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

Book

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

Chapter in a book

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

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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

Fracture resistance of root-filled teeth after cavity preparation with conventional burs, Er: YAG and Er,Cr: YSGG lasers

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Purpose
The aim of the present study is to compare the fracture resistance of teeth after access cavity preparation with conventional rotary burs, Erbium-doped yttrium aluminum garnet laser (Er:YAG) and Erbium, cromium: yttrium scandium gallium garnet laser (Er,Cr:YSGG) lasers.

Materials and methods
Fifty five intact mandibular molars were divided into 3 negative groups (groups 1, 2, 3; n=5 for each), 3 study groups (groups 4, 5, 6; n=10 for each) and 1 positive control group (intact teeth; n=10). Access cavities of groups 1, 2 and 3 were prepared with conventional burs, Er:YAG laser and Er,Cr:YSGG laser respectively. After root canal obturation, their coronal portions were left non-restored. Access cavities of groups 4, 5 and 6 were prepared by using the same equipment but their coronal portions were restored with composite resinafter root canal obturation. Following thermocycling, fracture strength was evaluated with a Universal Testing Machine. Mean force at which each sample is fractured was recorded in Newton unit and statistically analyzed.

Results
Fracture resistance of group 7 (intact teeth) was significantly higher than all other groups ($p<0.001$). Differences among the fracture resistance values of groups 4, 5 and 6 were not significantly different but they were significantly higher than those of groups 1, 2 and 3 ($p<0.001$). No significant difference was found between Groups 1, 2 and 3.

Conclusion
Preparing access cavities with either laser or bur has no effect on the fracture resistance of teeth with root canal treatment.

Keywords: *Bur; cavity preparation; Er,Cr:YSGG; Er:YAG; fracture resistance*

Introduction

Fracture is an important risk for endodontically treated teeth (ETT) (1). These teeth are more prone to fractures compared with those free of any endodontic application (2). Although the fractures of ETT have been traditionally associated with loss of elasticity and moisture (3), the main reason is the reduction of dental hard tissue bulk as a result of cavity preparation which is routinely done by using rotary burs (4). Furthermore, microcrack formation occurring during cavity preparation and/or root canal instrumentation renders teeth more susceptible to fractures (5).

Currently, laser technology is being used for many dental procedures including cavity preparation. The absence of vibration, noise and no or minimal need for local anesthesia have been emphasized as the advantages of laser over conventional rotary instruments in the cavity preparation (6, 7). Furthermore, lasers have been reported to allow minimal invasive approach (8). Particularly, Erbium lasers including Er:YAG (AT Fidelis, Fotona, Ljubljana, Slovenia) and Er,Cr:YSGG (WaterLase® iPlus, Biolase, Irvine, CA, USA) lasers are contemporary systems used in order to excavate dental

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hard tissues (7-9). Following absorption of laser light, dental hard tissues heat above melting point and explode by releasing their mineral content (10).

Microleakage of restorations after cavity preparation with lasers (7-9) and their effectiveness during cavity preparation (10-12) have been previously investigated. However, to the best of our knowledge, fracture strengths of cavities prepared with laser devices and conventional burs have not been compared before. The aim of the present study is therefore to examine the coronal fracture resistance of root-filled teeth after access cavity preparation either with Er,Cr:YSGG, Er:YAG lasers or conventional rotary burs. The null hypothesis of this study is that there is no significant difference between Er,Cr:YSGG, Er:YAG and bur groups in terms of fracture resistance.

Materials and methods

Sample preparation

The present study was approved by the ethical committee of Gaziantep University (Project number: 2015/125) and verbal constent was obtained from patient in order to use their extracted teeth for the present study. This experiment included 55 intact (N=55), human mandibular molars of nearly similar dimensions (15±1 mm mesiodistally; 8±1 mm buccolingually), extracted due to periodontal reasons having no decay, filling, or other hard tissue loss. Any remnants over the surface were removed with scalers. Specimens were kept in 0.1 M thymol solution for disinfection at room temperature until the experiment. The samples were randomly distributed into 7 groups including 3 study and 4 control groups by using an on-line randomizing software service. Negative control groups (group 1, 2 and 3) included 5 teeth per each group (n=5), while study groups (groups 4, 5 and 6) and positive control group (group 7) included 10 teeth per group (n=10). An easy inlet to the root canals is provided in all cavities. A size 15K-file (Sybron Endo, Scafati, Italy) was advanced throughout the canal until its tip was visible at the apical foramen. Working length was calculated as 0.5-1 mm shorter of this point. Following each instrument, the canals were rinsed with 2 mL of NaOCl solution. Root canals were prepared up to an apical diameter of size 40. Step-back preparation was continued by withdrawing 1 mm after each file until size 80 file. Coronal one-third was enlarged by using size 2-4 Gates-Glidden burs (Thomas, Bourges, France). Excess irrigants were dried with paper points (Dentplus, Choonchong, Korea). Root canal filling was achieved with lateral condensation technique by using gutta-percha (Dentplus, Choonchong, Korea) and sealer (AD seal, Meta-Biomed, Cheongwon, Korea). Excess gutta-percha was cut with a heating tool from canal tips (Gutta Cut, VDW, Munich, Germany). All cavities were modified to MOD configuration with cylindrical burs to reach a thickness of 2.5 mm at the buccal occlusal wall, 3.5 mm at the buccal cemento-enamel junction, 1.5 mm at the lingual occlusal surface and 2.5 mm at the lingual cemento-enamel junction by using caliper. Pulp chambers were filled with resin-modified glass-ionomer cement (GC Corporation, Tokyo, Japan) (Figure 1).

Cavity preparation

The teeth were grouped as follows: Group 1 and Group 4: Access cavities were prepared with diamond round burs (Medin, Nove Mestona Morave, Czech Republic) attached to a high-speed hand piece under water cooling. Group 2 and Group 5: Access cavities were prepared by using a non-contact tip (R02 Handpiece) attached to Er:YAG laser at a wavelength of 2940 nm (AT Fidelis, Fotona, Ljubljana, Slovenia). The energy settings were; 300 mJ 30 Hz (9 W) 6 water (65%) and 4 air (45%) in Medium Short Pulse (MSP=100 microseconds) mode for enamel, 225 mJ 15 Hz (3.35 W) 5 water (55%) and 3 air (35%) in MSP mode for dentin. Average energy used for each sample was approximately 2700 joule for enamel and 3000 joule for dentin. Group 3 and Group 6: Access cavities were prepared with a non-contact tip Turbo handpiece (MX7 tip) attached to Er,Cr:YSGG laser at a wavelength of 2780 nm (WaterLase® iPlus, Biolase, Irvine, CA, USA). The energy settings were; 8 W 20 Hz 70% Air and 80% water in H mode for enamel, 6W 15 Hz 50% Air and 70% Water in Hard Mode (H mode=60 microseconds) for dentin. Average energy used for each sample was approximately 2800 joule for enamel and 3200 joule for dentin. Group 7: No treatment was applied (intact teeth).

Coronal restoration

Coronal restorations of groups 4, 5 and 6 were done as follows: After applying self-etching bonding agent (Single Bond Universal Adhesive, 3M ESPE, St. Paul, MN, ABD) for 20 seconds, it was gently dried and light-cured for 10 seconds with light-emitting diode device (Valo Cordless, Ultradent Products Inc., South Jordan, UT, USA) at 1000 mW/cm^2 intensity. Cavities were restored with composite resin (Filtek Z550, 3M ESPE, St. Paul, MN, USA) by using incremental technique. Two mm resin was placed in each layer (Figure 2). To provide standardization, the light source was applied by positioning it just over the cusp tips. Following each 10 samples, the density of the light was checked with a dental radiometer (Demetron, Kerr, Orange, CA, USA) because the intensity of light source should not declineunder 1000 mW/cm^2. Coronal segments of the samples in groups 1, 2 and 3 (negative control) were left unfilled. Roots of all samples were embedded in cylinderic molds filled with self-curing polymethylmethacrylate (Imicryl, İstanbul, Turkey) up to cemento-enamel junction.

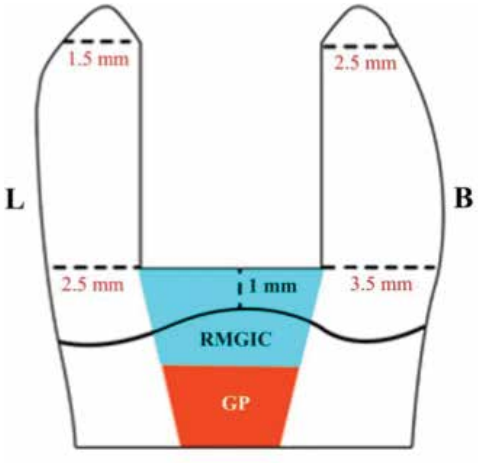

Figure 1. *Schematic representation of cavities without coronal restoration.*

B: Buccal, L: Lingual, RMGIC: Resin Modified Glass Ionomer Cement, GP: Gutta Percha)

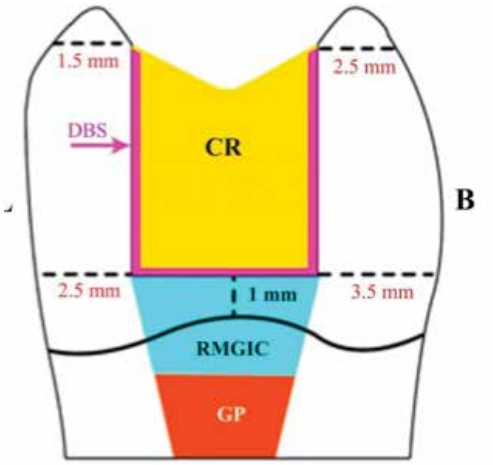

Figure 2. *Schematic representation of cavities with coronal restoration. B: Buccal, L: Lingual, RMGIC: Resin Modified Glass Ionomer Cement, GP: Gutta Percha, DBS: Dentine Bonding System, CR: Coronal Restoration)*

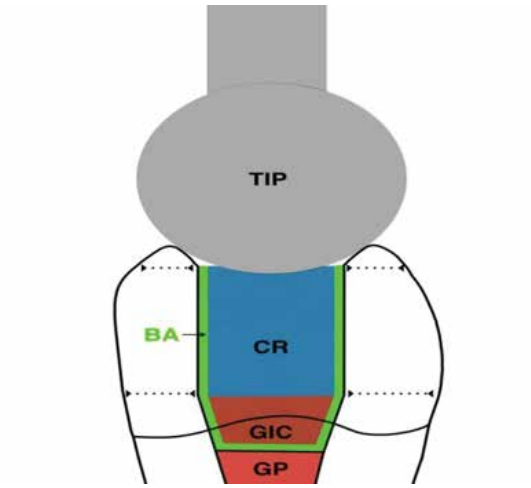

Figure 3. *Schematic representation of applying fracture test to the samples. GIC: Glass Ionomer Cement GP: Gutta Percha BA: Bonding Agent CR: Coronal Restoration TIP: Spherical tip of the testing machine)*

Fracture Test

All specimens were thermocycled for 5000 cycles between 5 and 55 ^{0}C, using a dwell time of 30 seconds in each bath. Following thermocycling process, the samples were placed in a Universal Testing Machine (AGS-X, Shimadzu, Kyoto, Japan). A round-shaped steel tip in 5 mm diameter was connected to the testing machine in contact with restoration surface, buccal and lingual walls of the teeth (Figure 3). Fracture resistance of each group was measured by applying force parallel to the long axis of each tooth at a crosshead speed of 1 mm/min (Figure 4). Force necessary to fracture each tooth was recorded in Newton. Fracture test was applied by another blinded researcher. Fracture modes were classified according to the study of Taha *et al.* (1); Type 1: Horizontal cuspal fracture above cemento-enamel junction (CEJ) (Restorable). Type 2: Vertical fracture of either lingual or buccal wall above CEJ (Restorable). Type 3: Vertical fracture of either lingual or buccal wall below CEJ (Non-restorable).

Statistical analysis

Prior to statistical analysis, the normality of the data was analyzed with Shaphiro-Wilk test. Due to normal distribution of the data, statistical analysis was performed with one way analysis of variance (ANOVA) and post-hoc Tukey's Honestly Significant Difference (HSD) tests by using Statistical Package for Social Sciences (SPSS) (IBM Corp.; Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY, USA) software. Confidence level was set to 95% and p values less than 0.05 were considered as statistically significant.

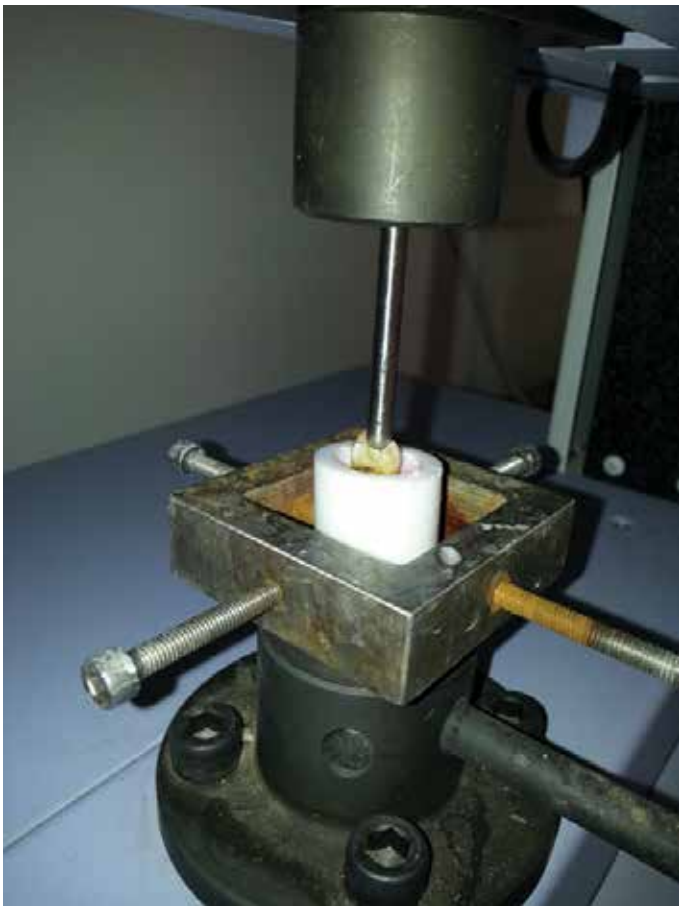

Figure 4. *Sample attached to the Universal Testing Machine for fracture test.*

Results

The mean force required for the fracture to occur in each group in Newton (N) and their standard deviations are presented in Table 1. Fracture resistance of group 7 (intact teeth) was significantly higher than all other groups ($p<0.001$). Differences between the fracture resistance values of groups 4, 5 and 6 were not significantly different, while they were significantly higher those of the groups 1, 2 and 3 ($p<0.001$). There were no statistically significant differences between the mean fracture resistances of Group 1, 2 and 3. Fracture modes of the groups are presented in Table 2. The percentage of non-restorable fractures were; 80% in group 1, 100% in group 2, 80% in group 3, 50% in group 4, 50% in group 5, and 60% in group 6. All fracture occurred in dental hard tissues while coronal restorations were observed to be intact.

Discussion

Erbium lasers work by ablating water either present within the structure of dental hard tissues or supplied as a spray by laser devices. Ablation causes microstructural changes that include flaking, charring, microcrack and pore formation in dental hard tissues which may lead to fractures. The studies of Meister *et al.* (13) and Ekwarapoj *et al.* (14) pointed out that Er:YAG laser ablates endogenous water found in collagen of intertubular den-

Table 1. *Mean fracture resistances and standard deviations of 7 experimental groups. Same superscript symbols indicate no significant difference*

Groups	N	Mean	SD
1 Bur cavity	5	375.56†	72.25
2 Er:YAG cavity	5	469.58†	129.18
3 Er,Cr:YSGG cavity	5	208.69†	74.08
4 Bur+composite	10	2249.99‡	402.94
5 Er:YAG+composite	10	1767.18‡	384.75
6 Er,Cr:YSGG+composite	10	1930.50‡	442.37
7 Intact	10	2745.83§	628.17
Total	55	1676.44	964.03

Er:YAG: erbium-doped yttrium aluminum garnet laser; Er,Cr:YSGG: erbium, cromium: yttrium scandium gallium garnet laser; SD: standard deviation

Table 2. *Fracture modes observed in each group.*

Groups	N	Type 1	Type 2	Type 3
1 Bur cavity	5	0	1	4
2 Er:YAG cavity	5	0	0	5
3 Er,Cr:YSGG cavity	5	0	1	4
4 Bur+composite	10	2	3	5
5 Er:YAG+composite	10	3	2	5
6 Er,Cr:YSGG+composite	10	2	2	6
7 Intact	10	8	1	1
Total	55	15	10	30

Er:YAG: erbium-doped yttrium aluminum garnet laser; Er,Cr:YSGG: erbium; cromium: yttrium scandium gallium garnet laser

tine more than exogenous water, while Er,Cr:YSGG laser uses exogenous water more than endogenous water. Franzen *et al*. (15) found that similar microstructural changes occur if adequate water supply is provided for both type of lasers. We assume that the similarity in the fracture strength values of laser groups is related to providing sufficient amount of water in the present study. In other words, using correct water settings for lasers resulted in similar microstructural changes and thus, similar fracture strength values. Furthermore, results of the present study revealed no significant difference between laser and bur groups. Thus, the null hypothesis was accepted. The results of this study, when combined with those of the previous researches suggest that the fracture of root-filled teeth may be correlated with either the amount of dental hard tissue loss or the micro crack formation during cavity preparation. We also need to highlight that the samples of the present study were prepared from intact teeth and cavities were modified as MOD type by using high power settings in order to provide standardization. Previous studies indicated a positive correlation of power settings with the amount of ablated tissue (14, 16). During clinical practice, lower power settings, which are only sensitive to decayed tissue, can be used and, by doing this, both microstructural damages can be minimized and more conservative cavities can be prepared. Thus, less hard tissue removal leads to increased resistance. This was also confirmed by Corona *et al*. (17) and Fornaini *et al*. (18). Franzen *et al*. (15) reported that minimal invasive cavities can be prepared and patient comfort can be enhanced by using Erbium lasers during cavity preparation. Accordingly, despite the present study found that fracture resistance did not vary either with burs or with laser, other advantages of cavity preparation with laser mentioned above may also motivate the clinicians to use Erbium lasers for cavity preparation. Further *in vivo* and *in vitro* studies with lower power settings may be beneficial to explore such advantages of laser devices.

The results of the present study further indicated that the reinforcing properties of the coronal restoration is more important than the technique used for access cavity preparation because as seen in Table 1, all coronally restored groups (4, 5 and 6) have significantly higher fracture strengths compared to non-restored groups (1, 2 and 3). In the study of Sengun *et al*. (19), it was reported that restoring the coronal portion of the root-filled teeth with appropriate materials compensates the loss of hard tissue bulk and reinforces dental hard tissues. Thus, it can be stated that the quality of the coronal restorative material are more important than the technique used for cavity preparation in terms of fracture strength.

Re-restorability of root-filled teeth following fracture is another issue of concern. If fracture occurs in non-restorable form, extraction may be required. However, according to our results, 50-60% of the fracture modes in coronally restored groups are non-restorable, while this percentage is 90-100% in non-restored groups. This may be due to the irrelevancy of cavity preparation techniques with the reinforcing capacity of coronal restoration. Better strengthening of coronal restoration results in more restorable fracture modes (20). Although different cavity preparation techniques have been used in the present study, all coronal restorations were performed with the same material in a similar manner.

Thermocycling was performed in the present study to simulate aging effects of intra-oral conditions. Eakle (21) stated that the thermocycling process reduces the strengthening capacity of resin restorations, therefore, long-term use of the root-filled and restored teeth can be simulated. The study of Kruzic *et al*. (22) reported that micro cracks leading to fractures occur as a result of fatigue cycling rather than the force load itself. For these reasons, samples of present study were subjected to thermocycling before the fracture strength tests.

Zadik *et al*. (23) stated that mandibular molars are the most likely to be extracted following endodontic treatment resulting from fractures compared to other teeth. For this reason, mandibular molars were included in the present study. Dental hard tissue bulk remained following caries removal and cavity preparation is another risk factor in terms of fracture occurrence. Tang *et al*. (24) found that MOD cavities carry more fracture risk compared to MO and OD cavities. In the present study, all cavities were modified to MOD configuration to increase fracture risk. Furthermore, all fractures have notably occurred between dental tissues and composite restoration (adhesive failure). This may be related to the low thickness of the cavity walls, particularly at the cavity base (3.5 mm for buccal, 2.5 mm for lingual) which may have led to fracture of these thin walls before composite restoration. Fracture resistance values were determined with a universal testing machine by applying force parallel to the long axis of teeth. However, magnitude and directions of physiological chewing forces may be different from those of simulators (25). Therefore, the results of *in vitro* studies must be confirmed by clinical trials.

Conclusion

Within the limitations of this *in vitro* study, it can be stated that preparing access cavities with either laser or bur does not have any deleterious effect on the fracture resistance of teeth with root canal treatment.

Ethics Committee Approval: The present study was approved by the ethical committee of Gaziantep University (Project number: 2015/125).

Informed Consent: Verbal informed consent was obtained from the parents of the patients/patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: UA, FA and ST designed the study and generated the data. FA and ST gathered the data. UA and DAB analyzed the data. FA and ST wrote the majority of the original draft. UA participated in writing the paper. All authors approved the final version of the paper.

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

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

Türkçe öz: *Konvansiyonel frez, ER:YAG ve ER,CR:YSGG lazerler ile kavite açılan kanal tedavili dişlerin kırılma dirençleri. Amaç: Bu çalışmanın amacı, konvansiyonel frezler, Er:YAG ve Er,Cr:YSGG lazerler ile giriş kavitesi açılmasının ardından dişlerin kırılma dirençlerinin karşılaştırılmasıdır. Gereç ve Yöntem: Elli beş alt çene azı dişi, 3 negatif grup (grup 1, 2, 3; n=5), 3 çalışma grubu (grup 1, 2, 3; n=10) ve 1 pozitif kontrol grubu (sağlam dişler; n=10) olmak üzere 7 gruba ayrıldı. Grup 1, 2 ve 3' ün giriş kaviteleri sırasıyla konvansiyonel frez, Erbium-doped yttrium aluminum garnet laser (Er:YAG) ve Erbium, cromium: yttrium scandium gallium garnet laser (Er,Cr:YSGG) ile açıldı. Kanalların doldurulmasının ardından koronal kısımlar restore edilmeden bırakıldı. Grup 4, 5 ve 6' nın giriş kaviteleri sırasıyla konvansiyonel frez, Er:YAG ve Er,Cr:YSGG ile açıldı. Kanalların doldurulmasının ardından koronal kısımlar kompozit rezin ile restore edildi. Termosiklüs sonrası, kırılma dirençleri, Universal Test Cihazı ile ölçüldü. Her bir örneği kırmak için gereken ortalama kuvvet Newton olarak kaydedildi ve veriler istatistiksel olarak değerlendirildi. Bulgular: Sağlam dişlerden oluşan Grup 7'nin kırılma direnci, diğer gruplardan anlamlı düzeyde yüksek bulundu (p<0,001). Grup 4, 5 ve 6'nın kırılma dirençleri arasında farklılık izlenmezken Grup 1, 2 ve 3'ten anlamlı düzeyde fazla bulundu (p<0,001). Grup 1, 2 ve 3 de ölçülen kırılma dirençleri arasında fark bulunmamıştır. Sonuç: Giriş kavitelerinin lazer veya frezler ile hazırlanması kırılma direncini etkilemedi. Anahtar kelimeler: Frez; koronal dayanım; Er,Cr:YSGG; Er:YAG; kırılma direnci*

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Original research

The influence of the irrigant QMix on the push-out bond strength of a bioceramic endodontic sealer

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Purpose
The aim of this study was to evaluate the effect of a commercially available irrigant, the Qmix 2 in 1, on the push out bond strength of Endosequence BC which is a bioceramic based root canal sealer.

Material and methods
Sixty extracted maxillary central incisors were prepared with Protaper Next rotary instrument up to X4 (# 40). The specimens were randomly divided into four groups of equal sample size (n=15), according to the final irrigation regimen as follows: Group 1: 2.5% sodium hypochloride (NaOCl) (control), Group 2: 17% ethylenediaminetetraacetic acid (EDTA), Group 3: 2% Chlorhexidine (CHX), Group 4: QMix 2 in 1. After rinsing, teeth were obturated using gutta-percha with Endosequence BC sealer. Slices were obtained from coronal, middle, and an apical section of the root canal. Push-out test was performed to evaluate the bond strength between the root canal dentin and the sealer. Data was statistically analyzed.

Results
The push-out bond strength of the root canal sealer was significantly affected by the type of irrigation solution. Highest bond strengths were found in canals irrigated with Qmix solution, and these results were regardless of anatomical section of the root canal ($p< 0.05$).

Conclusion
Final irrigation of the root canals with QMix had a positive effect on the bond strength of Endosequence BC sealer throughout the root canal.

Keywords: *Endosequence BC sealer; irrigant; push-out test; Qmix; endodontics*

Introduction

The main aim of endodontic treatment is to eliminate micro-organisms from the root canal system and inhibit reinfection (1). Mechanical instrumentation is definitely one of the important factors in reducing the bacterial load of the infected root canals (1), although it is not completely effective in removing all bacteria and debris (1). Ability of instrumentation alone to debride and clean the canal is limited (2-5). Peters *et al.* (2, 3) reported that large areas of the root canal walls could remain untouched, regardless of the instrumentation technique. Accurate instrumentation and debridement of root canals is often very complicated and difficult because of their anatomical characteristics. Most of them demonstrate oval morphology in shape and include the lateral canals, isthmuses, and deltas (2, 3). Rödig *et al.* (4) showed that debridement with the nickel-titanium (Ni-Ti) rotary instruments of buccal and lingual extensions of oval canals may still be inadequate. Similarly, Wu and Wesselink (5) reported that uninstrumented areas may be left in many oval canals after the instrumentation. Therefore,

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irrigation of root canals is the only way left to eliminate microorganisms and debris from the root canal walls, which could not be reached by mechanical instrumentation.

Sodium hypochlorite (NaOCl) solutions in concentrations ranging from 0.5% to 6.15% have been used as endodontic irrigants for more than 70 years, and they are still the most commonly used solutions for this purpose (5). Sodium hypochlorite solution has bactericidal and virucidal properties and it dissolves necrotic tissue (6). Moreover, NaOCl has a low viscosity and long shelf life (6). On the other hand, this solution may be toxic and cannot break up inorganic contents of the smear layer (7). Because of this, NaOCl has been used with chelating agents like ethylenediaminetetraacetic acid (EDTA) in 15-17% concentrations for smear layer removal. Combined used of these irrigants represents the current optimal irrigation protocol. However, NaOCl and EDTA should not be combined *in situ*, because EDTA strongly reduces free chlorine in NaOCl solutions, mostly rendering them ineffective (8, 9). Furthermore, it has been shown that using NaOCl as a final irrigant after the use of EDTA may compromise the structural integrity of the dentin (8, 9). Chlorhexidine (CHX) solution in 2% concentration is also a widely used root canal irrigation solution. It has long term antimicrobial effect, comparatively low toxicity (7). However, when CHX comes in contact with residual NaOCl, subsequent chemical reaction produces para-chloroaniline precipitate which is potentially toxic (10). Thus, it requires to remove any remaining NaOCl solution from the root canal with saline, alcohol or distilled water prior to CHX application (10).

To overcome these problems in the irrigation of the root canals, a new irrigation solution, QMix 2in1 (Dentsply Tulsa Dental, Tulsa, OK), has been introduced to market for smear layer removal with antimicrobial efficiency. It comprises of EDTA, CHX, a detergent and deionized water. It is designed as a final irrigant to replace 17% EDTA final wash protocol, and is used for 60-90 seconds (11). QMix is a ready-to-use clear solution, and requires no chair-side mixing. It has been shown that QMix 2in1 is effective in both removing the smear layer and killing bacteria such as *Enterococcus faecalis* in one application (11). Elnaghy (12) reported that QMix 2 in1 could remove the smear layer more effectively than 17% EDTA and 17% EDTA/2% CHX based on the completely opened dentinal tubules. Eliot *et al.* (13) have also stated that QMix removed more smear layer than EDTA. Furthermore, QMix eliminates some disadvantages of both EDTA and CHX. It does not interact with residual NaOCl if used for the purpose of final irrigation (14).

