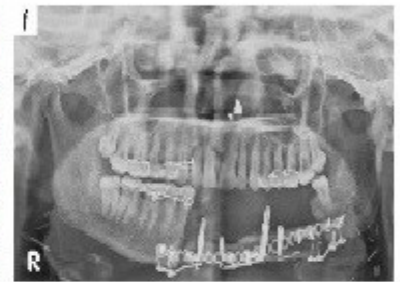
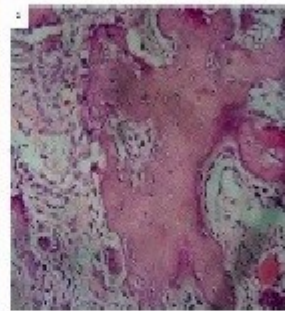
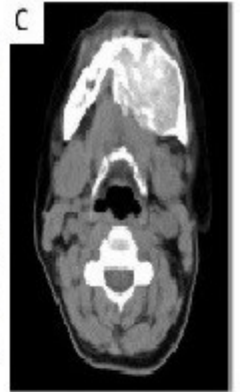




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The Effectiveness of Continuous Versus Sequential Chelation in the Removal of Smear Layer and Their Influence On Push-Out Bond Strength of Bio-C Sealer (An in vitro study)

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ABSTRACT

Objectives: Successful endodontic treatment outcome requires effective shaping and cleaning of root canals. This study aims to evaluate the smear layer removal after continuous chelation (CC) (NaOCl\HEDP) and sequential chelation (SC) (NaOCl\EDTA) and their influence on the push-out bond strength (POBS) of Bio-C sealer.

Material and Methods: Palatal roots of the maxillary first molar (n=72) were divided into four groups (n=18) as follows: 3% NaOCl, SC: 3% NaOCl followed by 17% EDTA, CC: 3% NaOCl \9% HEDP and Distilled water. Thirty-two roots (n=8/group) were split longitudinally for smear layer evaluation using SEM. Forty roots were obturated with Guttapercha and Bio-C sealer using a single cone technique. Three sections were taken horizontally from the coronal, middle, and apical third (1.5±0.1 mm thickness) for the push-out test using a universal testing machine. The Kruskal-Wallis and Mann-Whitney tests were used to analyze the SEM data, while the One-way analysis of variance (ANOVA) test and the Tukey test were used to analyze POBS data. Z test to compare failure mode.

Results: There was no difference between SC and CC in the smear layer removal at all thirds (p>0.05). The POBS in CC was significantly higher than SC in all thirds (p<0.05). Failure mode distribution was 53.3% mixed, followed by 26.6% adhesive and 20% cohesive.

Conclusions: NaOCl\HEDP was as effective as NaOCl\EDTA in removing the smear layer. Neither of the two protocols could eliminate the smear layer completely. The bonding strength of Bio-C sealer was optimized by using NaOCl\HEDP combination.

Key words: Bio-C sealer, dual rinse HEDP, EDTA, push-out, smear layer.

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Introduction

The goal of a root canal treatment is three-dimensional obturation with a complete seal of the root canal system.¹ Root canal irrigation is the most important factor in the healing of periapical tissues.² Root canal irrigation is used to eliminate the smear layer that contains organic and inorganic debris.³ Endodontic instruments during the shaping procedure causes smear layer to form and this layer may affect the success of endodontic therapy.^{4,5} By removing the smear layer, the root canal walls may be cleaned and disinfected more thoroughly, and the root canal filling materials can adapt more effectively.⁶

Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are often used to clean the root canal and remove the smear layer.⁷ Because NaOCl and EDTA cannot be used together; this rinse requires two separate irrigations known as "sequential chelation" Still, it has significant drawbacks, including that active chlorine is rapidly eliminated when NaOCl contacts EDTA.⁸ In addition, extended EDTA exposure to root dentin diminished flexure strength of dentin.⁹

Etidronic acid or ("1- hydroxyethane-1, 1-diphosphonic acid") (HEDP or less commonly HEBP) is nitrogen-free biphosphonate weak chelator that can be mixed with NaOCl to create a single irrigant solution during mechanical preparation and as a final irrigation and eliminating sequential rinses with chemically incompatible solutions. Etidronate is a salt of etidronic acid where cations are linked to the anion of HEDP (usually Disodium etidronate "Na₂HEDP" or Tetrasodium etidronate "Na₄HEDP").¹⁰

Zehnder *et al.*¹⁰ was the first investigator who used HEDP for smear layer removal. HEDP\NaOCl mixture was found to minimize smear layer production during instrumentation, rather than removing an existing smear layer, hence the term "continuous chelation." was used.¹¹ Based on this chemistry, Dual Rinse HEDP (Medcem, GmbH, Weinfelden, Switzerland) is a clinically certified product. It comes in a capsule (0.9 g etidronate), which must be mixed with 10 mL of sodium hypochlorite solution.¹²

Keeping the root canal's integrity and seal is dependent greatly on the endodontic sealers' ability to adhere to dentin.^{13,14} Bio-C sealer (Angelus, Londrina, PR, Brazil) is a newly introduced hydraulic tricalcium silicate sealer. Manufacturers stated that it has a short setting time, is effective at sealing the complex root canal system, and maintains its volume.¹⁵

Little information in the literature about the impact of Dual Rinse HEDP irrigation solution on smear layer removal and the Push-out bond strength (POBS) of calcium silicate-based sealers. No studies have been conducted to date to assess the effect of HEDP on the POBS of Bio-C sealer to root canal dentin. Thus, this study aimed to compare the influence of sequential chelation (3% NaOCl followed by 17% EDTA) and continuous chelation (3% NaOCl/9% HEDP) on the smear layer removal and its influence on POBS of Bio-C sealer at the different thirds of the root canal. The null hypothesis stated that there is no difference in smear layer removal after irrigation with NaOCl/HEDP and NaOCl/EDTA. Also, there is no difference in POBS of Bio-C sealer in root canals irrigated with NaOCl/HEDP and NaOCl/EDTA.

Material and Methods

The preferred reporting Items for Laboratory studies in Endodontics (PRILE) 2021 recommendations were followed in writing the study's manuscript.¹⁶ The PRILE 2021 flowchart summarizes the important aspects of this study (Appendix 1). The materials utilized in this study are described in Table 1.

