silver diamine fluoride/potassium iodide (SDF) for dentin pretreatment. They reported that SDF minimized the leakage score. In the present study, the Fluor Protector group showed similar nanoleakage scores to the control group. This can be explained by the differences between the fluoride contents. In another study, Nystrom *et al.* (46) applied 0.71% tin fluoride to class V restorations that comprised 50% cement and 50% enamel and restored the teeth with a composite by using a total etch adhesive. No significant differences were detected compared with the control group when the microleakage values were examined. No significant differences were detected between the Fluor Protector that contained fluoride and the control group in present study ($p=0.286$). However, higher nanoleakage values were detected compared with the Cervitec Gel and control groups ($p=0.001$). The cause might be fact that Cervitec were in gel forms, and the Fluor Protector was in a varnish form. Additionally, many differences were found in their contents.

Penetration into dentin tubules is one of the important factors for the selection of cavity disinfection methods. Chemical agents can penetrate only up to 130 µm into dentin, although bacterial penetration is 1100 µm. (47) Thus, lasers and PAD with high penetration capabilities gain importance (48, 49). Odor et al. (50) gave a possible explanation about the penetration of laser beams to dentin. According to their explanations, enamel and dentin are capable of acting as a light collecting and transmitting device. Therefore, emitted laser light to the deeper layers of dentin can be actualized (48).

Although PAD has strong antibacterial efficacy, limited data are available concerning its effects on marginal leakage. Oskee *et al.* (51) used a PAD, Nd:YAG and diode laser for cavity disinfection. They declared that diode lasers and PAD had no detrimental effects on marginal leakage. In another study, no adverse effects of PAD on marginal leakage were found, in accordance with the present study, which used FotoSan as a PAD system (52).

Laser irradiation on dental hard tissues has been widely studied in dentistry (8,12). Several advantages, such as the creation of an acid-resistant surface, antibacterial activity and enhanced bonding capacity, were shown in previous studies (30, 53). In the present study Diode and Er,Cr:YSGG laser were used. The limited studies were found about the diode laser usage for cavity disinfection (51, 54). They found that diose laser had no adverse effect on marginal leakage parallel to the results of the present study. After Er:YAG laser treatment, a honeycomb pattern similar to that following phosphoric acid application was observed (55). An increased bonding capacity can be explained with this pattern. It was reported in a previous study that Er,Cr:YSGG laser etching does not eliminate the need for acid etching (56). In the present study, a self-etched bonding system was used; thus, no additional acid etching was used. In the present study, decreased nanoleakage scores after Er,Cr:YSGG laser irradiation can be explained by the honeycomb pattern detected following phosphoric acid application; thus, the sealing ability of the restoration material may increase. Consistent with the results of this study, Baygin *et al.* (53) found decreased microleakage scores after Er,Cr:YSGG laser irradiation.

According to the results of the present study, the first null hypothesis, 'the systems and agents that were used in the study would have no effect on nanoleakage' was rejected. Corsodyl and Cervitec usage increased and Er,Cr:YSGG laser usage decreased the nanoleakage scores compared with the control group. Also, the second null hypothesis, 'nanoleakage would not differ between the systems or agents' was rejected. The Corsodyl use showed higher nanoleakage than in all the tested groups and the Er,Cr:YSGG laser group showed significantly less nanoleakage than all the tested groups.

Conclusion

Under the limitations of this study, Er,Cr:YSGG laser irradiation, which showed lower nanoleakage scores than either the control or tested groups, can be recommended for cavity disinfection. Also Diode laser, FotoSan and Fluor Protector which have no adverse effect on nanoleakage scores can be alternative system for cavity disinfection.

Türkçe Öz: *Kavite dezenfeksiyonunun kompomer restorasyonların nanosızıntısına etkileri: taramalı elektron mikroskobu ve enerji dağılımlı x-ray analizi. Amaç: Rutin çürük uzaklaştırma yöntemlerine ek olarak kavite dezenfeksiyonu mikroorganizmaların eliminasyonu için önerilmektedir. Bu çalışmanın amacı farklı sistemlerin; Er,Cr:YSGG lazer, diod lazer, FotoSan ve ajanların; Corsodyl, Cervitec, Cervitec Plus, Fluor Protector kavite dezenfeksiyonu amacıyla kullanımında kompomer restorasyonların nanosızıntısına etkilerinin incelenmesidir. Gereç ve yöntem: Bukkal ve lingual yüzlerinde mine sement sınırına paralel sınıf V kaviteler (3×2×1.5 mm) bulunan 40 adet insan süt azı dişi kavite dezenfeksiyon yöntemine göre 8 gruba ayrıldı. Antibakteriyel ajan ve sistemler üretici firmaların önerileri doğrultusunda uygulandı. Tüm örnekler kompomer dolgu materyali ile restore edildi. Restore edilen dişlere 30 saniye 5°C-55°C su banyosunda 500 tur termal siklus uygulandı. Termal siklus işlemlerinden sonra, nanosızıntı testleri için herbir kavitenin orta hattından 1 mm'lik kesit alındı. Örnekler bölündükten sonra %50'lik amonoikal gümüş nitrat solüsyonunda 24 saat, florosan ışık altında fotoğraf solüsyonunda 8 saat bekletildi. Örnekler SEM ile incelendi. İstatistiksel analizlerde non parametrik Kruskal Wallis ve Mann Whitney U Test'leri uygulandı. Bulgular: Er,Cr:YSGG lazer grubunda diğer gruplara göre anlamlı derecede daha az nanosızıntı görüldü (p<0.01). Diod lazer, Fluor Protector ve Fotosan grupları kontrol grubuna göre benzer nanosızıntı skorları gösterdiği bulundu (p>0.05). Corsodyl (p<0.01) ve Cervitec (p<0.001) gruplarında ise nanosızıntı miktarı kontrol grubuna göre daha yüksek olduğu tespit edildi. Sonuç: Antibakteriyel etkinliği bulunan ve hem kontrol hem de test gruplarına göre daha düşük nanosızıntı değerleri gösteren Er,Cr:YSGG lazer kavite dezenfeksiyonunda önerilebilir. Ayrıca antibakteriyel etkinliği bulunan ve sızıntıyı etkilemeyen diod lazer ve FotoSan da kavite dezenfeksiyonunda önerilebilecek yöntemler arasında yer alabilir. Anahtar kelimeler: Er,Cr:YSGG Lazer; Diod Lazer; FotoSan; Antibakteriyal Ajan; Nanosızıntı.*

Ethics Committee Approval: Ethical approval of the present study was obtained from the Ethics Committee of Karadeniz Technical University, Faculty of Medicine (Protocol # 2015/149).

Informed Consent: The informed consents were provided by the participants.

Peer-review: Externally peer-reviewed.

Author contributions: IA and OB designed the study. IA, FE and AC participated in generating the data for the study. IA and OB participated in gathering the data for the study. TT and FMK participated in the analysis of the data. IA wrote the majority of the original draft of the paper. IA and OB participated in writing the paper. All authors approved the final version of this paper.

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Original research

Prosthetically driven immediate implant placement at lower molar area; an anatomical study

Sabit Demircan[1]

Purpose
To examine the effectiveness and safety of immediate implant placement (IIP), we evaluated the risk of lingual plate perforation (LPP) and mandibular canal perforation (MCP) associated with posterior mandible anatomy using cone beam computed tomography (CBCT) images.

Materials and Methods
A morphological study of the molar sockets of 135 patients (age: 18–84 y) was done and its relationship to the mandibular canal was investigated. The risk of LPP and MCP was recorded as yes or no. Mandibular cross-sectional morphology was defined as one of three types (U-P-C) using the criteria of Chan et al.

Results
The risk of LPP was significantly higher for second molars ($p = 0.0001$), and the risk increased with age ($p = 0.039$). There was a strong relationship between the risk of LPP and cross-section type U ($p = 0.0001$). The mean root to alveolar canal (RAC) distance (mm) of males was significantly higher than that of females. The mean RAC value was 5.02 mm for males and 3.49 mm for females. There was no statistically significant relationship between the risk of MCP and sex. There was a significant relationship between the risk of MCP and cross-section type U ($p = 0.0001$). Although the MCP risk was higher in second molars, there was no statistically meaningful relation between MCP and tooth type.

Conclusion
The results suggest that IIP in the mandibular molar area carries a high risk of MCP and LPP. Based on the elevated level of risk, a delayed implant protocol should be considered.

Keywords: *CBCT; immediate implant; mandibular canal; lingual plate perforation; mandible*

Introduction

Molars, especially first molars, frequently decay, as they are the first permanent teeth to erupt. Loss of molar teeth is associated with neighbouring tooth movement, extrusion of opposing teeth and occlusal disorders (1, 2). Immediate implant placement (IIP), which was introduced into clinical practice in 1978 (3), is popular among patients due to the need for only a single surgical procedure and a reduced treatment time (4). In the past, the initial purpose of implant operations was to place the implant in an area of the bone that provided support to a functional prosthesis. In this concept, osseointegration was the primary goal, and prosthetic restorations did not always meet aesthetic ideals (5).

The mandible forms the lower portion of the jaw complex and supports mastication, speech and facial expressions. The alveolar processes of the mandible consist of buccal-lingual plates, inter-dental septa and inter-radic-

ORCID IDs of the authors: S.D. 0000-0001-6933-201X

[1]*Beykent University Vocational School Dental Services, Oral Health Program, Istanbul, Turkey*

Corresponding Author: Sabit Demircan

E-mail: sabitdemircan@hotmail.com

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ular septa (6). Several anatomical studies have shown major changes in the anatomy of the posterior mandible after tooth loss, with age and sex contributing little to these changes (7, 8).

The mandibular molar area is a challenging site for implant placement because of the inferior alveolar canal (IAC) and concavity of the submandibular fossa. Nerve injury can lead to a partial or permanent paraesthesia, lingual plate perforation (LPP) and sublingual or submandibular hematomas, with excessive bleeding or infection (9-11). (Figure 1) These anatomical structures not only give rise to surgical complications during implant operations but also cause fracture of the lingual plate during extractions, thereby facilitating the dissemination of microorganisms and infection to other areas (6). Furthermore, in cases of fenestration in the lingual plate, displacement of endodontic materials and iatrogenic subcutaneous emphysema are possible complications (6).

Cone beam computed tomography (CBCT) is an effective instrument to evaluate bone quality and anatomy, and it a reliable, objective method of determination of bone density values (12, 13).

To examine the effectiveness and safety of IIP, the present study evaluated the risk of LPP and mandibular canal perforation (MCP) associated with posterior mandible anatomy using CBCT images.

The null hypothesis tested in this study was that there would be no relationship between IIP and LPP and IAC perforation.

Materials and Methods

The study protocol was approved by the institutional review board of the Istanbul University Faculty of Dentistry (2016–83). In total, CBCT images obtained from 500 patients were evaluated for fully erupted mandibular permanent premolar and molar teeth and fully formed apexes. The exclusion criteria included uncontrolled periodontal problems, dental caries, alcohol or drug addiction, systemic/local conditions that affected bone metabolism, chemotherapy and a history of radiotherapy in the head and neck regions. Of the 500 images, 135 images met the inclusion criteria. A detailed morphological study of 292 molar sockets of these135 patients (mean age: 46.3 Y) was performed.

All CBCT data were obtained using the same CBCT scanner (Galileos; Sirona Dental Systems, Germany). The CBCT protocol was as follows: 98 kVp/6 mA and exposure time of 2–5 sec. The CBCT examinations of all patients performed for other causes and measurements were carried out using Galileos software (Sirona Dental Systems, Germany). In all the CBCT images, the field of view 12 cm with 1 mm slice thickness, as any change in the field of view could change the effective dose and affect the spatial resolution.

All the images were manipulated to provide the best resolution and magnification. In the CBCT images, the operator ensured the following:

The boundary of the mandible and IAC was clear;

Each tooth was normally positioned, and an imaginary line connecting the cusp tip of the canines and the central grooves of the premolars and molars was smooth;

The angulation of opposing maxillary teeth was correct.

Three types of mandibular cross-sectional morphologies were evaluated (U-P-C) using the criteria described by Chan et al. (11, 14). The U type consisted of a ridge with a narrow base, a wider crest and a lingual undercut on the lingual

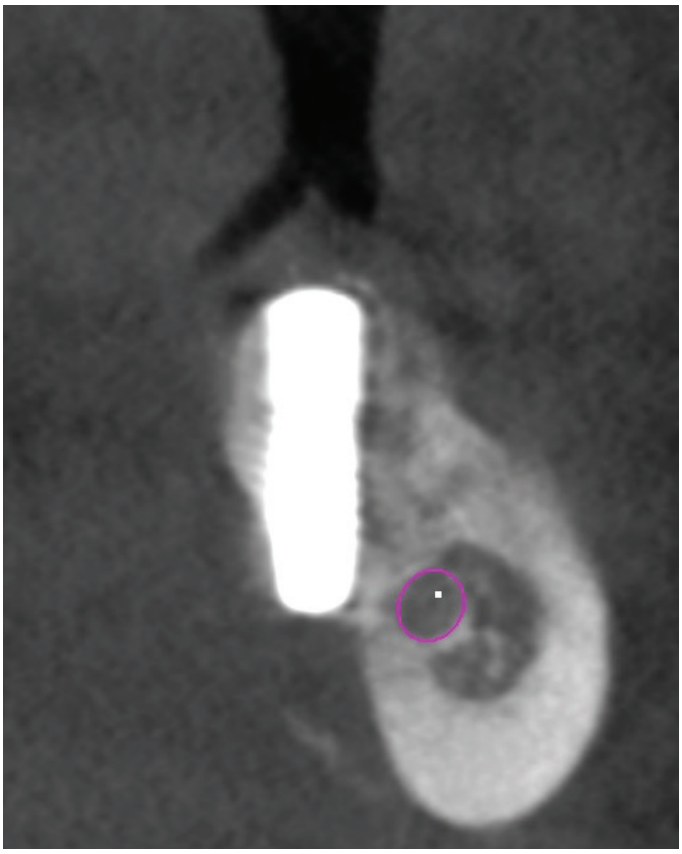

Figure 1. *Lingual plate perforation.*

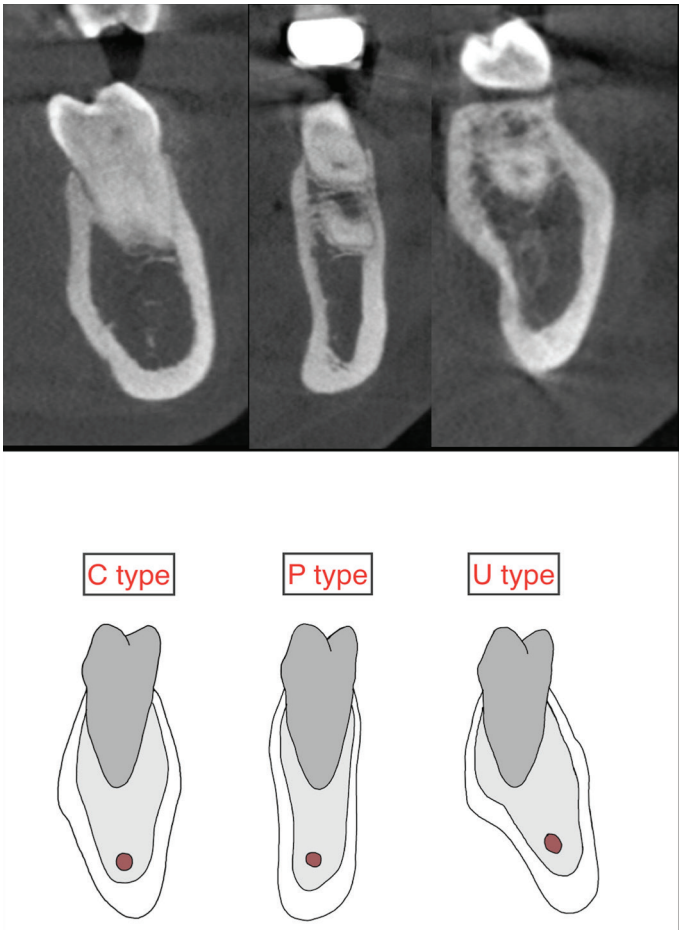

Figure 2. *Three types of mandibular cross-sectional ridge morphology (CST) were determined.*

plate. The C type was a ridge with no obvious lingual undercut. The P type was defined as parallel ridge boundaries of the mandible buccolingually (Figure 2).