Using gutta-percha with various types of sealers is the standard technique in endodontic obturations. Tricalcium silicate based Endosequence BC Sealer (Pulpdent, Watertown, Massachusetts USA) has recently been used to treat root canals. It is an aluminum-free material comprised of calcium, calcium phosphate, zirconium oxide and calcium hydroxide that requires the existence of water to harden. It shows alkaline pH, antibacterial activity, radio-opacity and biocompatibility (15).

The use of irrigants before the obturation of root canals is very important to dissolve organic and inorganic contents of the smear layer, to clean the dentin walls and enhance the bonding of root canal sealers. The data regarding the effects of QMix irrigation solution on the bond strength of root canal sealers is limited. Therefore, the aim of the present study was to assesment the effect of QMix irrigant on the push out bond strength of a bioceramic endodontic sealer. The main null hypothesis tested in the present study is that the push-out bond strength of Endosequence BC sealer in root canals irrigated with Qmix is not different from those irrigated with NaOCL, EDTA and CHX.

Materials and methods

Specimen selection

This *in vitro* study was conducted on 60 maxillary central incisors extracted within 6 months prior to the experiments and stored in 0.1% thymol solution at 4°C. The inclusion criteria were straight canals and completely formed apices. The exclusion criteria were teeth with root caries, cracks, resorption, incomplete apices, or those with root length less than 15 mm. This study was confirmed by the Research Ethics Committee of Medipol University (project no: 324).

Specimen preparation

The specimens were stored in 1% Chloramine T solution (Ricca Chemical Company, Arlington, TX) for 48 hours for disinfection. Then, the external root surfaces were scaled with ultrasonic instruments, and rinsed with distilled water for the elimination of remnants from the root surface. The crowns were sectioned transversally at the cementoenamel junction. and the root length was set to 15 mm, and later access cavity was established, the working length was detectioned by a direct method of withdrawing 1 mm from the real root length. Root canals shaping were done with Protaper Next (Dentsply / Maillefer, Ballaigues, Switzerland) up to X4 (#40) master apical file size. During the shaping, the root canal was irrigated by 2 mL 2.5% NaOCl (ImidentMed, Konya, Turkey) solution after preparation with each file. The roots were then randomly divided into four groups (n=15) according to the final irrigation ptotocol as follows: Group 1: 5 mL of 2.5% NaOCl for 60 seconds (control)**,** Group 2: 5 mL of 17% EDTA solution (Pulpdent, Watertown, Massachusetts USA) for 60 seconds, Group 3: 5 mL of 2% CHX (Consepsis, Ultradent, South Jordan, UT) for 60 seconds (washed with distilled water before CHX application)**,** Group 4: 5mL QMix 2in1 (Dentsply Tulsa Dental, Tulsa, OK) for 60 second.

Root canals irrigated with a 30-g side-perforated irrigation probe (Canal Clean, Biodent Co. Ltd, South Korea) and a syringe. Final rinsing was done for one minute in each canal. At the procedure of irrigation, the needle was used with up and down movements in the canal to within 1-2 mm of the working length. Subsequent to the procedures, all the canals were dried using paper points and obturated with Endosequence BC sealer (Brasseler USA, Savannah, GA) using the single cone technique with matching taper X4 gutta-percha cones (Dentsply Maillefer, Ballaigues, Switzerland) to achieve standard samples for push-out test. After root obturation, the coronal accesses of the root canals were sealed with temporary filling material. Teeth were stored at 37°C and 100% relative humidity for seven days to allow the sealer to set.

Push-out bond strength

Each samples was horizontally cut with a low-speed diamond. saw (Isomet, Buehler, Lake Bluff, IL, USA) under cold water irrigation. Three slices in 2±0.1 mm thickness were obtained from the coronal, middle, and apical sections of the roots. Apical and coronal aspects of each slice were examined under a stereomicroscope (Imaging Systems, Leica Ltd., Cambridge, England) to measure the diameter of each hole.

Push-out bonding strength was calculated with a universal testing machine (TSTM02500, Elista Inc., Konya, Turkey) at a crosshead speed of 1 mm/min. As for the tapered design of the root canal, three different sizes of cylindrical pins were used for the push-out test. The diameter. of the cylindrical pins was 1.2 mm for the coronal slices, 1.0 mm for the middle slices, and 0.8 mm for the apical slices to make sure that the strength was implemented as adequately as possible to the adhesion area during the loading phase. The highest load implemented to the obturation material before debonding was recorded in Newton (N). The bond strength was calculated in Mega Pascals (MPa) by dividing the load (N) by the adhesion area of root filling (A) (mm^2), with the equation: Mpa = F/A. The parameter A was calculated with this equation:$A = \pi (R + r) \times g$, where R is the coronal radius, r is the apical radius, and g is the height relative to the tapered inverted cone (mm). The parameter g was calculated with this equation: $g^2 = (R - r)^2 + (2.0)^2$.

Statistical analysis

Statistical Package for Social Sciences SPSS version 21.0 (IBM-SPSS Inc, Armonk, NY, USA) software was used for statistical analysis. Data was first examined by use of the Shapiro-Wilk test to verify the assumption of normality and with Levene's test to check for variance equality. As the data was found to be normally distributed, one-way analysis of variance (ANOVA) test for multiple groups and Tukey HSD test for pairwise comparisons were performed. Confidence limit was regulate to 95% and p values less than 0.05 were planned as statistically important.

Results

The effects of the type of irrigant on the push-out bond strength of the root canal sealer are summarized in Table 1.

Table 1. *Mean and standard deviations (SD) of push-out bond strength values of study groups in Megapascal*

	n	Mean±SD	Minimum	Maximum
NaOCl	45	1.18±0.59 A	0.23	2.36
CHX	45	2±0.56 B	0.58	2.95
EDTA	45	2.51±0.42 C	1.76	3.45
QMix	45	3.49±0.61 D	2.45	4.75

CHX: chlorhexidine; EDTA: ethylenediamine tetra acetic acid; NaOCl: sodium hypochlorite
Different uppercase letters in columns show statistically significant differences at p<0.05 level

There was a significant difference between the push out bond strengths of Endosequence BC sealer with respect to type the irrigation solution (p<0.05). Endosequence BC sealer showed the highest bond strength values when QMix 2in1 was used as the final irrigant (p<0.05). On the other hand, Endosequence BC sealer showed the lowest bond strength values when NaOCl was used as the final irrigation solution (p<0.05). These results were regardless of anatomical section of the root canal (p<0.05).

Discussion

Adhesion capability is a crucial factor for root canal sealers. An ideal endodontic sealer must bond to both the gutta percha and root dentin surface and must seal the root canal space (16). The effects of different irrigation solutions on the bond strength of resin based sealers to dentin have earlier been investigated (15-19). However, there is no comparative data regarding the effects of Qmix and other irrigants on the push out bond strength of a bioceramic based endodontic sealer. In the present study, we used Endosequence BC as a root canal sealer to test the effects of different irrigation solutions on its bond strength to the dentin surfaces.

The smear layer may negatively affect the bond strength of root canal sealers by acting as a barrier. As it also contains organic and inorganic contents, it should be effectively removed by different irrigation solutions (16). Studies have shown that, removing the smear layer makes it easier for the canal sealer to penetrate dentin tubules (20-23). EDTA and NaOCl solutions are commonly used to remove the smear layer from the root canals (24). Because of its residual antimicrobial activity, CHX has been proposed as a supplemental final irrigation procedure after smear layer removal (25). However, the concomitant use of CHX and NaOCl leads to color changes and formation of a possibly toxic, insoluble precipitate which reduces the sealing ability of the root canal filling procedure. Also, this precipitate involves a substantial amount of parachloroaniline, which has been shown to be carcinogenic and toxic (14, 24, 25). This substance acts as a chemical smear layer by coating the dentin surface and by changing permeability of dentin surface (26). Moreover, the mixture of CHX and EDTA was found to form a white precipitate which has a chemical structure of salt. This precipitate may also cover the dentin surface and alter dentin permeability. The chemical pattern of QMix prevents this, when CHX is combined with EDTA or NaOCl.

Previous research concerning the smear layer elimination efficacy of EDTA and Qmix revealed contradictional results (11, 12, 18, 27-29). Some studies reported that QMix could eliminate the smear layer as effectively as 17% EDTA (27-29), while others concluded that QMix was better than 17% EDTA (12, 13). QMix solution is composed of EDTA, CHX and a detergent. It not only penetrates and removes the smear layer in the dentin surface but also kills bacteria within the tubules. Tuncer (18) reported that the percentage of bond strength of sealer was importantly greater in the EDTA + CHX and QMix groups than the NaOCl group. On the other hand, author stated that there was no difference between the efficiency of QMix and EDTA + CHX irrigation for smear

layer elimination. Shokouhinejad *et al.*(30) showed that the existence of smear layer did not substantially affect the bond strength of obturation materials. In the present study, Endosequence BC indicated the highest bond strength in root canals irrigated with Qmix. This may be the result of more efficient elimination of the smear layer by the QMix when used as a final irrigant.

Different irrigation solutions may alter the permeability and solubility of the dentin surface and therefore influence the adhesion of root canal sealers to dentin surfaces (22). Adhesion process mainly depends on the wettability of the rigid surface which is provided by the internal dentin wetness as a result of water in dentinal tubules (31). Ballal *et al.* (24) indicated that the wettability of the root canal dentin which is filled with AH Plus sealer is higher in canals treated with Qmix compared to those flushed with EDTA, when both solutions are used as final irrigants. This may be owing to the combined reaction of CHX and the detergent existence in QMix. In addition, Uzunoglu *et al.*(32) showed that bond strength of AH Plus sealer to root canal dentin is improved with QMix. However, Aranda Garcia *et al. (27)* stated that the surfactant compound in Qmix did not increase the bond strength of AH Plus compared to 17% EDTA. Carvalho *et al.*(33) indicated that EDTA did not influence the push-out bond strength of MTA Fillapex and AH Plus. In the present study, various irrigants affected the bond strength of Endosequence BC to the root canal wall differently. Endosequence BC showed higher bond strength after irrigation with QMix than with EDTA and CHX.

Conclusion

Within the limitations of this *in vitro* study, our findings suggest that using QMix may lead to superior retention of Endosequence BC sealer when compared to EDTA and CHX. From clinical perspective, Qmix solution can be used for removing the smear layer and may be considered as an alternative to using EDTA following NaOCI as final irrigant.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Medipol University (project no: 324, date: 15.09.2017).

Informed Consent: This study was performed *in vitro* by exploiting exracted teeth. Therefore written and verbal informed consent was not obtained.

Peer-review: Externally peer-reviewed.

Author Contributions: MG, GPS, EE and ÖYÖ designed the study. MG generated the data. MG and GPS gathered the data. MG, EE and ÖYÖ analyzed the data. GPS wrote the majority of the original draft. MG and GPS participated in writing the paper. All authors approved the final version of the paper.

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

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Türkçe öz: *Qmix irriganının Bioseramik esaslı kök kanal patının dentine bağlanma dayanımı üzerindeki etkisi. Amaç: Bu çalışmanın amacı Qmix irriganının bioseramik esaslı kök kanal patı Endosequence BC'nin dentine bağlanma dayanımı üzerine etkisinin değerlendirilmesidir. Gereç ve Yöntem: 60 adet çekilmiş üst santral diş Protaper Next rotary enstrüman ile X4 (40#)'e kadar şekillendirildi. Numuneler rastgele eşit sayıda olacak şekilde, final irrigasyon yöntemine göre 4 gruba ayrıldı (n:15); Grup1:%2,5 sodyum hipoklorit (NaOCI) (kontrol), Grup2: %17 EDTA, Grup3: %2 klorheksidin (CHX), Grup4: QMix 2 in 1. İrrigasyondan sonra dişler Endosequence BC kanal patı ile güta-perka kullanılarak doldurulduu. Kök kanalının apikal, orta ve koronal üçte birlik kısımlarından kesitler alındı. Push-out testi ile kanal patı ve kök kanal dentini arasındaki bağlanma dayanımı ölçüldü. Datalar istatistiksel olarak analiz edildi. Sonuç: Kök kanal patının dentine bağlanmasını irrigasyon solüsyonunun tipi önemli ölçüde etkilemiştir. Qmix solüsyonu ile en yüksek bağlanma dayanımı oluşurken bu sonuçlar kök kanalının anatomik bölümlerine bakılmaksızın elde edilmiştir. Anahtar kelimeler: Endosequence BC kanal patı; irrigan; push-out test; Qmix; endodonti*

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Original research

The effects of different desensitizer agents on shear bond strength of orthodontic brackets after home bleaching: an *in vitro* study

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Purpose
The aim of this study was to test the null hypothesis that no difference exists between shear bond strength values of control and bleaching plus desensitizer applied groups.

Materials and methods
A hundred freshly extracted human premolar teeth were randomly divided into five groups. Group I served as the control group with no bleaching application, while only bleaching was achieved in Group II. Desensitizer containing potassium nitrate–fluoride and casein phosphopeptide amorphous calcium phosphate were applied in Groups III and IV, after bleaching respectively. A bleaching agent containing amorphous calcium phosphate was used in Group V. Shear bond strength tests were carried out using a universal testing machine (Instron Corp., Norwood, MA, USA). Remnant adhesive on the teeth and brackets was examined to score the adhesive remnant index. Kruskal-Wallis and Chi-Square tests were used for statistical analysis of the data.

Results
Statistically significant differences were found among the groups for shear bond strength values ($p<.001$). The shear bond strength of Group III (8.0±2.2 MPa) was significantly lower than the other groups ($p<.05$). The highest shear bond strength values were found for Group I (13.6±3.7 MPa) and Group IV (12.8±4.0 MPa). No statistically significant difference was observed between Group II (10.0±2.7 MPa) and Group V (10.8±2.9 MPa). The differences between adhesive remnant index scores of the groups were not statistically significant.

Conclusion
Casein phosphopeptide amorphous calcium phosphate gel application showed a similar shear bond strength value to the control group, while shear bond strength values decreased after using other desensitizers.

Keywords: *Orthodontic bonding; tooth bleaching; dentin desensitizing agents; casein phosphopeptide amorphous calcium phosphate; shear bond strength*

Introduction

Tooth color has been considered as one of the most important indicators of wealth, beauty and prosperity since ancient times. Discoloration can negatively impact quality of life, so tooth whitening procedures have become a popular dental application in recent years. Different combinations such as honey, burned salt and vinegar or calcium hypochlorite and oxalic acid were used for tooth whitening before peroxide was found in 1884 (1). In modern dental practice, bleaching procedures with various agents have been accepted as simple, safe, effective and predictable both for dentists and patients (2).

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Tooth sensitivity caused by bleaching procedures is commonly experienced as a side effect (3), which is characterized by a short, sharp pain in response to cold, hot or sweet stimulus (4). The "hypodynamic theory" is generally accepted to explain the cause of sensitivity. According to this theory chemical, thermal or physical changes create the movement of fluids in dentinal tubules and these movements generate a stimulus on the related nerve receptor causing the initiation of sensitivity or pain (5). For this reason, patients frequently benefit from desensitizing agents that can be applied easily and safely (6).

Because cosmetic dentistry showed progress, some patients with dental malocclusion are interested in both orthodontic treatment and dental bleaching (7). During initiation of fixed therapy, the enamel structure is very important for bonding success (7) and the enamel structure is affected by bleaching and desensitizing agents (6). Controversial findings have been reported in the literature about bonding success of bleached enamel. Some studies showed significant shear bond strength (SBS) decrease after dental bleaching (8, 9), while no significant difference was also found (10).

Amorphous calcium phosphate (ACP) is a biologically active material for repairing tooth structure and reducing dentin hypersensitivity. Casein phosphopeptide is a milk protein derivative used as a remineralizing agent and contains phosphoseryl sequences which are stabilized with ACP (11). Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) compound prevents the dissolution of calcium and phosphate ions and provides a supersaturated solution of bioavailable calcium and phosphates (12), so CPP-ACP has also been proposed by manufacturers for the prevention and treatment of dentin hypersensitivity (13, 14).

Different desensitizer agents have been used after bleaching, however it is important to determine the appropriate agent for increased bonding success. Although the effects of different desensitizers on SBS have been reported previously, to our knowledge, no study has compared ACP, CPP-ACP and potassium nitrate–fluoride agents in orthodontic bonding success. Therefore, the aim of this *in vitro* study was to evaluate the effects of different desensitizers on SBS of orthodontic brackets bonded to bleached teeth. The null hypothesis of this study was "no difference exists between SBS values of control and bleaching plus desensitizer applied groups".

Materials and methods

Sample size estimation

The experiment protocol was approved by *ethical committee* of Erciyes *University (approval code: 2016/73)*, and informed consent was obtained from all patients whose teeth were extracted for orthodontic purposes. A priori power analysis was completed using G*Power Ver 3.1.9.2 (Universität Kiel, Germany) software. Based on a 1:1 ratio between groups, a sample size of 20 specimens in each group is able to provide 90% power to detect significant differences with 0.86 effect size at a significance level of $\alpha = .05$. Totally 100 intact freshly extracted human permanent upper first premolar teeth were randomly allocated to five groups with 20 teeth in each.

Specimen preparation

The teeth were embedded in acrylic resin blocks with ten teeth to a block. Acrylic resin was formed 1 mm below the cervical lines of the teeth. Alginate impressions were taken from the blocks and plaster models were obtained. Nail varnish was applied on the vestibular surfaces of the teeth at a thickness of approximately 0.5 mm to 1.0 mm to provide reservoir spaces on the bleaching trays. Low-density polyethylene plates were applied to the plaster models and bleaching trays were prepared using a vacuum thermoforming machine (ProForm, Dental Resources Inc., Minn., USA) (Figure 1).

Bleaching and Bonding Procedures

The teeth surfaces were cleaned and polished with pumice and rubber cups for 10 seconds to simulate a routine clinical procedure.

Group I: This group was the control group and received no bleaching. Etching was performed with 37% orthophosphoric acid gel (3M Dental Products, Minn, USA) for 15 seconds. The etching material was removed from the teeth surfaces with air-water spray for 10 seconds, and teeth were dried for

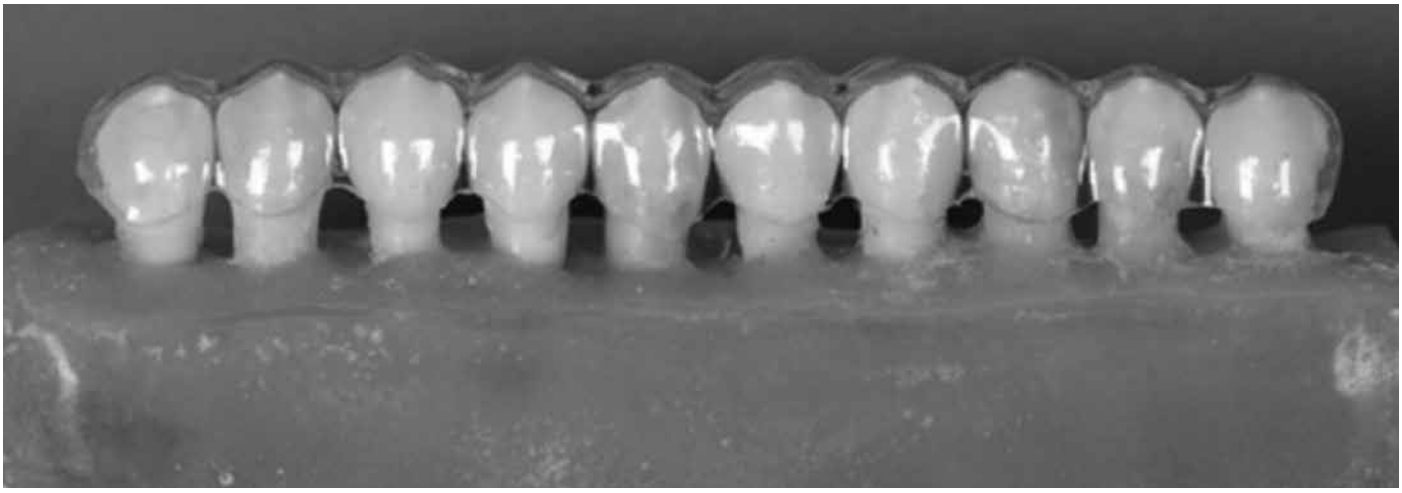

Figure 1. *Acrylic resin blocks and bleaching trays.*

10 seconds. A thin uniform coat of the sealant Transbond XT primer (3M Unitek, Monrovia, USA) agent was applied to the etched surface. Premolar brackets were bonded using Transbond XT (3M Unitek Monrovia, USA) composite and light cured with a light-emitting diode curing unit (Valo, South Jordan, USA) for 20 seconds according to the manufacturer's instructions (10 seconds per each approximal side). Teeth were stored in salivary buffer for 24 hours before the SBS test in order to obtain the highest adhesive bond strength (15).

Group II: Teeth were bleached with 22% carbamide peroxide (CP) agent (Hollywood Smiles Bleaching Pen; Onuge Oral Care Co, Henan, China) according to manufacturer's instruction. The procedure was repeated every day for one week. Bleaching material was applied to the vestibular surfaces of the teeth with a pen applicator and spread across the surface with the pen tip brush. Then, trays were placed onto the teeth and blocks were covered with salivary buffers. Half an hour later, trays were removed and kept for later use. The surfaces of the teeth were washed with saliva and stored in the salivary buffers.

Group III: 22% CP and desensitizer agent containing potassium nitrate–fluoride (UltraEZ; Ultradent Products Inc., South Jordan, USA) was applied to the teeth consecutively. The procedure was repeated daily for 1 week. For each application, the bleaching procedure was performed in the same manner as for Group II. Then, the teeth were washed and dried with a sponge. Desensitizer material containing potassium nitrate–fluoride (UltraEZ; Ultradent Products Inc., South Jordan, USA) was applied to the vestibular surfaces of the teeth with bonding brushes. Trays were placed for the desensitizer and blocks were covered with salivary buffers. Trays were removed after 30 minutes and the teeth were covered with salivary buffers until the next application.

Group IV: CPP-ACP gel (GC Tooth Mousse gel; *GC* Int *Corp*, *Tokyo*, *Japan*) was applied to the teeth after bleaching with 22% CP. The application procedures were repeated as for Group III.

Group V: 22% CP gel containing ACP (NiteWhite ACP; Discus Dental, Culver City, USA) was used. 22% CP and ACP combination gel (NiteWhite ACP; Discuss Dental, Culver City, USA) was applied to the teeth every day for a week. Bleaching material was applied to the vestibular surfaces of teeth and spread on the surface. After, trays were placed on the teeth and blocks were covered with salivary buffers. Half an hour later, trays were removed and the surfaces of teeth were washed with saliva and stored in the salivary buffers.

During the experimental procedure, blocks were stored in buffers with saturated artificial saliva (Table 1) to imitate intraoral conditions and the buffers were renewed twice a day.

At the end of the seventh day, all teeth were washed and pumiced. The bonding procedure was performed in the same manner as for the control group. Upper first premolar metal brackets were used (American Orthodontics Roth system, Master series, Sheboygan, Wisconsin, USA) for orthodontic bonding.

Shear bond strength testing

Shear strength tests were performed using an Instron Testing Machine (Instron Corp., Norwood, USA). A steel rod with one flattened and sharpened end was attached to an upper grip (movable head) connected to the load cell. The blade was positioned at the bracket-enamel interface (Figure 2) and an occluso-gingival directed load was applied. Cross-head speed was adjusted at 1 mm/min during recording the data.

After debonding, the teeth and brackets were examined to detect existence of any remnant adhesive after bracket removal. These results were scored according to the adhesive remnant index (ARI) (16). The examination of the enamel surface was performed by a blind investigator to group allocations. The ARI scores ranged from 0 to 3 (Table 2).

Table 1. *Composition of the artificial saliva*

Components	**Per cent**
NaCl	0.08
KCl	0.12
$MgCl_2$-$6H_2O$	0.01
K_2HPO_4	0.03
$CaCl_2$-$2H_2O$	0.01
Sodium Carboxymethyl Cellulose	0.10
Ion-Exchanged Water	99.6

Figure 2. *Test apparatus used for shear bond strength testing.*

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences, software (Version 20.0, IBM Corp.; Armonk, NY, USA). The Shapiro-Wilk W test was used to test the data for normality. Data was not normally distributed, so non-parametric tests were used. Group differences for SBS values and ARI scores were tested using Kruskal-Wallis and Chi-Square tests, respectively. A multiple comparison procedure (Student-Newman-Keuls Method) was used to isolate the group or groups that differ from the others. Confidence level was set to 95% and $p<0.05$ was considered statistically significant.

Results

The null hypothesis was rejected. A statistically significant difference was found between the SBS values of groups ($p<.001$, Table 3). SBS values of Group I (control; 13.6±3.7 MPa) and IV (12.8±4.0 MPa) were significantly greater than other groups ($p<.05$ for all comparisons). Group III had the lowest SBS value (8.0±2.2 MPa). No statistically significant difference was observed between Group II (10.0±2.7 MPa) and Group V (10.8±2.9 MPa).

Adhesive remnant index scores are presented in Table 4. No significant differences were found between groups in ARI evaluations.

Table 2. *The Adhesive Remnant Index (ARI) scores*

Index	Enamel Adesiv Remnant
0	No adhesive left on the tooth (_10%)
1	Less than half of the adhesive left on the tooth
2	More than half of the adhesive left on the tooth
3	All adhesive left on the tooth (_90%)

Discussion

The decreasing SBS values of orthodontic brackets after bleaching were attributed to the changes in the enamel structure. Conventional acid etching with 37% phosphoric acid leads to prism core demineralization, prism sheath demineralization or both types of demineralization (17). Composite resin adheres to etched enamel by mechanical bonding through unfilled resin penetration and polymerization in these surface irregularities. However, bleached teeth lose these retentive areas which are prepared by etching and required for bonding (6,17). Titley *et al*. (18) observed sparse, short, poorly defined enamel tags immediately after hydrogen peroxide (HP) bleaching. In this study, bleaching with 22% CP led to a statistically significant reduction in SBS. Oskoee *et al.* (19) reported reduction of SBS with bleaching, while Miles *et al*. (9) showed

Table 3. *Descriptive statistics and multiple comparisons of the shear bond strength values of groups*

Groups	Shear Bond Strength (MPa)						Significance	*Multiple Comparison
	N	25%	Median	75%	Mean	SD		
Control	20	10.0	13.5	17.0	13.6	3.7	p <.001	A
CP	20	9.0	9.5	12.0	10.0	2.7		B
CP+D	20	6.5	7.0	9.0	8.0	2.2		C
CP+CPP-ACP	20	9.5	13.0	17.0	12.8	4.0		A
CP+ACP	20	9.0	10.0	12.0	10.8	2.9		B

CP: carbamide peroxide; CP+D: carbamide peroxide plus potassium nitrate–fluoride containing desensitizer; CP+ACP: amorphous calsium phosphate containing carbamide peroxide; CP+CPP-ACP: carbamide peroxide plus casein phosphopeptide-amorphous calsium phosphate
*Groups with different letters are significantly different from each other

Table 4. *Frequency Distribution of the Adhesive Remnant Index (ARI) Scores*

		Adhesive Remnant Index Score				
Groups	n	0	1	2	3	Sig.
Control	20	1	2	7	10	NS
CP	20	3	4	5	8	
CP+D	20	4	3	6	7	
CP + CCP-ACP	20	2	2	6	10	
CP + ACP	20	2	3	7	8	

n: sample size; NS: non-significant; CP: carbamide peroxide; CP+D: carbamide peroxide plus potassium nitrate–fluoride containing desensitizer; CP+ACP: amorphous calsium phosphate containing carbamide peroxide; CP+CPP-ACP: carbamide peroxide plus casein phosphopeptide-amorphous calsium phosphate
Chi-square test revealed no significant differences between groups

decreased SBS values when bleaching was carried out with CP. In contrast, Bishara (20) showed that both in-office bleaching with 25% HP and at-home bleaching with 10% CP did not affect the SBS of orthodontic brackets to enamel.

Another reason for reduction in SBS with bleaching was accepted as residual peroxide on the enamel surface (21). Torneck *et al.* (22) found a significant decrease in bond strength related to the presence of residual peroxide or peroxide-related substances on the enamel surface. Neutralizing these substances with antioxidants increases the bond strength of bleached enamel (23).

Sensitivity after bleaching is a common adverse effect, and a desensitizer including potassium nitrate and fluoride ions decreases sensitivity complaints by obstructing the tubules (24), which can lead to the failing of the most important stage of bonding: adhesion between enamel and resin. Our findings on CP plus desensitizer containing potassium nitrate–fluoride indicated that using this desensitizer after CP bleaching can reduce SBS, and possible reason for this reduction may be the weakening of enamel adhesive linkage. Similar to our findings, Turkkahraman *et al.* (6) also reported that bleaching and desensitizer applications significantly reduce the SBS of orthodontic brackets.