The sample size calculation was performed using G*Power 3.1.9.4 (Heinrich Heine University, Düsseldorf, Germany) based on the results from previous studies^{17,18} with an effect size of 0.8, power 0.95, α error 0.05, thus requiring sixteen teeth for each group. Eighteen teeth were assigned for each group.

Ethical approval from The Research Ethics Committee of the College of Dentistry, University of Baghdad (Project No. 511522, Ref. No.511) was gained for the use of extracted human teeth. Seventy-two extracted human maxillary first molars were included. Each tooth should have a straight palatal root, a round form canal, the length of palatal root (12 mm), and without cracks, external root resorption, or fracture. Calcified debris on the teeth were removed by using ultrasonic scalers. Then, the teeth were immersed in a thymol solution 1% (sigma –Aldrich, steinheim, Germany) for 48 hours for disinfection and stored in distilled water up to the experiment time. The palatal roots were sectioned and a K-file size 10 (Dentsply Maillefer, Ballaigues, Switzerland) was inserted in each root until the file was observable through the apex, and the working length was established by subtracting 1 mm from this measurement (11mm).

The samples were randomly divided using the website (<https://www.randomizer.org/s>) based on irrigation protocols into four groups (n=18):

I. Positive control “NaOCl”: 2 mL 3% NaOCl for 1 minute after each instrument, as a final rinse 5 mL 3%

NaOCl for 1 minute. Then, 5 mL of distilled water for 1 minute.

II. Sequential chelation “NaOCl\EDTA”: 2mL 3% NaOCl for 1 minute after each instrument, as a final rinse 5 mL 17% EDTA for 1 minute. Then, 5 mL of distilled water for 1 minute.

III. Continuous chelation “NaOCl\ HEDP”: 2mL 3% NaOCl/9%HEDP for 1 minute after each instrument, as a final rinse 5 mL of 3% NaOCl/9% HEDP for 1-minute. Then, 5 mL of distilled water for 1 minute.

IV. Negative control “Distilled water”: 2mL distilled water for 1 minute after each instrument and a final rinse of 5mL distilled water for 1 minute.

Immediately before instrumentation, the NaOCl/HEDP mixture was prepared by mixing one capsule (0.9 g etidronate powder) with 10 mL of the 3% NaOCl. ProTaper NEXT system (Dentsply Maillefer, Ballaigues, Switzerland) was used for the canals' instrumentation up to size X3 (30\07), and an X-smart plus Endo-motor (Dentsply Maillefer, Ballaigues, Switzerland) was used with a rotation speed of 300 RPM and 200 gcm torque. A 30-gauge needle (United dental group, China) was used for the irrigation, which was inserted 2 mm shorter than the working length. All the procedures were performed by single operator.

SEM examination

Eight specimens from each group (32 samples) were chosen for SEM examination. Then, using a diamond disc and water irrigation, longitudinal grooves were created on the buccal and palatal surfaces. To prevent contamination during the splitting procedure, an X3 master cone was passively introduced into the root canals.

The roots were separated into two halves using a blade n° 15 and a hammer. The specimens were sputter coated with gold and analyzed by scanning electron microscope (Axia Chemisem, Thermo Scientific Fisher, USA, 2021) in the center of the apical, middle, and coronal of the canal at (2.5, 6.5, and 10.5 mm from the apex respectively) at 1500x.

Horizontal marks were made at the middle of the coronal, mid-root, and apical sections on the cut/split dentine surface outside the root canal, using a permanent marker. This was to objectively locate the center of each sections when examined under the SEM.

Photomicrographs were scored by two calibrated evaluators according to Hülsmann *et al.*¹⁹ criteria as follows: 1= indicated that the dentinal tubules were entirely open with no smear layer, 2= indicated that more than 50% of the dentinal tubules were open, 3= indicated that less than 50% of the dentinal tubules were open, and 4= indicated that more than 75% of the dentinal tubules were covered by the smear layer.

Canal filling

The remaining 40 roots (n=10/group) were obturated using the single cone obturation method using Guttapercha and Bio-C sealer (Angelus, Londrina, PR, Brazil). The master cone was inserted into the canal after

the sealer had been injected at 4mm from the apex. Radiographs with mesiodistal and buccolingual directions were taken to verify correct root canal obturation without any voids. Then, all the teeth were kept with gauze moistened in Phosphate-Buffered Saline (Chemical point, Egham, United Kingdom) at 37°C for one week for the complete set of sealer.

Push-out test

Each root was inserted in clear cold cure acrylic (Duralay; Reliance Dental, Alsip, IL, USA) and sectioned perpendicular to the long axis using low speed saw (Isomet; Buhler, Ltd Lake Bluff, NY) with a diamond disk (0.5mm) under continuous water cooling to gain three slices of (1.5 ±0.1mm) thickness for each root third at the following distance from the apex (2-3.5, 6-7.5 and 10-11.5mm) respectively. The thickness of each slice was verified using a digital caliper.

A digital microscope (Q-scope, Netherlands) captured images of each slice's apical and coronal sides. Push-out test was performed using a universal testing machine (Tinius Olsen, United Kingdom) in the apico-coronal direction at a crosshead speed of 0.5 mm/min. The area calculation was performed using the formula:¹⁸

$$\text{Area (mm}^2\text{)} = D1 + D2 \sqrt{2} \times \pi \times h$$

D1, and D2 represent the largest and the smallest canal diameter, respectively, π is the constant 3.14, and h represents the root slice thickness. Three plunger sizes (0.7, 0.5, and 0.4 mm) were used calculated as 90% of canal diameter in the apical aspect of each root slice and the plungers were provided complete coverage over the GP without touching the canal walls and sealer.

The following equation was used to determine the POBS in megapascals and the maximal force (F) in Newtons: POBS (Mpa) = Force (N) / surface area (mm²). Failure mode analysis was performed using a digital microscope at 60 X magnification. The failure mode was categorized into the following types: Adhesive failure, Cohesive failure, and Mixed failure.²⁰

Statistical analysis

The SPSS software version 26 (SPSS Inc., Chicago, IL, USA) was used for data analysis, with a significance level of ($p \leq 0.05$). Statistical tests included: The Kruskal-Wallis, Mann-Whitney U, Kappa, ANOVA, and Tukey post hoc tests. Z test to compare the failure mode.