In the literature, the presence of 4-mm native bones is considered the minimum requirement to provide primary stability for implant survival (14, 15). In the present study, the amount of bone apical to the socket that was deemed necessary for IIP was 6 mm to allow 4 mm for primary stability and 2 mm as a safety zone (11, 13, 16). To determine whether there was a high risk of nerve injury, a measurement was made on the coronal sections of the mandibular first and second molars. Using computer software, a vertical line was traced from the level of the apices of the mesial root of the mandibular first and second molars to the superior border of the IAC. This was designated as the root to alveolar canal (RAC) distance (mm) (Figure 3).

A 4-mm diameter single tapered implant was selected from the software database. Without considering the lingual plate and IAC, all implants were placed according to the following criteria. Mesiodistally, the implants were placed along an imaginary line connecting the central grooves of the teeth. Buccolingually, the centre of the implant was placed along a line passing through the middle of the marginal ridge of the buccal and lingual aspects of each tooth. The mesiodistal and buccolingual angulation of the implant depended on the axis of the implant parallel to the long axis of the existing tooth. The functional cusps of the opposing teeth were positioned at the centre of the implant. The software was used to verify the position of the virtual implant in different planes. A high risk of MCP was defined as a virtual implant placed within 4 mm of the native bone and in contact with the IAC. A high risk of LPP was defined as an implant that exceeded the outline of the lingual plate (Figure 3). All measurements were made by a dental surgeon (S.D) with 14 y of experience and a PhD degree in oral surgery.

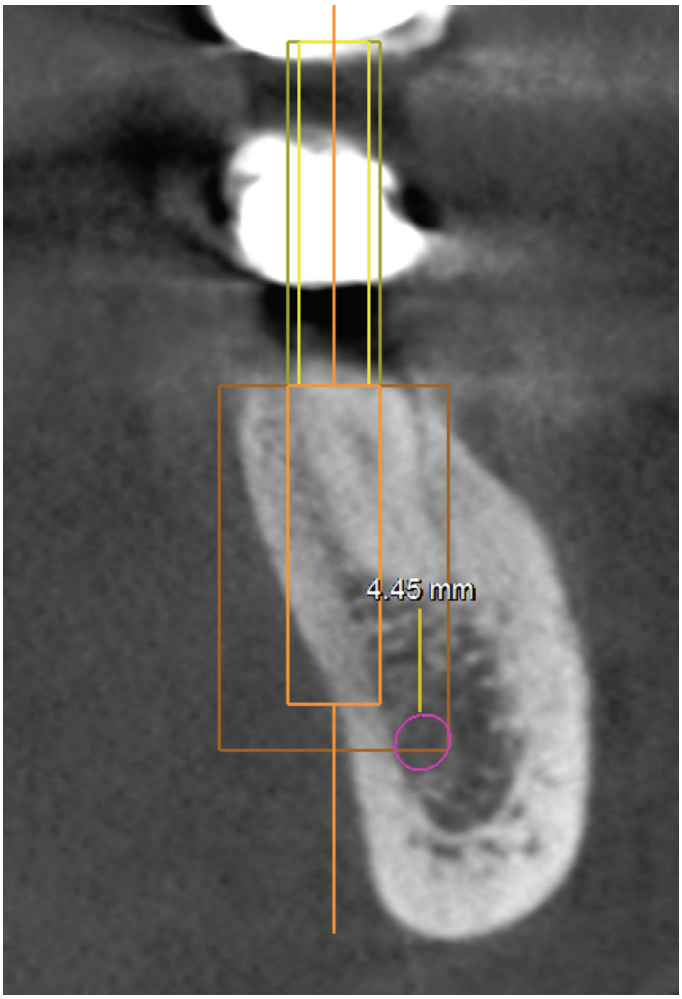

Figure 3. *The simulation was categorized as LPP if the virtual implant extruded the outline of cortical bone in the cross-sectional images.*

Statistical analysis

NCSS software (Number Cruncher Statistical System, 2007, UT, USA) was used for statistical evaluation. The normality of the data was assessed using Kolmogorov–Smirnov and Shapiro–Wilk tests. If the variables were distributed normally, a binary group comparison was undertaken with an independent *t*-test. For variables that were not distributed normally, a between-group comparison was conducted using the Kruskal–Wallis test. A Mann–Whitney *U* test was applied for the binary group comparison, qualitative data comparisons were conducted using a chi-squared test, and Spearman's rank correlation coefficient test was performed for identification of relationships between variables ($r < 0.2$: no correlation, 0.2–0.4: a weak correlation, 0.4–0.6: a moderate correlation, and 0.6–0.8: a strong correlation). The level of significance was considered as $P < 0.05$.

Results

In total, CBCT images obtained from 135 patients (males: $n = 62$, 46.5%; females, $n = 73$, 53.5%) aged 18–84 y were selected for inclusion in the study. The mandibular first molars were observed in 136 (46%) patients, and the mandibular second molars were studied in 156 (54%) patients. In the study group, the U type was the most common (50.65), followed by the P (36.9%) and C types (12.3%) (Figure 4).

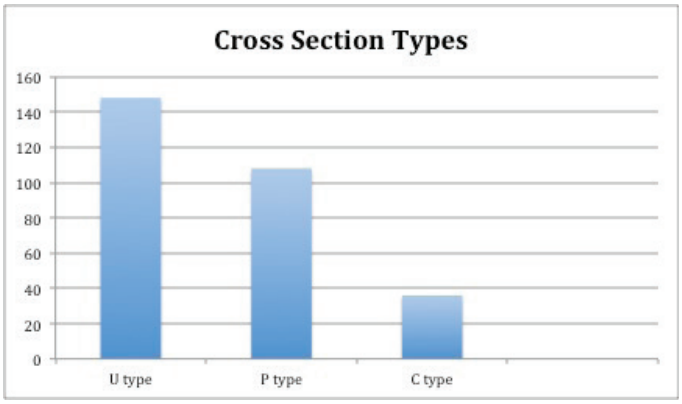

Figure 4. *Distribution of cross section types.*

Risk of LPP

The positive risk group of LPP was significantly older than negative risk group ($p = 0.039$, < 0.05). There was a significant relationship between the risk of LPP and cross-section type U ($p = 0.0001$, < 0.05). There was no statistically meaningful relationship between the risk of LPP and sex. The risk of LPP was significantly higher in second molars than first

molars ($p = 0.0001, < 0.05$). A high risk of LPP was observed in 24.6% of the study group (1.4% of first molars and 23.2% of second molars) (Table 1).

Risk of MCP

In the study group, 73.9% of the patients had a risk of MCP. Similar to the risk of LPP, the MCP risk increased with age ($p = 0.0001, < 0.05$). There was no statistically meaningful relationship between the risk of MCP and sex. However, there was a significant relationship between the risk of MCP and cross-section type U ($p = 0.0001, < 0.05$). Although MCP increased in second molars, there was no statistically significant relationship between MCP and tooth type (Table 2).

Cross-section type

There was no statistically meaningful relationship between cross-section type and sex.

RAC measurement

The mean RAC value of males was significantly higher than that of females (5.02 mm versus 3.49 mm). The RAC mea-

Table 1. *Frequency distribution of lingual plate perforation of each tooth type, sex and cross section type (*independent t-test, +Chi square test)*

		LPP yes	***LPP no***	***p value***
Age (*mean* ± SD)		47,95±13,55	42,39±14,76	0.039*
Sex	Male	12	51	0.287+
	Female	19	53	
Tooth	M1	4	112	0.0001+
	M2	68	108	
CST	U	68	80	0.0001+
	P	0	108	
	C	4	32	

M1: First Molar, M2: Second Molar, SD: Standart deviation, LPP: Lingual plate perforation, CST: Cross section type

Table 2. *Frequency distribution of nerve injury risk of each tooth type, sex and cross section type (* independent t-test, +Chi square test)*

		MCP yes	***MCP no***	***p value***
Age (*mean* ± SD)		55,21±14,21	43,54±12,67	**0,0001***
Sex	Male	43	19	0,104+
	Female	59	14	
Tooth	M1	88	28	0,673+
	M2	128	48	
CST	U	132	16	0.0001+
	P	58	50	
	C	26	10	

M1: First Molar, M2: Second Molar, SD: Standart deviation, CST: Cross section type, MCP: Mandibular canal perforation

Table 3. *Frequency distribution of three types of cross-sectional morphology, sex and tooth types and RAC values (†Mann Whitney U testi ‡Kruskal Wallis testi)*

	Number		***RAC, mm, mean ± SD***	***p value***
Sex	Male	62	5,02±2,63	**0,001**†
	Female	73	3,49±2,52	
Tooth	M1	136	4,30±2,74	**0,665**†
	M2	156	4,13±2,64	
CST	U	148	3,57±2,30	**0,0001**‡
	P	108	5,33±2,72	
	C	36	3,42±2,98	

M1: First Molar, M2: Second Molar, mm: milimeters, SD: Standart deviation, CST: Cross section type, RAC: Root to alveolar canal measurement

surement decreased with age (r=0.414)). The RAC values for cross-section type P were significantly higher than those of the other types. There was no statistically meaningful difference between the RAC values of cross-section types U and C (Table 3).

Discussion

Dental implant therapy commences with extraction, followed by healing of soft and hard tissue, osteotomy and implant placement. Maximum bone implant contact was thought to be achieved by adopting the aforementioned procedure (17, 18). The popularity of IIP is due to the need for only one surgical procedure and a reduced overall treatment time (19, 20). The implant survival rate is an added benefit of IPP, with an immediate implant survival rate of 95% in the posterior mandible reported in the literature (21, 22).

Initial stability is important for the survival of implants immediately after placement. The extraction socket must be examined to investigate whether it is suitable for IIP. Observations during surgery will determine whether the implant can be placed during or after surgery (i.e. after hard and soft tissue healing). Micro-movements between the implant and bone should be evaluated to assess the likelihood of successful healing. In the present study, the amount of bone apical to the socket that was deemed necessary for IIP was 6 mm. This allowed 4 mm for apical bone support and a 2-mm safety zone to avoid nerve damage (11, 13, 16). Although some studies have suggested implant placement in the inter-septal bone of multi-rooted mandibular molars during an IIP protocol, the quality of cancellous bone means it is not ideal for implant placement. Moreover, the bone between the roots will be lost while drilling (23). To avoid such problems, we ensured that our measurements were made at the mesial root apex. The diameter was fixed at 4 mm, representing the minimum implant diameter required to support an occlusal load in the posterior mandible while minimising the risk of LPP (24, 25).

Lin et al. (26) reported in a virtual implant placement study that 51.7% of 1,008 teeth had a risk of MCP in IPP procedures. They used an RAC value of 6 mm as the safety margin. In the present study, the mean RAC value was 5.02 mm for males and 3.49 mm for females. Only 26% of the subjects had an RAC distance of > 6 mm.

Previous studies examined the occurrence of LPP and sublingual or submandibular hematomas, excessive bleeding and infection (9, 10, 11). Froum et al. (15) reported that 9% of first molars and 31% of second molars had a high risk of LPP in cases of IMPs 4 mm in diameter. In the present study, 1.4% of first molars and 23.2% of second molars showed a high risk of LPP when placing an immediate implant 4 mm in diameter. In cases of implants with larger diameters, the probability of LPP would increase.

In the present study, the U type was the most common type (50.6%) of mandibular cross-sectional morphology in the study group. The P type was the second most common (36.9%), followed by the C type (12.3%). The findings of the present study are in accordance with those of Chan et al. (14), who reported that the U type (lingual concavity) accounted for 66% of cross-section types in their study population. Yu et al. (27) reported similar results in a Taiwanese study population (U type: 50%). However, Watanebe et al. (28) reported that the C type was the most common in their study of a Japanese population. The difference might be the result of the study design, analysed areas and ethnicity of the sample (14).

A number of systematic reviews and consensus documents have reported that the survival rates of short posterior mandible implants are comparable to those of conventional posterior mandible implants (29, 30). Thus, short implants may be an alternative to conventional implants in complicated cases.

Although this study was designed under the guidance of current scientific data, it has some limitations, , such as differences in the risk of LPP and MCP in implants with different diameters, different placement depths and various implant designs. Further studies that include both implants with different diameters and different types of implants are needed. As this study comprised a virtual simulation, translation of the data to the clinic may not be possible.

Conclusion

The results of the present study suggest that the IIP procedure in the mandibular molar area carries a high risk of LPP (1.4% for first molars and 23.2% for second molars) in cases of IMP where the diameter of the implant is 4 mm. These complications may lead to debilitating and even life-threatening situations for the patient. Based on the high level of risk, a delayed implant protocol should be considered.

Türkçe Öz: *Mandibular molar bölgede protezin yönlendirdiği immediate implant yerleştirilmesi; anatomik çalışma. Amaç: İmmediate implant yerleştirmenin(IIP) etkinliğini ve güvenliğini incelemek için, volumetrik bilgisayarlı tomografi görüntüleri kullanılarak posterior mandibula anatomisi ile ilişkili lingual plak perforasyonu (LLP) ve mandibular kanal perforasyonu (MCP) riskini değerlendirdik. Gereç ve yöntem: 135 hastanın molar soketlerinin morfolojik incelemesi ve bunun inferior alveolar sinir kanalı ile ilişkisi araştırıldı. LLP ve MCP riski evet veya hayır olarak kaydedildi. Altçene çapraz kesit morfolojisi, Chan et al ölçütlerini kullanarak üç tipten biri (u p c) olarak tanımlandı. Bulgular: LLP riski ikinci molar dişler (p=0.0001)için önemli derece daha fazlaydı, ve bu risk yaş (p=0.039) ile birlikte artmaktaydı. LLP riski ile U tipi kesit (p=0.0001) arasında güçlü bir bağlantı vardı. Erkeklerdeki alveolar kanal (RAC) mesafesi (mm) ortalamasının kökü kadınlarınkine göre önemli derece daha yüksekti. Erkekler için ortalama RAC değeri 5.02 mm ve kadınlarınki de 3.49 mm idi. MCP riski ve cinsiyet arasında istatistiki olarak önemli bir bağlantı yoktu. MCP riski ile U tipi kesit (p=0.0001) arasında önemli bir bağlantı vardı. MCP riski ikinci molarlarda daha yüksek olmasına rağmen MCP ve molarlar arasında istatistiki olarak anlamlı bir bağlantı yoktu. Sonuç: Sonuç, mandibular molar bölgede IIP'nin yüksek bir MCP ve LPP riski taşıdığını göstermektedir. Yüksek risk seviyesi düşünülerek, gecikmiş bir implant yerleştirme protokolü göz önünde bulundurulmalıdır. Anahtar kelimeler: CBCT; immediate implant; mandibular kanal; lingual plak perforasyonu; çene*

Ethics Committee Approval: The study protocol was approved by the institutional review board of the Istanbul University Faculty of Dentistry (2016–83).

Informed Consent: The informed consents were provided by the participants.

Peer-review: Externally peer-reviewed.

Author contributions: SD designed the study, generated and gathered the data, wrote and approved the final version of the study.

Conflict of Interest: The author had no conflict of interest to declare.

Financial Disclosure: The author declared that he received no financial support.