Amorphous calcium phosphate and CPP-ACP compositions treat hypersensitivity of the teeth by releasing free calcium and phosphate ions in a different way to the desensitizer mechanism (12, 14). According to results of this study, SBS values of samples which used CPP-ACP were similar to the control group. SBS values were higher in the ACP bleaching group compared to bleaching plus desensitizer containing potassium nitrate–fluoride applications. An increase in SBS values with CPP-ACP was also reported by Lu *et al.* (25). Similarly, Oskoee *et al.* (19) found the same SBS values between the control group and the group that received CPP-ACP after bleaching.

In this study, CPP-ACP application after bleaching provided higher SBS than the application of bleaching agent containing ACP. This difference may be attributed to the difference in application procedures for these two materials. Saliva can enhance the effectiveness of CPP-ACP, so more effective results can be obtained if increased contact of CPP-ACP and saliva is maintained (26). ACP was applied within the CP gel, so there is no direct contact between ACP and a salivary environment, while CPP-ACP gel was applied separately after CP gel. So, remineralized enamel following CPP-ACP application after bleaching may cause a higher SBS value than ACP samples. In addition, the obliterated enamel structure with calcium and phosphate ions on the destructed core and sheath of prisms during peroxide application in the CP-containing ACP group may cause a more acid-resistant surface for etching with CP-containing ACP, compared with CPP-ACP plus CP. Machado *et al.* (27) found similar lowering SBS with peroxide-ACP combined gel and peroxide gel applications, compared with the control group. Although no significant difference was found between CP and CP with ACP applications in this study, a slight increase was observed with CP containing ACP gel compared with only CP application.

The evaluation of ARI scores showed that the site of bond failure is not significantly affected by the experimental procedures of this study. Reynolds (28) reported that successful clinical bonding should provide bond strength of 5.9 to 7.8 MPa, and these results have been accepted as threshold values for SBS studies. The SBS values of our study ranged between 8 to 15 MPa, meaning there is no contraindication for using ACP, CPP-ACP and the desensitizers for the elimination of sensitivity caused by bleaching in terms of bonding failure. Even though SBS values of experimental groups of this study were higher than acceptable clinical limits, using the CPP-ACP agents for the elimination of sensitivity before the fixed orthodontic therapy can be more safer than CP alone, CP containing ACP, and CP plus desensitizer containing potassium nitrate–fluoride in terms of bonding success. In the light of these findings, the clinicians will be able to recommend patients who need orthodontic treatment to use CPP-ACP agents as a desensitizer.

Artificial saliva was used to simulate the oral environment, but heat and humidity conditions in the oral cavity are highly variable. A direct correlation with *in vitro* design and in vivo oral conditions might be inaccurate during the interpretation of results. Further studies simulating in vivo settings which provide real heat, stress, acidity and humidity are required for more valid and reliable results.

Conclusion

Bleaching with 22% carbamide peroxide, 22% carbamide peroxide containing ACP and 22% carbamide peroxide bleaching plus desensitizer containing potassium nitrate–fluoride applications significantly decreased shear bond strengths of orthodontic brackets bonded to human enamel compared with the control group. CPP-ACP application after 22% carbamide peroxide bleaching and the control groups showed similar shear bond strength values. No statistically significant difference was observed in the site of bond failure for all groups. According to these findings, clinicians may recommend patients who need orthodontic treatment to use CPP-ACP after 22% carbamide peroxide bleaching as a safer desensitizer for the prospective orthodontic bonding procedure.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Erciyes University (Approval code: 2016-73).

Informed Consent: Written and verbal informed consent was obtained from patients and/or patients' parents who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: NGA, AB and GK designed the study. NGA and YÜ generated the data. NGA gathered the data. NGA, AB, and GK analyzed the data. NGA wrote the majority of the original draft. NGA, AB and GK participated in writing the paper. All authors approved the final version of the paper.

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

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Türkçe öz: *Farklı hassasiyet giderici ajanların ortodontik braketlerin ev tipi beyazlatma sonrasındaki bağlanma dayanımı üzerine etkileri. Amaç: Bu çalışmada, ev tipi beyazlatma sonrası farklı hassasiyet giderici ajan uygulamalarının, ortodontik braketlerin bağlanma dayanımı üzerine etkilerinin değerlendirilmesi ve karşılaştırılması amaçlanmıştır. Gereç ve Yöntem: Çalışmamızda 100 adet insan premolar dişi 5 gruba ayrılmıştır. Grup I hiçbir beyazlatma uygulaması yapılmayan kontol grubu olarak belirlenirken; Grup II de yalnız beyazlatma işlemi gerçekleştirilmiştir. Grup III ve Grup IV'e beyazlatma işleminden sonra sırasıyla potasyum nitrat-florid içeren ve kazein fosfopeptit amorf kalsiyum fosfat içeren hassasiyet gidericiler uygulanmıştır. Grup V örneklerinde ise beyazlatma işlemi amorf kalsiyum fosfat içerikli beyazlatma ajanı ile gerçekleştirilmiştir. Braketlerin bağlanma dayanımı testleri için Instron Test Cihazı kullanılmıştır. Test sonrasında diş ve braket yüzeyinde kalan adeziv, artık adeziv endeksi ile skorlanmıştır. Veri analizinde Kruskal-Wallis ve Ki-kare testlerinden faydalanılmıştır. Bulgular: Gruplar arası bağlanma dayanımı karşılaştırmasında istatistiksel olarak anlamlı fark bulunmuştur (p<,001). Grup III'ün bağlanma dayanım değeri (8,0±2,2 MPa) diğer gruplardan anlamlı olarak düşüktür (p<,05). En yüksek bağlanma dayanımı değerlerinin Grup I (13,6±3,7 MPa) ve Grup IV'de (12,8±4,0 MPa) olduğu gözlenirken; Grup II (10,0±2,7 MPa) ve Grup V (10,8±2,9 MPa) arasında anlamlı farklılık yoktur. Gruplar arası artık adeziv endeks skorları arasında istatistiksel olarak anlamlı fark bulunmamıştır. Sonuç: Kazein fosfopeptit amorf kalsiyum fosfat uygulaması ile kontrol grubuna yakın braket bağlanma değerleri gözlenirken; diğer hassasiyet gidericiler braket bağlanma dayanımını azaltmıştır. Anahtar kelimeler: Ortodontik bonding; diş beyazlatma; dentin hassasiyet gidericiler; kazein fosfopeptit amorf kalsiyum fosfat; braket bağlanma dayanımı*

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Original research

In-vitro analysis of maxillary first molars morphology using three dimensional Micro-CT imaging: considerations for restorative dentistry

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Purpose
The aim of this study was to determine the differences between the positional relationship of the crown contour and the pulp chamber of left and right maxillary first molars, as well as their morphological characteristics by using micro-CT system with reconstruction from a volumetric rendering software.

Materials and methods
In total, 21 extracted maxillary first molars, including 11 left and 10 right teeth, were used. The positional relationship between the crown contour, pulp chamber and morphology of the teeth were investigated three-dimensionally by means of micro-CT imaging.

Results
Closest distance of mesio-buccal pulp horn to enamel surface in mm was calculated as 2.5±0.20 mm for right and 2.29±0.17 mm for left teeth. This difference was statistically significant (p=0.017). The means of closest distance of disto-buccal pulp horn to enamel surface were also significantly different between left and right teeth (p=0.001). The mean pulp volumes of right side and left side teeth were, respectively, 32.94±3.19 mm^3 and 33.71±2.82 mm^3 but this difference was not statistically significant.

Conclusion
These results suggest that right and left maxillary first molars should be treated differently during preparation of cavities. Further studies must be done with larger samples as well as for other molar teeth in different populations to reveal the morphology of the molar for further considerations in restorative dentistry.

Keywords: *Micro CT; maxillary molars; anatomy; pulp dimensions; restorative dentistry*

Introduction

The main aim of restorative treatment is to ensure the integrity of the teeth and their supporting tissues. Successful restorative procedures lies in the understanding the complex anatomy of the teeth (1). Minimal intervention has been proposed as the primary aim of modern caries therapy (2). Medical and dental interventions should be determined by the underlying scientific paradigms that guide the treatment and the progress of the disease (3). Moreover, it is crucial to select the appropriate treatment strategy in order to minimize the risk of creating pulp complications, as it can affect the quantity of caries excavation, risk of pulp injury and exposure, size of cavity preparation, and selection of capping materials (4). Various complications may occur during access cavity preparation or when locating the canal orifices because of the anatomical differences in maxillary molars.

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As maxillary molars usually represent complex anatomy and canal morphology, some studies assessed their anatomical characteristics to contribute to the treatment strategies (5, 6). These teeth may exhibit some anatomic variations and can be challenging cases while performing restorative treatment (7). Previous studies also indicated that access cavity preparation is performed subjectively, which mostly depends on the clinician's tactile perception and knowledge of dental anatomy (1, 4). Two-dimensional methods used for studying morphology of dental tissues are being replaced by three-dimensional ones. The conventional three-dimensional data is obtained by the *in vitro* reconstruction of the images of sample sections under light microscopy (8-10). Micro-CT is an innovative technique that provides three-dimensional data of the teeth, as it can produce this information without destruction of the dental tissue specimen (11). There is a lack of information concerning teeth morphology and pulp orifices in maxillary molar teeth in the literature (12, 13).

The present study therefore aims to evaluate the positional relationship between the crown contour and the pulp chamber as well as morphological characteristics of maxillary first molars using micro-CT system. The null hypothesis tested in this study is that the anatomical and morphological characteristics of right and left maxillary molars do not differ in any of the micro-CT based three dimensional measurements.

Materials and methods

Study sample

Based on the literature, (14-18) a power analysis (Power and Precision software, Biostat, Englewood, NJ, USA) was conducted to determine the sample size. At least 20 teeth at a power of 0.8 (alpha=0.05) was indicated. Thus, this study was conducted using 21 teeth (11 from left, 10 from right) of subjects aged between 20-30 years (mean age for left: 26 and for right: 25). The teeth used in this study had been extracted from the patients who had periodontal problems without carious lesions. Ethics committee approval and written consents from the patients were obtained before the study.

Micro-CT evaluation

A desktop, Micro-CT system in high resolution (Skyscan 1174, Skyscan, Kontich, Belgium) was used to scan the specimen. Before scanning, teeth were rinsed and stored in 0.9% saline solution within a tube. The teeth were placed in upright position on the scanning platform, to which the resorbed roots were fixed with wax. The teeth were scanned at 50 kvp, 100 mA beam current, 0.5 mm Al filter, 18.5 µm pixel size, rotation at 0.5 step, three frame averaging. Furthermore, after scanning of a tooth, in order to minimize ring artifacts, air calibration of the detector was carried out prior to each scan. A ring artifact correction of 0 and beam hardening correction of 40% were applied. Each sample was rotated 360° within an integration time of 5 min. Mean time of scanning was around 2 hrs.

Micro-CT image reconstructions

Reconstructions were performed using NRecon software (v 1.6.7.2, Skyscan, Kontich, Belgium), by means of Feldkamp *et al.* modified algorithm, obtained using a three-dimensional density function based on a series of two-dimensional projections. The NRecon software, by using this algorithm, created axial two-dimensional images. Other settings included beam-hardening correction and input of optimal contrast limits (0–0.0005) were set prior to teeth reconstructions. Contrast limits were applied according to the manufacturer's instructions. To obtain density scale of zero origin, the lowest limit was set to zero. The top of the brightness spectrum was the maximum limit, representing the highest density value. The image data set was approximately 900 axial tomographic slices, each measuring 1024x1024 pixels with a sixteen bit gray level (Figure 1). A 21.3-inch flat-panel color-active matrix TFT medical display (NEC MultiSync MD215MG, Muenchen, Germany) with a resolution of 2048 × 2560 at 75 Hz and 0.17-mm dot pitch operated at 11.9 bits was used to perform all reconstructions and measurements (Figure 1).

Volumetric rendering software analysis

After obtaining the axial images from Micro-CT scanning, the original grayscale images were processed with a Gaussian low-pass filter in order to reduce noise, and then for subtraction of teeth and pulp, an automatic segmentation threshold was used with CTAn (ver. 1.16.1.0, SkyScan, Kontich, Belgium). The images were rendered, and sagittal, axial, and coronal slices and the 3D models were reconstructed (Figure 2). The crown contour, pulp orifices and their positional relationships could be observed three-dimensionally by making the enamel and dentin translucent.

Three-dimensional measurements

The topographic relationship between the crown contour and the pulp orifices were measured. The crown volume/pulp volume and crown/pulp (ratio) was also calculated with the help of the software CTAn in which the user can designate

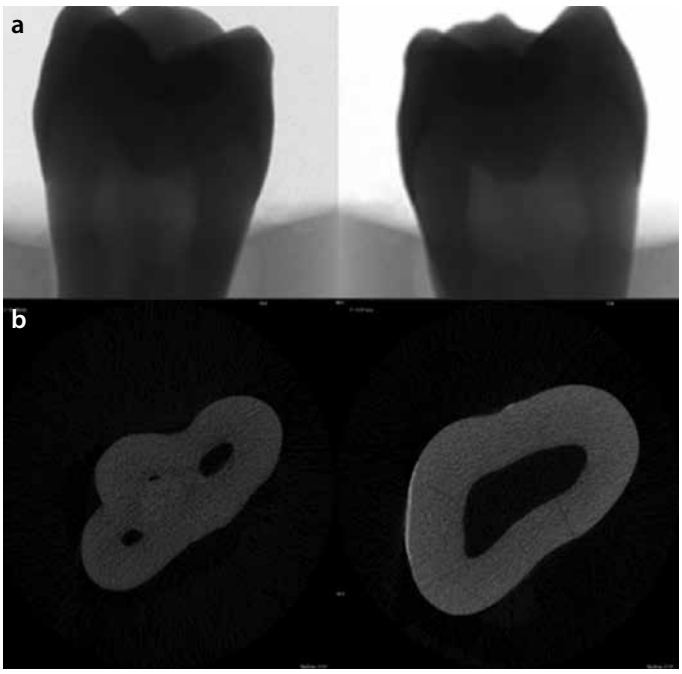

Figure 1. a, b. *(a) 3D scout and (b) axial micro-ct image showing one of the investigated tooth.*

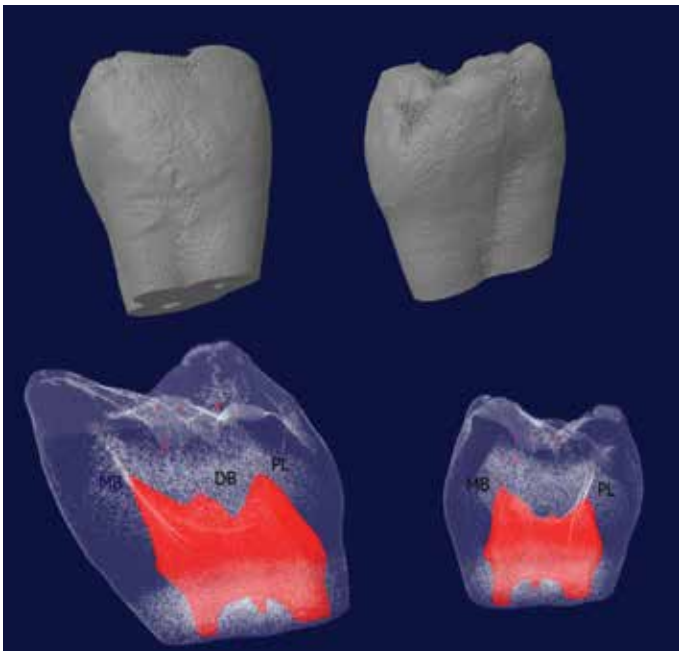

Figure 2. *Three representations of micro-CT image; showing the pulp chamber with hints extending to surface. Note the mesiobuccal horn protuberance.*

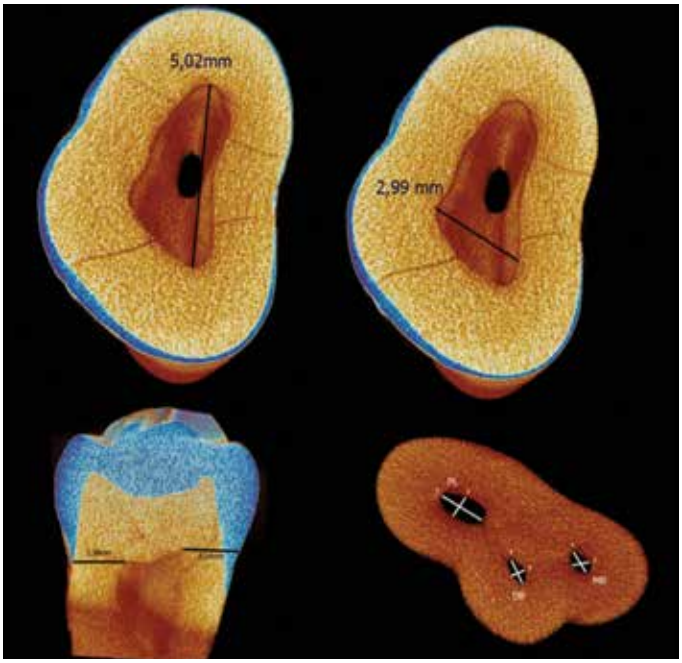

Figure 3. *The color cited representations of micro-CT images with measurements of the investigated parameters.*

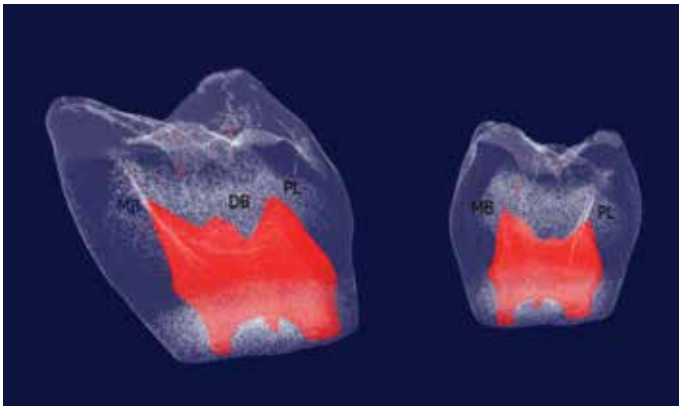

Figure 4. *3D images of the teeth and reflection of pulp and horns.*

the desired volume from the given three-dimentional structure. Also, the observer could remove the unwanted voxels before calculating the final volumes by adjusting brightness and opacity values (Figure 2). Mesial canal orifice length and width (mm), distal canal orifice length and width (mm), palatinal canal orifice length and width (mm), the closest distance from pulp chamber to mesial enamel surface (mm), the closest distance from pulp chamber to distal enamel surface (mm), max. pulp Chamber length and width (mm), closest distance of mesio-buccal pulp horn to enamel surface (mm), the closest distance of disto-buccal pulp horn to enamel surface (mm) (Figure 3, 4). All reconstructions and measurements images were done twice by a dentomaxillofacial radiologist with 18 year of experience (KO) and a restorative dentist with 10 years of experience (IHB) with Micro-CT own CTAn software. Software allows the operator to measure the distances, areas, and volume in life size without dependent of the operator skills. All measurements were done twice by the same observers. To detect intra-observer variability, observers performed their observations twice with an interval of 2 weeks.

Examiner reliability and statistical analysis

The Statistical Package for the Social Sciences 17.0.1 software (SPSS Inc.; Chicago, IL, USA) was used for statistical analyses. Intra- and inter-examiner validations were measured. To assess intra-observer reliability, the Wilcoxon matched-pairs signed rank test was used for repeated measurements. The inter-observer reliability was determined by the intraclass correlation coefficient (ICC) and the coefficient of variation (CV) [CV=(standard deviation/mean)x100%]. Values for the ICC range from 0 to 1. ICC values greater than 0.75 showed good reliability, and the low CV demonstrated the precision error as an indicator for reproducibility (19). Differences in side, dentin thickness and pulp volumes were evaluated using chi-square and paired t-tests. Confidence interval was set to 95% and p-values less than 0.05 were considered as statistically significant.

Results

Intra-observer consistency

Repeated CBCT evaluation and measurements indicated no significant intra-observer difference for both observers. Overall intra-observer consistency for observer 1 was rated at 92.2% and 96.4%, while the consistency for observer 2 was found 91.8% and 94.4% between the two evaluations and measurements, respectively. All measurements were found to be highly reproducible for both observers and no significant difference was obtained from two measurements of the observers.

Inter-observer consistency

The ICCs between Observer 1 and Observer 2 ranged from 0.940 to 0.992. There was a high inter-observer agreement, while a high ICC and low CV demonstrated that the procedure was standardized between the evaluations and mea-

Table 1. *Results of statistical analyses stratified by study variables*

Study Variables	Group							Mann Whitney U Test	
		n	Mean	Median	Min	Max	SD	Mean Rank	z	p
Pulp Volume (mm^3)	Right	10	32.94	32.91	27.84	38.65	3.19	10		
	Left	11	33.71	34.48	28.78	37.5	2.82	11.91	-0.704	0.481
	Total	21	33.35	33.48	27.84	38.65	2.95			
Crown Volume (mm^3)	Right	10	130.9	130.5	124	138	4.7	9.05		
	Left	11	133.82	132	128	141	4.4	12.77	-1.381	0.167
	Total	21	132.43	132	124	141	4.68			
Crown/Pulp (Ratio)	Right	10	3.64	3.95	0	4.6	1.31	11.2		
	Left	11	3.99	3.83	3.62	4.45	0.32	10.82	-0.141	0.888
	Total	21	3.82	3.92	0	4.6	0.93			
Mesial Canal Orifice length (x) (mm)	Right	10	1.14	1.13	1	1.29	0.08	9.6		
	Left	11	1.16	1.15	1.08	1.29	0.06	12.27	-0.992	0.321
	Total	21	1.15	1.15	1	1.29	0.07			
Mesial Canal Orifice Width (y) (mm)	Right	10	0.74	0.75	0.6	0.9	0.09	13.45		
	Left	11	0.66	0.66	0.5	0.82	0.09	8.77	-1.73	0.084
	Total	21	0.7	0.7	0.5	0.9	0.1			
Distal Canal Orifice Length (x) (mm)	Right	10	1.49	1.45	1.1	1.8	0.21	10.55		
	Left	11	1.51	1.52	1.2	1.8	0.2	11.41	-0.318	0.751
	Total	21	1.5	1.5	1.1	1.8	0.2			
Distal Canal Orifice Width (y) (mm)	Right	10	0.71	0.69	0.54	1.1	0.16	11.3		
	Left	11	0.67	0.68	0.5	0.8	0.1	10.73	-0.213	0.832
	Total	21	0.69	0.68	0.5	1.1	0.13			
Palatinal Canal Orifice Length (x) (mm)	Right	10	1.7	1.7	1.52	1.8	0.08	5.9		
	Left	11	1.87	1.9	1.79	1.9	0.05	15.64	-3.672	0.120
	Total	21	1.79	1.8	1.52	1.9	0.11			
Palatinal Canal Orifice Width (y) (mm)	Right	10	1.16	1.12	1	1.6	0.17	11.1		
	Left	11	1.13	1.1	0.98	1.2	0.07	10.91	-0.073	0.942
	Total	21	1.15	1.12	0.98	1.6	0.13			
Closest distance from pulp chamber to mesial enamel surface (mm)	Right	10	2.03	2	1.8	2.4	0.18	12.65		
	Left	11	1.91	1.92	1.5	2.2	0.19	9.5	-1.167	0.243
	Total	21	1.97	1.96	1.5	2.4	0.19			
Closest distance from pulp chamber to distal enamel surface (mm)	Right	10	1.91	1.94	1.72	2.2	0.15	11.65		
	Left	11	1.85	1.86	1.4	2	0.17	10.41	-0.464	0.643
	Total	21	1.88	1.9	1.4	2.2	0.16			
Maximum Pulp Chamber length (mm)	Right	10	5.09	5.1	4.8	5.4	0.21	13.45		
	Left	11	4.74	4.6	4.2	5.6	0.49	8.77	-1.731	0.083
	Total	21	4.91	5	4.2	5.6	0.42			

Table 1. *Results of statistical analyses stratified by study variables (continued)*

Study Variables		Group						Mann Whitney U Test	
		n	Mean	Median	Min	Max	SD	Mean Rank	z	p
Maximum Pulp Chamber width (mm)	Right	10	2.94	3.05	2.5	3.6	0.37	8.5		
	Left	11	3.33	3.1	2.7	4.1	0.48	13.27	-1.777	0.076
	Total	21	3.14	3.1	2.5	4.1	0.47			
Closest distance of mesio-buccal Pulp horn to enamel surface (mm)	Right	10	2.5	2.51	2.1	2.9	0.2	14.4		
	Left	11	2.29	2.29	1.9	2.56	0.17	7.91	-2.396	0.017
	Total	21	2.39	2.4	1.9	2.9	0.21			
Closest distance of disto-buccal Pulp horn to enamel surface (mm)	Right	10	3.62	3.61	3.52	3.78	0.08	15.8		
	Left	11	3.41	3.41	3.24	3.58	0.12	6.64	-3.387	0.001
	Total	21	3.51	3.54	3.24	3.78	0.14			
Closest distance of palatal Pulp horn to enamel surface (mm)	Right	10	3.46	3.54	3.50	3.68	0.06	14.2		
	Left	11	3.38	3.34	3.26	3.48	0.10	7.82	-2.986	0.001
	Total	21	3.42	3.44	3.34	3.52	0.08			

surements of the observers. No statistical differences were found among observer's evaluations and measurements. The means of all observer's evaluations and measurements were therefore calculated for further analysis.

Repeated measurements of images showed no significant intra-observer difference. Intra-observer consistency was 92.2% between two examinations, 96.4% between measurements.

Observation of the pulp chamber showed a clear morphological image of the mesiobuccal, distobuccal, mesiopalatinal and distopalatinal pulp horns. Moreover, , a pulp horn corresponding to Carabelli's cusp was seen under the mesiopalatinal cusp (Figure 2). The mesiobuccal pulp horn projected the most, followed by the distobuccal, the palatinal pulp horns (Table 1) (Figure 2, 4). The pulp horn of the mesiobuccal and distobuccal cusp showed protrusion to the crown.

Table 1 shows the results of the evaluations and measurements. There were no statistical significant difference between right and left maxillary teeth canal orifices' length and width. There were also no significant difference in terms of closest distance from pulp chamber to mesial enamel surface (mm) and closest distance from pulp chamber to distal enamel surface (mm) of the maxillary molar teeth.

However, significant difference was found in terms of closest distance of mesio-buccal pulp horn to enamel surface (mm). Mean distance was 2.5±0.20 mm for right; and 2.29±0.17 mm for left teeth ($p<0.05$). Similarly, closest distance of disto-buccal pulp horn to enamel surface (mm) was also significant between left and right teeth ($p<0.05$).

Table 1 also shows the crown, pulp volumes and crown/pulp ratio. There was no statistical significance between pulp volume ratios of pulp chamber and gender. This results indicated that the volume ratio of the pulp chamber to the total crown was approximately same for both sides.

Discussion

Knowledge of the structures of teeth and their relationships to each other contributes to the success of treatment, especially when treating dental caries. Before tooth preparation localization of the caries usually diagnosed radiographically but a 2-dimensional image may not always be accurate. Generally, conventional clinical radiography is used to examine the pulpal anatomy; however, this method only produces a 2D record rather than providing more realistic 3D information (20).

When considering restorative procedures on vital teeth; 3D information of the internal structures is crucial not only for having a proper seal of the remaining tissue under the restoration, but also affect the type of the restoration that will be applied to the teeth (21). The findings of this study suggested that right and left maxillary first molars can differ in terms of internal anatomical appearance. Hence, during tooth preparation in maxillary first molars, care must be taken before any restoration procedure. Moreover, based on the limited findings of the study for this particular population; mesio-buccal pulp horn in maxillary molars are more prominent than the other pulp horns, thus they are more likely to get exposed during tooth preparation.

Different restorative materials require varied thickness to provide resistance of the restorations. Conventional preparations require specific wall forms, depths, and marginal forms because of the properties of the restorative (22). Adequate thickness for amalgam restorations is 1.5–2 mm in occlusal surface and 0.75 mm in axial areas (23), depending on the region, cast metal restorations requires 1 to 2 mm and ceramics 2 mm thickness (24). In addition, these restorative materials require conventional tooth preparation. The use of adhesive restorations, primarily composites and glass ionomers, has allowed a reduced degree of precision of tooth preparations (25). Although some author suggest that composite materials

dimensional needs depends on the occlusal wear potential of the restored area, it is generally accepted that, in areas of occlusal loading, minimal thickness of resin composite restorations should be 1.5 to 2 mm (26).