Results

SEM examination

The Kappa test value was (0.89), indicating high agreement among the observers. The results of the smear layer scoring, including median and mean rank, are

presented in [Table 2]. Representative SEM photomicrographs of all thirds are presented in [Figure 1].

There was no significant difference in smear layer scores between all thirds in the NaOCl and distilled water groups ($P > 0.05$), whereas there was a significant difference in smear layer scores between thirds in the experimental groups ($P < 0.05$). In the NaOCl \ EDTA group, the apical third showed a significantly higher mean rank of smear layer scoring than the coronal third ($P < 0.05$). while in the NaOCl \ HEDP group, the apical third showed a significantly lower mean rank than the coronal third ($P < 0.05$), as shown in [Table 2].

There was a significant difference in smear layer scores between the groups at each third ($P < 0.05$). There was no significant difference between NaOCl \ EDTA and NaOCl \ HEDP groups in the mean ranks of the smear layer scores at all thirds ($P > 0.05$). Both NaOCl \ EDTA and NaOCl \ HEDP groups showed significantly lower mean ranks of smear layer scores compared to the control groups ($P < 0.05$), as shown in [Table 2].

Push-out test

The mean values and standard deviation of POBS (MPa) are summarized in [Table 3]. NaOCl \ HEDP group has the highest mean values at all thirds, followed by the NaOCl \ EDTA group. There was a statistically significant difference in POBS among the groups in all thirds ($P < 0.05$). The NaOCl \ HEDP group showed significantly higher POBS than the NaOCl \ EDTA and control groups at all thirds ($P < 0.05$). NaOCl \ EDTA group showed significantly higher POBS than the distilled water group at all thirds ($P < 0.05$), however, there was no difference in POBS between NaOCl \ EDTA and NaOCl groups in all thirds ($P > 0.05$).

There was a significant difference in POBS mean values among thirds in all groups ($P < 0.05$). In all groups, the coronal third showed significantly higher POBS as compared to the apical third ($P < 0.05$), while there was no difference in POBS between the middle and apical thirds ($P > 0.05$).

The distribution of failure mode is illustrated in [Figure 2]. The percentages of failure mode were 53.3% mixed, followed by 26.6% adhesive and 20% cohesive. An example of each failure mode is illustrated in [Figure 3]. Z-test revealed that there was a significantly higher cohesive failure in the NaOCl \ HEDP group compared to NaOCl \ EDTA ($z = -2.10$, $p = 0.034$), both NaOCl and distilled water groups didn't exhibit cohesive failure. Also, there was significantly higher mixed failure in the NaOCl \ EDTA group compared to NaOCl \ HEDP and distilled water ($z = -2.10$, $p = 0.034$) and ($z = -2.6$, $p = 0.009$), respectively and no difference to the NaOCl group ($z = -1.6$, $p = 0.1$). No significant difference was observed in the adhesive mode of failure between NaOCl and distilled water groups ($z = 1.03$, $p = 0.29$).

RATIONALE/JUSTIFICATION

A sequential chelation protocol of sodium hypochlorite followed by EDTA is recommended for the efficient removal of smear layer. According to the literature, this protocol has many disadvantages and negative effect on dentin. Continuous chelation by using soft chelator HEDP combined with sodium hypochlorite may exert minimal detrimental effect on dentin. There is minimal evidence concerning the effects of Dual rinse HEDP irrigation solution on smear layer removal and on the bond strength of calcium silicate based sealers. Up to now, no studies have evaluated the effect of continuous chelation by Dual rinse HEDP on POBS of bio c sealer to root canal dentin.

AIM/HYPOTHESIS

The aim of this study was to compare and assess the influence of sequential chelation protocol (3% NaOCl followed by 17% EDTA) and continuous chelation (3% NaOCl/9% HEDP) on the removal of the smear layer from the instrumented root canals and its influence on POBS of Bio c sealer at the different thirds of the root canal.

ETHICAL APPROVAL

Ethical approval was obtained for the collection of extracted human teeth in this study from The Research Ethics Committee of the College of Dentistry, University of Baghdad (Ref No. 511).

SAMPLES

72 freshly extracted human maxillary first molars were used. The teeth were selected according to the following criteria: Straight palatal root, Round shape canal, Palatal root length at least 12 mm and maximum apical diameter of ISO size #15, Patent and centrally located apical foramen and without cracks, external root resorption or fracture.

EXPERIMENTAL AND CONTROL GROUPS, INCLUDE INDEPENDENT VARIABLES

- I. Positive control (NaOCl): (n=18) 2 mL 3% NaOCl for 1 minute after each instrument change, 5 mL 3% NaOCl for 1-minute and final rinse of 5 mL distilled water for 1 minute.
- II. Sequential chelation (NaOCl\EDTA):(n=18) 2mL 3% NaOCl for 1 minute after each instrument change, 5 mL 17% EDTA for 1-minute and final rinse of 5 mL distilled water for 1 minute.
- III. Continuous chelation (NaOCl\ HEDP)(n=18): 2mL 3% NaOCl/9%HEDP for 1 minute after each instrument change, 5 mL 3% NaOCl/9% HEDP for 1-minute and final rinse of 5 mL distilled water for 1 minute.
- IV. Negative control (Distilled water): (n=18) 2mL distilled water for 1 minute after each instrument change and final rinse of 5mL distilled water for 1 minute.

OUTCOME(S) ASSESSED, INCLUDE DEPENDENT VARIABLES AND TYPE

- I. smear layer removal(ordinal variable)
- II. push-out bond strength values (continuous variable)
- III. mode of bond failure(nominal variable).

METHOD USED TO ASSESS THE OUTCOME (S) AND WHO ASSESSED THE OUTCOME(S)

Smear layer removal were examined using Scanning Electron Microscope and the photomicrographs at 1500 x magnification were scored independently by two blinded observers. The push-out bond strength were tested using universal testing machine at cross head speed 0.5mm/min. The mode of failure was assessed using digital microscope at 60 x magnification

RESULTS

There was no difference between SC and CC in removing the smear layer at all thirds ($p > 0.05$). The bond strength in the coronal third was higher than that in the middle and apical ($p < 0.05$). The highest mean of push-out bond strength was recorded in the CC (5.175 ± 0.93 Mpa), while the lowest mean was found with distilled water (0.574 ± 0.28 Mpa). The bond strength in NaOCl\HEDP group was significantly higher than NaOCl\EDTA at all thirds.