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Original research

Effect of zinc oxide nanoparticles on the flexural strength of polymethylmethacrylate denture base resin

Santhanam Vikram[1], N. Gopi Chander[2]

Purpose
This study evaluated the flexural strength of polymethyl methacrylate (PMMA) reinforced with various concentrations of zinc oxide (Zn O) nanoparticles

Materials and Methods
Nano ZnO was added in 0, 0.4, 0.6, 0.8, 1.2 and 1.4 percentage to PMMA denture base material. 60 specimens of heat cure polymerizing acrylic resin of dimensions 10mm x 4mm x 80mm were fabricated in accordance to ISO 20795-1-2013. The specimens were divided into 6 groups. Acrylic specimens were processed according to manufacturer's instruction. Three-point bending test was performed to evaluate the flexural strength. Surface analysis was performed with scanning electron microscopy (SEM) to observe the fracture surfaces of specimens. ANOVA and Tukey tests were used for the statistical analysis ($p < 0.05$).

Results
Statistical analysis revealed significant differences in strength between groups. The flexural strength improved with the addition ZnO nanoparticles. Highest mean value was observed in Group nZn -14 (91.31 MPa) and lowest in control Group nZn-0 (61.36 MPa). ANOVA and Tukey's honestly significance test found statistical significant differences among the groups ($p<0.001$).

Conclusion
The addition of ZnO nanoparticles in all concentrations increased the flexural strength of acrylic resin when compared to the control group.

Keywords: *Flexural strength; heat cure acrylic; nano particles; poly methyl metha acrylate; zinc oxide*

Introduction

Polymethyl methacrylate (PMMA) is the commonly used denture base material. It possesses a combination of favorable characteristics such as easy laboratory manipulation, light weight, inexpensive fabrication, stability in the oral environment, lack of toxicity and appropriate aesthetic and color matching ability (1,2). Limitations inherent in the resin are poor fatigue failure, high coefficient of thermal expansion, low thermal conductivity, dimensional inaccuracy, denture fracture and wear of the denture teeth (3,4). Clinicians encounter fracture of denture to low resistance to impact, flexural, or fatigue stresses (5). In order to prevent fracture of the dentures, the thickness of acrylic resin in susceptible regions was increased or reinforced (6). Copolymerization by rubber (7), reinforcement by incorporation of different forms like metallic wire (8), fibers (9-11) and the use of metallic oxides (12) were attempted to improve the properties of PMMA denture base resins.

Nanoparticles have been increasingly used in material science for its wear and tear resistance and anti-corrosion abilities. The alteration of filler

ORCID IDs of the authors: V.S. 0000-0001-7688-4875; N.G.C. 0000-0002-2040-4550

[1] SRM Dental College, Department of Prosthodontics, Ramapuram Chennai, India

[2] SRM Dental College, Ramapuram, Department of Prosthodontics, Chennai, India

Corresponding Author: N. Gopi Chander

E-mail: drgopichander@gmail.com

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size improves the properties of the material (13). The earlier studies conducted showed a marginal improvement of flexural strength but had several shortcomings in fatigue life, fatigue crack, propagation resistance and long term wear that restricted immediate clinical application (14-17). The search exists with more ideal reinforcement. The nano materials and technology provides wider opportunity in identifying the better reinforcement material.

Nano zinc oxide (ZnO) has excellent antibacterial, antifungal properties. ZnO in blending with denture base resins can improve the properties of denture base resins, significantly the biological properties of acrylic resins. This study was done with the objective to evaluate the flexural strength of PMMA with various concentrations of Zn O nanoparticles.

Material and Methods

A total of 60 heat cure acrylic denture base specimens (DPI Heat Cure, India) with dimension of 65mm x 40mm x 5mm were fabricated according to manufacturer's recommendations. The specimens were divided into six groups for flexural strength evaluation (n=10). Sample distribution and composition of material listed in Table 1.

The heat cure acrylic resin (DPI Heat Cure, India) with liquid monomer and polymer powder of 0.10mm particle was used for specimen fabrication. ZnO nanoparticles of 12 nm particles were procured from external source (Zigma Elhrich). Zinc oxide nanoparticles are incorporated into the heat cure polymer by twin screw extruder. Specimen groups were fabricated with 0.4 % (group nZn4), 0.6% (group nZn6), 0.8% (group nZn8), 1.2% (group nZn12) and 1.4% (group nZn14) nano ZnO by weight. The specimens were fabricated by mixing the nano ZnO with monomer in ratio of 25g/10ml.

Initially a master die was prepared according to ISO 20795.1.2013 with dimension of 65mm x 40mm x 5mm. The master die was duplicated with addition silicone impression material and resin specimen were fabricated with the specified dimensions by compression molding technique, processed by long heat cure polymerization cycle, trimmed with acrylic stone and finished with 600 grit sandpaper [Fig 1]. Each prepared specimen was cut lengthways with milling machine into three equal strips, 64 mm long, (10,0 ± 0,2) mm wide, and (3,3 ± 0,2) mm in height. The samples were subjected to a three-point bending test in universal testing machine (Autograph universal testing machine, Shimadzu Corp, Japan). The flexural strength was tested by applying a load until fracture at the midpoint of specimen by means of a hardened steel cylinder with a cross head of 1mm/min. The flexural strength in MPa was calculated using the equation, $M = 3WI/2bd^2$ Where M = flexural strength (MPa), W = fracture load (N), I = test span (center to center) distance between support points (mm), B = width of specimen (mm) and d = thickness of the specimen (mm). The mean flexural strength of each group was calculated, tabulated and statistically analyzed with ANOVA and Tukey HSD test.

Table 1: *Sample distribution*

Group	Sample	ZnO nanoparticles Conc.
nZn0	10	-
nZn4	10	0.4
nZn6	10	0.6
nZn8	10	0.8
nZn12	10	1.2
nZn14	10	1.4

Results

The mean flexural strength of specimen ispresented in Table 2. Group nZn0 control group showed lesser strength of 61.3 MPa and Group nZn14 – 91.31 MPa was the highest when compared to other groups. The flexural strength increased with the concentration of ZnO. Group nZn-4 to Group nZn-14 exhibited increase in strength of 71.73 MPa,77.05 MPa, 84.98 MPa, 86.92 MPa and 91.31 MPa. The data analysis was executed using statistical software SPSS Version 20.0 (SPSS Inc., Chicago, IL). ANOVA displayed statistically significant differences among the 6 groups ($p<0.5$). The Post hoc test multiple comparisons Tukey's HSD revealed significant differences. The scanning electron microscope (SEM) revealed the distribution of the nanoparticles in PMMA and the fracture of the material occurred in the midst of the nano particles (Figure 1-6).

Table 2: *Descriptive Analysis on flexural strength*

Groups	N	Mean	SD	Standard Error	95% Confidence Interval for Mean		Minimum	Maximum	P value
					Lower Bound	Upper Bound			
nZn0	10	61.36	4.91	1.55	57.85	64.88	52.48	67.38	0.000*
nZn4	10	71.73	3.49	1.10	69.23	74.22	66.49	77.52	0.000*
nZn6	10	77.05	2.41	0.76	75.33	78.77	73.36	81.22	0.000*
nZn8	10	84.98	2.49	0.79	83.57	86.77	80.79	88.02	0.000*
nZn12	10	86.92	1.89	0.59	85.57	88.28	83.16	89.32	0.000*
nZn14	10	91.31	1.15	0.36	90.48	92.13	89.59	92.68	0.000*
Total	60	78.89	10.62	1.37	76.15	81.64	52.48	92.68	

*Oneway $p < 0.001$ (99.9 % sig), SD: Standard Deviation

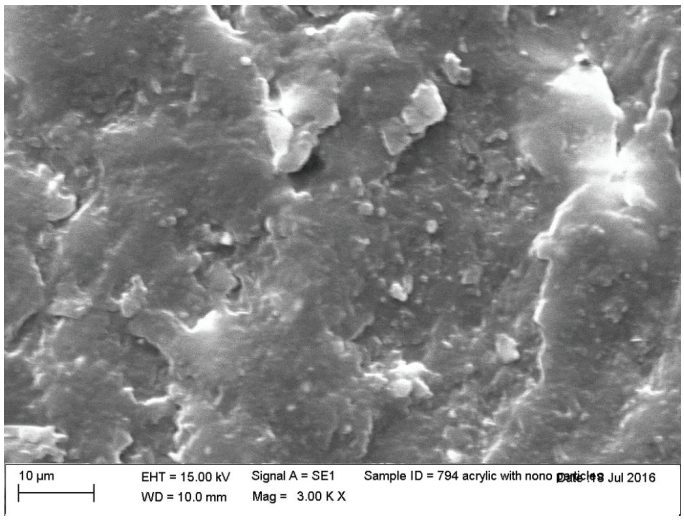

Figure 1. *SEM of heat cure acrylic with no reinforcement.*

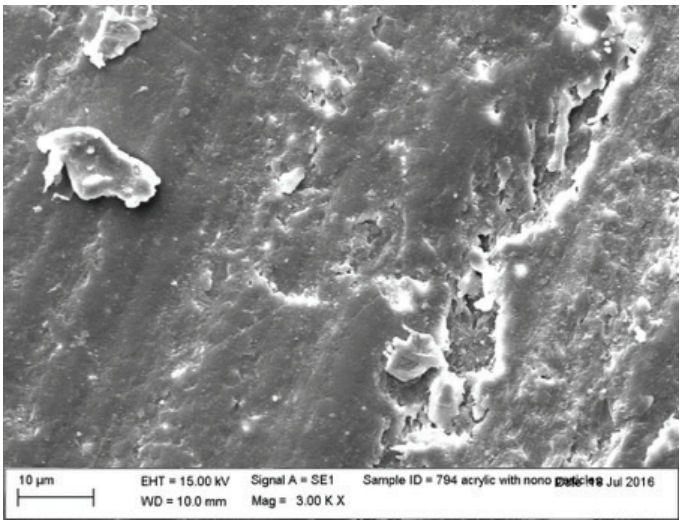

Figure 2. *SEM of n Zn 4 nanoparticles reinforcement specimen.*

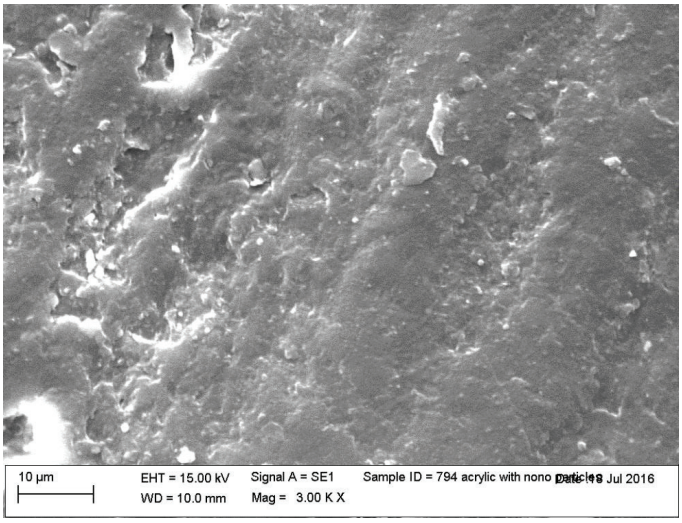

Figure 3. *SEM of n Zn 6 nanoparticles reinforcement specimen.*

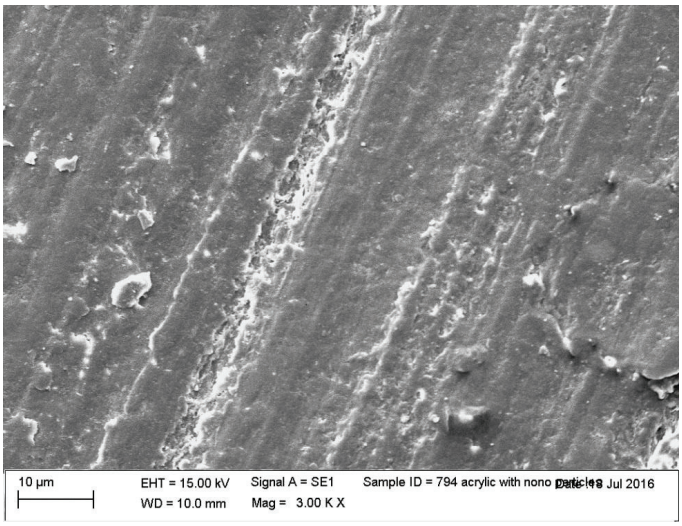

Figure 4. *SEM of n Zn8 nanoparticles reinforcement specimen.*

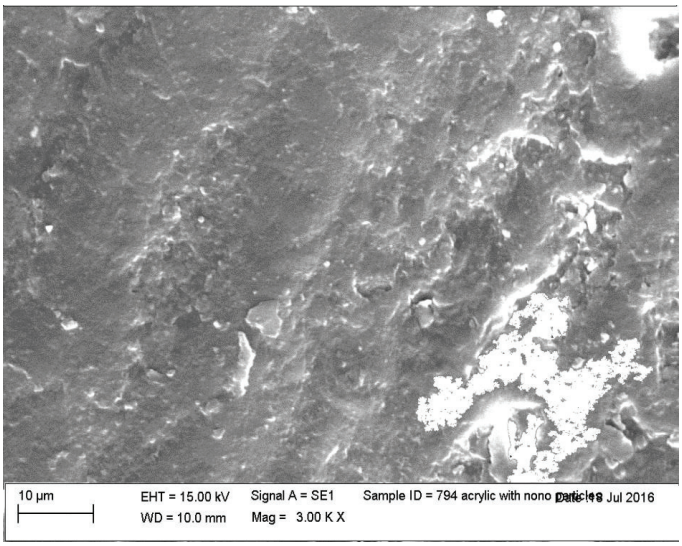

Figure 5. *SEM of nZn12 nanoparticles reinforcement specimen.*

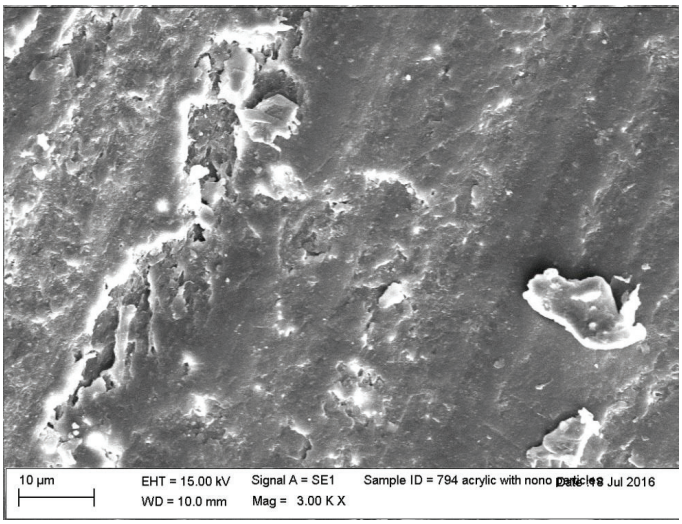

Figure 6. *SEM of nZn14 nanoparticles reinforcement specimen.*

Discussion

The flexural strength of PMMA denture base resins improved with the concentrations of nano Zn0. Rahim et al (18) established that the addition of metal nanoparticles increases the surface hydrophobicity and reduce the agglomeration of biomolecules. The studies on aluminum dioxide (19), cobalt-chromium (20), silver (21), zinc oxide (22), zirconia (23), titanium dioxide (24) nano particles have improved the flexural strength and documented the theory of surface hydrophobicity and decreased molecular agglomeration (25).

Nano particles are considered over macroscopic materials for their higher surface to volume ratios and an increased percentage of atoms at the grain boundary. The nano particles reduces the filler size increases the compaction of materials improves the mechanical properties of materials (15-17). Various nanoscale fillers, including silica, calcium carbonate, and metal oxides when added to dental -polymer matrix improved the properties. Nano-sized ZnO fillers was considered because of the unique physical properties, low cost and extensive applications in diverse areas (26-28). Xie et al. (27) observed the antibacterial properties of ZnO nanoparticles. Studies have indicated that ZnO nanoparticles at a concentration of between 3 and 10mM caused 100% inhibition of bacterial growth. Additionally, ZnO has superior biocompatibility properties and less likely to alter esthetics of denture base. The percentages of ZnO nano particles analyzed had effective antibacterial effect, obtained from the studies of Xie et al (27), Raj et al. (29).