The management of dental caries has evolved from G.V. Black's "extension for prevention" to "minimally invasive", because of advance in adhesive dentistry and restorative materials. Therefore, clinicians should prefer minimal tooth preparation with modified cavity designs and use adhesive dental materials (27). In the present study, it can be suggested that especially in maxillary first molars, adhesive restoration techniques should be preferred to conventional restorations. On the other hand, even when adhesive restoration techniques are used, minimal dentin thickness must be around 0.5 mm with capping or 2 mm without capping (28-30).

Over preparation may perforate pulp chamber or can cause reversible or irreversible pulpitis. It must be kept in mind that capping procedures may be necessary when remaining dentin thickness gets reduced. Hence, future studies must be done regarding modified techniques with newly manufactured materials.

Clinical knowledge on the anatomy of crown-root pulp structure is the key for successful endodontic treatment. Due to the complex root canal system of maxillary first molars, various errors could occur during access cavity preparation or when locating the canal orifices. Having three dimensional information on dental anatomy would definitely help clinicians in the preparation of access cavity. Also, 3-D measurements of pulp chamber, canal anatomy, root orifices will enhance their success in root canal treatment (1).

Conventional methods used for morphological studies on internal anatomy of teeth are destructive *in vitro* methods that generally result in irreversible changes to the specimen (15). As a non-destructive analysis technique, Micro-CT imaging provides objective data. Specimens can be evaluated both quantitatively and qualitatively. In addition, the volumes can be calculated and it is possible to pinpoint specific details with visual image analysis. Filling materials, voids, and tooth structures can be distinguished with high accuracy and spatial resolution (31). With proper lighting, color and texture use during rendering of the image, micro-CT is able to provide a better understanding of internal anatomy of the teeth that could be examined from different angles (14). For the reference, the internal crown anatomy of the teeth investigations especially with and without different restoration materials must be performed using this device.

The study had several limitations. Most importantly, its sample size can be considered low, even though the power analysis indicated that at least 20 teeth should be included in the study for the detection of differences. Micro-CT analysis is an expensive technique; therefore, we had to limit our sample size. The second limitation was the possible effects of sexual dimorphism which were not taken into account due to small sample size. Further studies with larger samples should focus on possible age-, gender- and population-related differences. Also, volumetric and morphologic changes of pulp chamber related to age, chronic caries lesions, and formation of tertiary or reparative dentin, calcifications and other factors were not considered in the present study, which acts as the third limitation factor.

Conclusion

Within the limits of this *in vitro* study, it can be suggested that right and left maxillary first molars should be treated differently during preparation of cavities. Further studies must be done with larger samples, as well as on other molar teeth, in different populations to reveal the morphology of the molars for considerations in restorative dentistry. Development of non-destructive analysis techniques such as micro-CT is of utmost importance to provide clinicians with accurate three dimensional information.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ankara University Faculty of Dentistry (02.02.2017, 36290600/07).

Informed Consent: Written and verbal informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: İHB and KO designed the study. GD and MEK generated and gathered the data. İHB and KO analyzed the data and wrote the majority of the original draft. GD and MEK participated in writing the paper. All authors approved the final version of the paper.

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

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Türkçe öz: *Üst birinci büyük azı dişlerinin morfolojisinin üç boyutlu mikro-BT görüntülemesi ile in-vitro analizi: restoratif diş hekimliği için değerlendirmeler. Amaç: Bu çalışmanın amacı üst sağ ve sol birinci büyük azı dişlerinin kron konturu ve pulpa odası arasındaki konumsal ilişkinin farklılıklarını ve morfolojik özelliklerini mikro-bilgisayarlı tomografi (BT) sistemi ve hacimsel yeniden yapılandırma programı kullanarak belirlemektir. Gereç ve Yöntem: Bu çalışmada toplam 21 üst büyük azı dişi (11 sol, 10 sağ) kullanılmıştır. Dişlerin; kron konturu, pulpa odası ve morfolojileri arasındaki konumsal ilişki mikro-BT görüntüleme yöntemi ile üç boyutlu olarak incelenmiştir. Bulgular: Dişlerde mezio-bukkal pulpa boynuzu ile mine yüzeyi arasındaki en yakın mesafe sağ bölge için 2,5±0,20 mm, sol bölge için 2,29±0,17 mm olarak ölçülmüş ve bu farklılığın istatistiksel olarak anlamlı olduğu belirlenmiştir (p=0,017). Disto-bukkal pulpa boynuzu ile mine yüzeyi arasındaki en yakın mesafede de anlamlı farklılık olduğu bulunmuştur (p=0,001). Sağ bölgeden alınan dişlerin ortalama pulpa hacmi (32,94±3,19 mm3) ile sol taraftan alınanların (33,71±2,82 mm3) ortalama pulpa hacimleri arasında anlamı bir fark bulunmamıştır. Sonuç: Bu çalışmanın bulguları kavite preparasyonu sırasında sağ ve sol dişlerdeki farklılıklara dikkat edilmesi gerektiğini göstermektedir. Diğer azı dişlerini de içeren, farklı toplumları inceleyen ve daha geniş örneklem hacmine sahip olan çalışmaların yapılması azı dişlerinin morfolojik özelliklerini ortaya çıkartarak restoratif tedavi alanına katkıda bulunacaktır. Anahtar kelimeler: Mikro BT, üst büyük azı dişleri; anatomi; pulpa boyutları; restoratif diş hekimliği*

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Original research

Effect of dentin pretreatment on shear bond strength of three resin-based luting cements

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Purpose
The aims of this study were; to compare the shear bond strength values of resin-based luting cements using etch-and-rinse, self-etching or self-adhesive techniques and to evaluate the effects of pretreatment with 0.2%, and 2% chlorhexidine (CHX) solutions on the bonding stability to dentin.

Materials and methods
Ninety specimens were divided into 9 groups of equal sample size (0.2% CHX, 2% CHX and no CHX application groups). Variolink N (multi-step etch-and-rinse technique), Panavia F2.0 (self-etching technique), or RelyX U200 (self-adhesive technique) resin-based luting cement was applied. All specimens were subjected to shear bond strength test (SBS) after bonding procedure. The mode of failure was analyzed by using a stereomicroscope.

Results
There were no significant differences among study groups for the dentin treatment factor. However, for luting cement factor, significant differences were found and Variolink N showed the highest SBS values.

Conclusion
CHX application has no immediate effect on the SBS values of any resin cements. Despite the development of simplified cementation techniques, etch-and-rinse technique is still the most reliable technique because of its high bond strength to dentin.

Keywords: *RelyX U200; Panavia-F 2.0; Variolink; chlorhexidine; shear strength*

Introduction

Chlorhexidine digluconate (CHX) is one of the most commonly used antimicrobial agent in dentistry and is commercially available as mouth-wash, irrigation solution, gel, spray, and aerosol formulations (1). Previous studies (2-6) have shown that CHX demonstrates anti-microbial activity, substantivity, biocompatibility and it also inhibits proteolytic enzymes referred to as metalloproteinases (MMPs) and cathepsins (CTs). These enzymes are responsible for the degradation of bonding interface and can compromise the longevity of the luting cement (7-9). Degradation retarding effects of CHX on the bonding interface have been previously examined by different authors. Kul *et al.* (5) reported the efficacy of different irrigation solutions on the bond strength of a fiber post attached with a self-adhesive resin cement and authors found no difference among the CHX, phosphoric acid, and distilled water groups. In addition, CHX activity on the bonding process of root dentin on different luting agents has been widely examined. De Araújo *et al.* (7) investigated the influence of 2% CHX solution on the bond strength of glass fiber posts to root dentin

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Table 1. *Commercial brands, compositions and manufacturers of luting cements and chlorhexidine digluconate (CHX) solutions used in this study*

Material	Composition	Manufacturer
Panavia F 2.0	ED Primer II: liquid A: 10- methacryloloxydecyl dihydrogenphosphate, 2-hydroxyethyl methacrylate, N,N-diethanol-p-toluidine, N-methacryloyl 5-aminosalicylic acid, water; liquid B: N,N-diethanol-p-toluidine; sodium benzen sulphinate, N-methacryloyl 5-amino-salicylic acid, water. Panavia F: paste A: silanated barium glass, colloidal silica, bisphenol A polyethoxy dimethacrylate, 10- methacryloloxydecyl dihydrogenphosphate, hydrophilic dimethacrylate, hydrophobic dimethacrylate, benzoil peroxide, dl- camphoroquinone; paste B: silanated barium glass, silanated titanium oxide, sodium fluoride colloidal silica, bisphenol A polyethoxy dimethacrylate, hydrophilic dimethacrylate, hydrophobic dimethacrylate, N,N-diethanol-p-toluidine, sodium	Kuraray, Osaka, Japan
Variolink N	Monomer matrix: bis-GMA, urethane dimethacrylate, triethylene glycol dimethacrylate; inorganic fillers: barium glass, ytterbium trifluoride, Ba-Al-fluorosilicate glass, spheroid mixed oxide, initiators, stabilizers, pigments. Syntac primer: triethyleneglycol methacrylate, polyethyleneglycol dimethacrylate, maleic acid, ketone; syntac adhesive: polyethyleneglycol dimethacrylate, glutaraldehyde. Heliobond: bis-GMA, triethyleneglycol dimethatcrylate, stabilizers, initiators.	Ivoclar Vivadent, Schaan, Liechtenstein
RelyX U200	Base paste: glass powder treated with silane, 2-propenoic acid, 2-methyl 1,1′-(1-[hydroxymetil]-1,2-ethanodlyl) ester dimethacrylate, triethylene glycol dimethacrylate (TEGDMA), silica treated silane, glass fiber, sodium persulfate, per-3,5,5-trimethyl hexanoate t-butyl. Catalyst paste: glass powder treated with silane, substitute dimethacrylate, silica-treated silane, sodium p-toluenesulfonate, 1-benzyl-5-phenyl-acid barium, calcium, 1,12-dodecane dimethacrylate, calcium hydroxide, titanium dioxide.	3M ESPE, Seefeld, Germany
Klorhex (0.2% CHX)	0.2% Chlorhexidine digluconate	Drogsan Pharmaceuticals, Ankara, Turkey
Cavity Cleanser (2% CHX)	2% Chlorhexidine digluconate	Bisco Inc., Schaumburg, IL, USA

using two luting cements. Authors reported that CHX did not improve the bond strength of any luting cement.

Post and core restorations are mainly luted to the root dentin with adhesive resin-based luting agents to provide a reliable bonding to the tooth structure. Furthermore, resin-based luting cements have popularized all-ceramic systems because of their bond strength and for increasing the fracture resistance properties of ceramics (10). Various studies investigated CHX activity on bond strength of resin-based luting cements to root dentin. However, the influence of CHX on the bond strength between self-adhesive luting cements and dentin has not been clearly examined.

The aims of this study were twofold. First, to investigate the influence of different concentrations of CHX on the bond strength of luting cements to prepared teeth using three resin-based luting cements, two different dual-cured composite resin cements and a self-adhesive dual-cured resin cement, and second, to compare the shear bond strength of these luting cements to dentin specimens. The null hypothesis tested in this study were; there are no differences in bond strength according to luting cement and the use of different concentrations of CHX has no effect on the luting cements' shear bond strength.

Materials and methods

This *in vitro* study involved the analysis of two main factors: type of resin-based luting cement (three types); and dentin pre-treatment using different CHX concentrations (0.2% and 2%) (Table 1).

Specimen preparation

Ninety mandibular third molar teeth (N=90) were collected, cleaned and stored in 0.5% Chloramine-T (9.0 g sodium chloride and 5.0 g chloramine–trihydrate dissolved in 1000 mL distilled water) solution to prevent dehydration at 4°C for a maximum of 1 month until use. Inclusion criteria were the absence of caries and/or restorations. After cleaning and drying, teeth were embedded in autopolymerizing acrylic resin (Simplex Rapid, KemDent, Wiltshire, UK). Specimens were wet-ground flat with silicon carbide abrasive papers ending with 600 grit (11) to obtain flat dentin surface at 1 to 2 mm distance from the pulp, which was measured by using a digital caliper. For each type of resin-based cement and concentration of CHX, the specimens were randomly divided into nine groups of ten specimens each (n=10) (0.2% CHX, 2% CHX and no CHX groups for each of the three resin-based luting cements) (Table 2).

Ninety disk shaped specimens, 4 mm in diameter and 2 mm in height, were produced with IPS e.max Press lithium disilicate glass ceramic system (IPSe.maxPress, Ivoclar Vivadent, Schaan, Liechtenstein). IPS e.max Press ingots (MO1 shade, IPSe.maxPress, Ivoclar Vivadent, Schaan, Liechtenstein) were heat pressed with the lost wax/heat pressed technique according to the manufacturer's instructions. Ceramic disks were then allowed to bench cool at room temperature and divested by 50-μm Al_2O_3 at 0.2 MPa pressure from a distance of 10 mm. Ceramic disks were ultrasonically cleaned (Invex-Liquid, IvoclarVivadent, Schaan, Liechtenstein) for 10 minutes to remove the reaction layer and polished with 600 grit silicon carbide paper under water-cooling to adjust final thickness

Table 2. *Group descriptions stratified by dentin pre-treatment solutions and luting cements*

Luting cement	Dentin pre-treatment with CHX	Group description
Panavia F 2.0	0.2% CHX	GR1
	2% CHX	GR2
	-	GR3
Variolink N	0.2% CHX	GR4
	2% CHX	GR5
	-	GR6
RelyX U200	0.2% CHX	GR7
	2% CHX	GR8
	-	GR9

CHX: chlorhexidine digluconate

Figure 1. *All specimens prepared and embedded in autopolymerizing acrylic resin.*

Figure 2. *All specimens were stabilized and shear bond strength tests were performed in an universal testinh machine.*

and to standardize surface roughness. All ceramic disk surfaces were acid-etched for 20 seconds with hydrofluoric acid (HF) in less than 5% concentration (IPS Ceramic Kit Etching Gel, Ivoclar Vivadent, Schaan, Lichtenstein), rinsed thoroughly under running water for 60 seconds and dried for pre-cementation surface treatment (Figure 1).

Bonding procedure

Three resin-based luting cements (Panavia F2.0/ Kuraray, Osaka, Japan; Variolink N/ Ivoclar Vivadent, Schaan, Liechtenstein; RelyX U200/ 3M ESPE, Seefeld, Germany) were used in this study. All ceramic disks received pre-treatment in the bonding areas, according to the luting cement, study group, and manufacturer's instructions (Table 3). After pre-treatment, bonding areas were isolated by adhesive tape with a 4 mm in diameter circular hole to prevent excess flash adhering to the specimens. Finger pressure was used for cementing all specimens with an approximate thickness of 5 µm. Dentin-cement-ceramic specimens were left in the air for polymerization of the cement according to the manufacturer's instructions. Before shear bond strength test (SBS), all specimens were stored in distilled water for 24 hours at 37°C.

Shear bond strength (SBS) test

All specimens were mounted to a universal testing machine (Autograph AG-IS Series, Shimadzu, Japan) and SBS tests were performed at a crosshead speed of 1mm per minute until fracture occurred. Bond strength was recorded in Newtons (N) and converted into Megapascals (MPa). Average shear bond strength (MPa) was calculated by dividing the load (N) at which failure occurred by the bonding area (mm^2) (Figure 2).

Fracture types

Bond failure sites and fracture analysis on all specimens were performed visually with a stereomicroscope (OlympusSZ61, Olympus Optical Co., Tokyo, Japan) at 40x magnification. Fractures were classified into one of the three categories as: adhesive failure (if the complete fracture was seen at the luting-dentin interface) or cohesive failure (if the cohesive fracture was seen in the luting cement) or mixed failure (if the adhesive fracture was seen at the resin-based luting cement-dentin interface combined with cohesive fracture in the luting material).

Ethics committee approval and informed consent were not considered to be necessary.

Statistical analysis

Statistical Package for Social Sciences (SPSS) software version 15.0 (SPSS Inc.; Chicago, IL, USA) was used for statistical analysis. The Kolmogorov–Smirnov test was used to determine whether the distribution characteristics of the data meet the requirements of normality assumptions. Levene's test was employed to check the homogeneity of variances. As the data is normally distributed and the variances are homogenous, two-way analysis of variance (ANOVA) and post-hoc Tukey's Honestly Significant Difference (HSD) tests were used for multiple and pairwise comparisons, respectively. Confidence interval was set to 95% and p values less than 0.05 were considered as statistically significant.

Table 3. *Details of surface treatments and luting protocols used in this study*

Luting cement	Dentin pre-treatment	Ceramic surface pre-treatment	Protocol
Panavia F 2.0	0.2% CHX was applied with microbrush for 1 min and dried with absorbent paper; ED primer II: drop each of liquid A and liquid B mixed, 30 s, dried with gentle air flow.(GR1) 2% CHX solution was applied by lightly scrubbing with a microbrush for 5 s and surface was rinsed with distilled water for 2 s; ED primer II was applied according to previous description.(GR2)	Acid-etched for 20 s with <5% HF acid (IPS Ceramic Kit Etching Gel, Ivoclar Vivadent, Schaan, Lichtenstein), rinsed with water for 60 s and dried. CCP (Kuraray, Osaka, Japan) was applied and left to dry for 5 min.ED primer II was applied according to previous description.(GR3)	Mixed paste A + B (1:1) for 20 s. Oxyguard II (Kuraray, Osaka, Japan) was applied and light cured for 20 s. Oxyguard II was removed after 3 min.
Variolink N	Total etch for 15 s, rinsed with water, gently air-dried; 0.2% CHX was applied according to previous description; Syntac primer for 15 s, Syntac adhesive for 10 s; Heliobond blown to a thin layer, light cured for 20 s.(GR4) Total etch for 15 s, rinsed with water, gently air-dried; 2% CHX solution was applied according to previous description; Syntac primer for 15 s, Syntac adhesive for 10 s; Heliobond blown to a thin layer, light cured for 20 s.(GR5) Total etch for 15 s, rinsed with water, gently air-dried; Syntac primer for 15 s, Syntac adhesive for 10 s; Heliobond blown to a thin layer, light cured for 20 s.(GR6)	Acid-etched for 20 s with <5% HF acid (IPS Ceramic Kit Etching Gel, Ivoclar Vivadent, Schaan, Lichtenstein), rinsed with water for 60 s and dried. Monobond S (Ivoclar Vivadent, Schaan, Liechtenstein) was applied for 60 s and dried with air.	Transparent base paste and high viscosity transparent catalyst paste was mixed (1:1). Liquid strip (Ivoclar Vivadent, Schaan, Liechtenstein) was applied and light cured for 40 s.
RelyX U200	0.2% CHX was applied according to previous description.(GR7) 2% CHX solution was applied according to previous description. (GR8) No dentin pre-treatment.(GR9)	Acid-etched for 20 s with <5% HF acid (IPS Ceramic Kit Etching Gel, Ivoclar Vivadent, Schaan, Lichtenstein), rinsed with water for 60 s and dried. RelyX Ceramic Primer (3M/Espe, St. Paul, MN, USA) was applied for 5 s and dried with air.	Base and catalyst paste was applied through an automix syringe and light cured for 40 s.

CHX: chlorhexidine digluconate

Results

Shear bond strength to dentin

The results of the two-way ANOVA of SBS data are presented in Table 4. Results of two-way ANOVA indicated that the type of luting cement affected the bond strength values ($p<0.05$). Tukey's HSD test (Table 5) showed that there were significant differences between Variolink N and the other luting cement groups. Variolink N exhibited significantly higher SBS value than Panavia F2.0 ($p=0.021$) and RelyX U200 ($p=0.031$). There was no significant difference between the SBS values of Panavia F2.0 and RelyX U200. As presented in Table 5, dentin pre-treatment with any of the two CHX solutions demonstrated no significant difference in the SBS values.

Fracture types

Figure 3 presents the distribution of the various fracture types in percentage. Majority of the fractures occurred during the SBS tests were adhesive type failures (56.67%). Adhesive failures were associated with the lowest SBS values while cohesive failures were associated with greater SBS values. 40% cohesive and 70% adhesive failure rates were found in GR6 GR2 groups, respectively.

Table 4. *Two-way analysis of variance test results for luting cement and dentin pre-treatment with chlorhexidine digluconate (CHX)*

Source	Sum of Squares	df	Mean squares	F	p
Luting cement	116.573	2	58.286	3.304	0.042
Dentin pre-treatment with CHX	13.805	2	6.903	0.391	0.677
Luting cement*dentin pre-treatment with CHX	22.883	4	5.721	0.324	0.861

Statistically significant p values are written in bold

Table 5. *Pairwise comparisons of the study groups*

Factor	Luting system or pre-treatment	p
Luting cement	Panavia F2.0 / Variolink N	0.021
	Panavia F2.0 / RelyX U200	0.873
	Variolink N / RelyX U200	0.031
Dentin pre-treatment with CHX	0.2% CHX / 2% CHX	0.966
	0.2% CHX / No dentin pre-treatment with CHX	0.82
	2% CHX / No dentin pre-treatment with CHX	0.672

CHX: chlorhexidine digluconate
Statistically significant p values are written in bold

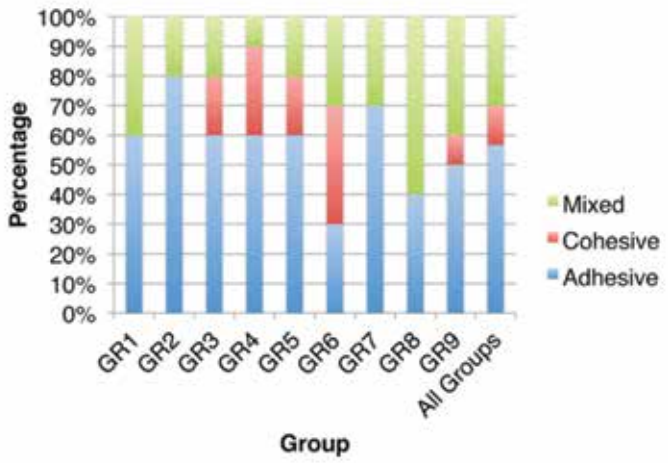

Figure 3. *Percentage distribution of the failure types in study groups.*

Discussion

This study investigated the shear bond strength of three resin-based luting cements to dentin with and without prior CHX application. Because there were statistically significant differences in bond strength according to luting cement in dentin, the first null hypothesis was rejected. In the present experimental settings, three resin-based luting cements with different dentin pre-treatment protocols in terms of the number of steps of adhesive application were used according to the manufacturer's instructions. Variolink N luting cement was used with multi-step etch-and-rinse technique including etching and rinsing, application of primer and bonding agent. Panavia F2.0 luting cement was used with self-etching technique in which application of self-etching primer and application of adhesive paste applied step by step. RelyX U200 luting cement system uses a self-adhesive application technique and no dentin pre-treatment is required according to the manufacturer's claim.

When compared to that of RelyX U200 Variolink N revealed significantly higher bond strength to dentin (p=0.031). This can be explained by the removal of the smear layer and dissolving of the mineral during the rinsing step of etch-and-rinse technique. According to the manufacturer, RelyX U200 luting cement consists of methacrylate monomers modified with phosphoric acid that can mineralize the dentin, and cement infiltrates the hybrid layer with resin tags, thus, no prior removal of the modified smear layer is needed. In contrast, it was reported that adhesive resin composites which contain phosphoric acid, have very low pH and these systems appear not to have a chemical affinity for bonding to the dentin (12, 13). In addition, this poor micromechanical infiltration for attachment to the dentin can be explained by inadequate demineralization on dentin layer and weak formation of the hybrid layer (14).

Variolink N also revealed significantly higher bond strength to dentin, compared to that of Panavia F2.0 (p=0.021), which is in accordance with the results of previous studies (15-18). This may be explained by, firstly, the high filler content and viscosity of the Panavia F2.0 luting cement, which may decrease infiltration depth of the adhesive into the primed dentin (18, 19). Secondly, water diffusion may occur from the dentin across the ED Primer during the slow polymerization in the dual cured mode and water droplets along the primer-cement interface may affect adhesive permeability (20), and finally, the residual acids of ED primer may impede the chemical curing of the luting cement (21).The results of the present study showed that RelyX U200 specimens' bond strength to dentin was not statistically different from those of Panavia F2.0. These results are in agreement with previous literature. Using the microtensile bond strength (μTBS) test of simplified resin-based luting cements, Bacchi *et al.* (22) observed no statistically significant difference and the self-etching primer along with a conventional dual-curing cement (ED Primer+Panavia F2.0) led to μTBS similar to that of the self-adhesive resin cement (RelyX U200).

The results of the present study showed that dentin pre-treatment with CHX did not significantly affect the bond strength to dentin in any group; therefore, the second null hypothesis was accepted. CHX is a non-specific MMP inhibitor and it has shown success in inhibiting both MMPs and cysteine cathepsin; thus preserving the integrity of the hybrid layer (23). To improve bond strength, CHX can be applied to dentin as a primer after phosphoric acid-etching for rehydration (24, 25), be incorporated in the acid etchant (26, 27), or with CHX-containing dental adhesives (28, 29). However, the effectiveness of using CHX as a primer after phosphoric acid application on bond strength in previous studies is controversial. The aforementioned studies reported that CHX used as a therapeutic primer on acid-etched dentin does not interfere with immediate bond durability and significantly higher bond strengths were observed after only 6 to 12 months (24, 25). A recent study controversially reported that the application of the 2% CHX as dentin pretreatment decreased the number of adhesive failures compared with untreated controls after 9 months of aging, however, this effect was not significant and was seen on the etch-and-rinse adhesive but not on the self-etching adhesive (30). In contrast, Ricci *et al.* (31) found that 2% CHX application significantly increased the μTBS values of adhesives to the acid-etched dentin and positive immediate efficacy on bond durability.

The bond strength test results of the present study confirmed that different concentrations of CHX used as a therapeutic primer in etch-and-rinse adhesive, primer self-etching adhesive and self-adhesive groups have no positive or negative immediate effect on bond strength, which is mostly in accordance with the literature. On the other hand, authors show, for the first time, that RelyX U200, a self-adhesive luting cement that consists of methacrylate monomers modified with phosphoric acid, produced similar improvement in SBS values when applied to CHX-treated dentin. Although no negative or positive effects of 0.2% or 2% CHX solutions were observed, further studies are needed to ascertain the time-dependent efficacy of CHX on dentin bond strength.

Conclusion

Within the limitations of this *in vitro* study, the etch-and-rinse technique shows highest bond strength to dentin and CHX is not effective on the bond strength of luting cements to dentin. The benefits of CHX application prior to bonding may still be observed after long-term clinical studies, encouraging further clinical investigations in the evaluation of bond strength over longer periods of time. Despite the development of simplified cementation techniques, etch-and-rinse technique seems to be the most reliable one because of high bond strength to dentin.

Ethics Committee Approval: Ethics committee approval was considered not to be necessary.

Informed Consent: There were no participated patients in this study and written informed consent was not obtained.

Peer-review: Externally peer-reviewed.

Author Contributions: NBB and GE designed the study and generated the data. NBB, GE and BGR gathered and analyzed the data. NBB, GE, BGR and TÇ wrote the majority of the original draft. All authors approved the final version of the paper.