CONCLUSION(S)

The ability of NaOCl\ HEDP to remove the smear layer was as effective as NaOCl\EDTA and neither of the two protocols could render all root canals free of smear layer. HEDP\NaOCl mixture optimized the bond strength of Bio c sealer to dentin as compared with NaOCl\EDTA. Continuous chelation protocol may be considered an alternative to the commonly used irrigation with NaOCl\EDTA.

CONFLICT OF INTEREST

The authors deny any conflicts of interest related to this study.

Figure 1. PRILE 2021 Flowchart

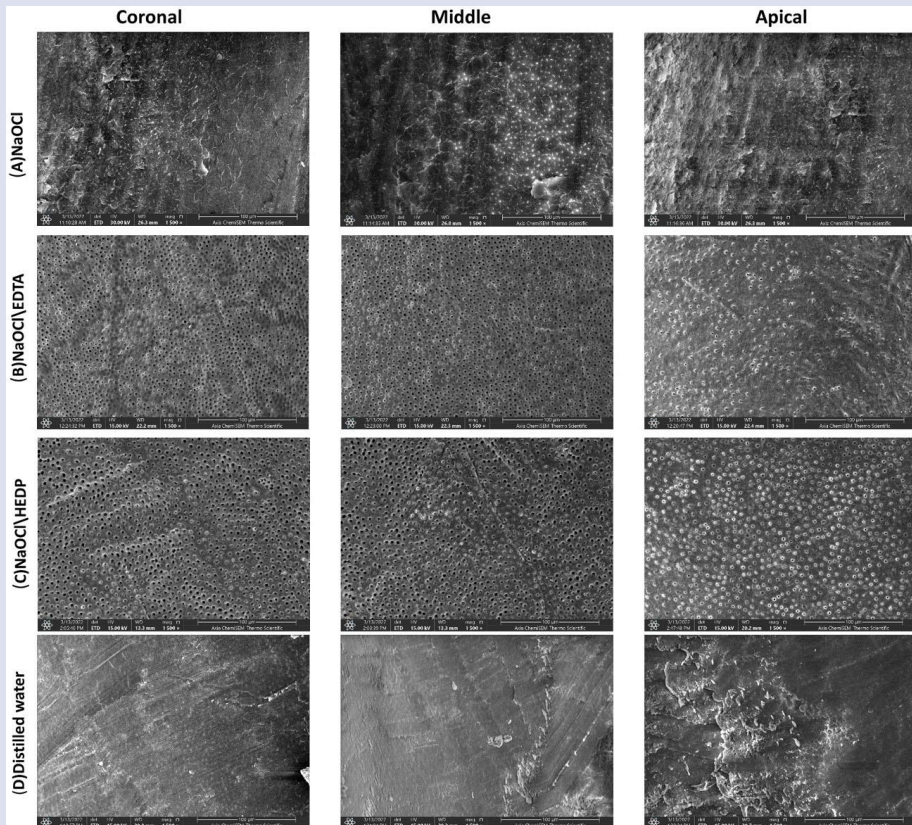


Figure 1. Scanning electron microscope photomicrographs at 1500x showing; root specimens irrigated with NaOCl (A), NaOCl \ EDTA (B), NaOCl \ HEDP (C), and Distilled water (D) at different thirds of root canal (Coronal, middle, and apical).

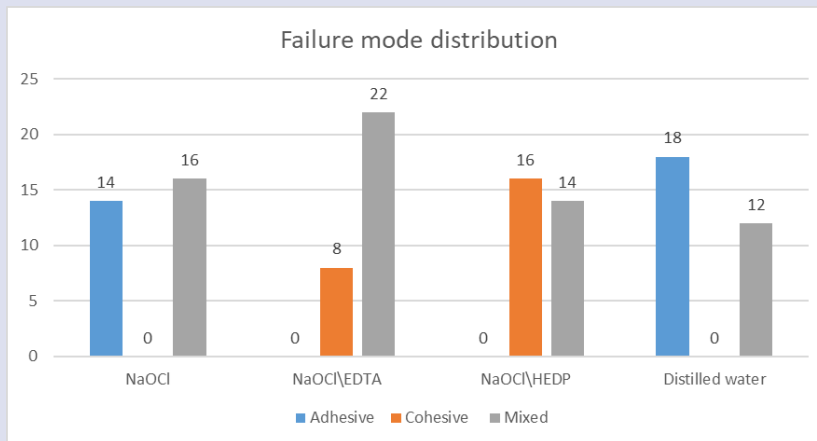


Figure 2. Bar chart illustrating the failure mode distribution.

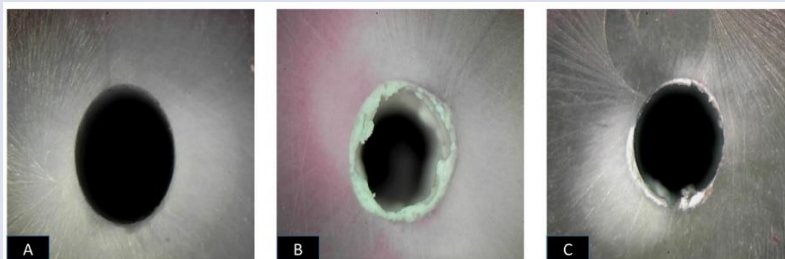


Figure 3. Images were obtained by digital microscope at 60 x magnification for the examination of failure mode, (A) adhesive failure, (B) cohesive failure, and (C) mixed failure.

Table 1. Materials' composition, manufacturers, and Lot numbers.

Materials	Composition	Manufacturer	Lot numbers
9% HEDP (Dual Rinse)	1-hydroxyethane 1,1-diphosphonic acid (etidronate powder)	(Medcem, GmbH, Weinfelden, Switzerland)	DR210419
Bio-C Sealer	Calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, and dispersing agents.	(Angelus, Londrina, Parana, Brazil)	60123
NaOCl 3%	Ionizes in water into Na ⁺ and OCl ⁻	(Modern medical equipment LLC, Dubai, UAE)	PK2122464
EDTA 17%	Disodium edetate (ethylene Diamine Tetra acetic acid 17%), water, and excipients.	(Cerkamed, Stalowa wola, Poland)	1408201
PBS (7.4 ±0.2 pH)	Sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate	(Chemical point, Egham, United Kingdom)	9066

NaOCl: Sodium hypochlorite; OCl⁻: hypochlorite ion; Na⁺: sodium; EDTA: ethylene Diamine Tetra acetic acid; PBS: Phosphate Buffered Saline.