The polymerization reaction is significant in determining the mechanical properties of denture base resin. The availability and generation of free radicals, the control of temperature during polymerization are some of the significant factors that influence the properties of the material. The compression molding technique and long curing polymerization cycle enabled to obtain to optimize the procedure and aided in obtaining the superior flexural strength.

The nanoparticles where incorporated by twin stage extruder. It aided is better dispersion in polymer matrix and homogenous distribution of the particles. The addition of nano particles to resin matrix is significant in improving the properties. The technique adapted aided in better distribution and it is visualized in SEM (28).

The distribution and dissolving of nano ZnO in PMMA aided in obtaining improved strength properties. Earlies studies on different nanoparticles emphasized on the need importance of homogenous distribution of particles for improved strength. Additionally, the particles should have displayed improved wettability with PMMA monomer. The SEM images displayed uniform distribution and no voids were observed. The images confirmed the blending of materials that improved the strength of the materials.

The study followed stringent testing protocol. Fewer limitations were unavoidable in the testing set up. In future, different concentrations of particles, size of particles, custom made nano particles, other forms of nano rods, tubes, polymerization techniques, types of PMMA resins can be evaluated. More studies are required to evaluate thermal properties , impact strength, mechanical, physical, antifungal and antibacterial spectrum for better interpretation.

Conclusion

Within the limitation of this study it can be concluded that the flexural strength of PMMA denture base increased with the addition ZnO nano particle to the PMMA denture base.

Türkçe Öz: *Çinko Oksit nanopartiküllerinin Polimetilmetakrilat protez kaidelerinin bükülme direncine etkisi. Amaç: Bu çalışmada, farklı konsantrasyonlarda çinko oksit (Zn O) nanopartkülleriyle güçlendirilmiş polimetilmetakrilatın (PMMA) bükülme direnci değerlendirilmiştir. Gereç ve yöntem: Nano Zn O yüzde 0, 0.4, 0.6, 0.8, 1.2 ve 1.4 oranlarda PMMA protez kadie materyaline ilave edilmiştir. ISO 20795-1-2013 standardına uygun olarak 10mm x 4mm x 80mm boyutlarında 60 adet ısı ile polimerize olan akrilik örnek hazırlanmıştır. Örnekler, 6 gruba bölünmüştür. Akrilik örnekler üreticinin önerileri doğrultusunda hazırlanmıştır. Bükülme direncinin ölçümü için 3 nokta eğme testi uygulanmıştır. Örneklerin yüzeylerindeki kırılmaları tespit etmek için yüzey analizi scanning electron microscobu(SEM) ile gerçekleştirilmiştir. İstatistiksel analiz için ANOVA and Tukey testleri kullanılmıştır ($p<0.05$). Bulgular: İstatistiksel analiz gruplar arasında anlamlı değişiklikler göstermiştir. Zn O nanopartküllerinin ilavesi bükülme dayanuımını arttırmıştır. En yüksek ortalama değer nZn -14 (91.31 MPa) ve en düşük değer control grubu nZn-0 (61.36 MPa) de bulunmuştur. ANOVA and Tukey testleri gruplar arası farklılıklar tespit etmiştir ($p<0.001$). Sonuç: Zn O nanopartküllerinin ilavesi her konsantrasyonda control grubuna göre akrilik rezinin bükülme direncini arttırmıştır. Klinik sonuç: PMMA'ın çinko oksit nanopartkülleriyle güçlendirilmesi protezlerin bükülme direncini arttırabilir. Anahtar kelimeler: Bükülme dayanımı; ısı ile polimerize olan akrilik; nanopartkül; polimetilmetakrilat; çinko oksit*

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: NGC designed the study. SV and NGC participated in generating the data for the study. SV and NGC participated in gathering the data for the study. SV participated in the analysis of the data. NGC wrote the majority of the original draft of the paper. NGC participated in writing the paper. All authors approved the final version of this paper.

Conflict of Interest: The author had no conflict of interest to declare.

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Original research

Contrast-to-noise ratios of different dental restorative materials: An in-vitro cone beam computed tomography study

Seval Bayrak[1], Emine Sebnem Kursun-Cakmak[2], Hakan Kamalak[3]

Purpose

In radiological views, strong beam hardening and streaking artifacts occur due to high-density structures and polyenergetic X-ray beams, and these lead to misdiagnosis. This study was performed in vitro to compare the contrast-to-noise ratio (CNR) of commonly used dental restorative materials by using Cone Beam Computed Tomography (CBCT) images with and without artifact reduction (AR) mode.

Materials and Methods

A total of 108 molar teeth were restored with nine different groups of restorative materials, with each group containing 12 teeth. Teeth were placed in a dry human mandible and scanned, one by one, via Planmeca 3D ProMax (Planmeca, Helsinki, Finland) with and without AR mode. Images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD) to calculate the CNR.

Results

CNR was calculated to be the highest in compomer (Glassiosite) images without AR mode (mean: 3.36) and with AR mode (mean: 3.61). CNR was calculated to be the lowest in amalgam (Tytin) images without AR mode (mean: 0.21) and with AR mode (mean: 0.23). A significant difference was found between materials in terms of CNR measurements ($p \leq 0.05$). CNR measurements were increased after the AR mode application ($p \leq 0.05$).

Conclusion

AR mode was effective in reducing artifacts arising from dental materials on CBCT images, so it is necessary to use AR mode for correct diagnoses.

Keywords: *Cone-Beam Computed Tomography; contrast-to-noise ratio; dental materials; artifacts; image quality*

Introduction

Cone Beam Computed Tomography (CBCT) has been used in dentistry for dental, maxillofacial, and various head and neck examinations (1). This technology supplies 3D images for regions of interest with high spatial resolution, geometric accuracy, and lower ionizing radiation doses than other tomographic devices (2, 3). Many parameters affect image quality; these include the field of view (FOV), X-ray beam quality and quantity, voxel sizeand rotation arc in CBCT images (4).

Imaging technology should obtain high-quality images with sufficient contrast-to-noise ratio (CNR) and soft-tissue differentiation while minimizing the required dose of radiation (5).

Since CNR is considered a standard factor in the evaluation of image quality, it has been measured in previous studies. Researchers have stated that the same material exhibits different CNR values with different exposure parameters (6, 7).

ORCID IDs of the authors: S.B. 0000-0003-0819-4547; E.S.K.C. 0000-0002-7113-5450; H.K. 0000-0002-1497-2009

[1]Abant İzzet Baysal University, Dentistry Faculty, Dentomaxillofacial Radiology Department, Bolu, Turkey

[2]Ministry of Health, Türkiye Public Hospitals Agency, Ankara, Turkey

[3]Kahramanmaraş Sütçü İmam University, Dentistry Faculty, Department of Restorative Dentistry, Kahramanmaraş, Turkey

Corresponding Author: Seval Bayrak

E-mail: dtseval@hotmail.com

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CBCT manufacturers are developing artifact reduction (AR) software to decrease the influence of beam hardening and streaking artifacts caused by high-density materials; this result in an increased CNR value (8, 9). Although these types of software programs eliminate streaks far from metallic objects, the details around metal-tissue interfaces, which might be the main regions of interest, still may not be visible to clinicians (10).

As a result of dental materials having been developed very rapidly, many studies have been planned to analyze their biological, physical, and mechanical characteristics, all of which can affect and predict their performance. Knowledge of the characteristic properties of dental materials is essential to support their correct application and to expect the long-term performance of these materials (11).

Materials containing metal have been reported to cause artifacts that inhibit the diagnostic quality of CBCT images by decreasing contrast, concealing structures, and consequently impairing estimates of the region of interest. In radiographic images, beam hardening and streaking effects occur due to high-density structures and polyenergetic X-ray beams; these lead to dark streaks that are known as image artifacts, resulting in misdiagnosis (12). The intensity of these artifacts increases in CBCT images as the percentage of radio-opacifying materials increases (13).

This study aimed to compare in-vitro the CNR of commonly used dental restorative materials by using CBCT images with and without AR mode.

Materials and Methods

Preparation of teeth

This in vitro study protocol was in accordance with the Declaration of Helsinki approved by the Local Ethical Committee of Fırat University (Review No. 15.06.17/02).

108 noncavitated human permanent molar teeth extracted for periodontal or orthodontic reasons independent from this study were used. The teeth had no cavitations, restorations, or hypoplastic pits as judged by the naked eye. Extracted teeth were cleaned and kept in distilled water for 24 hours at 37°C.

Class I (occlusal) cavities (7 mm × 3 mm × 4 mm) were made with a carbide bur (#330, Mani; MANI Inc., Tokyo, Japan) for 108 molar teeth and randomly divided into nine groups each containing 12 teeth.

After cavity preparation, the groups were restored with 9 different restorative materials. These materials are summarized with their specifications in Table 1.

CBCT imaging

A dry human mandible covered with a pink wax layer to simulate soft tissues was used in the imaging procedures, and the restored teeth were placed in the same socket throughout the study. A hexagonal plexiglass box filled with

Table 1. *Restorative materials and their characteristics evaluated in the study*

Material Name	Manufacturer	Material type	Matrix type	Filler content	Filler ratio %
Estelıte® Sıgma Quıck	Tokuyama Tokyo, Japan	Submicron filled composite resin	Bis-GMA, TEGDMA	Spherical silica-zirconia filler and silica-zirconia prepolymerized fillers	82
Estelıte® Flow Quıck	Tokuyama Tokyo, Japan	Low viscosity, medium flow, light cured, radiopaque composite resin	Bisphenol A polyethoxy Methacrylate (Bis-MPEPP), TEGDMA, UDMA	Silica- zirconia filler and silica-titanium filler	71
Filtek Bulk Fill	3M-Espe (St. Paul, Mn, USA)	Bulk-fill flowable composite	Bis-GMA, UDMA Bis-EMA	Procrylat resins	64.5
Surefil SDR	Dentsply Caulk, Universal	Bulk-fill composite	Modified UDMA TEGDMA, EBPDMA	Ba-Al-F-B-Si glass and St-Al-F-Si glass as fillers	68
Ionoseal	VOCO Gmbh, Cuxhaven, Germany	Resin-reinforced glass ionomer cement	Bis-GMA, HEMA, TEDMA	Fluoroaluminum silicate, champherechinon, amine	-
Tytin	Kerr Manufacturing Co., Romulus, MI, USA	Spherical high-copper amalgam alloy	-	Silver 59 %, Tin 28%, Copper %13, Hg 42.5 %	-
GCP Glass Fill	Gcp Dental Elmshorn Germany	New carbomised nano-particles	Modified Polysiloxanes	Fluoro-aluminosilicate Glass, Apatite, Polyacids	-
CAD/CAM Katana	KATANA® Noritake Dental Supply Japan	CAD/CAM inlay restorations	-	-	-
Glassiosite	VOCO Gmbh Cuxhaven, Germany	Compomer	BisGMA, di-UDMA,TEGDMA, BHT	Glass ceramics, silicates	77.5

Figure 1. *Dry human mandible positioned in a hexagonal plexiglass box with soft base plate wax covering the mandibular crest.*

water to mimic clinical conditions was used to position the machine. Colored markers were made to place the mandible into the same position after changing the teeth (Figure 1).

The mandible was radiographed with a Planmeca 3D ProMax (Planmeca, Helsinki, Finland). This device was operated with and without AR mode parameters at 76 kVp, 4.5 mA, 13.5 sn, 20 x 10.2 cm FOV, and 0.4 mm voxel size.

For each of the restorative materials, the mandible was scanned 24 times (12 times with the AR option and 12 times without the AR option) after changing the restored teeth (n = 12). A total of 216 scans were acquired for the nine different restorative materials.

All images were evaluated by one maxillofacial radiologist on two separate sessions with at least a one-week interval.

CNR measurement

Volumes were exported using multislice DICOM format. Identical images were chosen at the same level from each restored tooth image, and measurements were made using ImageJ software (National Institutes of Health, Bethesda, MD). Two separate areas were selected for each image, and mean gray value and standard deviation were measured. The first area was selected on the wax located lingual to the dental restorative material; the second area, called the control area, was chosen where the artifact was minimal (Figure 2). The CNR was calculated using the following formula: (9)

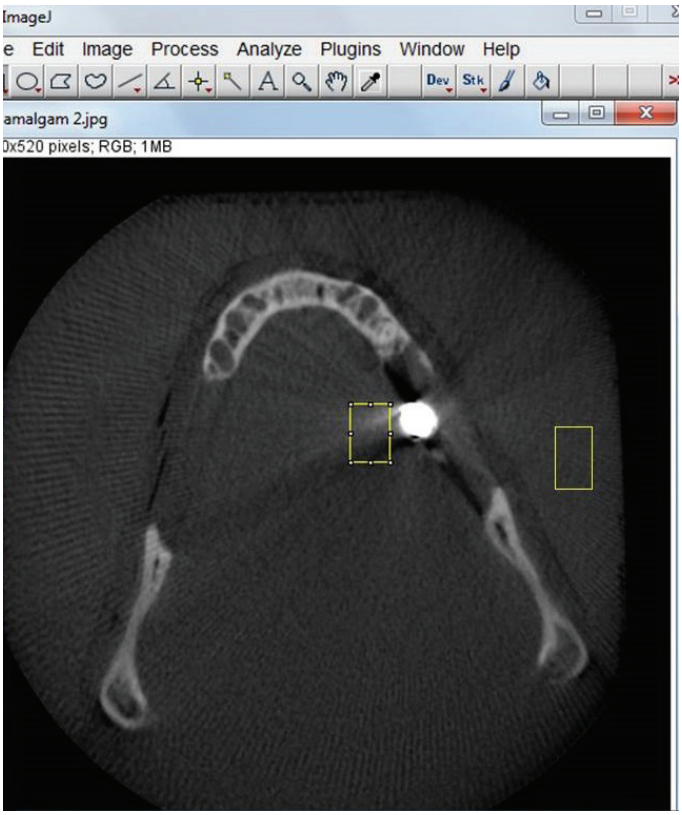

Figure 2. *Areas of interest used for CNR calculation.*

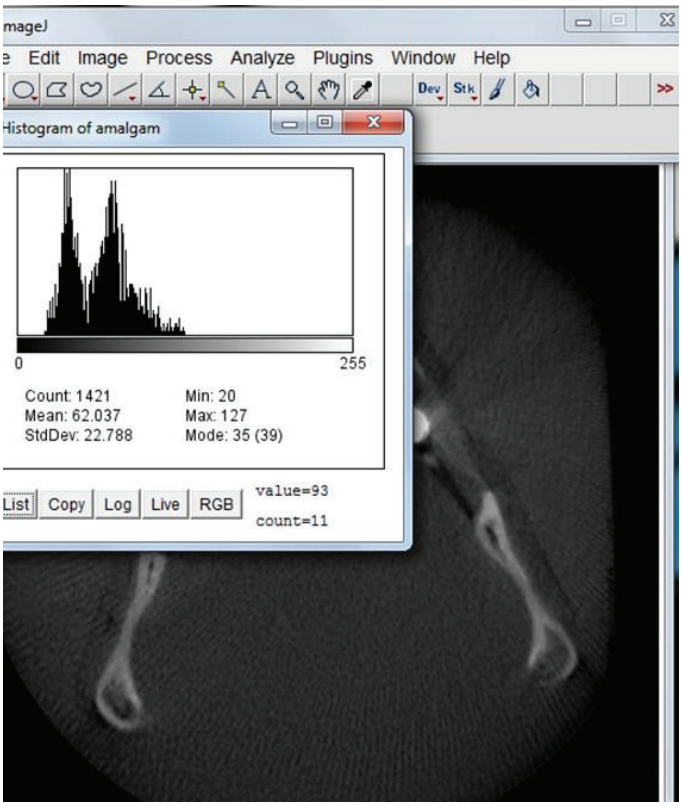

Figure 3. *Histogram showing CNR values of restorative materials.*

Table 2. *Contrast-to-noise ratio (CNR) measurement values with and without AR mode for various restorative materials*

Materials	N	Without AR Module				P*	With AR Module				P*
		Mean	SD	Median	Range (min-max)		Mean	SD	Median	Range (min-max)	
Gcp Glass Fill	12	0.49[a]	0.07	0.51	0.30-0.57	**0.001**	0.52[a]	0.08	0.55	0.31-0.61	**0.001**
Ionoseal		0.58[a]	0.12	0.57	0.42-0.92		0.62[a]	0.13	0.59	0.49-0.98	
Estelite Sigma Quick		1.29[b]	0.17	1.25	1.13-1.65		1.44[b]	0.20	1.37	1.25-1.84	
Cad-Cam/Katana		0.25[a]	0.50	0.25	0.19-0.37		0.29[a]	0.07	0.27	0.21-0.49	
Tytin		0.21[a]	0.03	0.22	0.14-0.27		0.23[a]	0.03	0.24	0.16-0.29	
Estelite Flow Quick		2.30[c]	0.25	2.20	1.92-2.69		2.47[c]	0.23	2.42	2.11-2.84	
Filtek Bulk Fill		3.04[d]	0.39	2.97	2.40-3.69		3.20[d]	0.43	3.10	2.51-2.89	
Glassiosite		3.36[d]	0.12	3.36	3.14-3.64		3.61[d]	0.15	3.59	3.28-3.90	
Surefil SDR		2.41[c]	0.44	2.46	1.24-2.85		2.57[c]	0.47	2.64	1.32-3.10	

*P-values refer to statistically significant differences between groups ($p < 0.05$). SD: Standard Deviation

$$\mathrm{CNR} = \frac{|Mean_{wax} - Mean_{control}|}{\sqrt{SD_{wax}{}^2 + SD_{control}{}^2}}$$

Where mean is the mean gray value and SD is the standard deviation gray value in the same region of interest as seen in Figure 3.