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

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Türkçe öz: *Dentin yüzeyine uygulanan ön işlemlerin üç farklı rezin bazlı yapıştırma simanının makaslama bağlanma dayanım kuvvetlerinin üzerindeki etkileri. Amaç: Bu çalışmanın amaçları; "etch-and-rinse", "self-etch" ya da "self-adeziv" teknikleri kullanılarak, rezin bazlı yapıştırma simanlarının dentine olan makaslama bağlanma dayanım kuvvetlerinin karşılaştırılması ve dentin yüzeyine ön işlem olarak uygulanan %0.2 ve %2'lik klorheksidin solüsyonlarının simantasyon esnasındaki bağlanma stabilitesine olan etkisinin değerlendirilmesidir. Gereç ve Yöntem: 90 adet numune eşit örnek büyüklüğüne sahip 9 gruba ayrılmıştır. (%0.2 klorheksidin grupları, %2 klorheksidin grupları ve ön işlem görmeyen gruplar). Variolink N (çok aşamalı "etch-and-rinse" tekniği), Panavia F2.0 ("self-etch" tekniği), ya da RelyX U200 ("self-adeziv" tekniği) rezin bazlı yapıştırma simanları uygulanmıştır. Tüm örneklere yapıştırma aşamalarından sonra makaslama bağlanma dayanım testi (SBS) uygulanmıştır. Kırılma tipleri stereomikroskop kullanılarak analiz edilmiştir. Bulgular: Dentin yüzeyine işlemine göre değerlendirildiğinde, grupların kırılma dirençleri arasında anlamlı bir fark bulunamamıştır. Kullanılan simana göre değerlendirildiğinde, gruplar arası istatistiksel olarak anlamlı farklılıklar bulunmuştur ve Variolink N en yüksek kırılma değerlerini vermiştir. Sonuç: Dentin yüzeyine uygulanan klorheksidin ajanının kırılma değerlerine erken dönem etkisi bulunmamaktadır. Bununla birlikte "etch-and-rinse" tekniği, güncel teknolojiler ile geliştirilmiş olan basitleştirilmiş simantasyon tekniklerine göre, dentine yüksek bağlanma kapasitesi sebebiyle en güvenilir yöntemdir. Anahtar kelimeler: RelyX U200; Panavia-F 2.0; Variolink; klorheksidin; makaslama kuvveti*

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Original research

Evaluation of deformation and fracture rates for nickel-titanium rotary instruments according to the frequency of clinical use

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Purpose
To evaluate the deformation and fracture rates for ProTaper Universal (PTU) nickel-titanium rotary instruments according to the frequency of clinical use.

Materials and Methods
A total of 619 PTU instruments (S1, S2, F1, F2, and F3) that have been used in the clinic by a single endodontist were collected over a period of 4 years. These instruments were grouped on the basis of one to three (Group A), four to six (Group B) and seven to nine (Group C) clinical uses (one canal = one use). All instruments were evaluated by a blinded investigator under a stereomicroscope at 15×–45× magnification for the presence of deformation and fracture.

Results
The overall rates of deformation and fracture were 10% and 1.2%, respectively. The deformation and fracture rates for the S2, F1, and F2 instruments showed no significant differences among groups. However, fracture rate for S1 instruments in Group A was significantly higher than for those in Group B (p=0.025) and Group C (p=0.004). In Group C, the S1 instruments showed a significantly higher deformation rate compared with the S2 (p=0.04), F1 (p=0.008) and F2 (p=0.049) instruments; there were no other significant differences within groups.

Conclusion
Under the conditions of the current study, frequency of use seemed to influence the deformation rates of PTU rotary instruments. Except S1, these instruments could be used without any fracture or deformation in up to 9 clinical cases by an experienced endodontist.

Keywords: *NiTi rotary system; ProTaper Universal; dental instruments; deformation; fracture*

Introduction

Nickel-titanium (NiTi) rotary instruments have gained increasing popularity since their first introduction and are now widely used in endodontic practice. Currently, a variety of NiTi rotary systems marketed by various manufacturers are available. However, despite improvements in their cutting efficiency and flexibility, the possibility of unexpected separation during use remains a major concern (1, 2). Separation can be caused by torsional failure or flexural fatigue (3); the former is generally accompanied by macroscopic distortion or unwinding of the flutes, whereas the latter often presents unexpectedly with no unwinding defects (4).

It is widely accepted that NiTi rotary instruments fail with incorrect or excessive use (5). Furthermore, there is a perception among clinicians and researchers that the frequency of use may be an important factor affecting instrument failure (6). Most manufacturers advocate that their NiTi rotary files should be used only once to minimize the frequency of fracture. Others recommend that the instrument should be regularly discarded after a certain number of uses, generally one to 10, to prevent fracture (7-11).

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However, there is no consensus regarding the optimal number of uses.

The ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) system is originally comprised six instruments, including three shaping files (SX-optional, S1, and S2) and three finishing files (F1, F2, and F3). Subsequently, F4 and F5 finishing files for wider canals were introduced. These files have a convex triangular cross-sectional shape with sharp cutting edges and no radial lands. A unique design element is the presence of varying tapers over the length of the shaft of each instrument. The taper of the three shaping files increases coronally, while that of the finishing files increases apically (12).

The aim of this retrospective study was to evaluate the deformation and fracture rates for ProTaper Universal NiTi rotary instruments with regard to the frequency of clinical use.

Materials and methods

A total of 619 ProTaper Universal NiTi rotary instruments (S1, S2, F1, F2, and F3) were included in this retrospective study. These instruments were collected after clinical use by a single endodontist at the Department of Endodontics, Faculty of Dentistry, İstanbul University over a period of 4 years (2007–2010). The risk of instrument fracture during treatment was explained to all patients, and written informed consent was obtained from each patient as a routine clinical procedure.

Instrumentation technique

After appropriate access cavity preparation, root canals were cleaned and shaped using the ProTaper Universal rotary instruments attached to an air-driven Endo NiTi WD 73M handpiece (W&H Dentalwerk, Bürmoos, Austria) at 150–200 rpm by an endodontist with nearly seven years of experience with this system. The operative sequence is described below.

i. Number 10 and 15 K-type hand files (Dentsply Maillefer) were pre-curved and passively inserted into the coronal two-thirds of the root canal as pathfinding files.
i. The coronal two-thirds were prepared using ProTaper Universal S1 and S2 files with a brushing motion.
ii. Hand files were then used to secure a glide path and determine the working length with an electronic apex locator and/or radiograph.
iii. S1 and S2 files were used up to the full working length.
iv. The preparation was finished with F1, F2 or F3 files according to the complexity of the root canal anatomy. The finishing files were used with a non-brushing motion (12).
v. During preparation, only light apical pressure was applied to the rotary instruments.
vi. Glyde File Prep (Dentsply Maillefer) was used as a lubricant, and 2 ml of 2.5% sodium hypochlorite solution was used for irrigation after the use of each file.

All instruments were autoclaved under standardized conditions before use. Following instrumentation, they were ultrasonically cleaned and autoclaved under standardized conditions.

Collection of discarded instruments

After each use, the instruments were wiped with a piece of gauze soaked with isopropyl alcohol and examined with the naked eye for the presence of any defects. The instrument was discarded when it showed deformation and was classified into one of the groups based on the frequency of clinical use. In severely curved or calcified canals, a new set of ProTaper Universal rotary instruments was used and discarded even if they showed no deformation. The decision regarding the frequency of use was at the discretion of the endodontist. Other routinely used instruments were randomly discarded without any signs of deformation under the naked eye and grouped accordingly.

All collected instruments were classified into three groups on the basis of one to three (Group A; n=113), four to six (Group B; n=168) and seven to nine (Group C; n=338) clinical uses. Instrumentation of a single canal represented one clinical use. Accordingly, instrumentation of one tooth with three canals represented three clinical uses.

Examination of discarded instruments

All groups of instruments were evaluated for the presence of deformation and fracture under a stereomicroscope (Leica MZ75, Leica Imaging Systems Ltd, Cambridge, UK) at 15×–45× magnifications by another experienced pre-calibrated endodontist who was blinded to the number of clinical uses. All defects such as unwinding, curving or bending were simply defined as deformation, regardless of the magnitude (Figure 1).

During the stereomicroscopic evaluation, a new, unused, sterilized ProTaper Universal rotary instrument of the same type was placed beside the used instrument for easy and accurate assessment of deformation or fracture in the same screen (Figures 2, 3).

Statistical analysis

The data were analyzed using NCSS 2007 Statistical Software (NCSS, LLC, Kaysville, Utah, USA). Frequency values were used for the descriptive statistics. All data were statistically analyzed using Fisher's exact test. A p-value of <0.05 was considered statistically significant.

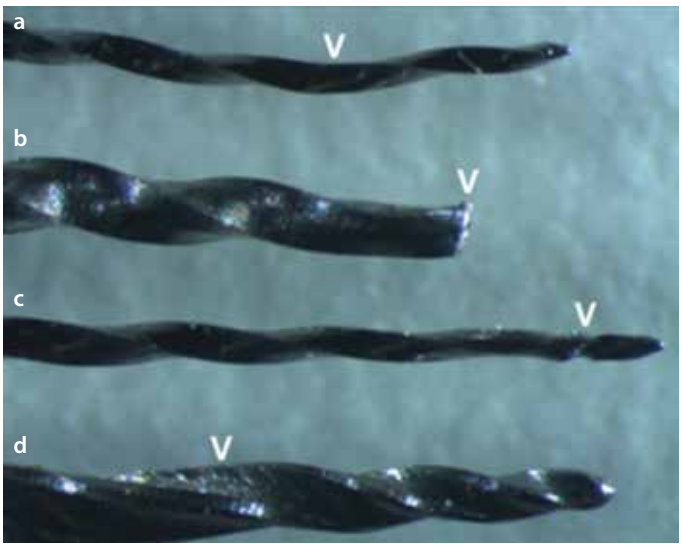

Figure 1. a-d. *Stereomicroscopic images of ProTaper Universal instruments. Deformation (a, c, d) and fracture (b) can be observed (arrowheads) under 24× magnification.*

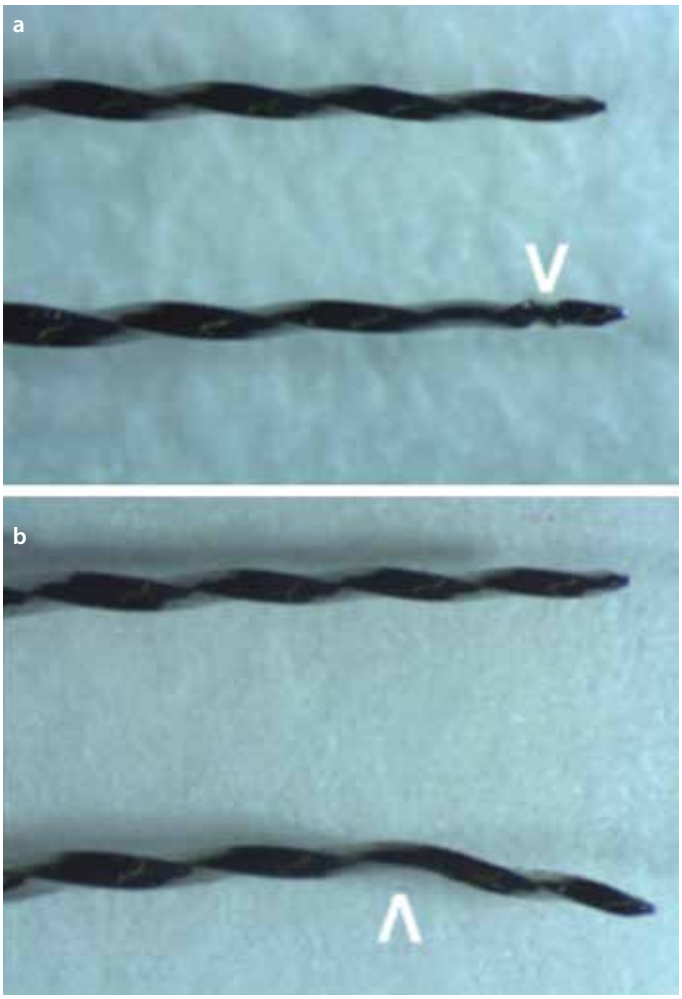

Figure 2. a, b. *Stereomicroscopic images of unused and used, sterilized ProTaper S1 instruments. Deformations can be observed (arrowheads) under 24× magnification.*

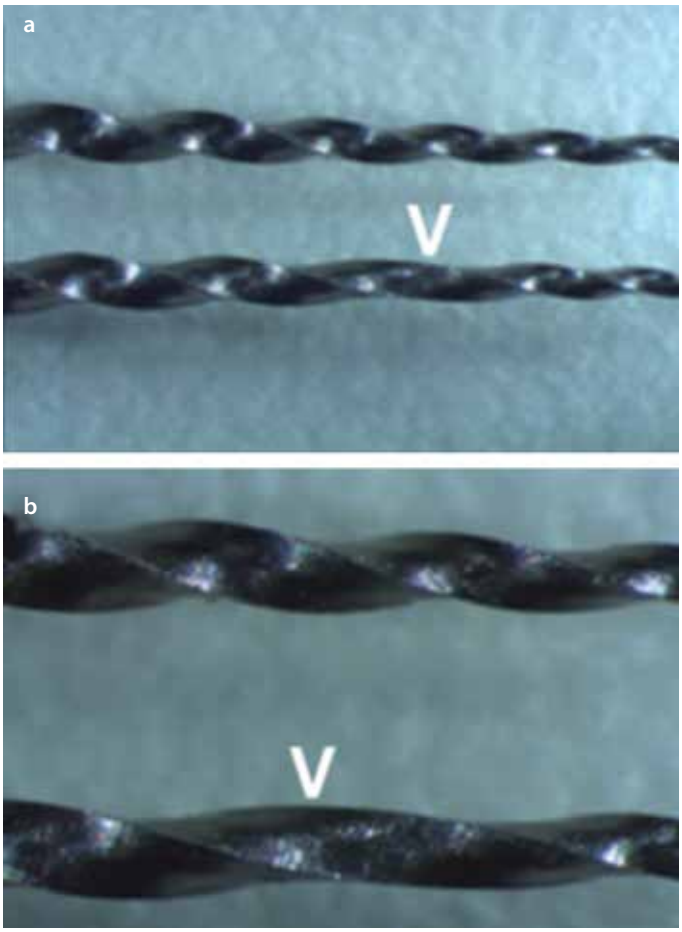

Figure 3. a, b. *Stereomicroscopic images of an unused and a used, sterilized ProTaper F1 instruments. (a) Slight deformation (arrowhead) of the used instrument is noted under 15× magnification, (b) Image of the same instrument under 30× magnification reveals considerable deformation (arrowhead).*

		Instruments				
Groups		**S1 n (%)**	**S2 n (%)**	**F1 n (%)**	**F2 n (%)**	**F3 n (%)**
Group A	No defect	26 (76.47)	29 (100)	25 (89.29)	14 (100)	7 (87.5)
	Deformation	4 (11.76)	0 (0.00)	2 (7.14)	0 (0.00)	1 (12.50)
	Fracture	4 (11.76)	0 (0.00)	1 (3.57)	0 (0.00)	0 (0.00)
Group B	No defect	43 (87.76)	44 (89.80)	51 (94.44)	15 (93.75)	0 (0.00)
	Deformation	6 (12.24)	5 (10.20)	2 (3.70)	1 (6.25)	0 (0.00)
	Fracture	0 (0.00)	0 (0.00)	1 (1.85)	0 (0.00)	0 (0.00)
Group C	No defect	79 (79.00)	88 (88.89)	99 (92.52)	29 (90.63)	0 (0.00)
	Deformation	21 (21.00)	10 (10.10)	8 (7.48)	2 (6.25)	0 (0.00)
	Fracture	0 (0.00)	1 (1.01)	0 (0.00)	1 (3.13)	0 (0.00)

Group A: 1–3 clinical uses (n=113), Group B: 4–6 clinical uses (n=168), Group C: 7–9 clinical uses (n=338)

Results

The overall rate of instrument deformation was 10%, while that of instrument fracture was 1.2% (Table 1).

The S2, F1, and F2 instruments showed no statistically significant differences with regard to the deformation and fracture rates among the three groups (p>0.05). However, the S1 instruments showed a significantly higher incidence of fracture when used one to three times than when used four to six (p=0.025) and seven to nine (p=0.004) times; the deformation rate was not significantly different (p>0.05).

When instruments were compared within each group, the S1 instruments showed a higher rate of deformation compared with the S2 (p=0.04), F1 (p=0.008) and F2 (p=0.049) instruments when used seven to nine times. No other significant differences were noted.

Because larger and stiffer ProTaper Universal instruments were reported to be reused with caution (4, 9), to use F3 in-

struments more than three times was not preferred, and therefore, these instruments were not included in the statistical analyses.

Discussion

The aim of this retrospective study was to evaluate the deformation and fracture rates for ProTaper Universal NiTi rotary instruments with regard to the frequency of clinical use. The frequency of use of NiTi rotary instruments depends on the complexity of the root canal anatomy and structure (4). One of the main causes of instrument fracture is a severely curved root canal (13, 14). However, one study has reported that there is no correlation between the frequency of use and the fracture rate (15). In the present study, the overall rate of instrument fracture was 1.2%, and S1 instruments showed a higher incidence of fracture when used one to three times than when used four to six (p=0.025) and seven to nine (p=0.004) times. The protocol for challenging cases is to use a new set of ProTaper Universal rotary instruments and discard these instruments after a single clinical use; possible deformation of only S1 instruments from the set after single use in challenging cases seems to be a plausible explanation for the statistical results obtained in the current study.

The overall rate of instrument deformation was 10% in the present study, with no significant differences among any instrument types in each group except Group C (seven to nine uses), where S1 showed the highest incidence. Shen *et al.* (4) reported a 0% failure rate for all ProTaper Universal files except S1 files, and their findings corroborated with those in studies showing a high incidence of distortion and separation with smaller NiTi instruments (11, 15). Ullmann and Peters (16) also found that SX and S2 files were more resistant to torque than S1 files. These findings support those of the present study, where a higher deformation rate was found for S1 than for S2 (p=0.04), F1 (p=0.008) and F2 (p=0.049) with seven to nine uses. This was possible because S1 has the smallest diameter among the ProTaper files and is the first rotary instrument used in canals as per the routine protocol (12).

The fracture rate is reported to vary according to the evaluation method (17). Wolcott *et al*. (9) reported a 2.4% rate of instrument fracture after the treatment of five teeth, including retreated teeth. In contrast, Wei *et al*. (18) found a 12% incidence of instrument separation after the treatment of 30 canals. In addition, Vieira *et al*. (6) mentioned that the use of ProTaper rotary instruments by an experienced endodontist allowed for the cleaning and shaping of up to 24–32 root canals without fracture. In their study, operator experience was found to affect the incidence of fracture and plastic deformation of files during shaping (6). In the present study, the fracture rate was 1.2%, which was lower than that in the abovementioned studies. Taken together, these findings indicate that the endodontist who performed all procedures in our study performed the treatment meticulously.

The deformation rate has also been reported to vary from 2.9% (19) to 12% (15) with different evaluation methods. In the present study, the deformation rate was higher than that in a study by Shen *et al.* (19), who evaluated instruments used only once. On the other hand, our findings were similar to those of Parashos *et al.* (15), who evaluated instruments used multiple times.

Wu *et al.* (17) mentioned that the fracture rate of reused ProTaper Universal rotary instruments remains low in endodontic practice, where the separation rate based on the number of treated canals is more reliable than that based on the number of treated teeth. In the present study, assessments were made on the basis of the number of treated canals.

Gambarini (20) reported that torque-controlled motors, which have been used for several years, increase operational safety. However, some authors reported that torque-controlled motors might be primarily useful for inexperienced clinicians (21), whereas others have found no significant difference in the instrument fracture rate between air-driven and electrical handpieces (22). In the present study, ProTaper Universal instruments were used with an air-driven W&H Endo NiTi handpiece according to the preference of the endodontist.

To avoid the risk of cross-contamination with the increased frequency of use, we sterilized each instrument before each use, which is mandatory. Some authors (23) have pointed out the negative effects of sterilization on the instrument durability, although others have reported contradictory results (24). In future studies, the sterilization process of the files may be undertaken after each use in a canal rather than a tooth to standardize the number of sterilizations and avoid the possible variable effects on the instrument durability.

Several recommendations have been proposed to prevent NiTi rotary instrument fracture, such as following a specific instrumentation protocol, shaping coronal third before the negotiation of the root canals, avoiding application of excessive apical pressure on the rotary instruments, preventing the rotation of the file at a single spot and the use of lubricants. In previous studies on the defect rates for clinically used NiTi rotary instruments, the most important factor was reported to be the skill of the operator (25-27). Parashos *et al.* (15) also concluded the same, and associated the defect rate with clinical skills or a conscious decision to use the instruments for a specified number of times or until the defects were evident. There is no agreement in the literature with regard to an association between the frequency of use and instrument fracture. Many authors have accepted that the failure of NiTi rotary files is influenced more by the manner of use than by the frequency of use (19). Although all clinical procedures were performed by an experienced endodontist and all recommendations and criteria were fulfilled with great care, the lack of comparisons with results obtained by an inexperienced user was a limitation of the present study.

Conclusion

ProTaper Universal has been one of the most extensively studied NiTi rotary instrument systems in the field of endodontics. However, the deformation and fracture rate results remain equivocal because of the presence of several influencing factors such as sterilization, handpiece use instead of torque-controlled motors, root canal anatomy, operator skill, and frequency of use. Under the conditions of the current study, frequency of use seemed to influence the deformation rates of PTU rotary instruments. Except S1, these instruments could be used without any fracture or deformation in up to 9 clinical cases by an experienced endodontist.

Ethics Committee Approval: Not required.

Informed Consent: Written informed consent was obtained from all patients involved in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: AY and IKK designed the study. AY, SSG, RD and IKK generated the data. AY, SSG and IKK gathered and analyzed the data, wrote the majority of the original draft. All authors approved the final version of the paper.

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Türkçe öz: *Nikel-titanyum döner aletlerin klinik kullanım sıklığına göre kırılma ve deformasyon oranlarının değerlendirilmesi. Amaç: Bu çalışmanın amacı, ProTaper Universal (PTU) nikel-titanyum döner aletlerinin deformasyon ve kırılma oranlarının klinik kullanım sıklığına göre değerlendirilmesidir. Gereç ve Yöntem: Çalışmada 4 yıl boyunca, bir endodontistin klinik kullanımı sonrası toplanan 619 PTU (S1, S2, F1, F2 ve F3) alet değerlendirilmiştir. Bu aletler; 1-3 (A grubu), 4-6 (B grubu) ve 7-9 (C grubu) klinik kullanım miktarı olacak şekilde 3 gruba ayrılmıştır (1 kanal=1 kullanım). Kullanılan aletler deformasyon ve kırık varlığı açısından kullanım sayısını bilmeyen bir araştırıcı tarafından stereo mikroskop ile 15×–45× büyütmede değerlendirilmiştir. Bulgular: Bütün grupların oranları birlikte değerlendirildiğinde, deformasyon yüzdesi %10 ve kırık yüzdesi %1,2'dir. Deformasyon ve kırılma yüzdeleri oranları açısından S2, F1 ve F2 aletleri arasında istatistiksel olarak anlamlı bir fark saptanmamıştır. A grubundaki S1 aletlerinin kırılma oranı, B (p=0,025) ve C gruplarındakilere (p=0,004) kıyasla daha yüksek bulunmuştur. C grubunda, S1 aleti, S2 (p=0,04), F1 (p=0,008) ve F2 (p=0,049) aletlerine kıyasla istatistiksel olarak anlamlı derecede yüksek deformasyon oranı göstermiştir; B ve C gruplarında ise anlamlı bir fark saptanmamıştır. Sonuç: Bu çalışmanın şartları altında, PTU aletlerinin deformasyon ve kırık oranları kullanım sıklığına göre değişiklik göstermektedir. Tecrübeli bir endodontist tarafından klinik uygulamada S1 haricindeki aletlerin kırık ve deformasyon oluşmadan 9 olguya kadar kullanılabildiği gözlemlenmiştir. Anahtar kelimeler: NiTi döner alet; ProTaper Universal; dental aletler; deformasyon; kırık*

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Original research

Skeletal changes following surgically assisted rapid maxillary expansion (SARME)

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Purpose
Surgically assisted rapid maxillary expansion (SARME) is a common treatment technique in the correction of maxillary transverse deficiency. The purpose of this study was to evaluate nasal and palatal skeletal changes following SARME using Cone Beam Computed Tomography (CBCT) and posterior anterior (PA) cephalograms.

Materials and Methods
In this retrospective study, the radiographic images obtained from 14 patients with transverse maxillary deficiency before treatment and 6 months after SARME operations were evaluated. The changes in nasal bone width and palatal bone width were measured on CBCT. The changes in basal maxillary width, nasal cavity width and angular measurements were evaluated on PA cephalograms.

Results
Nasal floor width was measured at the levels of upper first premolar teeth and molar teeth which significantly increased following SARME (p=0.005 and 0.017 respectively). Palatal bone width between first premolar teeth and molar teeth also significantly increased (p=0.003 and 0.002 respectively). Basal maxillary width (p=0.026), nasal cavity width (p=0.024) and other angular measurements also significantly increased (p<0.05).

Conclusion
Nasal and palatal skeletal transverse dimensions increased following SARME. Due to the enlargement of the nasal floor and nasal cavity, it is likely to improve air pass through the nose.

Keywords: *Transvers deficiency; maxilla; rapid expansion; skeletal change; CBCT*

Introduction

Maxillary transverse deficiency is a skeletal deformity characterized by unilateral/bilateral cross bite, crowded teeth, and a constricted maxillary arch. Transverse deficiency can either be managed by orthodontic treatment or by combination of orthodontics and surgery depending on patient's age, and bone growth (1). Surgically assisted rapid maxillary expansion (SARME) is a recognized treatment approach in patients with transverse maxillary deficiency. Its primary goal is to achieve skeletal expansion rather than dental expansion and to minimize dental tipping by separating the midpalatal and lateral maxillary sutures. In this technique, the expansion procedure is based on distraction osteogenesis of palatal bones after a surgical operation. SARME also causes craniofacial structural changes such as enlargement of nasal cavity width, nasal volume and palatal vault (2-5). The influence of SARME on the nasal cavity is based on the separation of the nasal lateral walls. The increase in the distance between the nasal cavity lateral walls enlarges the cross-sectional area and increases nasal volume

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and thus facilitates breathing. The aim of this study was to evaluate the short-term palatal and nasal changes following surgically assisted rapid maxillary expansion. Null hypothesis tested in this study is that the SARME procedure does not affect any of the skeletal measurements.

Materials and methods

Study sample

Fourteen patients with transverse maxillary deficiency underwent SARME procedure under general anesthesia at Medipol University School of Dentistry. All the patients were referred from orthodontics department after initial examination and treatment planning. This study was approved by the university local ethical committee (No: 10840098-604.01.01-E.21268) and written consents were obtained.

Surgical technique

The surgical technique was similar to that of Le-Fort 1 osteotomy with an exception of performing down fracture. Surgical technique was performed in all patients by the two oral and maxillofacial surgeons. A mucoperiosteal incision in the maxillary vestibule extended from the right first molar to the left first molar. Maxillary corticotomies were achieved with reciprocating micro-saw. A horizontal cut was done from pyriform aperture to pterygomaxillary fissure, midpalatal and pterygomaxillary sutures were separated and anterior nasal wall was osteotomized. Nasal septum was also separated to prevent deviation during activation period. To prevent irregular fractures, midpalatal suture was separated as well. The tooth borne Hyrax appliance was activated 8 turns (2 mm) at the end of the surgery to verify success of osteotomy and symmetrical separation of the bone segments. The appliance was then deactivated 4 turns and the wound was primarily closed. After one week of latency period the appliance was activated two times a day. Activation was carried on until the planned expansion was achieved. Distractor appliance was left *in situ* to prevent relapse.

Imaging protocols and measurements

All patients underwent a CBCT scan using i-CAT Next Generation Cone Beam Computed Tomography (Imaging Sciences International, Hatfield, PA, USA) (16 x 8 cm FOV, 0.2 mm slice thickness) being seated and in standard head position. The CBCT scans were taken from all the patients immediately before (T0) and 6 months after the surgery (T1). To assess the skeletal and nasal changes after SARME, the following distances were measured on the coronal CBCT images with the method used by Zandi *et al.* (6) (Figure 1). NFW4: Nasal floor width at the area of first premolars, 5 mm above the most inferior part of the nasal floor. NFW6: Nasal floor width at the area of first molars, 5 mm above the most inferior part of the nasal floor. PBW4: Palatal bone width at the level of a line connecting the palatal root apex of the first premolars. PBW6: Palatal bone width at the level of a line connecting the palatal root apex of the first molars. Postero-anterior (PA) radiography was taken from the patients immediately before (T0) and 6 months after surgery (T1).

To assess the nasal changes, the following measurements were performed on PA images with the same methods by Altug-Atac *et al*. (7, 8) and Krykanides *et al.* (9). Following parameters were measured. (Figure 2). MxR-MxL: Basal maxillary width MxR/cg/MxL: Angle between crista galli and maxillary base points. NC/Lom/VL: Right nasal cavity angle. CN/Lom/VL: Left nasal cavity angle. NC/Lom/CN: Total nasal cavity angle. NC-CN: Nasal cavity width. sn/Lom/VL :Nasal septum angle.

Statistical analysis

The results were analyzed using IBM Statistical Package for Social Sciences (SPSS) version 22 (SPSS IBM Corp.; Armonk, NY, USA) software. The coherence of parameters to normal distribution was evaluated with Shapiro-Wilks test. Parameters that display normal distribution were evaluated with Paired Samples test, whereas those display asymmetrical distribution were evaluated with Wilcoxon sign test. Confidence interval was set to 95% and p values less than 0.05 were considered statistically significant.