Table 2. Smear layer (median, mean rank) of all groups in the coronal, middle, and apical thirds of the root canal.

Groups	Root canal thirds					
	Coronal		Middle		Apical	
	Median	Mean rank	Median	Mean rank	Median	Mean rank
NaOCl	4.00	24.00 ^{a,b}	4.00	24.50 ^{a,b}	4.00	22.69 ^{a,b}
NaOCl\EDTA	2.00	7.50 ^{a,c,A}	2.00	8.18 ^{a,c}	3.00	10.88 ^{a,c,A}
NaOCl\HEDP	2.00	9.50 ^{b,d,A}	2.00	8.88 ^{b,d}	3.00	8.00 ^{b,d,A}
Distilled water	4.00	25.00 ^{c,d}	4.00	24.50 ^{c,d}	4.00	24.50 ^{c,d}

NaOCl: sodium hypochlorite; EDTA: ethylene Diamine Tetra acetic acid; HEDP: 1-hydroxyethane 1,1-diphosphonic acid.

Identical superscript uppercase letters represent a statistically significant difference between thirds in each row (in each group). Identical superscript lowercase letters represent a statistically significant difference between groups in each column.

Table 3. Means ± SD of push-out bond strength values (Mpa) in the study groups.

Group	Coronal	Middle	Apical
NaOCl	1.911±0.552 ^{a,b,A,B}	1.264±0.646 ^{a,B}	0.865±0.410 ^{a,A}
NaOCl\EDTA	2.361±0.963 ^{c,d,A}	1.813±0.695 ^{b,c}	1.376±0.692 ^{b,A}
NaOCl\HEDP	5.175±0.932 ^{a,c,e,A,B}	3.519±1.113 ^{a,b,d,B}	2.832±0.906 ^{a,b,c,A}
Distilled water	0.995±0.399 ^{b,d,e,A,B}	0.636±0.271 ^{a,b,c,d,B}	0.574±0.281 ^{b,c,A}

SD: standard deviation; MPa: MegaPascal; NaOCl: sodium hypochlorite; EDTA: ethylene Diamine Tetra acetic acid; HEDP: 1-hydroxyethane 1,1-diphosphonic acid. Identical superscript uppercase letters represent a statistically significant difference among thirds within each group in each row. Identical superscript lowercase letters represent a statistically significant difference between relevant groups in each column.

Discussion

This study aims to compare the effectiveness of CC protocol by a mixture of NaOCl\ HEDP to SC by NaOCl\EDTA on smear layer removal from dentinal tubules and their influence on POBS of Bio-c sealer. In this study, both protocols have comparable effectiveness in removing the smear layer at all thirds. Thus, the first hypothesis was accepted. Whereas, the second hypothesis was rejected because the CC protocol showed significantly higher POBS as compared to SC at all thirds.

Scanning electron microscopy (SEM) is the most widely used method for assessing cleaning efficiency as it aids in the examination of the whole area of the canal using a numeric evaluation score for the smear layer.

The findings of this study revealed that there was no significant difference between sequential chelation (NaOCl\EDTA) and continuous chelation (NaOCl\HEDP) in terms of the smear layer removal. SEM photomicrographs showed that in both groups clean dentin areas could be seen in the coronal third and middle thirds, and most of the dentinal tubules were opened, whereas in the apical third, most dentin areas were covered with a smear layer,

and there were fewer opened dentinal tubules than in the coronal and middle thirds. These results agree with the previous study by Kfir *et al.*¹⁷ which revealed that both protocols have comparable effectiveness in removing the smear layer from dentinal tubules but neither of the two protocols evaluated could completely remove the smear layer from all root canal thirds.

Conversely, it was claimed that HEDP has shown a greater capacity for smear layer removal than EDTA.²¹ Additionally, this may be explained by the fact that HEDP minimizes the formation of the smear layer and lowers the deposition of hard tissue debris during root canal instrumentation.¹²

On the other hand, at neutral pH, Deari *et al.*²² found that HEDP was less effective than EDTA at removing the smear layer. HEDP's higher pH and lower stability constant compared to EDTA explained this finding. Because of the low concentration of HEDP and the short duration of application, Zehnder *et al.*¹⁰ found that 17% EDTA is more effective than 3.5% Na₄ HEDP\0.5% NaOCl over 1 minute in eliminating smear layer.

In this study, root samples irrigated using NaOCl alone showed a statistically significant heavy smear layer at all thirds as compared to the experimental groups. The ineffectiveness of NaOCl alone to remove the smear layer was previously reported²³ and this is because of its limited action that is restricted to the organic constituent of the smear layer. SEM photomicrographs revealed that both NaOCl and distilled water groups showed a significant amount of smear layer in all thirds (coronal, middle, and apical) and nearly all of the dentinal tubules were covered with the smear layer.

Despite the non-statistically significant difference between EDTA and HEDP groups, HEDP removed slightly more smear layer in the apical third than EDTA. This contrasts with the results of the study done by Yadav *et al.*²⁴ who reported the ineffectiveness of etidronic acid in cleaning the apical third of the root canal and this might be because of its weak chelating action.²⁵

In this study, the apical third showed the least smear layer removed compared to the middle and coronal, which was attributed to decreased permeability, and sclerotic nature of the dentin at this region.²⁶ Additionally, vapor lock formation hinders the circulation and action of the irrigating solutions.²⁷

The POBS test is used to assess the bond strengths of root canal sealers to dentin.²⁸ The POBS of Bio-C sealer was significantly higher after irrigation with (NaOCl \ HEDP) as compared to other groups. This result agrees with a previous investigation that reported that pre-conditioning dentin with NaOCl \ HEDP mixture improved the POBS of calcium silicate-based sealers.¹⁸ The better performance of HEDP can be ascribed to a variety of reasons. Firstly, HEDP exhibited no negative effect on the hydration characteristics of calcium silicate types of the cement.²⁹ Secondly, the continuous chelation facilitated the dissolution of organic dentin components, conditioning of the inorganic component, resulting in a reduction of smear layer and smear plugs, enhanced micromechanical retention, and a greater bond strength to root canal dentin.³⁰ Thirdly, HEDP had deeper sealer penetration than (NaOCl/EDTA) and a higher proportion of sealer covering canal walls.³¹ NaOCl \ HEDP may provide better conditioning of root canal walls for root filling materials which in turn enhancing the bond strength of endodontic materials to root canal dentin with favorable prognosis for endodontic treatment.