Statistical analysis

A non-parametric test (Kruskal-Wallis Test) was used due to unequal variances across groups, and another non-parametric test (the Mann-Whitney U test) was performed for comparisons between groups. The SPSS 10.0© software (SPSS Inc., IBM Company Headquarters, Chicago, IL) was used for analyzing data. The significance level value was set at 0.05.

Results

The compomer (Glassiosite) exhibited the highest CNR (mean: 3.36), and amalgam (Tytin) exhibited the lowest CNR (mean: 0.21), showed statistically significant difference without AR mode ($p \leq 0.05$, Table 2).

The compomer (Glassiosite) exhibited the highest CNR (mean: 3.61), and amalgam (Tytin) exhibited the lowest CNR (mean: 0.23), showed statistically significant difference with AR mode ($p \leq 0.05$, Table 2).

When we compared CNR using the AR module, significant differences were found between groups ($p \leq 0.05$). The AR application enhanced the CNR values of all the tested materials.

Discussion

Contrast resolution, which is identified as the capability to discriminate between different contrast levels in an acquired image, is a substantial aspect of image quality in CBCT scans (14). The CNR is a factor associated with image quality rather than image noise. It is one factor among many other factors that depend on an acceptable level of lesion-to-background contrast (15, 16).

In the present study, AR mode indicated positive effects on CBCT images of dental restorative materials with a significant increase of CNR results. Applying the AR algorithm to CBCT scans prolongs the reconstruction time; therefore, when high-density materials are in the FOV, applying the AR algorithm is indicated to increase the image quality (6).

Bechara *et al.* (17) showed that the CNR is increased if the AR algorithm is used, but metal artifacts cause a decrease in the CNR. Previous studies report that artifacts and low CNR, particularly in the presence of gutta percha and canal sealers, lead to misdiagnoses of root fractures and voids and also lead to false-positive diagnoses (9).

In studies conducted with a Planmeca ProMax CBCT system, Kamburoğlu *et al*. (18) estimated no difference in the examination of peri-implant and periodontal defects among CBCT scans with and without the AR mode. Bechara *et al.* (12) found the highest accuracy of detecting root fractures in endodontically treated teeth without the AR mode.

It has been stated that using AR modes may be helpful if there is no need for high contrast and spatial resolution. This was stated as a result of a study that proved that the accuracy of root fracture detection in endodontically treated teeth was reduced after using the AR modes in two different CBCT devices (9).

Demirtürk *et al.* (9) investigated the CNR of multiple exposure parameters for different types of retrograde filling materials in CBCT scans with and without AR mode and stated that although there was no statistically significant difference between Biodentine, SuperEBA, MTA, and amalgam, the highest CNR was seen in Biodentine. Also, they reported that AR mode reduced the effect of the beam hardening and streaking artifacts caused by filling materials, resulting in a significant increase in the CNR seen with all four root-end filling materials (9).

Querioz *et al*. (6) studied the efficacy of AR mode in different dental materials and observed a significant reduction of artifact expression in tested materials except for gutta-percha, which may be explained by its having a low number of atoms. Parsa et al. (19) investigated if the AR tool can increase the gray value levels in CBCT images which were obtained

after implant placement and reported that the software did not correct the voxel gray values caused by the metal artifact around the implant in dry human mandibles.

Pauwels *et al.* (20) stated that CNR values are dependent on specific machines due to variations in hardware and software. This study was designed with a single CBCT with single exposure settings. Therefore, these results cannot be compared with those obtained from other CBCT units. Future studies will focus on other CBCT units and several exposure settings.

We recommend that CBCT imaging of patients with high-density dental materials be obtained using AR mode. In this way, the diagnostic quality is protected with an increase in CNR values. In examinations of CBCT images obtained for various reasons, it should be borne in mind that pathological conditions, such as caries, fracture, resorption, and other conditions, can be overlooked near restorations with lower CNR values, so it is better to support the diagnosis with an additional imaging modality (15).

Conclusions

Within the limitations of this study, we conclude that the CNR is affected by different restorative materials in CBCT scans. High-density materials exhibited lower results, and the application of the AR algorithm enhanced the CNR values. AR can be used on CBCT images of objects containing high-density restorative materials due to its efficacy in enhancing image quality.

Türkçe Öz: *Farklı dental restoratif materyallerin kontrast noise oranı: bir in- vitro konik ışınlı bilgisayarlı tomografi çalışması. Amaç: Radyografik görüntülerde, kuvvetli ışın sertleşmesi ve saçılma artefaktları yüksek dansiteli yapılardan ve polienerjik X ışını demetinden kaynaklanır ve yanlış tanıya neden olur. Bu çalışma yaygın kullanılan dental restoratif materyallerin in vitro olarak Konik Işınlı Bilgisayarlı Tomografi (KIBT) görüntülerinde artefakt azaltma (AA) modu kullanılarak ve kullanılmadan kontrast noise oranını (KNO) kıyaslamak için yapıldı. Materyal ve Metod: Her biri 12 dişten oluşan toplamda 108 molar diş dokuz farklı grup restoratif materyalle restore edildi. Dişlerin her biri kuru insan mandibulasına yerleştirilerek AA modu kullanılarak ve kullanılmadan Planmeca 3D ProMax(Planmeca, Helsinki, Finland) ile tarandı. Görüntüler ImageJ (National Institutes of Health, Bethesda) programı kullanılarak analiz edilip KNO hesaplandı. Bulgular: KNO, AA modu kullanılmadan (ortalama: 3,36) ve AA modu kullanılarak (ortalama:3,61) en yüksek kompomer (Glassiosite) görüntülerinde; AA modu kullanılmadan (ortalama: 0,21) ve AA modu kullanılarak (ortalama:0,23) en düşük amalgam (Tytin) görüntülerinde hesaplandı ($p \leq 0.05$). KNO ölçümleri AA modu kullanıldığında artış gösterdi. ($p \leq 0.05$). Sonuç: AA modu KIBT görüntülerinde dental materyallerden kaynaklanan artefaktları azaltmada etkilidir ve AA modunu kullanmak doğru tanı için gereklidir. Anahtar Kelimeler: Konik ışınlı bilgisayarlı tomografi; kontrast noise oranı; dental materyal; artefakt; imaj kalitesi*

Ethics Committee Approval: This in vitro study protocol was in accordance with the Declaration of Helsinki and the study protocol was approved by the Local Ethical Committee of Fırat University (Review No. 15.06.17/02).

Informed Consent: The informed consents were provided by the participants.

Peer-review: Externally peer-reviewed.

Author contributions: ESKC designed the study. ESKC and HK participated in generating the data for the study. SB participated in gathering the data for the study. ESKC participated in the analysis of the data. SB wrote the majority of the original draft of the paper. SB and ESKC participated in writing the paper. All authors approved the final version of this paper.

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Original research

Horizontal continuous and apical stretching sutures does not reduce FGG shrinkage: A split-mouth randomized controlled clinical trial

Amir Shammas[1], Hadi Ranjbar[2], Mohadeseh Arab Solghar[3], Najme Asghari[4], Mohammad Mohammadi[5]

Purpose
This study aimed to evaluate whether horizontal continuous and apical stretching sutures could reduce FGG shrinkage.

Materials and Methods
In this randomized controlled clinical trial ten patients (20 sites, seven females and three males) ranging from 18 to 53 years (average 39 years) with insufficient keratinized and attached gingiva received FGG in two quadrants of mandible (split-mouth design). Horizontal continuous and apical stretching sutures were used in test sites in addition to common suturing techniques. Clinical parameters including probing depth (PD), the width of keratinized and attached gingiva (KG, AG), the horizontal and vertical dimension of the graft (HD, VD), and graft area (GA) were recorded at baseline and 1, 3, and six months after the operation.

Results
PD did not differ significantly for six months. The average change of other parameters in test and control sites respectively was as follows: KG increased 5.5 mm and 5.1 mm, AG increased 5.3 mm and 5.1 mm, HD shrinkage was 21.6% and 15.8%, VD shrinkage was 33.7% and 33.2%, GA shrinkage was 47.3% and 43.3%. There were no significant differences between test and control sites in clinical parameters six months after surgery.

Conclusion
Application of horizontal continuous and apical stretching sutures does not reduce FGG shrinkage.

Keywords: *Free gingival graft; graft shrinkage; continuous horizontal suture; apical stretching suture; probing depth*

ORCID IDs of the authors: A.S. 0000-0001-6389-6420 H.R. 0000-0002-3672-7266; M.A.S. 0000-0003-1152-1314 N.A. 0000-0002-9908-0981; M.M. 0000-0001-6257-6126

[1]University of Medical Sciences, Dental School, Department of Periodontics, Birjand, Iran

[2]Iran University of Medical Sciences, Tehran Institute of Psychiatry- School of Behavioral Sciences and Mental Health, Mental Health Research Center, Tehran, Iran

[3]Kerman University of Medical Sciences, Dental School, Department of Periodontics, Kerman, Iran

[4]Kerman University of Medical Sciences, Kerman, Iran

[5]Kerman University of Medical Sciences, Dental School, Department of Periodontics, Kerman, Iran

Corresponding Author: Mohammad Mohammadi

E-mail: m_mohammadi@kmu.ac.ir

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Introduction

The free gingival graft (FGG) is a surgical procedure which is used to increase the keratinized tissue around the teeth (1, 2). It is the oldest surgical technique in periodontal surgery (3). In this procedure, the graft is obtained from the palate or the maxillary tuber. The FGG is the gold standard for the gingival augmentation procedures a procedure that is used to increase keratinized tissue around teeth (4). Dentists use FGG in the clinical situations that thin gingiva might be less protective in the presence of inflammation and gingival recession. Orthodontic treatment, inadequate plaque control, high frenulum attachment, and shallow vestibular depths are the leading causes of mucogingival problems (5).

After utilization of FGG, the vestibular depth of recipient area may be diminished by contraction of wound and reinsertion of muscle fibers in postoperative stage (6). Therefore, dimensional changes including the

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contraction of transplanted tissue may occur, especially in long-term follow-up, and as a result, expected treatment outcome including a satisfied amount of new keratinized tissue may not be achieved (6). The other factors affecting on FGG shrinkage are phenotype of the periodontium, the thickness of graft, and type of periosteal bed in the term of preserving periosteum or not (7-9). Suturing technique has been considered as an essential factor affecting graft shrinkage. According to Oschenbein, stretching the graft by continuous horizontal suture may counteract the first contraction and make the graft more receptive to revascularization by influencing the collapsed blood vessels (10). Furthermore, Miller proposed apical stretching suture to prevent coronal movement of the graft and reduce shrinkage by keeping the vascular channels patent within the graft (11). It is essential to accomplish this operation with as low as a possible number of sutures in order to reduce trauma to the graft (10). To our knowledge so far, no study has analyzed the effect of horizontal continuous and apical stretching sutures on graft contraction. Therefore the purpose of this study was to evaluate whether horizontal continuous and apical stretching sutures could reduce FGG shrinkage.

Materials and Methods

Study design

In this split-mouth randomized controlled clinical trial, 10 patients (7 females and 3 males) ranging from 18 to 53 years (average 39 years) with insufficient keratinized and attached gingiva (keratinized gingiva < 2 mm, attached gingiva < 1 mm) in premolar regions of mandible were selected based on their need to bilateral gingival augmentation due to oral hygiene procedures or prosthetic or orthodontic reasons or two-stage root coverage. Exclusion criteria were systemic diseases contraindicated periodontal surgery, pregnancy, breastfeeding, taking medications affecting on periodontium such as phenytoin, smoking, and full mouth plaque index and bleeding index> 20%. All patients received the treatment protocol and accepted the detailed informed and written consent. The ethics committee approved this research in Kerman University of Medical Science (Ethics code: KA/90/180) and registered through the IRCT website with the number IRCT201608205305N5.

Full mouth scaling and root planing and oral hygiene enhancement were performed for all patients one month before surgery. The width of keratinized gingiva (distance from gingival margin to the mucogingival junction) by Roll technique and the probing depth in the midbuccal region was measured with a Michigan-O-Probe (Hu-Friedy, USA) to the nearest 1 mm. The width of the attached gingiva in the midbuccal region was evaluated by subtracting probing depth from the width of keratinized tissue. All measurements were performed by an experienced periodontist who was blind to the study. Two sites in each patient were randomly assigned to test or control with throwing a coin (simple randomization).