Results

Images obtained from 14 patients (6 male, 8 female) aged between 18 to 30 years (mean 21.3 years) before SARME (T0) and at 6 months postoperatively (T1) were evaluated. Nasal floor width at the level of upper first premolars and first molars increased following SARME (p=0.005 and 0.017 respectively). The increase in palatal bone width at the level of

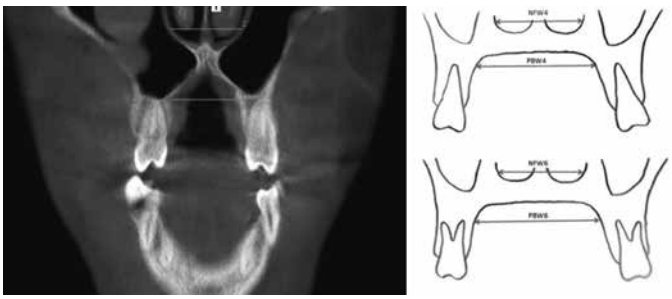

Figure 1. *Measurement of nasal and palatal dimensions on cone beam computed tomography (left) and illustration (right).*

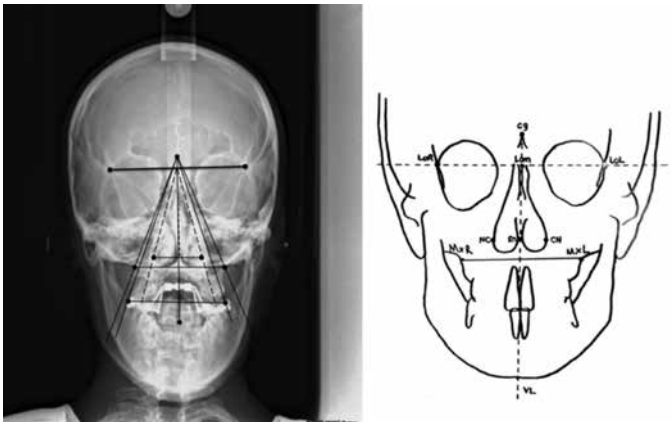

Figure 2. *Measurement of orthodontic points on posteroanterior cephalogram (left) and illustration (right).*

Table 1. *Findings of cone beam computed tomography measurements*

	T0	T1	
	Mean±SD	Mean±SD	p
NFW4	27.42±3.61	30.63±4.61	0.001*
PBW4	18.52±4.37	21.9±2.79	0.001*
NFW6	29.28±3.91	31.09±3.73	0.003*
PBW6	26.97±4	30±4.04	0.002*

*:p<0.05in paired samples t test; SD: standard deviation

Table 2. *Findings of posteroanterior cephalometric measurements*

	T0	T1	
	Mean±SD	Mean±SD	p
MxR/mxL	41.70±3.56 (41.5)	44.75±4.02 (43.8)	0.027*
MxR/cg/mxL	47.17±3.27 (47.5)	50.58±3.47 (51.5)	0.024*
NC/Lom/VL	1.25±0.69 (1)	0.67±0.52 (1)	0.066
CN/Lom/VL	17.12±2.31 (18)	19.63±2.65 (19.8)	0.027*
NC/Lom/CN	33.22±3.48 (33)	37.67±3.67 (39)	0.027*
NC-CN	20.58±3.06 (21.3)	22.92±2.93 (23.5)	0.026*
SN/Lom/VL	16.1±2.78 (15)	18.04±2.59 (16.9)	0.028*

*: p<0.05in Wilcoxon signed rank test; SD: standard deviation

both first premolars and first molars was found statistically significant (p=0.003 and 0.002 respectively). Basal maxillary width (p=0.026), angle between crista galli and maxillary base (p=0.02), left nasal cavity angle (p=0.026), right nasal cavity angle (p=0.027), total nasal cavity angle (p=0.027), nasal cavity width (p=0.024) also increased significantly. Nasal septum deviation was evaluated measuring the nasal septum angle at T0 and T1 did not yield any significant difference. The findings of the study were presented in Table 1 and Table 2.

Discussion

SARME is a well-known technique for the correction of transversal maxillary discrepancies. After SARME, skeletal alterations occur in the maxilla and in the midfacial bones. In the present study we evaluated the outcomes of SARME with posterior-anterior cephalometric radiographs (PA) and cone beam computed tomography (CBCT). Computed tomography is a more precise method for capturing the extent of expansion than the two-dimensional imaging. Three-dimensional visualization of the structures and their movements can be easily inspected with CT images with eliminating the magnification and distortion (7, 10). In this study we also used PA cephalograms for angular measurements since the area of Field of View (FOV) in CBCTs was not wide enough to capture crista galli to minimize radiation dose.

The main resistance to distraction movement occurs in the pterygomaxillary, zygomaticomaxillary, and frontomaxillary sutures. These are the buttress areas which are effecting the center of rotation during expansion. Inverted V-shaped horizontal opening movement of the maxillary segments was reported in previous studies (1, 5, 11) demonstrated a higher amount of expansion in the first premolar than in the molar area following tooth borne SARME. Seeberger *et al.* (12) reported a V shaped opening of the nasal floor and the palatal arch, but a parallel expansion of the alveolar crest with tooth borne SARME devices. Goldenberg *et al.* (13) suggested that the greatest expansion occurred in the most inferior and anterior region of the maxilla; however, Zandi *et al.* (6) reported that transpalatal distractor placed at the molar level provided parallel widening of dental arch, palatal vault and nasal floor. There is also an inverted V shaped opening that becomes smaller in superior parts as described in other studies with SARPE (14, 15). In the present study the alteration of nasal width at the level of first premolars and first molars was statistically significant. SARME provides transversal distraction of lateral nasal walls as well as the maxillary segments.

Posterior anterior cephalometric radiographies were useful in the evaluation in the transversal changes of maxilla and nasal bones. Altug-Atac *et al.* (7) used PA radiography to evaluate the outcomes of rapid maxillary expansion and orthopedic rapid maxillary expansion. In our study basal maxillary width, angle between crista galli and maxillary base points, right nasal cavity angle, left nasal cavity angle, total nasal cavity angle, nasal cavity width, nasal septum angle were evaluated in PA cephalometric radiographies.

In several studies it was demonstrated that the increase in transversal dimensions of maxilla, enlarges the nasal cavity and decreases the degree of nasal obstruction (3, 16, 17). However the presence of any nasal deformities or diseases such as polyps or hypertrophic mucosa may prevent air passage through the nasal cavity. Warren *et al.* (18) found that nasal volume increased 45% after rapid maxillary expansion and 55% after SARME. Basciftci *et al.* (19) reported that both maxillary width and nasal cavity width increased significantly after both RME and SARPE. Enoki *et al.* (20) presented the results of their study investigating the effect of RME on the dimension of the nasal cavity and on nasal air resistance. They reported an increase in the bony dimension of the nasal cavity and significant improvement of breathing due to decrease in nasal resistance. Seerberger *et al.* (12) applied tooth borne distraction device after SARME and their results showed that transverse shift of the segments can be achieved over the whole bony plate.

Nasal airflow resistance and nasal volume can alter regarding by horizontal transversal changes of the maxilla. However individual responses vary and it is hard to correlate the nasal changes and subjective improvement. Magnusson *et al.* (21) reported the alterations in the volume of nasal cavity and the patient's subjective sensation of nasal obstruction. Their results showed nasal volume increased and subjective sensation of nasal obstruction improved. Their findings presented that the improvement in subjective nasal obstruction evaluation was significant in both short and long terms; however, no correlations were reported between subjective parameters and nasal minimum cross sectional area and nasal airway resistance (21). In our study we did not evaluate change in nasal or mouth breathing in our patients; therefore we cannot comment on impact of surgery.

Intervention to the nasal septum during SARME is variable. The nasal septum is frequently released from palatal base to prevent septal deviation and nasal airway changes. It is believed that unseparated nasal septum is prone to septal deviation during distraction procedure (22); but there are some studies reporting the ineffectiveness of septum osteotomy to septal deviation (8, 12, 23). Reinbacher *et al.* (23) investigated the need for releasing the septum during SARME. The deviation of the nasal septum was evaluated by comparing measurements between pre and postoperative CTs. They reported low degrees of septal deviation or side shifting in non-released nasal septa. They declared that there was no compelling reason to release the septum (23). In our study the difference in the nasal septum angles between T0 and T1 radiographies was not statistically significant. We did not encounter septum deviation in our patients due to separation of the septum. SARME is a predictable surgical procedure for the correction of maxillary transverse deficiency. Its effect on nasal airway and breathing should be further evaluated particularly in patients with mouth breathing.

Conclusion

Nasal and palatal skeletal transverse dimensions increased following SARME. Due to the enlargement of the nasal floor and nasal cavity, it is likely to improve air pass through the nose.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul Medipol University (No: 10840098-604.01.01-E.21268- 13/07/2017).

Informed Consent: This is a retrospective radiographical study. We have ethical approval but we did not need informed consent from the patients.

Peer-review: Externally peer-reviewed.

Author Contributions: GG and ÇD designed the study. NKA and İK generated and gathered the data. GG, NKA and İK analyzed the data. GG and ÇD wrote the majority of the original draft. All authors approved the final version of the paper.

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

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

Türkçe öz: *Cerrahi destekli hızlı maksiller genişletme (SARME) sonrası iskeletsel değişiklikler. Amaç: Cerrahi destekli hızlı maksiller genişletme (SARME) maksiler transvers yetersizliğin tedavisinde sık kullanılan bir yöntemdir. Bu çalışmanın amacı, SARME uygulanan hastalarda nazal ve palatal iskeletsel değişikliklerin konik ışınlı bilgisayarlı tomografi (KIBT) ve posteroanterior (PA) sefalogramla değerlendirilmesidir. Gereç ve Yöntem: Bu retrospetif çalışmada, maksiler transvers yetmezliği bulunan 14 hastadan SARME öncesi ve girişim sonrası 6. ayda alınan radyografik görüntüler incelendi. Nazal kemik genişliği ve palatal kemik genişliği KIBT üzerinde ölçüldü. Bazal maksiller genişlik, nazal kavite genişlik ölçümleri ve açısal ölçümler PA sefalogramlar üzerinde yapıldı. Bulgular: Üst birinci premolar dişler ve birinci molar dişler seviyesinde ölçülen nazal taban genişliği SARME sonrası anlamlı olarak arttı (sırasıyla p=0,005 ve p=0,017). Palatal kemik genişliği de birinci premolar ve birinci molar dişler seviyesinde anlamlı olarak arttı (sırasıyla p=0,003 ve p=0,002). Bazal maksiller genişlik (p=0,026), nazal kavite genişliği (p=0,024) ve diğer açısal ölçümlerin anlamlı derecede arttığı* görüldü (p≤0,*05). Sonuç: SARME sonrası nazal ve palatal transvers boyutlar artmıştır. Nazal taban ve nazal kavite genişlemesine bağlı olarak burundan hava akışının iyileşmesi beklenebilir. Anahtar kelimeler: Transvers yetmezlik; maksilla; hızlı genişletme; iskeletsel değişiklik; KIBT*

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Original research

Evaluation of Shore A hardness of maxillofacial silicones: the effect of dark storage and nanoparticles

Pınar Çevik

Purpose
Little has been reported how the addition of nanoparticles could affect the hardness of maxillofacial silicones. The purpose of this study was to evaluate the effects of different types of nanoparticle additives and dark storage for 2-years on the Shore A hardness of two types of maxillofacial silicone elastomers.

Materials and Methods
A-2000 and A-2006 Room Temperature Vulcanized (RTV) silicone elastomers were tested in this study. Silaned silica, fumed silica and titanium dioxide nanoparticles at concentrations of 10% by volume were used as fillers for the maxillofacial silicone elastomers. A total of 64 silicone specimens were fabricated, which includes 8 samples, 30 x 10 mm in size, from each silicone elastomers for each subgroup and also controls. After the initial Shore A hardness measurements, specimens were kept in dark conditions at room temperature for 2 years. The final measurements were then taken from the silicone specimens. All data were statistically analyzed.

Results
For both types of silicones, there was statistically significant differences among study groups ($p<0.001$). Fumed silica group showed the lowest hardness values in A-2000 after storage. However, no significant difference was observed between control and fumed silica groups. Control group showed the lowest hardness values in A-2006, while the highest hardness values were found in fumed silica group; there was no significant difference between silica and silane groups.

Conclusion
Shore A values of the specimens were within the acceptable range for the maxillofacial silicones after aging. Nanoparticle addition did not prevent hardening of the silicone elastomers with time.

Keywords: *Aging; hardness; nanoparticles; silicone elastomers; Shore A*

Introduction

Maxillofacial prostheses are still being used to treat congenital and acquired defects of the head and neck region, despite advances in plastic surgery (1, 2). Maxillofacial prostheses provide a practical alternative by giving patients a normal appearance, esthetics, and social acceptance (3, 4). Currently, several types of materials can be used in maxillofacial prosthodontics such as chlorinated polyethylene, polymethylmethacrylates, polyurethanes, latex, and silicone elastomers. Silicone elastomers are widely used because of their favorable properties, including acceptable tear and tensile strengths, chemical inertness, high elongation percentage, ease of manipulation and biocompatibility (5, 6). Despite their wide use, they also suffer from deterioration of color and loss of physical, mechanical as well as dynamic properties in clinical practice. The average service life of a maxillofacial prosthesis ranges therefore from 6 to 18 months (6).

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Limited research has been conducted to enhance the mechanical and physical properties of maxillofacial silicones by adding nanoparticles (1, 7). Nanoparticles could diffuse the silicone matrix, which leads to the formation of the three-dimensional network within the silicone chain (3, 7).

Surface hardness can be described as the resistance of the material against vertical perforation (8). The hardness of the maxillofacial silicone elastomer is determined by the density of cross-links and the surface characteristics of polymer network (9). An ideal maxillofacial silicone elastomer should have an optimum hardness value to mimic the surrounding tissues (10).

Previous studies have evaluated the effect of artificial aging and real time aging on the mechanical and physical properties of different types of maxillofacial silicones. However, no previous study evaluated the effect of long-term dark storage on the hardness of nanoparticle added two types of room temperature vulcanized (RTV) maxillofacial silicone elastomers. Therefore, the purpose of this study was to evaluate the effect of long-term dark storage on the hardness of nanoparticle added two types of RTV maxillofacial silicone elastomers. The null hypotheses tested in this study were twofold; first, the natural aging would not affect the hardness of nanoparticle added maxillofacial silicone elastomers and second, Shore A hardness of the nanoparticle added specimens would be reduced after dark storage.

Materials and methods

Specimen preparation

The materials used in this study are given in Table 1. Platinum based A-2000 and A-2006 RTV type silicone elastomers were tested. A total of 64 silicone elastomer specimens (N=64) were fabricated from A-2000 and A-2006 silicones. Silane treated silica, fumed silica and titanium dioxide (TiO_2) nanoparticles were used as fillers. A metal mold was fabricated in accordance with the American Society for Testing and Materials (ASTM) D2240-68 standard for the standardization of the silicone specimens for the hardness tests.

For the fabrication of specimens in control group (Group 1), the homogenous silicone mixtures were prepared from A-2000 and A-2006 silicone elastomers, respectively. The ratio of the silicone elastomers were 1:1 for part A and part B of the silicones. A thixotropic agent, which prevents air bubble formation, was added to the silicone mixture and mixed by hand, according to the manufacturer's instructions. Thus, Group 1 with no addition of nanoparticles served as the control group for A-2000 and A-2006 silicone elastomers. For Group 2, 12 nm hydrophilic fumed silica nano particles were added at 10% concentration by volume to the silicone mixture. For Group 3, 12 nm silane-treated hydrophobic silica nanoparticles were incorporated to the silicone mixture at 10% concentration by volume. For Group 4, 30 nm TiO_2 nanoparticles were added to the silicone mixture at 10% concentration by volume. The final silicone mixture was poured to the metal mold and silicone specimens were polymerized. The manufacturer recommends the polymerization to take place at 75°C for 3 or 4 hours in stone molds and dry oven. They also recommend higher polymerization degree and lesser time if the metal molds used. For standardization of the specimens, metal molds, instead of stone molds, and constant pressure with proper degree of temperature were selected. Therefore, the polymerization process was set for the specimens as 6 minutes in 60°C under a hydraulic press (HD80; Motor Operated, Hidroliksan, Konya, Türkiye) in metal molds, according to the method described in a previous study (1). Eight silicone specimens were fabricated for each study group (n=8). Thus, a total of 64 silicone specimens from A-2000 and A-2006 silicone elastomers (32 in each) were used in this study for the hardness tests.

Shore A hardness measurements

Silicone specimens in 30 mm diameter and 10 mm thickness were prepared in a metal mold in compliance with the ASTM D2240-68 standard (Figure 1). The hardness measurements of the specimens were made by using a digital Shore A durometer (Shore Leverloader, Duratronic, Akron, OH, USA). Three hardness measurements were taken from each specimen as Shore units and the average values were calculated as the final Shore A value, before and after the dark storage of the specimens.

Storage conditions

Dark storage performed for the silicon specimens included storing the specimens in room temperature 23°C±2°C and relative humidity in pigment free plastic bags. Specimens then placed in a lightproof wood box for 2-years. At the end of this period, specimens were removed and tested by using the digital Shore A durometer (Figure 2).

Statistical analysis

The collected data from all groups were imported to Statistical Package for the Social Sciences software version 20

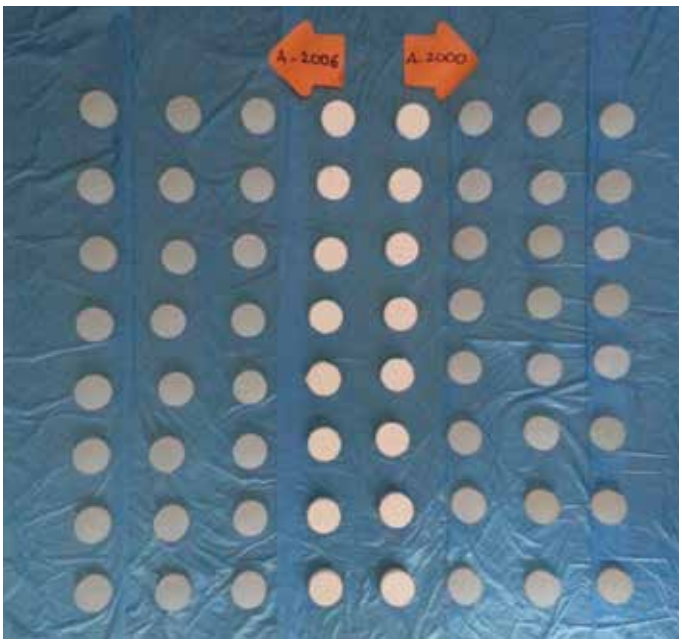

Figure 1. *Silicone specimens after polymerization process.*

Figure 2. *Shore A testing of the specimens.*

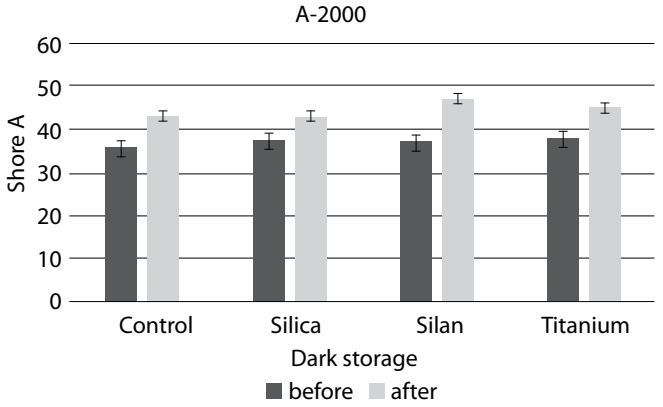

Figure 3. *Paired t-test results, hardness (Shore A) values of A-2000 silicone before and after dark storage.*

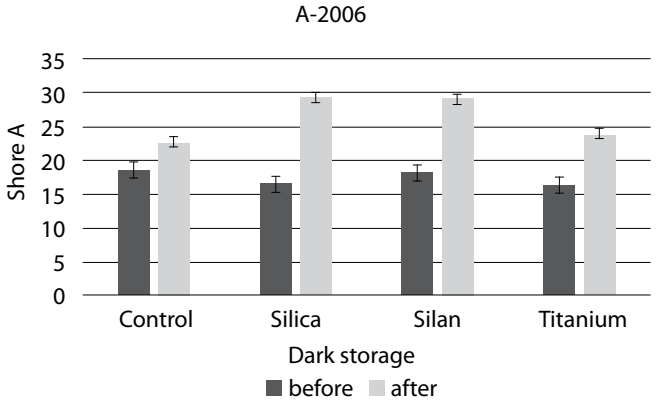

Figure 4. *Paired t-test results, hardness (Shore A) values of A-2006 silicone before and after dark storage.*

(SPSS IBM Corp.; Armonk, NY, USA). The standard descriptive methods such as the mean, standard deviation, minimum and maximum were applied to determine the characteristics of the sample. All data were first evaluated with Levene's test for homogeneity of variances and with Kolmogorov-Smirnov for normality. Paired t-test was used to evaluate the differences between the initial and the final hardness values of each nanoparticle groups in each silicone material. Because the data was normally distributed, one-way analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) tests pairwise comparisons was used to compare the nanoparticle groups in each silicone group, respectively. Confidence interval was set to 95% and p values less than 0.05 statistically significant.

Results

ANOVA results are presented as the mean and standard deviation in Table 2 and Table 3, and t-test results are presented in Figure 3 and Figure 4.

As can be seen from Table 2, ANOVA results revealed that statistically significant difference was found among groups after dark storage ($p<0.001$). The lowest hardness value was observed in fumed silica group in A-2000 silicone elastomer. However, there was no significant difference between control and silica groups. While the highest hardness value was observed in TiO_2, the significant difference was found between control and TiO_2 groups ($p=0.003$). According to ANOVA results for the hardness values of A-2006 silicone (Table 3), there was statistically significant difference among the groups ($p<0.001$). The lowest hardness value was observed in control group. Furthermore, while the highest hardness value was observed in silica group, there was not statistically significant difference between silica and silane groups in A-2006 silicone elastomer. According to paired t-test results, there was a significant difference between the hardness of each type of silicone and silicone subgroups before and the after storage ($p<0.001$). Shore A hardness of all specimens significantly increased after dark storage.

Discussion

In this study, it was found that 2 years of dark storage adversely affected Shore A hardness of the silicone specimens. Furthermore, regarding after-storage specimens, nanoparticle incorporation did not reduce the hardness of the silicone elastomers as compared to control groups. The hardness values of silicone elastomers after 2-years dark storage varied from 47.28 to 22.75, for A-2000 and A-2006, respectively. Both silicone elastomers, with or without nanoparticles, demonstrated significant hardness increase after dark storage. However, Shore A values of stored specimens were found to be consistent with the hardness values of silicone elastomers reported by Veres *et al*. (1, 11).

Many studies evaluated Shore A hardness of silicone elastomers for periods of 2 (12), 6 (13-15) or 12 (16, 17) months with measurements taken at the initial and the final observation periods. Because the standard clinical longevity of the

Table 1. *Information on the materials used in this study*

Material	Type	Manufacturer
RTV Silicone	A-2000	(Factor II Inc. Lakeside; AZ, USA)
RTV Silicone	A-2006	(Factor II Inc. Lakeside; AZ, USA)
Fumed Silica	Cab-O-Sil	(Factor II Inc. Lakeside; AZ, USA)
TiO2	30nanoTi	(Imicryl, Turkey)
Silanated silica	S-812	(Imicryl, Turkey)
Thixotropic agent	A-300-1 Thixo	(Factor II Inc. Lakeside; AZ, USA)

RTV: room temperature vulcanized

Table 2. *Final Shore A values for A-2000 silicone elastomer*

Groups	N	Mean	SD	Min	Max
Control	8	43.32[a]	1.0	41.23	44.43
Silica	7	43.12[a]	1.3	40.23	44.60
Silan	8	47.28[b]	1.0	45.26	48.86
Titanium	8	45.39[c]	.7	44.20	46.44
Total	31	44.70	1.9	40.23	48.86

Different superscript letters indicate the significant differences between groups
Means with same lowercase letters are not significantly different
SD: standard deviation

Table 3. *Final Shore A values for A-2006 silicone elastomer*

Groups	N	Mean	Std Dev	Min	Max
Control	8	22.75a	.7	21.42	23.72
Silica	8	29.45b	.6	28.53	30.16
Silan	8	29.26b	.5	28.38	30.06
Titanium	7	23.91c	1.0	22.50	25.22
Total	31	26.24	3.1	21.42	30.16

Different superscript letters indicate the significant differences between groups
Means with same lowercase letters are not significantly different
SD: standard deviation

facial prosthesis averages from 7 to 24 months (6, 18-22), it would be useful to evaluate the effect of time passage of at least 12-months on Shore A hardness of such silicone elastomers (22, 23). Therefore, the effect of long-term dark storage on the hardness value of silicone elastomers was evaluated in this study.

The relative hardness of a maxillofacial silicone elastomer should imitate the surrounding tissues and resemble the human skin (7). Facial prostheses during clinical service should be resistant and yet to soft and flexible to mimic the facial movements (24, 25). The acceptable limits of Shore A values for a facial silicone elastomer range from 25 to 55 units (26). The reason of different values considered to be acceptable clinically is due to the hardness variations that exist in the maxillofacial area.

It was reported that physical changes of maxillofacial elastomers that occur during natural aging could result from polymerization by-products, initiators (15), pigments and other fillers (2, 27). In this study, nanoparticle fillers adversely affected the hardness values of silicones after dark storage. However, hydrophilic fumed silica nanoparticles decreased the hardness value of A-2000 silicone as compared to control group. Hydroxyl groups of the hydrophilic fumed silica could generate hydrogen bonds between the silica and silicone. Thus, fumed silica could increase the mechanical properties of silicones (28). Most studies reported that fumed silica nanoparticles could increase the mechanical properties of silicone elastomers (1, 29, 30).

Nano-oxide particles have been used as fillers in silicone elastomers to strengthen the mechanical properties of elastomers. By cross-linking reactions, nano-oxide particles could increase the surface energy of silicone matrix, which leads to a reinforced matrix structure (31, 32). In recent studies, titanium dioxide nanoparticles were found to increase the mechanical properties of silicone elastomers (1, 7, 33). According to the results of this study, it could be stated that fumed silica nanoparticles might be effective on the mechanical properties of A-2000 silicone elastomer while TiO_2 nanoparticles might be effective on the mechanical properties of A-2006 silicone elastomer.

Most studies on silicone elastomers subjected to dark storage are generally based on color stability (2, 23, 25, 34, 35). However, limited research investigated the mechanical properties of silicone elastomers subjected to dark storage (17, 25, 27, 36). Furthermore, our study has a main difference with recent studies in which we investigated the effect of nanoparticle addition on the hardness of silicone elastomers after being subjected to dark storage.

Hardness increment during dark storage could result from continuous vulcanization (12, 36, 37) of the silicone elastomers due to the presence of nanoparticles. A possible explanation of hardness increment could be a mechanism that may start due to oxygen intake during dark storage and generates cross-linkers which cluster in the silicone matrix, leading to denser network and higher hardness for silicone materials (17, 38).

The hardness of the silicone elastomers is controlled by the surface characteristics of the polymer network and the density of crosslink as reported by Polyzois *et al.* (17) Furthermore, the density of the cross-linkers could affect the length of the polymer chain (39) which results in degradation of mechanical properties of silicon elastomers in time. Therefore, another possible reason for increased hardness of nanoparticle added silicones could be that nanoparticles maximize the network of silicone matrix, lengthen of the polymer chain, leading to increased hardness values.

Lai and Hodges (40) and Raptis *et al*. (41) reported that the silicone elastomers are not completely vulcanized in stone molds. Furthermore, stone molds have more surface irregularities than the metal molds do (1). Accordingly, new generation silicone elastomers are polymerized in metal molds (40).

Although stone molds can imitate the clinical fabrication techniques, metal molds were preferred for the polymerization process of the silicone elastomers to provide accurate and standard data in this study.

Various studies have used nano particles as fillers at different concentrations. Fillers used in this study were at 10 % by volume, which is consistent with previous studies (30, 33) and it is based on the results of a recently published article (1), in which the mechanical properties of silicones were evaluated after nanoparticle incorporation. Thus, different concentrations of nano particles could lead to differences in mechanical and physical properties of silicone elastomers. Therefore, future research should address the effects of adding such nanoparticles at different concentrations on the mechanical and physical properties of maxillofacial silicone elastomers subjected to aging process.