The POBS in the group of EDTA was significantly lower than that of HEDP at all thirds. This could be linked to the effect of EDTA on apatite formed during the Bio-C sealer setting reaction. After chemomechanical preparation, Lee *et al.*³² found that EDTA residues continued to chelate the ions of calcium that formed from MTA during hydration, preventing the precipitation of the hydrated product and reducing its microhardness. Atmeh *et al.*³³ hypothesized that calcium decrease at the sealer-dentin interface or degradation of the calcium silicate component in the sealer would impede the creation of the "mineral infiltration zone".

Another finding is that POBS decreases in a coronal-apical direction, which agrees with Anju *et al.*³⁴ This is because apical dentin has fewer patent tubules than coronal dentin⁷ and more sclerotic dentin, which is not favorable to sealer infiltration.

The low POBS in (NaOCl) group could be due to NaOCl only removing the organic portion of the smear layer and cannot dissolve the inorganic materials thereby reducing the bonding of Bio-C sealer to the root canal walls. This agrees with previous study³⁵ that reported a decrease in POBS of endodontic sealer following irrigation with NaOCl. Studies have also revealed that NaOCl interacts with calcium silicate cement, which can impact its adhesion.³⁶

The lowest POBS values were in the distilled water group. This agrees with previous studies²⁹ that showed inferior smear layer removal of distilled water, indicating the negative effects of undisturbed smear layer on POBS values by preventing the penetration of sealer inside dentinal tubules.

Regarding the modes of failure, in this study the mixed mode was the predominant one and this finding was in agreement with Falakaloğlu and Gündoğar.³⁷ This related to Bio-C sealer's bioactivity, which encourages hydroxyapatite formation, and its excellent flow rate¹⁵, which enables the sealer's penetration. Similarly, Caceres *et al.*³⁸ discovered that Bio-C sealer demonstrated uniform tubular penetration with few gaps and this may be attributed to its hydrophilic nature, which contains nanometric particles which enables deeper penetration.

The study is limited by in vitro environment. As a result, the obtained results cannot be generalized to clinical scenarios. The effectiveness of chelating irrigants may be affected by agitation devices. Also, digital image analysis can be used to score the smear layer. It takes less time, can measure other important characteristics including density and average dentinal tubule diameter, and can overcome evaluator bias. Furthermore, the applied load was static which is not resemble the dynamic load in the oral cavity, therefore the performance of the material in this in vitro study may differ from the clinical situation.

Conclusions

Continuous chelation did not vary from sequential chelation in smear layer removal. However, neither of the two protocols could eliminate the smear layer completely from root canals. The present study strengthens the fact that the POBS of calcium silicate-based sealers was differentially influenced by the irrigation protocol used. NaOCl \ HEDP protocol improved the bond strength of the Bio-C Sealer.

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Conflicts of Interest Statement

All authors hereby deny any conflicts of interest in connection with this article.

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Peri-Operative Hemodynamic Changes and Anxiety in Patients Undergoing Surgical and LASER Assisted Periodontal Therapies- A Randomised Clinical Trial

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ABSTRACT

Objectives: Hemodynamic changes, such as changes in blood pressure, heart rate, and hypoxia, are observed during surgical procedures and may be related to pre-operative anxiety. As a result, the current study aims to compare the hemodynamic changes, hypoxia, and anxiety in LASER assisted periodontal therapy and surgical periodontal therapy for pocket reduction.

Methods: This single blinded randomized controlled observational clinical trial included 30 patients with Stage II, III and Grade B, C periodontitis randomly allocated to surgical periodontal therapy (SurPT) and LASER assisted periodontal therapy(LAPT) groups. The systolic blood pressure(SBP), diastolic blood pressure(DBP), Pulserate(PR), oxygen saturation (SpO₂) were assessed before injection of local anaesthesia (LA), after LA, during and at the end of procedure. Pre-operative anxiety(POA) was assessed using Amsterdam pre-operative anxiety and information scale(APOAI) before starting the procedure. Statistical analysis was done using student t-test for intergroup and repeated measures of ANOVA for intragroup analysis.

Results: SBP and HR were significantly greater in SurPT group at all-time intervals compared to LAPT group (P<0.05). SpO₂ levels reduced significantly in LAPT group at T₂ (P=0.002) with no significant intergroup differences. POA especially anxiety score was significantly higher in SurPT(P=0.017) group with no difference in information scores (P=0.100).

Conclusions: Greater pre-operative anxiety was observed in SurPT group and significant increase in SBP, HR at all-time intervals compared to LAPT.

Key words: Blood pressure, dental anxiety, heart rate, laser, periodontitis, spO₂.

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Introduction

Dental anxiety and dental fear are both severe negative emotions connected with dental treatments, and the terms are frequently used interchangeably in the dental literature.¹ Dental anxiety, according to Klingberg and Broberg, is the fear that something bad may happen as a result of dental therapy or certain components of dental treatment.² Patients' compliance may be hampered by their fear of dental treatment, resulting in worsening dental, periodontal, and overall health.

Dental anxiety is the fifth most common cause of anxiety, according to Agras *et al.*³ It is an emotional state brought on by an unknown threatening stimulus. Dental anxiety and stress both cause physical, cognitive, emotional, and behavioural responses in people. Anxiety over surgery is widely acknowledged as a typical emotion in pre-operative patients. Waiting for surgery or invasive treatments is stressful, according to research, and anxiety aggravates and affects both physiological and psychological factors. Many factors, including previous

dental experience, dental experience of friends or relatives, and congenital factors, influence dental patients' anxiety or fear of treatment.⁴

Periodontal pocket surgery/Surgical periodontal therapy (SurPT) is a time-consuming procedure that involves manipulating diseased tissues like gingiva and alveolar bone, which can cause changes in blood pressure and oxygen saturation.⁵ Acute hypertension is a common complication of major surgical procedures. Blood pressure elevation raises the risk of intraoperative complications. As a result, to avoid complications, peri-operative blood pressure management is still required.^{6,7} The negative impact of suboptimal blood pressure on outcomes is most likely determined not only by the magnitude of the abnormality but also by its duration.⁸ Both a significant but brief change and a minor but ongoing change have the potential to be harmful.⁹ Additionally, patients with medical conditions like cardiovascular disease are more likely to have periodontal disease.¹⁰ As a result, there is a

growing need for routine periodontal therapy in hypertensive patients as well as for developing a safe protocol for managing periodontal pockets and lowering patient anxiety without affecting hemodynamics. The justification for routine and consistent BP monitoring in peri-operative care is crucial because BP can be extremely volatile, can cause unfavourable outcomes that are easily treatable, and can lead to complications that are difficult to treat.