Surgical procedure

Each patient received free gingival graft in test and control sites at the same visit. The local anesthetic (Lidocaine 2% with epinephrine 1/80000) was administrated to recipient and donor sites. At the recipient site, a 15C scalpel (Hu-Friedy, USA) was utilized to make a 10 mm horizontal incision in mucogingival junction with two 9 mm vertical incisions in mesial and distal extensions. A partial thickness bed was prepared with a scalpel. A Mucotome (PR4, DEPPELER, Swiss) was used to harvest a 9 × 10 mm free gingival graft with homogenous thickness (1.5 mm) from the palatal area of premolar and first molar teeth. The donor site was sutured by the arbitrary technique with 4-0 silk suture (SUPA medical devices, IRAN) to control bleeding and protected with periodontal dressing (Coe-Pak, GC, Japan). The size of the graft was measured midway in vertical and horizontal directions using a Michigan-O-Probe (Hu-Friedy, USA). The graft was sutured to the recipient region by SERALON 4-0 suture (SERAG-WIESSNER Co., Germany). The usual suturing techniques (conventional sutures) which have been used at test and control sites included two interrupted sutures in coronal and two interrupted sutures in mesial and distal of the graft and one circumferential suture (10). The latter was placed through the periosteum apical to the recipient bed slightly below the inferior border of the graft and carried around the cervical region of the tooth and tied (Figure 1). Two additional suturing techniques which considered only at test sites included horizontal continuous and apical stretching sutures. So we used combined sutures in test sites (Figure 2). The continuous horizontal suture was placed approximately midway corono-apically across the graft. First, the graft was tied at its distal margin to the underlying periosteum. The suture was not cut, but it was carried anteriorly across the

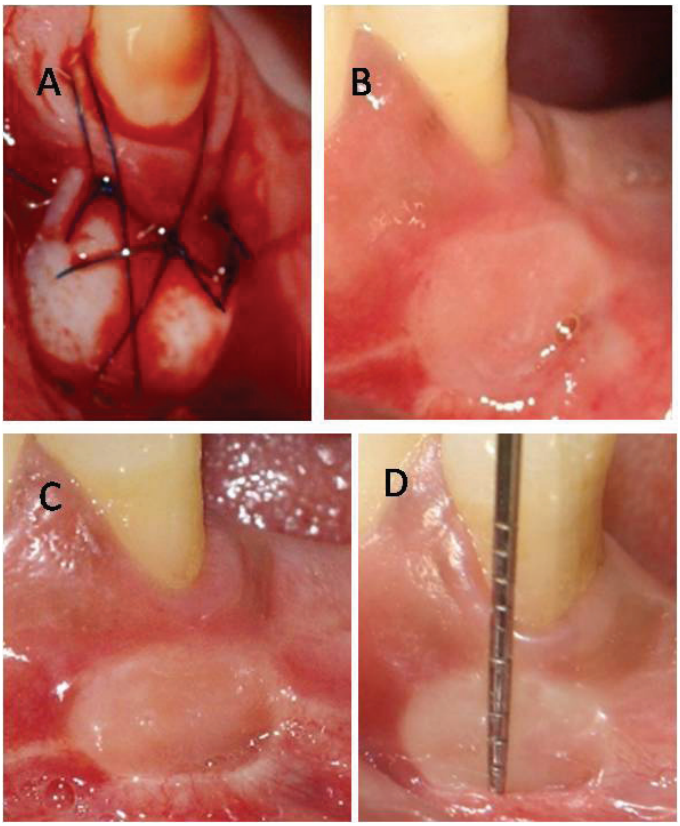

Figure 1. *Interrupted sutures in coronal, mesial and distal of the free gingival graft and one circumferential suture at control site (A). Free gingival graft dimensions 1(B), 3 (C), and 6 (D) months after the operation.*

graft with the needle passing through the mesial margin of the graft and exiting at its undersurface. Slack was left in the portion of the suture that extended across the body of the graft. The needle entered the periosteum at a sufficient distance from the mesial border of the graft so that the graft could be stretched adequately, ordinarily 2-3 mm. The suture end that had exited from the periosteum was tied to the loop formed by the slack. In the apical stretching technique two interrupted sutures with distal angulation tied the periosteum to the mesio-apical and disto-apical borders of the graft. The periodontal dressing (Coe-Pak, GC, Japan) was finally placed on the graft at test and control sites. All surgery procedures were performed by a periodontist who was not blind to the study.

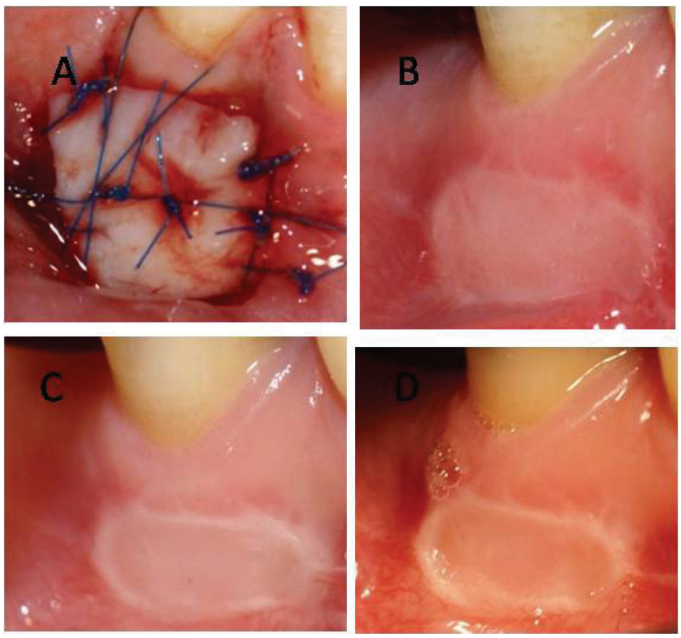

Figure 2. *Two additional suturing techniques which considered only at test sites were horizontal continuous and apical stretching sutures (A). Free gingival graft dimensions 1(B), 3 (C), and 6 (D) months after the operation.*

Postsurgical care

Patients have prescribed 0.2% chlorhexidine mouthwash and instructed to rinse gently twice daily for two weeks. Tooth brushing activities in the operated sites were discontinued during this time. They were also given ibuprofen (400 mg, four times daily in the case of feeling pain) and amoxicillin (500 mg, three times daily for one week). The periodontal dressing and sutures were removed two weeks after surgery, and then mechanical supra-gingival plaque control was permitted.

Postsurgical evaluation

Clinical parameters which were evaluated after surgery included probing depth (PD), the width of keratinized and attached gingiva (KG, AG), the horizontal and vertical dimension of the graft (HD, VD), and graft area (GA). KG and PD in the midbuccal region were measured with a Michigan-O-Probe (Hu-Friedy, USA) to the nearest 1 mm. AG in

Table 1: *Clinical parameters at baseline, 1,3, and six months after surgery at test and control sites, and comparison of these clinical parameters between test and control sites.*

Clinical parameters	Baseline Mean ± SD	1 month Mean ± SD	3 months Mean ± SD	6 months Mean ± SD	Within groups P value	Between groups P Value
Probing depth						
Test	1.30±0.67	1.30±0.48	1.30±0.48	1.10±0.31	0.258	0.800
Control	1.10±0.31	1.20±0.42	1.10±0.31	1.10±0.31	0.343	
The width of keratinized gingiva						
Test	1.50±0.84	7.70±1.25	7.20±1.61	7.00±1.63	0.00	0.323
Control	1.90±0.56	7.30±1.15	6.70±1.15	7.00±1.49	0.00	
The width of attached gingiva						
Test	0.6±0.69	6.40±1.42	5.90±1.91	5.90±1.85	0.00	0.805
Control	0.80±0.63	6.10±1.44	5.60±1.34	5.90±1.66	0.00	
Horizontal dimension of graft						
Test	9.70±0.94	8.90±1.10	8.00±1.24	7.60±1.57	0.005	0.505
Control	9.50±0.70	9.10±1.10	7.90±1.44	8.00±1.41	0.040	
Vertical dimension of graft						
Test	8.90±0.99	6.40±1.42	5.90±1.91	5.90±1.85	0.007	0.952
Control	8.70±0.82	6.10±1.44	5.60±1.34	5.90±1.66	0.001	
Graft area						
Test	86.60±15.39	57.30±15.07	48.40±21.39	45.60±18.33	0.003	0.821
Control	82.90±12.35	55.40±14.48	44.10±13.16	47.00±15.07	0.00	

the midbuccal region was evaluated by subtracting PD from KG. The size of the graft in two dimensions was determined midway mesiodistally and corono-apically with a Michigan-O-Probe (Hu-Friedy, USA). The surface area of the transplanted tissue was calculated by multiplying the vertical and horizontal dimensions. The shrinkage of the graft in horizontal and vertical dimensions and the shrinkage of its surface area were also calculated according to this formula:

$$\text{Shrinkage} = \frac{\text{Postoperative value} - \text{preoperative value}}{\text{Preoperative value}} * 100$$

The same investigator recorded these parameters at baseline and 1, 3, and six months after the operation to evaluate the subsequent contraction of the graft.

Statistical analysis

One sample Kolmogorov Smirnov test revealed a normal distribution of the data ($p>0.05$). Repeated measures ANOVA, was utilized in order to analyze the data.

Results

All patients had mild to moderate pain after the surgery but there was no complication. Clinical parameters including PD, KG, AG, HD, VD and GA were recorded at baseline and 1, 3, and six months after the operation (Figure 1 and Figure 2) (Table 1). PD was relatively fixed in test and control sites and did not differ significantly during six months ($p=0.258$, and $p=0.343$ respectively). The average change of other parameters in test and control sites (comparison between baseline and 6 months after surgery) respectively was as follows: KG increased 5.5 mm, and 5.1 mm, AG increased 5.3 mm and 5.1 mm, HD shrinkage was 21.6% and 15.8%, VD shrinkage was 33.7% and 33.2%, GA shrinkage was 47.3% and 43.3%. The increase of KG and AG and a decrease of HD and VD and GA were observed in test and control sites during the time, and these changes were statistically significant in each site according to the repeated measure test (Table 1). However, in general, there were no significant differences between test and control sites in any clinical parameter six months after surgery (Table 1).

Discussion

FGG is the most common technique for increasing keratinized, and attached gingiva (8, 12, 13). After the FGG surgery, tissue shrinkage and dimensional changes occur, especially in long-term follow-up and may affect the outcome of treatment (6). One of the factors that influence the contraction of the FGG is suturing technique. In the present study, the effect of apical stretching and horizontal continues suturing on graft contraction was examined for the first time. The results of our study showed increasing of attached and keratinized gingiva in both test and control groups. The amount of keratinized gingiva was 5.5 mm and 5.1 mm in test and control groups respectively. Therefore, the average of keratinized gingiva width in the test was 0.4 mm more than control. The amount of attached gingival augmentation was 5.3 mm in the case and 5.1 mm in control groups. In other words, the average increase in width of attached gingiva in test sites was 0.2 mm more than control sites. There was no statistical difference in the amount of keratinized and attached gingiva between test and control groups.

Silva (19) evaluated the healing of FGG in the anterior portion of the mandible in smokers in comparison with non-smokers. After three months, the mean width of keratinized gingiva was 5.4 and 4.8 mm in non-smokers and smokers respectively, but the difference between the two groups was not statistically significant. Wei, Laurell (14) compared the clinical results of FGG and alloderm regarding achieving increased attached gingiva. After six months, the keratinized gingival was 5.57 mm in FGG group and 2.59 mm in alloderm group. The difference was significant.

Agudio, Nieri (15) evaluated the long-term efficacy of FGG in 224 sites (103 patients). After one year, an average of keratinized gingiva was 4.2 mm, and after 10-25 years, it was reduced to about 0.7 mm. Harris (7) evaluated three different techniques (FGG, free connective tissue graft, and Allograft) and increasing the width of keratinized gingiva was statistically significant in all three groups. The result of FGG and Allograft was 4.1 mm, and connective tissue graft was 3.6 mm. After the free gingival grafts in 53 patients in Kovacevic (16) study, the gain of keratinized gingival was 5.41 mm that this gain was significant.

In the present study, the average of sulcular depth was reduced about 0.2 mm in the test group in comparison of a control group that no change was seen in sulcular depth. However, change of sulcular depth was not significant. In Egli, Vollmer (17) study, FGGs in 42 teeth ($n = 12$) evaluated for a year and did not see any change in probing depth. In the same study that performed by Rateitschak, Egli (18), no significant difference was recorded after four years. In Aquido study, sulcular probing depth remains constant after 10-25 years old (15).

Our results showed that horizontal, vertical dimensions and the graft area were reduced in both the test and control group compared to pre-operative measurement, but it was not statistically significant. Therefore, the results of this study revealed that horizontal continuous and apical stretching did not have any effect in reducing shrinkage of the gingival graft. In the present study, the amount of horizontal shrinkage was 2.1 mm (21.6%) while it was 1.5mm (15.8%) in the control site. So in a clinical view, the amounts of horizontal contraction in the test group were 0.6 mm (5.8%) more than the control group. The most percentage of horizontal contraction was 10.1% in the test group and 13.2% in the control group that occurred between the first and third months after the surgeries.

These results were comparable to Hatipoglu, Keceli (8) study that estimated horizontal shrinkage 10.2% after six months, and it is not statistically significant while it was in contrast with our study that was significant. In Silva study, the percentage of horizontal shrinkage was 22% in non-smokers and 25% in smokers that looks considerable, but the difference between the two groups was not significant (19). In the present study, vertical shrinkage was 3mm (33.7%) in test and 2.8 mm (32.2%) in control groups. In other words, the test group had more vertical graft contraction (1.5%) than control groups. Most vertical shrinkage was 28.1% in the

intervention group and 29.9% in the control group in the first month after surgeries. It was also observed that Vertical shrinkage was greater than horizontal contraction that was similar to Hatipoglu, Keceli (8) study. In the study above, average vertical shrinkage was 24.8% after six months and they reported statistical significant like our study.

Silva, Ribeiro Edel (19) showed vertical shrinkage in smokers was 44% versus in non-smokers reported 31% that the difference was not significant. The average vertical contraction was reported 25% within a year in Egli investigation (17). The results of Jung study showed vertical shrinkage in classic and strip technique was 29% and 28% respectively that did not find any significant difference (20).

In six months study of Wei, vertical shrinkage was reported 16% in FGG group and 71% in Allograft group that was statistically significant (14). In Kim study, the average vertical contraction after six months of free gingival grafts, connective tissue graft was 29% and 55% respectively. This difference was statistically significant (11). Rateitschak, Egli (18) estimated 25% vertical shrinkage after one year follow up.

In our study, area shrinkage was 47.3% in test and 43.3% in the control group. Therefore, in clinical view, the percentage of area shrinkage in the test was 4% more than the control group. The most percentage of area shrinkage was 33.8% in test and 33.1% in control sites after one month after surgery. These results are similar to Hatipoglu, Keceli (8) study that the average contraction of 35.3% was achieved six months after transplantation. In comparing smokers and non-smokers in Siva study, graft area shrinkage was 58% and 44% respectively. The changes in both groups were statistically significant, but the difference between the two groups was not statistically significant (19).

In the present study, using of Horizontal Continuous and Apical Stretching sutures does not seem to be efficient. Although the difference was not statistically significant between test and control groups, in term of clinical view, the percentage of graft shrinkage was more in intervention group that the most important factor could be trauma from an additional entry needle into the tissue. When the number of are increased to stabilize the graft, more trauma may exerted to the graft, and therefore, the probability of graft necrosis will increase. We can conclude that, using minimum number of sutures to stabilize the graft can be a factor which decrease the graft shrinkage.

One of our study limitations was low sample size which was because of budget and time shortage. The power analysis showed that a study with larger sample size can show the differences between two methods more accurately.

Conclusion

The findings of our study showed that using of apical stretching and continuous horizontal sutures do not provide any advantage in reducing graft shrinkage. Therefore, the use of the aforementioned sutures is not recommended.

Türkçe Öz: *Türkçe başlık Yatay devamlı ve apikal esnetici dikişler SDG büzülmesini etkilemiyor: Split-Mouth randomize kontrollü klinik çalışma. Amaç: Bu çalışmada, yatay devamlı sutur ve apikal germe suturlarının Serbest diş eti greftinin büzülmesini azaltıp azaltmayacağı değerlendirildi. Gereç ve Yöntem: Bu randomize kontrollü klinik çalışmada, yetersiz keratinize ve yapışık diş eti bulunan, 18-53 (ort:39) yaş aralığında, 10 hastada (7K,3E) alt çene her iki kadranda Serbest diş eti grefti uygulandı. Test bölgelerinde, yaygın kullanılan sutur tekniklerine ek olarak yatay devamlı sutur ve apikal germe sutur kullanıldı. Sondalama derinliği(PD), keratinize ve yapışık diş eti genişliği (KG,AG), greftin yatay ve dikey boyutu (HD,VD), greftin alanı (GA) da dahil klinik parametreler başlangıçta,1. Ve 3.ayda, ameliyattan 6 ay sonra kaydedildi. Bulgular: Sondalama derinliğinde (PD) 6 ay boyunca anlamlı fark görülmedi. Test ve kontrol bölgelerinde klinik parametrelerin ortalama değişimi sırasıyla, KG 5.5-5.1 mm arttı, AG 5.3-5.1 mm arttı, HD büzülme %21.6-%15.8, VD büzülme %33.7-%33.2, GA büzülme %47.3-%43.3 olarak belirlendi. Ameliyattan 6 ay sonra test ve kontrol bölgeleri arasında klinik parametrelerde anlamlı bir fark yoktu. Sonuç: Yatay devamlı sutur ve apikal germe sutur uygulaması serbest diş eti greftinin büzülmesini azaltmaz. Anahtar Kelimeler: Serbest diş eti grefti; greft büzülmesi; yatay devamlı sutur; apikal germe sutur; sondalama derinliği*

Ethics Committee Approval: The ethics committee approved this research in Kerman University of Medical Science (Ethics code: KA/90/180) and registered through the IRCT website with the number IRCT201608205305N5.