Conclusion

Within the limitations of this *in vitro* study, it can be stated that the hardness of A-2000 and A-2006 silicone elastomers increased after 2-years dark storage. On the other hand, both silicone elastomers, with or without nanoparticles, showed clinically acceptable Shore A hardness values even after dark storage. Nanoparticle addition did not prevent silicone elastomers from hardening effects of time and, finally, A-2000 silicone revealed maximum hardness values in all study groups.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of 12.01.2012 with the number 2012/01.

Informed Consent: There is no need for an informed consent form for the current in vitro study.

Peer-review: Externally peer-reviewed.

Author Contributions: PÇ designed the study, generated, gathered and analyzed the data, wrote and approved the final version of the paper.

Conflict of Interest: The author have no conflicts of interest to declare.

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Türkçe öz: *Maksillofasiyal silikonlarda Shore A sertlik ölçümü: karanlık ortam ve nanopartiküllerin etkisi. Amaç: Nanopartikül ilavesinin maksillofasiyal silikonların sertliğine olan etkisi hakkında yeterince çalışma bulunmamaktadır. Bu çalışmanın amacı, farklı nanopartikül ilavesi ve iki yıl karanlık ortam yaşlandırmasının maksillofasiyal silikon elastomerlerin sertliğine olan etkisini incelemektir. Gereç ve Yöntem: Çalışmada RTV tip A-2000 ve A-2006 silikon elastomerler test edilmiştir. Silanlanmış silika, tütsülenmiş silika ve titanyum dioksit nanopartikülleri hacimce %10 oranında doldurucu olarak kullanılmıştır. Her bir silikondan 30 x 10 mm boyutlarında sekiz örnek olmak üzere, kontrol grubu dahil toplam 64 silikon örnek hazırlanmıştır. İlk Shore A sertlik değerleri alınan örnekler oda sıcaklığında karanlık ortamda iki yıl boyunca saklanmış ve örneklerin yaşlandırma sonrasına ait ikinci Shore A değerleri kaydedilmiştir. Veriler istatistiksel olarak analiz edilmiştir. Bulgular: Her tip silikon için, gruplar arasında sertlik değerleri açısından istatistiksel olarak anlamlı farklar gözlenmiştir (p<,.001). Yaşlandırma sonrası; A-2000'e ait tütsülenmiş silika en düşük sertlik değerleri verirken, kontrol grubu ile arasında anlamlı bir fark bulunamamıştır. A-2006 için, kontrol grubu en düşük sertlik değeri verirken, en yüksek sertlik değeri tütsülenmiş silika grubunda gözlenmiştir. A-2006 'ya ait tütsülenmiş silika ve silanlanmış silika grupları arasında istatistiksel olarak anlamlı bir fark gözlenmemiştir. Sonuç: Yaşlandırma işlemi sonrasında maksillofasiyal silikonlara ait sertlik değerleri klinik olarak kabul edilebilir değerler arasında kalmıştır. Bununla birlikte, nanopartikül ilavesi silikon elastomerleri yaşlandırma işlemi sırasında ortaya çıkan sertlik artışına karşı koruyamamaktadır. Anahtar kelimeler: Yaşlandırma; sertlik; nanopartiküller; silkon elostomerler; Shore A*

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Original research

Comparative assessment of 3D reconstruction technique and Cavalieri's principle in predicting the mandibular bone defect volumes

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Purpose
The objective of this study was to compare the accuracy of the Cavalieri's principle and 3D reconstruction in predicting the volume of a bony defect.

Materials and Methods
Defects of the same approximate size were created on nine artificial mandibles. The actual volume of the defect on each mandible was measured by water displacement, and served as the control. Each mandible was then scanned using a CBCT and volume measurements were made for each defect using two techniques: Cavalieri's principle and 3D reconstruction. For each defect, the volume obtained by each of the two techniques was compared to the control volume using the analysis of variances (ANOVA) with $p<0.05$.

Results
ANOVA between the control, 3D reconstruction and Cavalieri's principle groups showed no statistically significant differences ($p=.058$). When the control group was further analyzed by Dunnett's post-hoc test, the results from Cavalieri's principle were found to be statistically different than the control group ($p=.035$), whereas the results of 3D reconstruction technique did not reach the level of significance ($p=.523$).

Conclusion
Cavalieri's principle significantly underestimates the actual control volume, and is less accurate than the 3D reconstruction technique. The 3D reconstruction method is a reliable technique in measuring volume of bony defects.

Keywords: *Cone-beam CT; three-dimensional imaging; Cavalieri's principle; defect; image reconstruction*

Introduction

Many surgical advances have been made in the last several decades that have given patients excellent options to regain esthetics and functionality after dentition has been lost. As an example, dental implant therapy has been widely accepted to be a predictable procedure with good long-term results. However, some clinical parameters should be considered prior to surgery, including the quantity of bone surrounding the implant in both vertical and horizontal dimensions (1).

Implant success rates are strongly associated with adequate bone volume, which ensures the placement of dental implants at the correct position and encourages osseointegration (2). Sufficient buccal bone volume around implants is essential, especially for achieving esthetic results in the anterior region (3, 4). Following extraction, the remodeling of the alveolar process leads to a predictable pattern of resorption in both the apico-cor-

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onal and bucco-lingual dimensions. The presence of buccal bone defects increases the risk of mechanical implant failure (1, 5). Pre-prosthetic reconstruction techniques have been proposed to re-establish the anatomic morphology to optimize prosthetic outcome.

Of the various augmentation techniques that have been described for the reconstruction of buccal bone defects, autogenous bone grafting is currently considered the gold standard for alveolar ridge augmentation, although resorption of the graft always remains a concern (6-8). Thorough preoperative planning and evaluation is essential to determine the precise amount of bone graft needed to reconstitute a defect. This will assist in proper selection of the donor site for bone grafting and minimize surgical morbidity.

Cone beam computed tomography (CBCT) is an emerging technology, which provides essential three dimentional (3D) information about the maxillofacial region for preoperative planning. The use of CBCT to quantify the volume of a bony defect in pre-prosthetic surgery can be very useful in determining the quantity of graft to be harvested, which aids in the selection of the appropriate donor site. It has been shown that CBCT technology can accurately measure periodontal defects as well as the depth and diameter of artificially created defects using Cavalieri's principle and volume extrapolation (8-12).

No attempt has been made in the literature to compare different techniques of measuring bony defects using CBCT technology to gauge whether one method provides more accurate measurements. In-Vivo Dental Image Analysis Software (Anatomage Inc, San Jose, CA) has developed a program that approximates the volume of a bony defect, referred to as the 3D reconstruction tool. An alternative method to measure volume was proposed by Italian mathematician Bonaventura Cavalieri, and is referred to as Cavalieri's principle. Using this principle, the volume of an arbitrary shaped object or defect can be measured by adding the surface area of each slice of the object of interest on a CBCT cross sectional view, and multiplying by the thickness of each slice. Several studies have incorporated this method to measure volume (8, 12-14). Figure 1 demonstrates the method and its application in volume estimation of an organ, a commonly applied stereological method in biomedical research (15).

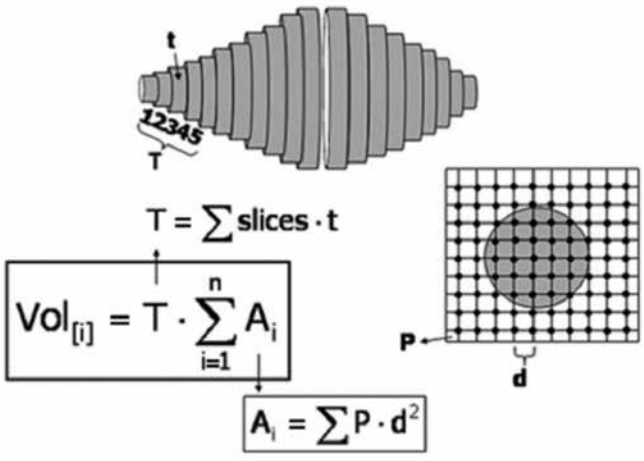

Figure 1. *Cavalieri's principle. (∑ cross-sectional area of each slice) X S, where S is the thickness of each slice on the CBCT, given that S remains constant. (from: Mandarim-de-Lacerda CA. Stereological tools in biomedical research. An Acad Bras Cienc. 2003 Dec;75(4):469-86. Epub 2003 Nov 4).*

The purpose of the current study is to compare the 3D reconstruction technique to Cavalieri's principle in regards to measuring the volume of bony defects in the maxillofacial region from CBCT imaging to determine if one method gives a more accurate representation of the actual bony defect. Furthermore, once the most accurate method for measuring volume is elucidated, we assess the degree of inter-user reliability of the technique. In the light of the findings of the present study, it is anticipated to enable clinicians to use CBCT imaging more accurately to predict the volume of defects in the maxillofacial area, which will aid in the planning of maxillo-mandibular surgery.

Materials and methods

Sample preparation

Nine artificial, adult-sized, foam cortical shell artificial mandibles (Sawbones; Vashon Island, WA, USA) were obtained. A surgical drill was used to create a geometric defect in the buccal aspect of the alveolus in each of these mandibles (Figure 2a).

A standard rectangular defect of the same size was made in the same location on each artificial mandible. A putty material was molded to fill in the defect for each artificial mandible (Figure 2b).

The putty material was then placed into a graduated cylinder filled with a known volume of distilled water. The amount of water that was displaced after placement of the putty was removed from the graduated cylinder and weighed. Hence, the volume of each defect (in cubic centimeters, which was converted to cubic millimeters) was obtained. This volume was recorded as the control volume for the defect on each artificial mandible.

Image acquisition and 3D reconstruction

Once the control volume for each mandible was acquired, CBCTs were obtained with CB Mercuray® (CB Mercuray; Hitachi Medical Corporation, Tokyo, Japan) at the Craniofacial Imaging Center. Artificial mandibles were positioned in the center of a scanning table with the mandibular plane horizontal and the mid-sagittal plane vertical, and imaging was performed using 15 mA, 120, kVp, a 4-inch field of view, and resulting in a voxel size of 0.38 mm. All CBCT imaging data were stored in the DICOM (Digital Imaging and Communication) format and then imported to be viewed using InVivo Dental Image Analysis Software. (InVivo Dental Image Analysis Software-Anatomage Inc; San Jose, CA, USA) (Figure 3).

Two techniques were used to measure the volume of bony defects on the artificial mandibles using CBCT (to minimize inter-user error, all measurements were taken by the same individual, who was trained how to obtain measurements using the two techniques, but was blinded to the purpose of the study):

1. The volumetric Reconstruction tool of In Vivo Dental Image Analysis Software was used, as described, to measure the volume of the defect created in each mandible (Figure 4).

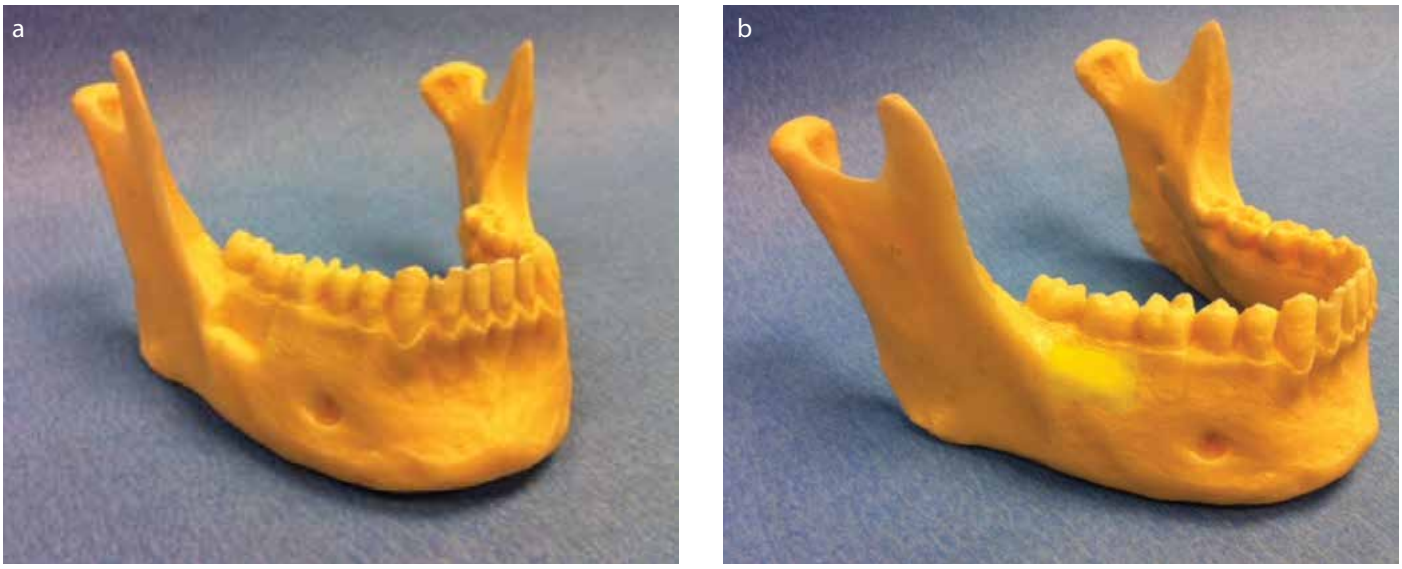

Figure 2. a, b. *On an artificial mandible, a defect has been created on the buccal alveolar aspect with a bur. The defects were made to be clinically proportional in all mandibles (a). Putty is molded into the defect to reconstitute the volume (b).*

Figure 3. *A CBCT image of the defect created in one of the artificial mandibles.*

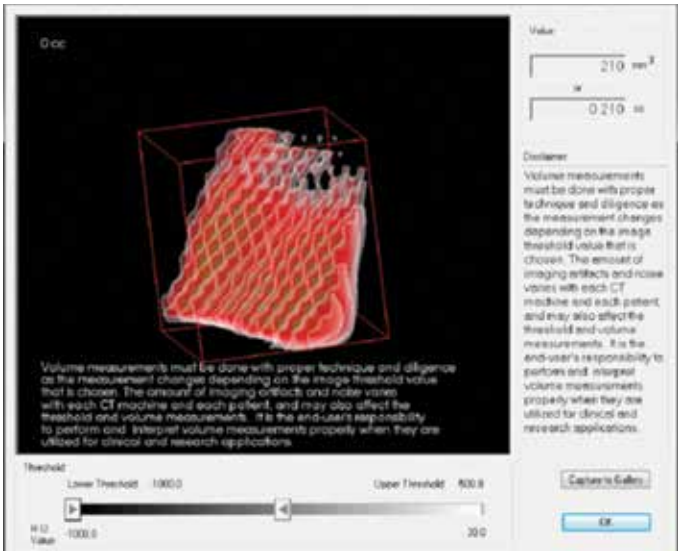

Figure 4. *Using the 3D reconstruction method, the defect being isolated. Once the defect has been completely isolated, the image is inverted, leaving only the volume of the defect. The program measures the volume of the isolated defect.*

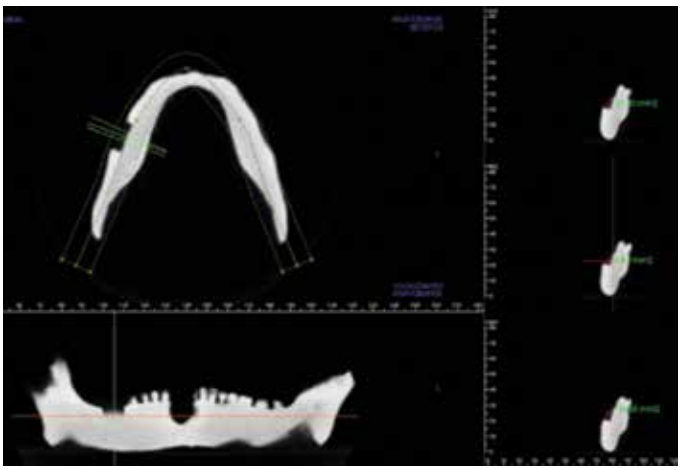

Figure 5. *Application of Cavalieri's principle. The cut for each slice is set at 2 mm. At each cross section a geometric measurement tool is used to measure the area in each defect. The sum of the areas is then multiplied by 2, the total thickness of each slice, to give the volume of the defect.*

After the defect was completely isolated, the program was set to measure the defect at -1000 Hounsfield units to -500 Hounsfield units (HU). This was done to ensure the program measured only the volume of defect filled with air, and not any tissue that was not part of the defect (air can be detected at -1000 HU, and tissues start to be detected at -500 HU) (16).

2. Next, the volume of the bony defect in each artificial mandible was measured by using Cavalieri's principle. All imaging was again opened via In Vivo software. The thickness of slices was selected to be 2 mm. A scroll tool was used to navigate through all the sections with the defect on the artificial mandible, and in each slice, the two dimensional cross sectional area of the defect was measured. The areas were added, and multiplied by the thickness of each slice to obtain the measured volume of the bony defect (Figure 5).

Ethics committee approval or informed consent was not required for this study.

Statistical analysis

The collected data from all groups were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 22.0 (SPSS Inc.; Chicago, IL, USA). Normality and homogeneity of the data were tested using Shapiro-Wilk and Levene's tests, respectively. The standard descriptive methods such as the mean and standard deviation were applied to determine the characteristics of the sample. The analysis of variances (ANOVA) was used to determine differences between control, 3D and Cavalieri groups, for which the results were further compared by Dunnett's post-hoc analysis. Data reliability was assessed by an intraclass correlation analysis. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

Table 1. *Control, 3D reconstruction and Cavalieri's principle volume measurements*

Mandible	Control Volume	3 D	Cavalieri's principle
1	860 mm^3	790 mm^3	727.12 mm^3
2	1060 mm^3	866 mm^3	704.12 mm^3
3	1170 mm^3	1173 mm^3	896.94 mm^3
4	630 mm^3	601 mm^3	628 mm^3
5	830 mm^3	769 mm^3	619.62 mm^3
6	830 mm^3	769 mm^3	708.32 mm^3
7	1140 mm^3	972 mm^3	895.12 mm^3
8	1130 mm^3	1160 mm^3	798.84 mm^3
9	1050 mm^3	902 mm^3	937.18 mm^3
Mean	966.66	889.11	768.36
Standard Error	61.89	62.71	39.66
Standard Deviation	185.67	188.15	118.99
Sample Variance	34475	35403.61	14160.87
		p=0.39	p=0.017

Results

It was hypothesized that both methods of volumetric measurement utilizing CBCT technology would give accurate results, and that there would be no statistical difference between the control volumes and the results obtained by 3D reconstruction and Cavalieri's principle. Table 1 displays results for control defect volumes in the artificial mandibles as compared to the volume of defects obtained by 3D reconstruction method and Cavalieri's principle. The results showed that at a 95% confidence interval, there was no statistical difference between the control group and the 3D volumetric reconstruction group. However, Cavalieri's principle underestimates the actual control volume by 21%, an amount determined to be statistically significant ($p<0.05$) (Table 2, 3).

Discussion

The clinical importance of buccal bone volume around implants has been emphasized by several authors (1-5). Defects in the buccal aspect of the alveolar bone are not only unfavorable for the initial planning and placement of implants, but may also jeopardize clinical success when encountered during the maintenance phase. Bony defects in the maxillo-mandibular complex may arise due to several reasons and the reconstruction of these defects commonly requires consideration to a number of factors for ideal prosthetic rehabilitation with dental implants. These factors include the type of graft being considered for reconstruction, patient preferences, morbidity associated with each surgical procedure, and the amount of graft needed to reconstitute a defect. Knowledge of the volume of bony defect can guide pre-prosthetic surgery by helping evaluate which donor site is appropriate for bone grafting if considering an autogenous graft. Hence, an accurate measurement of the defect to be filled can aid tremendously in pre-operative planning and in justification of appropriate surgical grafting procedures. Two-dimensional techniques are regarded as the mainstays for radiologic evaluations of jaws. However, they provide limited information regarding bone morphology, and are not suitable for

Table 2. *Multiple comparisons*

					95% Confidence Interval	
(I) Technique	(J) Technique	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
3D	Control	-77.556	78.900	.523	-262.92	107.81
Cavalieri	Control	-198.304*	78.900	.035	-383.67	-12.94

*The mean difference is significant at the 0.05 level

[a]Dunnett t-tests treat one group as a control, and compare all other groups against it

Dependent Variable: Volume, Dunnett t (2-sided)[a]

Table 3. *Intraclass correlation coefficient*

		95% Confidence Interval		F Test with True Value 0			
	Intraclass Correlation	Lower Bound	Upper Bound	Value	df1	df2	Sig
Average Measures	.810	.259	.956	11.741	8	16	.000

volumetric assessment of alveolar defects. Though multi-slice CT (MSCT) scan is an established method to assess the bone morphology in implant planning and is commonly regarded as the gold standard, it is associated with 40-60 times more radiation than CBCT systems that have been introduced more recently. In addition to lower dose of exposure, CBCT systems offer several other advantages over a medical CT, including shorter acquisition times, low cost, and adequate image quality of the mineralized tissues (17, 18).

Different studies have utilized various techniques using CBCT to approximate defects, but no study has directly compared these techniques. It was hypothesized in this study that there would be no difference between the control values and the actual measurements obtained for both techniques. No statistical difference was found using only the 3D volumetric reconstruction method. The study showed that, compared to Cavalieri's method of measuring facial bony defects, the 3D reconstruction method was more accurate, although both methods underestimated the actual volume of a maxillofacial defect. One would expect that the mathematically based Cavalieri's principle would be more accurate compared to the 3D volumetric reconstruction method used, however results from Table 1, show the Cavalieri's principle frequently underestimated the actual volume significantly.

It has been shown that jawbone width measurements on dry mandible using CBCT and spiral tomography are reliable, and on average, they are slightly underestimated; a similar trend was seen in this study (16). Anatomic variations seen in the bone itself can be one possible explanation for the consistent underestimation of volumes using both the 3D volumetric reconstruction and Cavalieri's principle. Trabecular pattern in bone may be misinterpreted as air rather than part of the osseous anatomy that potentially may lead to exclusion of such spaces in the calculation of bony volumes, leading to an underestimation of volumes (13). Although anatomic variations and trabecular pattern of bone may lead to underestimation of bony volumes, it did not explain the underestimations recorded in this study. In a previous study by Kayipmaz *et al*. (13), trabecular structure of bone on artificially created "lesion" margins was reported to complicate determination of boundaries of radiographic CBCT sections. In effort to address this issue, defects in this study were created in artificial mandibles that were uniform in internal structure. We believe that the clear internal margins of these defects enabled us to obtain radiographic sections with dimensions similar to those of the actual defects.

Further studies have investigated the dependence of intra-cranial volume (ICV) measurements upon section thickness when using Cavalieri's principle. In a previous study Sahin *et al*. (19) reported lower ICV values with wider section thicknesses and an underestimation of ICV measurements in planimetry evaluations derived from sections greater than 2 mm in thickness and recommended selection of thin sections. Conversely, Gadeberg *et al*. (20) reported overestimation with wider section thickness in MR imaging. Another possible reason for the inaccuracy encountered with Cavalieri's principle in the present study is the assumption that the area of each section measured is the same throughout the 2 mm slice, and this may not be the case. It should, however, be noted that utilizing a section thickness of 2 mm in the present study represents a technique that is reproducible and applicable in the dental office setting, which would otherwise remain impracticable if thinner sections were used.

After comparing the different techniques to actual control volumes, we believe CBCTs remain useful in predicting the volume of bony defect, especially using the 3D reconstruction method, which was found to be a valid and precise method of volume approximation. This study suggests that Cavalieri's principle is less accurate than 3D reconstruction in determining the volume of bony defects, although other studies have successfully used this principle to give an accurate representation of volume (Table 2) (8, 12-14). In a previous study by Smolka *et al*. (8), volumetric measurements of calvarial bone grafts were successfully performed using software based on Cavalieri's principle. Pinsky *et al*. (12) applied a similar technique for determining osseous defect sizes using 3D CBCT and suggested that clinically acceptable accuracy can be obtained with such a technique when performing volumetric analysis of small osseous defects of the human mandible. Moreover, Cavalieri's principle was also used in several other medical fields to assess the volume of lesions or to evaluate the regression of tumors after chemo-radiotherapy (21, 22).

The lack of soft tissue simulation and the relatively small sample size are among the main limitations of this study that need to be taken into consideration when interpreting its findings. Further studies with qualified simulation of both hard and soft tissues on larger sample sizes may provide clinicians with a better understanding of the comparative evaluation of these two techniques. Additionally, Hounsfield's scale, the validity of which remains an issue of debate in CBCT was used in the present study. Although controversial, the authors of the study believe that there is sufficient evidence in the current literature supporting the use of HUs in dentistry, which conveniently provide an insight to the mineral density of the hard tissues of the maxillofacial complex (23, 24).

Conclusion

This study did not reveal statistically significant difference in volumetric analysis performed with 3D CBCT reconstruction and the control values, whereas statistically significant underestimation was observed with the method based on Cavalieri's principle. When our findings are evaluated in consideration of contradictory findings reported in a limited number of previous studies, we believe that further research is required to determine the true efficacy of the techniques applied in this study.

Ethics Committee Approval: Ethics committee approval was not required for this study.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author Contributions: DAB designed the study. SKN, JFT and MPH generated and gathered the data. MAA, FAQ, SKN, JFT and MPH analyzed the data. MAA, FAQ and NY wrote the majority of the original draft. All authors approved the final version of the paper.

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

Financial Disclosure: Dr. Altay has provided consultancy for Checkpoint Surgical LLC. in 2014, and Dr. Baur is a paid consultant for Novartis Pharmaceuticals and Checkpoint Surgical LLC. Other authors declare that they have no competing financial interests.

Türkçe öz: *Alt çene kemiğindeki defektlerin hacimlerinin ölçülmesi amacıyla kullanılan 3 boyutlu rekonstruksiyon ve Cavalieri yöntemlerinin karşılaştırmalı olarak değerlendirilmesi. Amaç: Bu çalışmanın amacı kemik defektlerinin hacimsel tahmininde Cavalieri prensibi ve 3 boyutlu rekonstrüksiyon tekniğinin doğruluklarının karşılaştırılmasıdır. Gereç ve Yöntem: Dokuz adet yapay mandibula üzerinde yaklaşık olarak aynı boyutlarda defektler oluşturulmuştur. Her mandibulaki defektin gerçek hacmi suyun yer değiştirmesi ile ölçülmüş ve kontrol grubu olarak belirlenmiştir. Ardından, her mandibula konik ışınlı bilgisayarlı tomografi (cone-beam computed tomography – CBCT) ile taranmış ve defektlerin hacimsel ölçümleri iki teknik kullanılarak yapılmıştır: Cavalieri prensibi ve 3 boyutlu rekonstrüksiyon. Defektlerin her iki teknikle elde edilen hacimsel ölçümleri, kontrol hacimleri ile karşılaştırılmış ve istatistiksel anlamlılık düzeyi p<0.05 olacak şekilde varyans analizi ile (ANOVA) değerlendirilmiştir. Bulgular: Kontrol, 3 boyutlu rekonstrüksiyon ve Cavalieri'nin prensibi gruplarının ANOVA ile değerlendirilmesi sonucunda gruplar arasında istatistiksel olarak anlamlı bir farklılık bulunamamıştır (p=.058). Kontrol grubu Dunnett'in post-hoc testi ile ileri analize tabi tutulduğunda, Cavalieri'nin prensibi ile yapılan ölçümlerin kontrole kıyasla anlamlı farklılık gösterdiği (p=.035); ancak 3 boyutlu rekonstrüksiyon tekniğinin anlamlılık seviyesine ulaşmadığı görülmüştür (p=.523). Sonuç: Cavalieri prensibi, kontrol hacminin olması gerekenden daha az ölçülmesine neden olmaktadır. Bu prensibinin doğruluğu, 3 boyutlu rekonstrüksiyon tekniğine göre daha düşüktür. Üç boyutlu rekonstrüksiyon metodu, kemik defektlerinin hacimsel ölçümünde daha güvenilir bir tekniktir. Anahtar kelimeler: Konik ışınlı bilgisayarlı tomografi; üç boyutlu görüntüleme; Cavalieri prensibi; defekt; görüntü rekonstrüksiyonu*

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Case report

Management of regional odontodysplasia: a 10-year-follow-up case report and literature review

Mine Koruyucu[1], Duygu Yaman[2], Figen Seymen[1], Korkud Demirel[2], Koray Gençay[1]

The aim of this article was to review the literature and present a case of regional odontodysplasia (ROD) with special emphasis on clinical and radiographic features. A 6-year-old girl was referred to our department with the chief complaint of missing her permanent maxillary left central incisor, lateral incisor, and both of her canines. The gingiva of the involved region was enlarged, fibrous, and tense. Radiographic examination showed abnormal tooth formation and shortened roots. After 3 years of follow up with temporary prosthetic rehabilitation, periodontal surgery was performed. Following forced eruption and levelling, abnormal tooth eruption and root development were observed. ProRoot MTA (Dentsply-Maillefer, Ballaigues, Switzerland) was used for root canal treatment. Intracanal fiber posts selected and access cavities were restored with composite resin. Prosthetic rehabilitation was completed with zirconia ceramic crowns. The time of diagnosis, characteristics of the present/existing symptoms, and functional and esthetic needs of the patient should be considered to determine the optimal treatment modality for ROD.