Non-surgical periodontal therapy with LASER assistance is a commonly used and preferred minimally invasive procedure with reduced bleeding tendency and post-operative pain.¹¹ Many patients and professionals prefer LASER aided treatment techniques because they have less intraoperative and postoperative complications.^{12,13} However, no studies have been conducted so far to assess the hemodynamic alterations caused on by LASER assisted periodontal therapy (LAPT). A treatment protocol is required to ensure that any postoperative problems brought on by perioperative hemodynamic alterations are kept to a minimum. Therefore, the current study was designed to evaluate and compare the variability in hemodynamics, Oxygen saturation and patient anxiety during SurPT and LAPT.

Material and Methods

Study design and setting

This randomized controlled clinical trial was conducted in patients attending department of Periodontics and Implantology, Vishnu Dental College seeking treatment for moderate to severe periodontitis. Thirty subjects including 16 males and 14 females in the age range of 25-70 years were included. A single calibrated (with intra-examiner correlation coefficient $k=0.82$) and blinded examiner performed the clinical assessments throughout the study period. The study was conducted in the Department of Periodontology at Vishnu Dental College, Bhimavaram from March 2019 to June 2019. The institutional ethics committee (ref.No. IECVDC/15/F01/PI/IVV/47) accepted the trial protocol. Before the trial began, all patients were told about the study's design and methodology, and signed informed permission was acquired.

Sample size and patient selection

Using G*Power software (version 3.1.9.4), the required sample size was calculated to be 15 patients in both groups with a statistical power of 80, an alpha of 0.05, and effect sizes of 0.42 considering blood pressure as the primary variable.

Selection Criteria

Patients diagnosed with periodontitis stage II and Stage III, Grade B and C (according to 2017 classification of periodontal diseases by world workshop of periodontal and peri-implant diseases) and in a good state of general health without any systemic disorder were included in the study.¹⁴ Patients with systemic diseases that affects the heart rate and Blood pressure (BP), using medication that affects the

heart rate and BP, on medication for anxiety and stress, Sensitive to LA, Bronchial asthma or any existing respiratory disorder, anemia, Severe uncontrolled pain, Pregnant and lactating women, with physical and mental disability and Patients with a known history of psychiatric illness were excluded.

Randomization, allocation concealment and blinding

All the selected participants underwent an initial examination and non surgical periodontal treatment was performed during the subsequent visits. The participants were explained about the study protocol, the advantages and disadvantages of both the techniques and written informed consent was obtained. 4 to 6 weeks after initial therapy the participants were re-assessed and eligible participants i.e., those with persistent probing pocket depths of ≥ 5 mm and clinical attachment loss of ≥ 3 mm in at least three teeth in a single quadrant were randomly assigned to the study groups. i.e., SurPT group (received surgical periodontal therapy for pocket reduction) and LAPT group (received LASER assisted non surgical periodontal therapy). Allocation concealment was done by using computer generated random numbering system which is sealed in opaque envelopes. All the hemodynamic changes were monitored by a single calibrated examiner (intra-examiner calibration was done) and a single investigator performed all the clinical procedures in both the groups.

Assessment of parameters

The hemodynamic changes including the monitoring of Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Heart rate (HR) and Oxygen saturation (SpO₂) was done at four different stages i.e., before injecting LA (TI1), after injecting LA (TI2), during the procedure (TI3), at the end of procedure (TI4). BP was monitored using sphygmomanometer and HR, SpO₂ using digital finger pulse oxymeter. Pre-operative anxiety was assessed using Amsterdam Preoperative Anxiety and Information Scale (APAIS). The APAIS consists of six item questionnaire which is twofold consisting of anxiety and information scale. Each question has a five point likert scale ranging from 1 to 5.¹⁵

Treatment procedure

All the participants received upto two cartridges of Local anesthesia containing 2% Lignocaine with 1:2,00,000 adrenaline. In the SurPT group intracrevicular incisions were given and full thickness mucoperiosteal flap was elevated after which thorough debridement and root surface planning was done. Regenerative procedures or osteoplasty were performed as required and the flaps were approximated using 4.0 resorbable suture material.

In the LAPT group, a diode laser (Denlase®, Pune, Maharashtra) with a wavelength of 810 ± 10 nm and a power output of 2.5W was equipped with a probe tip, which was inserted at the depth of the pocket and moved circumferentially around the tooth in a continuous mode for 1 minute.

Statistical analysis

A sample size of 15 individuals was used in each group, with a 5% alpha error and an 80% power of the test to detect a significant difference. All collected data was tallied in Microsoft Excel (MS Office version 2010). SPSS 26.0 was used for statistical analysis of the results (IBM Inc. Chicago, IL, USA). The intergroup comparison of the data was done using the student t test and the intra group comparisons were performed using repeated measures of ANOVA.

Results

Demographic characteristics

A total of 30 participants were allocated to the intervention including 8 males and 7 females in each group. The mean age of the participants was 42.56 ± 5.60 and 47.52 ± 4.9 in SurPT and LAPT groups respectively with no significant difference in the distribution of participants in different age groups in both the protocols ($P=0.904$) (Table 1).