Informed Consent: The informed consents were provided by the participants.

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Author contributions: MM designed the study. AS participated in generating the data for the study. NA participated in gathering the data for the study. HR participated in the analysis of the data. AS and MM wrote the majority of the original draft of the paper. AS, MAS and MM participated in writing the paper. All authors approved the final version of this paper.

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Original research

Saliva profiles in children with congenital heart disease*

Mine Koruyucu[1], Sule Batu[2], Merve Bayram[3], Ezel Uslu[4], Yegane Güven[2], Figen Seymen[1]

Purpose
The low salivary pH and buffering capacity are caused by using heart failure medications. For this reason oral health should be supported in cardiac patients, it is necessary that they attend dental clinics for regular follow up. The aim of this study is to evaluate the relationship between the salivary oxidative stress markers and salivary pH, salivary buffering capacity, salivary flow rate and dental caries of children with congenital heart disease (CHD).

Material and Methods
This cross sectional study was carried out with 42 CHD and 42 healthy children. The participants' gender, age, general health and medications, and caries scores (dfs/DMFS) were written down, then their unstimulated saliva samples were collected. These specimens were evaluated in terms of the salivary secretion rate, salivary buffering capacity, pH, protein levels, superoxide dismutase (SOD), ferric reducing antioxidant power (FRAP), the thiobarbituric acid reactive substances (TBARS), protein carbonyl, protein thiols, total sialic acid.

Results
Both groups showed caries at similar levels. The salivary pH and buffering capacity were significantly less in the children with CHD than in the controls. The levels of TBARS and protein carbonyl were significantly higher in the children with CHD than in the controls. There was not any significant difference relating to the mean salivary secretion rate, protein levels, SOD, FRAP, protein thiols and total sialic acid.

Conclusion
The elevated TBARS and protein carbonyl levels in the patients with CHD were observed as an indicator of the free radical damage leading to oxidative stress.

Keywords: *Saliva composition; antioxidant activity; caries risk factors; congenital cardiac diseases; pedodontics*

Introduction

Congenital heart disease (CHD) is the one of the most common congenital anomalies in children, approximately 8-10 of the 1000 children around the world are born with CHD (1,2). Oral health problems are known to be common in children with severe heart disease, especially in the early ages, and accordingly an increasing number of patients require careful dental care (3,4). This group of children often required heart failure medications. It is well known that these medications change the saliva composition and biochemical properties in saliva such as levels of antioxidant and free radicals, but it is unknown whether there is a correlation between these biochemical properties and oral health problems (3).

With a better understanding of specific concentrations of salivary composition and its main components, both immunological and biochemical, different systemic and/or local pathologies can be located and assessed through the analysis of salivary composition and the flow rate (5).

*The study was registered at ClinicalTrials.gov (NCT03457974).

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ORCID IDs of the authors: M.K.0000-0002-2077-5095; S.B. 0000-0002-6834-477X; M.B. 0000-0002-8440-367X; E.U. 0000-0003-3925-0851; Y.G. 0000-0003-4718-927X; F.S. 0000-0001-7010-2035

[1]*Istanbul University, Faculty of Dentistry, Department of Pedodontics, Istanbul, Turkey*

[2]*Istanbul University, Faculty of Dentistry, Department of Biochemistry, Istanbul, Turkey*

[3]*Istanbul Medipol University, Faculty of Dentistry, Department of Pedodontics, Istanbul, Turkey*

[4]*Istanbul Cerrahpasa University, Faculty of Medicine, Department of Biochemistry, Istanbul, Turkey*

Corresponding Author: Mine Koruyucu

E-mail: mine.yildirim@istanbul.edu.tr

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A number of researchers have studied oral fluids to assess the aspects of systemic diseases, including CHD. Various serum biomarkers in oral fluids are known to be associated with inflammation, atherosclerosis, and myocardial damage (6,7).

Various researches have found evidence that a lot of cardiovascular diseases such as hypertension, type II diabetes, hypercholesterolaemia, atherosclerosis, and heart failure are influenced by oxidative stress in their pathogenesis. However, it is not clear that cardivascuar disease pathogenesis is primarily caused by oxidative stress (8,9). The researches have found a close relationship between the salivary biochemical and antioxidants systems in several pathological conditions and salivary components (5).

As formerly reported that the components in saliva can protect the oral cavity against the negative effects of endogenous and exogenous free radical damage as there are many antioxidant mechanisms in saliva (10).

One of the most important functions of antioxidants is the control of the oral bacteria that forms dental plaque, leading to an imbalance in ecology which causes dental caries and chronic inflammatory periodontal diseases. Salivary antioxidant defense systems and its relation to oral diseases have not been studied enough and there have been no studies conducted on total antioxidant status of saliva and whether this has any affect on caries protection in children (11).

In this study, we evaluated salivary oxidative stress status of children with CHD We included caries scores, gender, age, salivary flow rate, salivary pH, salivary buffering capacity and drug intake such as angiotensin-converting enzyme (ACE) inhibitors and tried to conclude a direct relationship between them.The null hypothesis is that the use of drugs such as angiotensin converting enzyme (ACE) inhibitors in children with CHD may affect salivary flow rate, pH, buffering capacity, salivary oxidative stress status and dental caries. If such relationships exist, they might be employed to patient caries –prevention treatment.

Material and Methods

The study was approved by the Ethics Committee of the Istanbul University, Medical Faculty (No:2010/1109-378) and was carried out in agreement with the Declaration of Helsinki principles. The study was registered at ClinicalTrials.gov (NCT03457974). This cross-sectional study was carried out 42 CHD and 42 healthy children who applied to Istanbul University Faculty of Dentistry. Patients in the high caries risk group were included in the study who were diagnosed in the cardiology department. Before participation to the study, the parents gave their informed consents in writing.

The children with congenital heart disease, aged 3-12 years who agree to give saliva were included in the study group. The children with no systemic disease, aged 3-12 years who agree to give saliva were included in the control group.

Gender, age, general health and medications, dfs/DMFS scores (total s=surface of d=decayed, m=missing, f=filled teeth) were recorded. Unstimulated saliva was collected.

In the presented work, the protein carbonyl and protein thiol-markers of the oxidative damage to the proteins and the thiobarbituric acid reactive substances (TBARS)- markers of lipoperoxidation were analysed. The antioxidant activities were evaluated by ferric reducing antioxidant power (FRAP) and superoxide dismutase (SOD) assays. The total protein concentration, sialic acid content and flow rate, pH and buffering capacity of the saliva were also assessed.

The saliva samples were collected in morning hours and at least 2 hours after the last food or drink. Prior to saliva collection mouths were rinsed out with distilled water and unstimulated whole saliva was collected for 5 min with the subject leaning forward and spitting saliva into a graded sampling tube. The flow rates were evaluated visually from graded test tubes (as ml/min). The Ericsson's method was used to measure the buffering capacity (12).

After the samples were collected, they were centrifuged instantly (4000 rpm for 10 min at 4°C), and the upper parts were drawn and stored in small aliquots at –80°C until analyzed.

Bovine serum albumin was used as standard in bicinchoninic acid (BCA) method to determine the salivary total protein concentrations (13). In short, 10 µL of saliva was mixed with the 200 µL of BCA working reagent, incubated 30 min at 37°C, and measured at 562 nm. The results were specified in mg/mL.

The measurement of salivary SOD activity was done with a modified method of Sun *et al*.(14). A superoxide generator, the xanthine oxidase was used in the inhibition of the nitroblue tetrazolium (NBT) reduction in this analysis. There are 40 ml of 0.3 mmol/l xanthine solution, 20 ml of 0.6 mmol/l EDTA solution, 12 ml of 400 mmol/l Na_2CO_3, and 6 ml of bovine serum in the reaction mixture. The final concentration of xanthine oxidase was 167 U/l. The SOD enzyme inhibited this reaction by scavenging the superoxide anion. There was a level of enyzyme that was able to inhibit the optical density at 560 nm of NBT reduction by 50% in 1 minute under the assay conditions, which defined the unit of SOD enzyme activity. The results were specified as U/ml of the saliva.

The measurement of salivary FRAP levels were done as stated by Benzie and Strain (15).

Briefly, in this test, antioxidant activity occured following the exposion of the medium to Fe^{3+} as the antioxidants in it begin to produce Fe^{2+} afterwards.

Shortly before use, 300 mM acetate buffer (pH 3.6), 10mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution and 20 mM $FeCl_3.6H_2O$ were mixed in a 10:1:1 ratio to become the working FRAP agent. Then this mixtuare was heated to 37°C. 10 mM TPTZ was added to 40 mM HCl to get the TPTZ solution. The reagent that was preaped and heated to 37°C was mixed with ten µL of H_2O-diluted sample afterwards. Blue color was observed when Fe^{2+} and TPTZ became a complex. 593 nm was the absorption rate of this complex. Ascorbic acid was used to prepare the FRAP standards (100-1000µM), which was the same in all samples. Ascorbic acid was used as standard, and the concentration of FRAP was expressed in µmol/L.

The oxidation of the polyunsaturated fatty acids creates MDA (Malondialdehyde) as the end product. The extent of lipid peroxidation's established measure is MDA's concentration in the medium. In this test, spectrophotometrically determined complex was achieved through the reaction of MDA with the thiobarbituric acid (TBA). The amount of TBARS produced was used to assess the lipid peroxidation in samples (16).

In short, 15% w/v trichloroacetic acid, 0.375% w/v TBA, and 0.25 N hydrochloric acid was mixed as a stock solution, whose two volumes were mixed with one volüme of the sample all through.The mixture was incubated for 30 min in

a boiling water bath. When the mixture cooled off, centrifugation was applied at 1000xg for 10 min to remove the flocculent precipitate. The absorption of the supernatant was recorded at 535 nm. The TBARS concentration was calculated using 1.56 x 10^5 M^{-1} cm^{-1} as molar extinction coefficient. The results were expressed in nmol/ml.

Spectrophotometric measurement of the saliva protein carbonyl (PCO) levels was done by using the method of Reznick *et al*. Chromophoric dinitrophenylhydrazones can be generated when the PCO groups react with 2,4-dinitrophenylhydrazine (DNPH) (17). After the dissolvement of DNPH in HCl and its reaction, precipition of the proteins with trichloroacetic acid (with an equal volume of 20% (w/v)) occured. 4 ml of an ethanol/ethyl acetate mixture (1:1) was used to wash it three times and afterwards it centifugared at 6000 × g for 5 min. In the end, 6 M guanidine–HCl solution was used for dissolution of the precipititates, and measurement of the absorbances at 360 nm was observed. The molar extinction coefficient of DNPH, ε = 2.2 × 10^4 M^{-1} cm^{-1} was used for the determination of the carbonyl content. The results were specified in µmol/L.

A modified Ellman's method was used for measurement of the salivary protein thiol levels (18). The complex of 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB), also known as Ellman's reagent, andthiol compounds (-SH groups) observed as yellow because of the reaction with a maximum peak at 412nm.

In short, the mixture of, 0.2 ml of saliva, phosphate buffer (pH 8.0), 40 µl of 10 mM DTNB and 3.16 ml of methanol was incubated for 10 min, and the absorbance was measured at 412 nm against appropriate blanks. The protein thiol content was calculated by using 13.6x10^3 cm^{-1} M^{-1} as the molar extinction coefficient (19). The results were specified in µmol/L.

A modified method which gave advanced sensitivity and high reproducibility to sialic acid (SA) were proposed by Tram *et al*. (1997) and this TBA method was used to determine the SA concentrations (Aminoff, 1961) t The saliva samples were incubated with 0.1 N H_2SO_4 at 80°C for 60 minutes, and the total SA were determined in hydrolysate by measuring the absorbance 549 nm. The calculation of the sialic acid content in the sample was performed by a comparison with a standard curve prepared with *N*-acetylneuraminic acid (20-22).

Statistical analysis

Statistical analysis was done with SPSS (Statistical Package for Social Sciences) for Windows 15.0 program. Kolmogrov-Smirnov test was used for the normal distribution suitability of the parameters. As well as the descriptive statistical methods (Mean, Standard Deviation, frequency), Oneway Anova test, Kruskal Wallis test, Student T test, Mann Whitney U test, Chi-square test, Pearson correlation and Spearman's rho correlation ayalsis were used to examine the relationships between variables. The significance was assessed at p<0.05 level.

Results

The study population comprised of 84 patients (42 cardiac-42 healthy), 28 (33.3%) female, 56 (66.7%) male, and 3-to 12-year-old (mean age 7.19±2.37) children. Gender and ages were selected same for study and control groups. There was 14 female and 28 male in both groups. In both groups there were 17 children between the ages of 3-6 and 25 children between the ages of 7-12 years. The significant difference

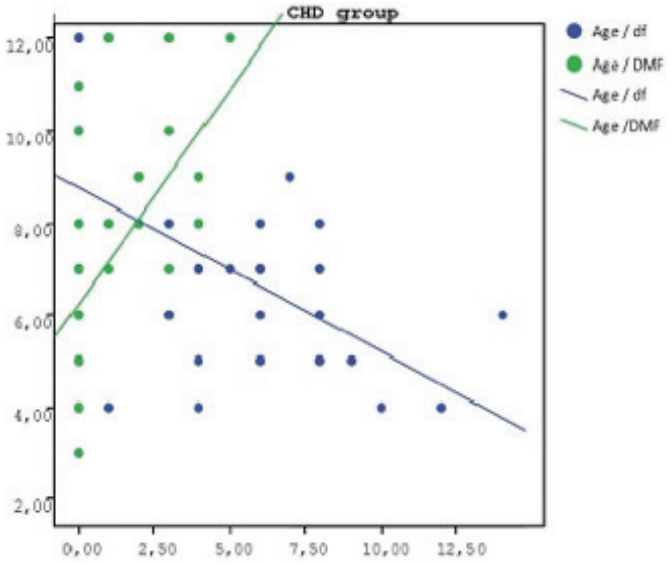

Figure 1. *Correlation between age and caries scores.*

Table 1. *Distribution of the dfs-DMFS scores and saliva profiles of CHD and control groups*

	Groups		*p value*
	Study group (CHD)	**Control group**	
Dfs	4.40 ± 3.49	5.00 ± 2.94	***0.274***
DMFS	1.02 ± 1.53	1.14 ± 1.63	***0.744***
Salivary flow secretion rate (ml/min)	0.42 ± 0.33	0.40 ± 0.29	***0.683***
Salivary buffering capacity	4.40 ± 0.63	4.84 ± 0.55	***0.001****
Salivary pH	6.63 ± 0.67	6.85 ± 0.41	***0.041****
Protein levels (mg/ml)	0.16 ± 0.07	0.14 ± 0.06	***0.162***
+SOD (U/ml)	1.50 ± 0.58	1.24 ± 0.58	***0.055***
+FRAP (µmol/L)	627.26 ± 168.12	571.05 ± 221.39	***0.221***
+TBARS (nmol/ml)	0.70 ± 0.19	0.55 ± 0.15	***0.001****
+Protein carbonyl (µmol/L)	53.06 ± 18.30	19.93 ± 16.23	***0.001****
+Protein thiols (µmol/L)	56.70 ± 25.96	65.23 ± 51.38	***0.390***
+Total sialic acid (mg SA/dL)	7.43 ± 2.41	6.14 ± 4.07	***0.099***

Mann Whitney U test, +Student t test, *p <0.05, ** p<0.01

Table 2. *SOD, FRAP, protein thiols, total sialic acid, TBARS ve protein carbonyl relationship with age in the CHD group*

CHD group	Age	
	r	*p*
SOD (U/ml)	-0.251	***0.109***
FRAP (µmol/L)	0.055	***0.746***
Protein thiols (µmol/L)	0.108	***0.544***
Total Sialic Acid (mg SA/dL)	-0.014	***0.931***
TBARS (nmol/ml)	0.021	***0.894***
Protein Carbonyl (µmo l/L)	-0.109	***0.492***
Pearson correlation analyses, p<0.05		

was not found between the groups according to the ages and gender (p>0.05).