Keywords: *Odontodysplasia; ghost teeth; tooth eruption; shell teeth; developmental anomaly*

Introduction

Regional odontodysplasia (ROD) is first described by Hitchin (1) in 1934. ROD can also be defined as ghost teeth, nonhereditary amelogenesis imperfecta, odontogenesis imperfecta, and odontogenic dysplasia (2). ROD is a rare and unique dental abnormality that involves enamel, dentin, pulp, and follicle of both primary and permanent dentitions, generally affecting the teeth of one quadrant (3-6).

Diagnosis of this nonhereditary disorder is made by clinical as well as radiographic examination and, occasionally, supported with microscopic examination of the affected teeth (1, 2). An irregular surface contour with pitting and grooves is observed in the affected teeth during clinical examination (2, 5-7). The affected teeth appear hypoplastic and hypocalcified (3). These teeth also show extreme erosion and underlying periapical abscesses, with discoloring to yellow, or even yellowish brown. Radiographs display these teeth with having large pulp spaces and short roots with open apices. Due to lack of contrast between enamel and dentin tissues, the ROD tooth appears ghost-like. Eruption of the affected teeth is delayed or may not occur (1-10). Generally, affected permanent teeth replace affected primary teeth; however, normal permanent teeth may also replace affected primary teeth (5).

There are histological alterations present in ROD. Enamel structure is hypoplastic and hypocalcified, with changing thickness and uneven surfaces. Enamel prisms are randomly distributed, possibly including aprismatic regions with degenerated globular calcifications. Another common feature

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of ROD includes the presence of different degrees of pulpal calcification. Reduction of the dentin layer yields randomly distributed tubules that are lesser in amount. Extensive interglobular and amorphous dentin areas, predentin layer enlargement, and clefts, which might enable the pulp and oral cavity to communicate, were additional characteristics observed. Dense fibrous connective tissue constructs the dental follicle, which might demonstrate calcification areas (2-4, 8, 9, 11). The affected enamel has been detected to have more mineral content than dentin in microradiographic studies (9).

The prevalence of this condition is still not clear since the literature have mainly been based upon case reports (1, 4). Males have been less affected than females. The mandible has been less frequently affected than maxilla (ratio, 1.6:1). The condition is usually unilateral, rarely crosses the midline, and is more common in the anterior dentition. Teeth may be affected in different degrees, even in the same arch. A tooth's inability to erupt has been the most distinctive clinical symptom of ROD (1, 2, 4, 6, 9, 10).

The etiology of ROD is unknown. Various assumptions such as local circulatory disorders, viral infections, teratogenic drugs, neural crest cell defects, vascular defects, irradiation, Rh incompatibility, local trauma, local somatic mutation, hypophosphatasia, hypocalcemia, hyperpyrexia, nutritional deficiency, circulatory disorders, and idiopathic factors have been discussed (1, 2, 4, 9, 11, 12). Systemic anomalies, like facial asymmetry, may be present in some patients (9). In addition, this anomaly has been found to be associated with hemangioma, epidermal nevus, vascular nevi, ectodermal dysplasia, hydrocephalus, hypophosphatasia, and gingival swelling (2, 11). As Courson *et al.* (13) stated, there may be a link between odontodysplastic changes and metalloproteinases (MMPs), besides their inhibitors (TIMPs), in relation to ROD.

Regional odontodysplasia may be a separate syndrome or a symptom of oculodentodigital syndrome or epidermal nevus/Schimmelpenning-Feuerstein-Mims syndrome (7). A differential diagnosis of ROD includes hereditary conditions, such as dentin dysplasia, dentinogenesis imperfecta, amelogenesis imperfecta, oculodentodigital dysplasia, segmental odontomaxillary dysplasia, odonto-onycho-dermal dysplasia, or odontochondrodysplasia (1, 6).

The aim of this paper was to review the literature, present a case of regional odontodysplasia, and to discuss the clinical and radiographic characteristics and treatment of odontodysplasia.

Case report

In 2007, a 6-year-old girl was referred to İstanbul University Faculty of Dentistry Department of Pedodontics with the chief complaint of a missing or unerupted permanent maxillary left central incisor, lateral incisor and both canines.

There was no previous history of dental anomalies in either parent and no similar cases among family members. Her prenatal, natal, and medical history was unremarkable. Family is nonconsanguineous. The mother of the patient had a natural pregnancy and childbirth. The patient's past and medical history were not significant for any systemic abnormalities. When she was 18 months old, the patient fell on the left side of her face causing trauma. No history of previous extraction was seen in

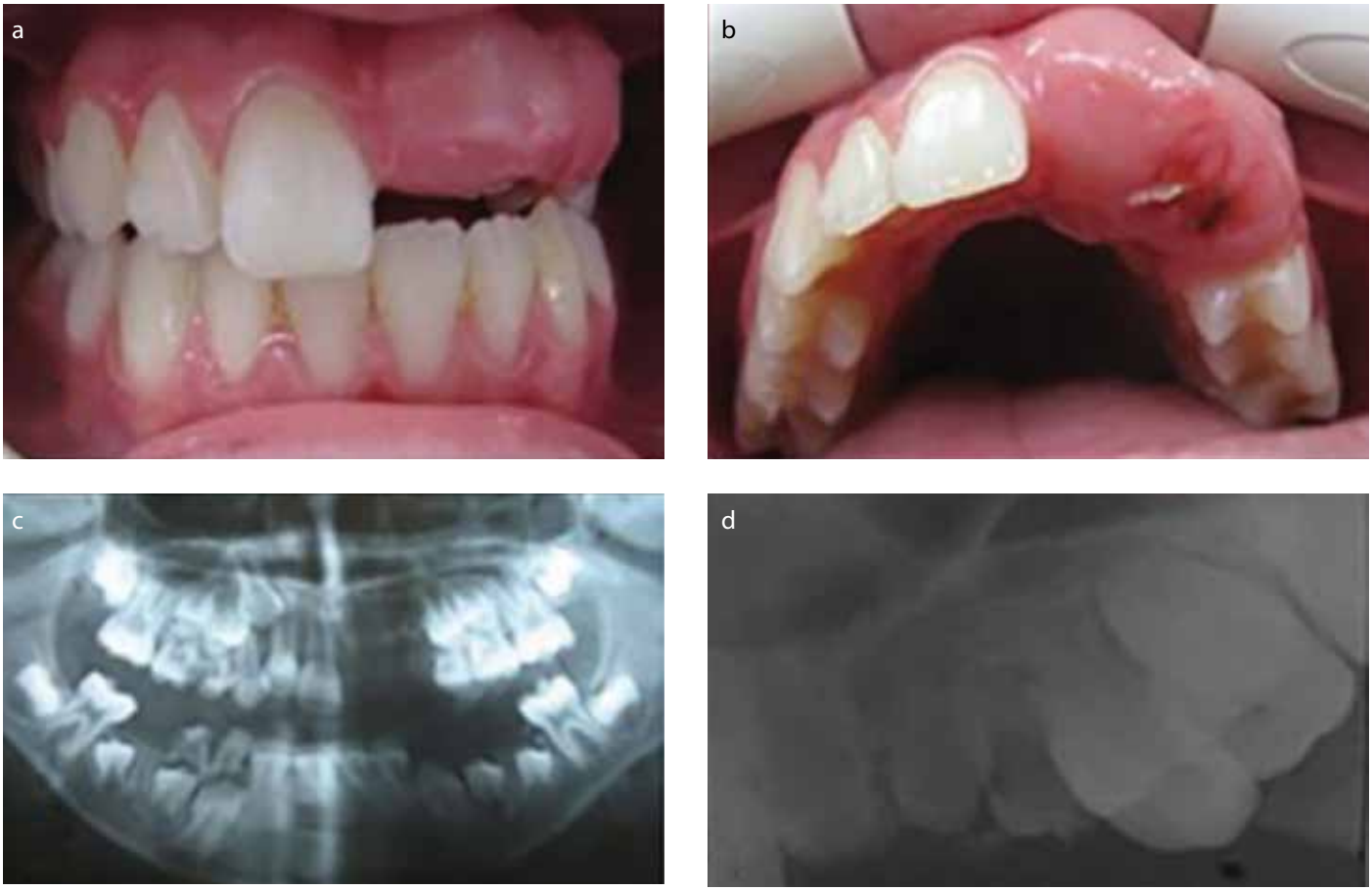

Figure 1. a-d. *(a, b) Intraoral (c, d) radiographic view during the first presentation.*

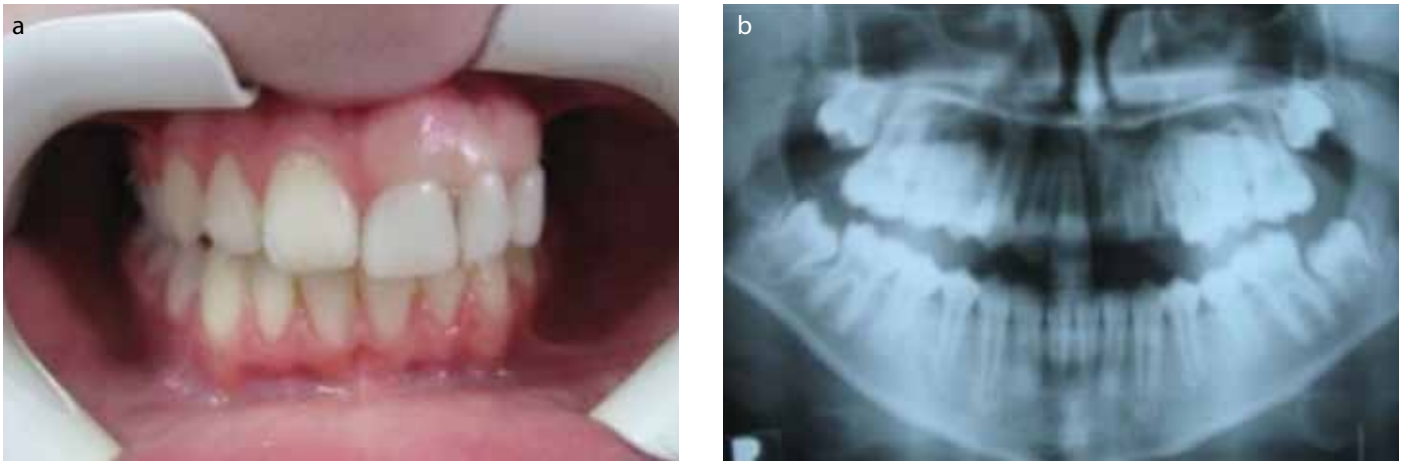

Figure 2. a, b. *(a) Temporary prosthetic rehabilitation (b) 1 year follow-up panoramic radiograph.*

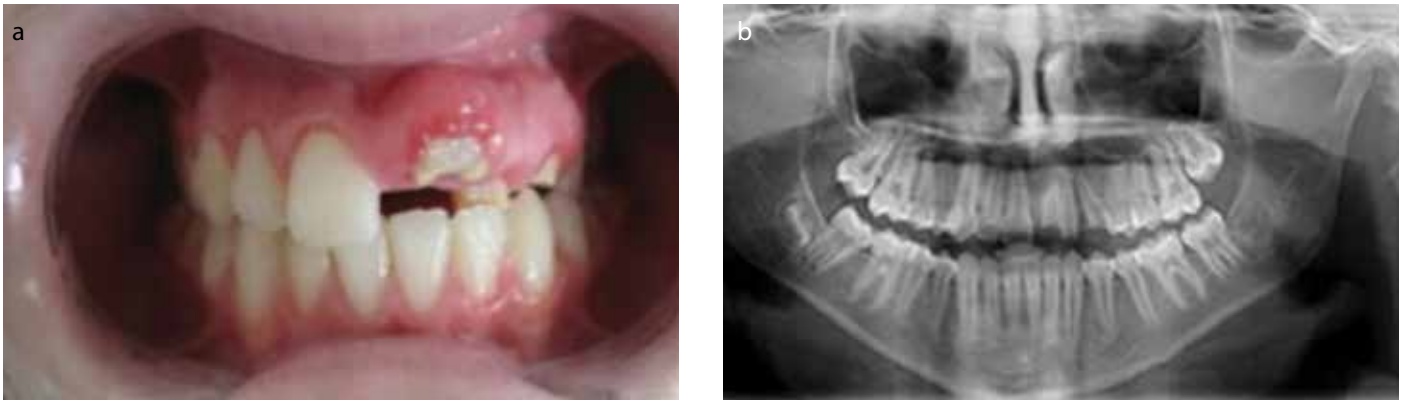

Figure 3. a, b. *(a) Intraoral (b) radiographic view after periodontal surgery.*

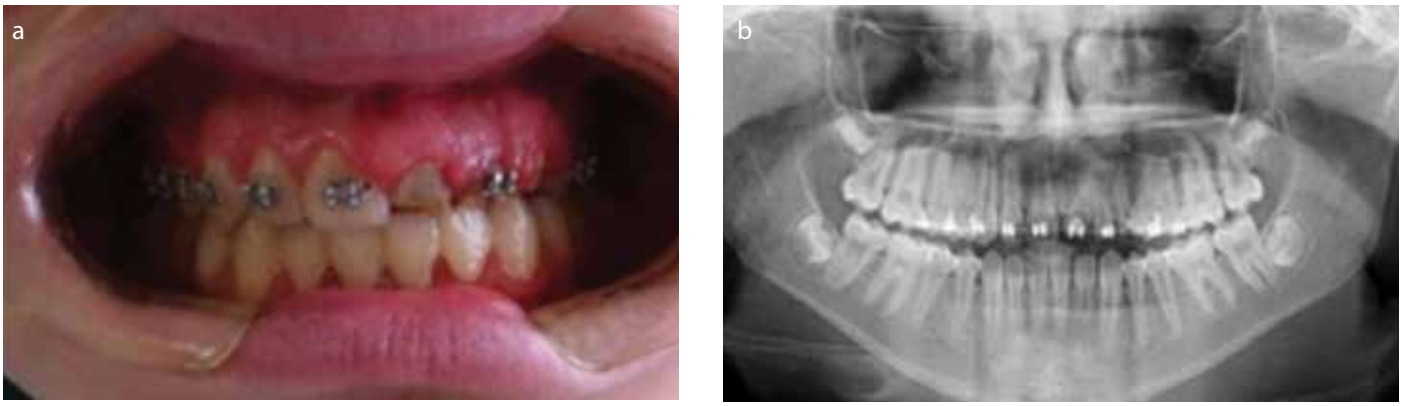

Figure 4. a, b. *(a) Intraoral (b) radiographic view during orthodontic treatment.*

the patient. It was learned from the mother that the patient's primary teeth were normal and exfoliated on time.

The patient showed normal maxillary dentition for her age during intraoral examination. The gingiva was enlarged, fibrous, and tense in the affected area. Permanent maxillary left central, lateral, and both canine teeth were unerupted. No dental abscess was seen clinically. All other teeth in the maxilla and mandible were normal. Radiographically, development of the maxillary left incisors and canine teeth was delayed, and these teeth showed abnormally formed and shortened roots, with a ghostlike appearance due to their reduced radiodensity (Figure 1).

After written informed consent was obtained from family, ROD was diagnosed and without extraction, a temporary prosthetic rehabilitation was applied (Figure 2). In order to keep track of the development of dentition and craniofacial growth, a periodic recall was offered.

The patient had periodontal surgery in the affected teeth after 3 years of temporary prosthetic rehabilitation follow-up. The teeth were misshapen, pitted, hypoplastic, and yellow or yellowish brown (Figure 3).

The teeth (#21, #22, #23) showed abnormal eruption and root development. Orthodontic treatment was planned for tooth positioning. Brackets were applied to maxillary teeth for forced eruption and levelling for a period of 2 years (Figure 4). Periodontal surgery was then applied during levelling procedures.

When the patient was 11 years and 5 months old, root canal treatment was begun due to the apical lesions. A calcium hydroxide paste was applied to the teeth for reinstrumentation and redressing. After the removal of the intracanal dressing af-

Figure 5. a, b. *(a) Intraoral (b) radiographic view after endodontic treatment and post-core application.*

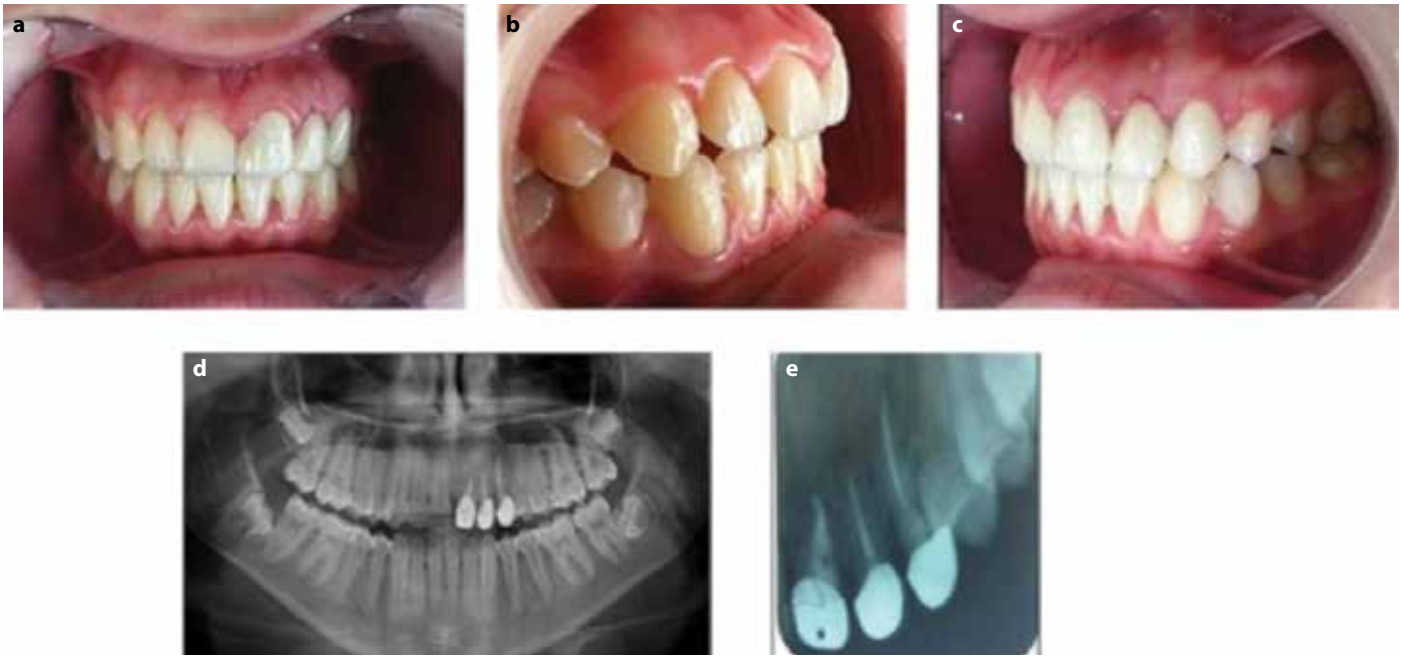

Figure 6. a-e. *(a-c) Intraoral (d, e) radiographic view after zirconium crown in 5 year follow-up.*

ter 1 year, ProRoot MTA (Dentsply-Maillefer, Ballaigues, Switzerland) was used as an apical plug. Intracanal fiber posts were selected considering the diameter of each canal. Composite resin restorations were used for the access cavity as a core (Figure 5). Each tooth was treated with a separate zirconium crown.

At the end of 10 years of treatment, the teeth presented no signs of external root resorption. Crowns compatible with gum and periodontal tissue were normal in color and shape (Figure 6).

Discussion

Approximately 200 cases have been reported in the literature regarding ROD. These articles describe the clinical, radiographic, and histological features of ROD as well as treatment alternatives.

For instance, endodontic approaches are considered conservative, while prosthetic rehabilitation is a less-conservative alternative. A fixed restorative prosthesis might be used in less affected patients, while patients with more severe cases must wait for the placement of implants or the final prosthetic reconstructions. Therefore, in more severe cases, extraction and removable temporary prostheses are more favorable (4, 6).

Canela *et al.* (11) presented a regional odontodysplasia case which occurred in the maxilla of a 10-month-old. After radiographic examination, the diagnosis was confirmed and follow-up went on for 5 years. Canela *et al*. (11) case supports the theory that an early diagnosis is critical to be able to determine the most convenient moment to intervene as well as to follow up and monitor ROD.

Gondim *et al.* (3) reported a case of a 2.5-year-old patient. There was a fistula in the related area of the central incisor apex, which caused damage in the crowns of the right central and lateral incisors. The partially erupted right canine was healthy and hypoplastic. Initially, the maxillary right incisors underwent endodontic treatment, thus a conservative approach was adopted. The maxillary right incisors were then cleared from the radicular remnants. Prophylaxis was used as a treatment for the canine, and professional fluoride was applied topically at monthly follow-ups (3).

Babu *et al.* (1) presented a delayed eruption of teeth in the mandible of a 33-month-old boy. Intraoral examination revealed an adequate maxillary arch with fully erupted primary teeth. All the maxillary teeth appeared normal. Although all the primary teeth were visible in the mandibular arch, they were not fully erupted and seemed to be embedded in abnormal, slightly hyperplastic alveolar mucosa. Furthermore, these teeth revealed abnormal crown morphology with yellowish discoloration and hypoplastic enamel. Radiographically in the mandibular arch, all the primary teeth showed a striking "ghost-like" appearance. The treatment plan for the child was mainly conservative. Maintenance of proper oral hygiene and regular follow-up examinations for monitoring the developing dentition was advised (1).

Mukhopadhyay *et al.* (2) reported a 3½-year-old girl with a chief complaint of noneruption of maxillary teeth. Extraoral examination of the patient showed a bilaterally symmetrical face. On intraoral examination, the patient presented with primary dentition. On the left quadrant of the maxilla, a hypoplastic, partially erupted primary central incisor and the tip of the adjacent lateral incisor were seen. As the condition was asymptomatic, a conservative treatment approach was followed (2).

An 8-year-old male from Turkey presented with a yellowish-brown color of the permanent canine tooth on the right maxilla. There was no indication of gingival swelling or abscess formation in the unerupted maxillary right permanent central incisor. In contrast to the unaffected teeth, in the radiographs, radiodentisity was lowered in the maxillary right deciduous canine tooth and germs of the maxillary right permanent central, lateral, and canine teeth. The extraction of maxillary right permanent central and canine teeth was performed, and an acrylic partial denture was used to rehabilitate the affected quadrant (12).

Gurunathan *et al.* (10) reported an RO case in an 11½-year-old with condition of noneruption of the maxillary right permanent incisor. At 1-2 years old, the child had trauma to the maxillary anterior region. Generally, this tooth abnormality affects teeth in a single quadrant. The posterior teeth are less influenced than central and lateral incisors, along the midline or with possible involvement of each tooth (10). Likewise Ramakrishnan and Menon (14) presented a case with RO in involving single tooth, the maxillary right central incisor, which showed a ghost-like appearance. The patient's mother gave a history of intrusive trauma to the maxillary anterior region at 2 years old, and the history of extraction of the corresponding primary tooth (14).

Thimma Reddy *et al.* (15) reported a case with RO in the entire maxillary right quadrant. A temporary partial acrylic denture was used for rehabilitation, and periodic recalls followed treatment. After extraction was performed, dental implants were used for rehabilitation (15, 16). A similar case was in the right maxillary quadrant and left maxillary central incisor, which were affected by RO. The treatment was planned to follow the eruption process (17).

The progress of the development of teeth are described in many articles involving transplantation. However, orthodontic treatment such as autotransplantation of a mature tooth has been reported in few cases. The reviewed case is a rare one in that regards odontodysplasia as affecting only a single tooth.

Arx *et al.* (5) reported a case with RO in left mandible for a 6-year follow-up. In the treatment plan, the patient was given general anesthesia and underwent surgery to remove the primary molars, the permanent canine, premolars, and first and second molars of the right mandible. Immediately following, autotransplantation of the first premolars of the three other quadrants was done in the right mandible. Despite being affected by RO as well, the incisors that erupted fully were not extracted. This aggressive treatment approach was chosen due to recurrent infections (5).

An uncommon case of RO involving three quadrants of the jaws was reported. An 8-year-old boy had no history of hereditary anomaly in his family. Intraorally, the crowns of the maxillary and mandibular right permanent first molars were hypoplastic. The anterior region of the mandible was affected, and gingival overgrowth was observed. The maxillary and mandibular permanent molars and the mandibular permanent incisors were submitted to endodontic treatment. Prosthetic restoration will be performed after their eruption (18). Badger (19) presented a case with RO in maxillary left second primary molar. All these authors planned the treatment of RO by following long-term follow-up of tooth development.

There is an ongoing controversy regarding the treatment of ROD. ROD cases require constant and multidisciplinary treatment approaches. Many clinicians support the idea of removing the tooth affected by ROD immediately and placing a prosthetic replacement. Restorative procedures are preferred by some other clinicians so as to preserve the affected erupted teeth. In this present case, the affected teeth demonstrated most of the distinctive features of ghost teeth as characterized in the literature, both clinically and radiographically. The treatment was planned according to the age of the case, the development of the teeth, and the aesthetic need. A long-term follow-up after tooth development with orthodontic, endodontic, periodontal, and restorative treatments were performed. The development of root has been found to have been affected by orthodontic treatment forces and periodontal treatment.

Conclusion

There are some factors to consider in determining the best treatment option for ROD; whether it is an early diagnosis, what the present symptoms and signs consist of, what the functional and esthetic needs of the patient are, and which treatment modalities are available. A delay or failure in the process of tooth eruption may be caused by the noneruption of the affected teeth. Additionally, structural defects and the

bacterial infection may often be seen as well. The age of the patient, the degree of anomaly, and the functional and esthetical needs of an individual case should all be taken into consideration when treatment plan is discussed. In addition to all, treatment of ROD requires a multidisciplinary approach.

Informed Consent: Written informed consent was obtained from the parents of the patient and patient who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: MK designed the study. KD and KG generated the data. FS and KD gathered the data. FS, KD and KG analyzed the data. MK and DY wrote the majority of the original draft. All authors approved the final version of the paper.

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Türkçe öz: *Rejyonel odontodisplazi tedavisi: 10 yıllık olgu takibi ve kaynak derlemesi. Bu makalenin amacı bir rejyonel odontodisplazi (ROD) olgusunun klinik ve radyografik özelliklerini sunmak ve bu konu ile ilgili güncel kaynakları derlemektir. 6 yaşındaki kız hasta üst çene sürekli orta kesici, yan kesici ve kanin dişilerindeki sürme problemleri ve bu dişlerin eksikliği şikayetleri ile kliniğimize başvurmuştur. Klinik muayenede etkilenen bölgede dişetinin büyümüş, fibröz ve gergin olduğu izlenmiştir. Radyografik muayenede kısa köklü anormal şekilli dişler izlenmiştir. Hasta geçici protez ile 3 yıl takip edildikten sonra periodontal cerrahi uygulanmıştır. Dişlerin sürdürülmesi ve ortodontik olarak sıralanmasını takiben anormal diş sürmesi ve anormal kök gelişimi izlenmiştir. ProRoot MTA (Dentsply-Maillefer, Ballaigues, Switzerland) materyali ile kök kanal tedavisi sonrası fiber post seçilmiş ve zirkonyum seramik kuronlar uygulanmıştır. ROD olgularında en uygun tedavi yaklaşımını seçmek için tanı zamanı, mevcut belirtiler ve hastanın işlevsel ya da estetik gereksinimleri değerlendirilmelidir. Anahtar kelimeler: Rejyonel odontodisplazi; hayalet dişler; diş sürmesi; Shell dişler; gelişimsel anomali*

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