The mean dfs and DMFS scores, were found respectively 4.40±3.49, 1.02±1.53 for the cardiac group; 5.00±2.94, 1.14±1.63 for the control group. According to the groups statistically significant differences were not found between scores (p>0.05) (Table 1). In the CHD group, statistically significant negative relationship was found between age and dfs scores (r=-0.500, p<0.01). Also statistically significant positive relationship was found between age and DMFS (r=0.701, p<0.01) (Figure 1).

Statistically significant differences were not found between the mean salivary flow secretion rate (p=0.683, p>0.05) (Table 1).

The salivary buffering capacity (p=0.001) and salivary pH (p=0.041) were significantly less in the CHD than in the controls (p<0.05, p<0.01) (Table 1).

The statistically significant difference was not found between the mean protein levels (p=0.162, (p>0.05) (Table 1).

The statistically significant difference was not found between the mean SOD (p=0.055) and the FRAP (p=0.221) values (p>0.05) (Table 1). However, the SOD values of the children with CHD were found to be higher than the control group.

The levels of TBARS and protein carbonyl (p=0.001) were significantly higher in the CHD than in the controls (p≤0.01) (Table 1).

The statistically significant difference was not found between the mean protein thiols (p=0.390) and the total sialic acid (p=0.099) (p>0.05) (Table 1).

Table 3. *Evaluation of salivary secretion rate, pH and salivary buffering capacity according to age groups*

	CHD mean±SD (median)	Control mean±SD (median)	*p*
Age 3-6			
Salivary secretion rate (ml/min)	0.34±0.32 (0.22)	0.35±0.34 (0.25)	***0.966***
pH	6.55±0.68 (6)	6.73±0.45 (7)	***0.196***
Salivary buffering capacity	4.08±1,59 (4.5)	4.12±1.88 (4.5)	***0.478***
SOD (U/ml)	1.38±0.78	0.97±0.64	***0.084***
TBARS (nmol/ml)	0.59±0.31	0.47±0.19	***0.157***
Age 7-12			
Salivary secretion rate (ml/min)	0.47±0.36 (0.4)	0.42±0.24 (0.38)	***0.950***
pH	6.63±0.77 (7)	6.92±0.38 (7)	***0.082***
Salivary buffering capacity	4.13±0.96 (4.5)	4.94±0.53 (5)	***0.001****
SOD (U/ml)	1.41±0.59	1.27±0.61	***0.428***
TBARS (nmol/ml)	0.71±0.19	0.55±0.18	***0.004****
Student t test, Mann Whitney U test, **p<0.01			

Table 4. *Evaluation of SOD, FRAP, protein thiol, total sialic acid, TBARS, protein carbonyl, dfs and DMFS according to ACE inhibitor usage in the CHD group*

CHD group	Medication		+*p*
	usage+ Mean±SD	usage - Mean±SD	
SOD (U/ml)	1.41±0.64	1.53±0.56	***0.545***
FRAP (µmol/L)	593.25±169.72	638.19±169.25	***0.493***
Protein thiol (µmol/L)	59.02±24.85	55.98±26.74	***0.778***
Total Sialic Acid (mg SA/dL)	8.51±3.24	6.99±1.89	***0.066***
TBARS (nmol/ml)	0.73±0.18	0.68±0.19	***0.482***
Protein Carbonyl (µmo l/L)	26.63±18.06	17.25±14.92	***0.091***
+dfs	3.91±3.39	4.60±3.57	***0.672***
+DMFS	0.66±1.07	1.16±1.68	***0.482***
+Student t test, Mann Whitney U test, p<0.05			

There was no statistically significant relationship between age and SOD, FRAP, protein thiols, total sialic acid, TBARS and protein carbonyl levels in the CHD group (p>0.05) (Table 2).

The salivary buffering capacity and TBARS were significantly greater in the CHD groups, especially in the 7-12 years group (p≤0.01; p<0.05) (Table 3).

There was no statistically significant difference between ACE inhibitors usage and SOD, FRAP, protein thiol, total sialic acid, TBARS, protein carbonyl, dfs and DMFS in the CHD group (p>0.05) (Table 4).

The statistically significant difference was not found between cardiac status and SOD, FRAP, protein thiol, total sialic acid, TBARS, protein carbonyl, dfs and DMFS in the CHD group (p>0.05) (Table 5).

There was statistically significant positive relationship between SOD and TBARS levels in the control group children (r=0.323, p=0.042, p<0.05) (Table 6).

Discussion

The risk of caries is especially important for young children and those with systemic disease. The frequency of high caries in these patients is unacceptable. . Apositive correlation was found between the caries prevalence and age in this study.

Statistically significant differences were not found between df, dfs, DMF, and DMFs scoresaccording to the groups. The cardiac group received more interest in the efforts to prevent caries because they were being monitored by health proffesionals most of the time. Also, the parents tended to be attentive and care for their children's dental care. One of the factors that may lead to caries in CHD children has been found to be medication-induced xerostomia. Heart failure medication may lead to reduced salivary secretation, and therefore caries. Clinically, the children who experince heart diseases are given antibiotics more than the healthy ones, although no higher *Streptococcus Mutans (MS)* have been observed in children with heart disease. On the other hand, children between the ages 5 to 12 who had used antibiotics at an earlier age had higher MS levels (23).

Although long term medication need for CHD children is a common knowledge, the knowledge of how this regular use of medication affects the medically comprimised children's oral health is inadequate. Long term use of medications with low ph, high acidity, and fermentable sugars may create a

Table 5. *Evaluation of SOD, FRAP, protein thiol, total sialic acid, TBARS, protein carbonyl, dfs ve DMFS according to cardiac status in the CHD group*

CHD group	Cardic status			+p
	ASD Mean±SD	VSD Mean±SD	other Mean±SD	
SOD (U/ml)	1.55±0.64	1.47±0.63	1.49±0.56	***0.961***
FRAP (µmol/L)	662.80±146.08	614.03±155.53	623.88±186.27	***0.849***
Protein thiol (µmol/L)	51.51±24.38	58.60±27.97	57.37±26.60	***0.866***
Total sialic acid (mg SA/dL)	8.31±3.08	7.82±2.72	6.89±1.91	***0.300***
TBARS (nmol/ml)	0.68±0.10	0.65±0.17	0.72±0.21	***0.576***
Protein Carbonyl (µmo l/L)	28.71±18.34	18.65±15.85	17.43±15.28	***0.235***
+dfs	3.63±3.40	4.37±4.24	5.83±2.94	***0.131***
+DMFS	1.13±1.69	1.50±1.69	0.50±1.00	***0.377***

+Oneway ANOVA test, Kruskal Wallis test, p<0.05

Table 6. *Relationship between SOD-FARP and TBARS, protein carbonyl, protein thiol, total sialic acid in the groups*

		SOD (U/ml)		FRAP(µmol/L)	
		r	*p*	*r*	*p*
CHD group	**TBARS (nmol/ml)**	-0.151	***0.339***	0.286	***0.086***
	Protein carbonyl (µmol/L)	0.210	***0.182***	0.094	***0.579***
	Protein thiol (µmol/L)	0.218	***0.217***	0.073	***0.682***
	Total Sialic Acid (mg SA/dL)	-0.013	***0.936***	-0.208	***0.216***
Control group	**TBARS (nmol/ml)**	0.323	***0.042****	-0.202	***0.223***
	Protein Carbonyl (µmol/L)	0.066	***0.733***	-0.222	***0.257***
	Protein thiol (µmol/L)	0.081	***0.647***	-0.090	***0.626***
	Total Sialic Acid (mg SA/dL)	0.120	***0.486***	-0.306	***0.078***

Pearson correlation analyses, *p<0.05

direct oral health concern on thedental caries and/or erosive lesions apart from the concerns raised by salivary secretion (3,24). In this study, the groups showed no statistically significant differences in terms of the mean salivary secretion rate.

The results of the studies in paediatric cardiology indicate some of the pharmaceutical preparations may cause caries and erosions, thus badly affecting oral health. The amount of saliva and its quality have significant importance for oral health as they might prevent dental carries and erosion. The salivary buffering capacity neutralizes acids in plague, dilute acids, transport the acid from the oral cavity, thus preventing a harmful pH change, and helps remineralization process by providing some minerals. Bicarbonate levels are important in maintaining the neutralization of acids and when salivary secretion is low the salivary buffering capacity also decreases (3). In this study the salivary buffering capacity and salivary pH were significantly less in the CHD than in the controls.

Many major drugs are not available in paediatric form. The most commonly used method is grinding the drug inside the syringe. Then by mixing the powder with tepid tap water in a plastic cup, it is made ready to administer. Pharmaceutical preparations with acidic pH like captopril could be a reason for low salivary pH and buffering capacity in children with CHD when used several times each day over long periods of time (25).

The first hypertension medicine taken orally to be released to the market was Captopril. It is an ACE inhibitor that prevents angiotensin I from turning into angiotensin II.Captopril includes thiol groups which allow specific binding to ACE and it suppresses the expression of the gene encoding ACE indirectly, therefore it is known to have specific inhibitory properties. Moreover, it has reaction ability to superoxide anion radicals, acting as a scavenger and to hydroxyl radicals, and it can improve the oxidative balance as well Ahmed *et al*. used captopril for the treatment of portal vein-ligated rats and reported significant elevations in glutathione (GSH) content and SOD activity (26).

Lipid peroxidation products MDA accumulates in the heart due to oxidative stress, which also leads to impaired cell function. On the other hand, antioxidant enzyme SOD helps to defend cells against oxidative stress. Sheng *et al.* measured the myocardial levels of the MDA content and SOD activities in order to prove that the cardiac hypertrophy model included oxidative stress. They've suggested that oxidative stress occurs during the development of cardiac hypertrophy, because the pressure overload is in the cardiac hypertrophy rat model, after aortic constriction, continious increase ocuured in the level of MDA contents in the hypertrophic myocardium at three, five, and 7 weeks. Meanwhile the SOD activities reduced slowly (27).

In this study, the TBARS and protein carbonyl were significantly higher in the CHD patients. This may be regarded as an indicator of oxidative stress due to heart disease. Salivary SOD activity was not statistically significant, even though a tendency towards increase was noticed.

SOD, FRAP, and protein thiols (i.e. enzymatic and non-enzymatic antioxidants), being no different between the groups, and it may depend on the use of the captopril and enalapril.

The results show that how oxidative stress and antioxidant systems in saliva of persons with caries affect each other is only partly understood. In this study, we also investigated levels of the sialic acids in whole saliva of the participants. Sialic acids on the terminal position in the mucins are believed to be important in antioxidant defense (28). The salivary total SA levels were correlated with the dental caries status, oral hygiene, and the gingival status in the children with CHD, but in only one study (29).

High levels of TBARS and protein carbonyl related to a higher oxidative stress present in saliva of children with CHD. Further investigations into the connection between these factors and development of oral diseases are required in cardiac patients. For this reason, researches in antioxidant and anti-inflammatory strategies may provide new treatments that reduce the damage caused by the imbalance in the oxidative system.

This research can be considered as a pilot study.The limitation of this study is the lack of the number of cases. In addition, the number of drug users and the degree of cardiological disease should be defined as subgroups. Evaluation of parameters should not only be limited to saliva and should provide more reliable results. More studies are needed to evaluate cardiac diseases and saliva composition. However, as far as we know, this is the first study in children to evaluate salivary oxidative stress parameters and dental caries. There have been many studies on dental caries and on periodontal diseases in subjects with cardiovascular disease. These studies have generally only investigated the pH and the flow rate. Studies about salivary composition of subjects with cardiovasccular diseases are limited. Paediatric cardiologists and paediatric dentists should work more closely for better dental care for cardiac patients. Children with cardiac diseases should be consulted to a paediatric dentist at an early age. Each child with a cardiac disesase should be able to receive an individual treatment plan to maintain oral health, based on risk assessment. The first aim of these programs should be to prevent caries with dietary counselling, oral hygiene, and fluoride applications if necessary. The challenges of the cardiac diseases and families with the cardiac patients's experience should not be underestimated and should be recognized.

Conclusion

The elevated TBARS and protein carbonyl levels in the patients with CHD were observed as an indicator of the free radical damage which may lead to oxidative stress.

Türkçe Öz: *Konjenital kalp hastalığına sahip çocuklarda tükürük profili. Amaç: Kalp hastalıklarında düzenli ilaç kullanımı ile düşük tükürük pH'sı ve tamponlama kapasitesi ortaya çıkmaktadır. Bu nedenle kardiyak hastalarda ağız sağlığı desteklenmelidir ve düzenli takip şarttır. Bu çalışmanın amacı, konjenital kalp hastalığı olan çocuklarda tükürük oksidatif stres belirteçleri ile tükürük pH, tükürük tamponlama kapasitesi, tükürük akış hızı ve diş çürüğü arasındaki ilişkiyi değerlendirmektir. Yöntemler: Bu kesitsel çalışma 42 konjenital kalp hastası ve 42 sağlıklı çocuk ile yapılmıştır. Katılımcıların cinsiyet, yaş, genel sağlık ve kullandıkları ilaçları ile çürük skorları (dfs / DMFS) kaydedilmiş, uyarılmamış tükürük örnekleri toplanmıştır. Bu örnekler tükürük sekresyon miktarı, tükürük tamponlama kapasitesi, pH, protein düzeyleri, süperoksit dismutaz (SOD), ferrik indirgeyici antioksidan güç (FRAP), tiyobarbitürik asit reaktif maddeler (TBARS), protein karbonil, protein tiyolleri ve toplam sialik asit açısından değerlendirilmiştir. Bulgular: Her iki grup da benzer oranlarda çürük olduğu saptanmıştır. Konjenital kalp hastalığına sahip çocuklarda tükürük pH'sı ve tamponlama kapasitesi*

kontrol grubuna göre anlamlı olarak daha az bulunmuştur. Konjenital kalp hastalığına sahip çocuklarda kontrol grubuna göre TBARS ve protein karbonil düzeyleri anlamlı olarak yüksek bulunmuştur. Ortalama tükürük sekresyon miktarı, protein düzeyleri, SOD, FRAP, protein tiyolleri ve toplam sialik asit ile ilgili anlamlı bir fark bulunmamıştır. Sonuç: Konjenital kalp hastalığına sahip çocuklarda yükselmiş TBARS ve protein karbonil düzeyleri, oksidatif strese yol açan serbest radikal hasarının bir göstergesi olarak gözlenmiştir. Anahtar Kelimeler: Tükürük kompozisyonu; antioksidant aktivite; çürük risk faktörleri; konjenital kalp hastalığı; çocuk dişhekimliği

Ethics Committee Approval: The study was approved by the Ethics Committee of the Istanbul University, Medical Faculty (No:2010/1109-378) and was carried out in agreement with the Declaration of Helsinki principles. The study was registered at ClinicalTrials.gov (NCT03457974).

Informed Consent: The informed consents were provided by the participants' parents.

Peer-review: Externally peer-reviewed.

Author contributions: MK, SB, MB and FS designed the study. MK, MB and EU participated in generating the data for the study. MK, SB, MB and EU participated in gathering the data for the study. SB, EU, YG and FS participated in the analysis of the data. MK, SB and MB wrote the majority of the original draft of the paper. MK and MB participated in writing the paper. All authors approved the final version of this paper.

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