Hemodynamic changes and SpO₂

A significant rise in the SBP was observed in both the groups after injection of LA with P value of 0.001 and 0.000 in the SurPT and LAPT groups respectively (Table 2, 3). Intergroup comparison of SBP showed significant difference at all time points with greater SBP in SurPT group except at T12. No significant difference in DBP was observed in both the groups at any time point. Similarly intergroup comparison of DBP showed no considerable difference between the groups at any time point (Table 4). Heart rate was highest at T12 which reduced significantly towards T14 in both the groups ($P<0.000$). In SurPT group the HR reduced from 81.40 ± 3.22 (T12) to 78.06 ± 3.32 (T14). Similarly in LAPT group HR reduced from 79.00 ± 2.13 (T12) to 76.00 ± 1.60 (T14). HR was considerably greater in SurPT group compared to LAPT group at all time points. Reduction in SpO₂ levels was observed in both the groups at T12. However LAPT group showed a statistically significant reduction ($P=0.002$) in SpO₂ at T12 which increased towards T14. Intergroup comparison showed no difference in SpO₂ levels at any time point (Tables 2-4).

Pre-operative anxiety

The APAIS was assessed before starting the treatment in both groups. The anxiety scale showed greater scores in SurPT group (6.8667 ± 2.133) compared to LAPT group (5.0667 ± 1.70) depicting greater anxiety in SurPT group ($P=0.017$). However, on information scale there was no significant difference ($p=0.100$) between both the groups. Overall anxiety scores were highest in SurPT group (12.40 ± 3.35) compared to LAPT group (9.66 ± 3.03) (Table 4).

Discussion

Hemodynamic changes are influenced by a number of variables, including the use of LA, patient-specific variables such as age, gender, hypertension, anxiety, stress,

prior dental treatment experience, and psychological response. Blood pressure changes with age and is found to be greater in males compared to females. The results show an equal distribution of gender in both the groups and all the patients included were in the age range of 30-50 years showing uniformity in the patient selection. Cardiovascular events during and after surgery are linked to intraoperative hemodynamic alterations. Even healthy patients are at serious risk from severe perioperative hypertension, especially if their blood pressure rises by more than 20% from preoperative levels.⁷ Acute circulatory irregularities, bleeding, and uncontrollable haemorrhage are all effects of such pressure surges. As a result, monitoring hemodynamic changes while providing is critical to ensuring patient safety, identifying circumstances of elevated risk quickly, establishing an early diagnosis, preventing potential problems, and operating with enhanced safety. Although hemodynamic changes associated with dental treatment, surgical therapy and use of LA with vasoconstrictors has been well established no clinical trials evaluated the association of the same with LASER therapy.¹⁶⁻¹⁹ Hence the current trial evaluated and compared the hemodynamic changes, HR and SpO₂ variability in SurPT and LAPT.

In the present study SBP and HR increased after giving LA. This correlates with the study done by Matsumura *et al.* (1998) and Babak A *et al.* (2013), Matsumura *et al.* found that administering LA (2% lidocaine) containing 1:80,000 epinephrine increased both blood pressure and pulse rate.^{16,17} The authors also reported that middle-aged and older patients have a greater increase in blood pressure during dental surgery than younger patients, owing to differences in the regulation of the autonomic nervous system during dental surgery.¹⁶ Similarly Babak A *et al.* reported a significant increase in SBP, DBP and HR after injection of LA (2% Lignocaine) containing 1:80,000 epinephrine. However, greater changes in SBP (137.84 ± 1.729) and HR (101.14 ± 1.590) after LA were reported by these authors which could be due to the higher concentration (1:80,000) of epinephrine in LA compared to our study (1:2,00,000).¹⁷ The vasoconstrictor i.e., epinephrine used in the LA increases the potency and duration of anaesthesia, reduces the plasma concentrations of the anaesthetic, and improves local control of bleeding. However, use of epinephrine containing LA is found to induce hemodynamic changes during surgical procedures. In normotensive individuals, sympathetic activation during anaesthesia induction can cause blood pressure to rise by 20 to 30 mmHg and heart rate to rise by 15 to 20 beats per minute.⁹ Contrary to the results of the current study Gedik RG *et al.* reported reduction in BP and HR after periodontal surgery.⁵

Shakeel AM *et al.* examined the cardiovascular effects of 2% lignocaine with two different adrenaline concentrations, 1:80,000 and 1:2,000,000. They reported a considerable increase in pulse rate immediately after LA with 1:80,000 adrenaline concentrations, which progressively decreased to the normal rate after 15 minutes, and no significant increase in pulse rate with a

1:2,00,000 adrenaline concentration.²⁰ However when compared to earlier studies the rise in hemodynamics was slightly less in our study and the rise has reduced at a faster rate to pre-operative values, which may be due to the 1:2,00,000 concentration of epinephrine used which is a commonly used concentration in regular practice.²¹ Another important factor in the hemodynamic changes associated with LA is the amount of LA injected, hence in the current study a standard amount of up to two cartridges was considered as inclusion criteria.

No significant variation in the SpO₂ levels were observed during the different steps of periodontal therapy which is in accordance to an earlier study which evaluated the changes during surgical periodontal therapy.¹⁷

Intergroup comparison of the SBP and HR scores at different time intervals showed greater elevation in the values in SurPT group compared to LAPT group. And also individual anxiety scores and overall APAIS scores were significantly greater in SurPT group. These hemodynamic changes could be attributed to the anxiety of the patients towards surgery which results in a surge of adrenaline and rise in BP and HR. Anxiety caused by dental treatment can stimulate the release of endogenous catecholamines. In comparison to less invasive LASER therapy, the prospect of a planned surgical procedure causes a physiologic stress response in patients, manifesting as corticoid release, BP

change, and hemodynamic and cardiovascular reactions. When this condition is paired with the use of local anaesthetics and vasoconstrictors, the negative effects on the cardiovascular system may be exacerbated. Furthermore, the anxiety associated with minor surgery and local anaesthetic injection may cause a catecholamine rush, which may raise myocardial oxygen demand and be arrhythmogenic.^{22,23} Local anaesthetics' possible effect on cardiac rhythm has also been described in the literature.²² Incomplete or inappropriate awareness regarding the treatment procedure can be considered as one of the factor for increased anxiety. However, the results of the current study dictate no difference in the information scale in patients in both the groups. The assessment of postoperative anxiety and patient reported outcomes was not within the confines of the current study, however may influence the postoperative hemodynamic changes. Hence, further studies may be necessary to prove the possible association between postoperative anxiety, patient reported outcomes and hemodynamic changes. Strategies for reduction of preoperative anxiety i.e., pharmacological and non-pharmacological may be necessary to prevent perioperative complications. Non pharmacological cognitive-behavioral therapy, music therapy, pre-op preparation video, aromatherapy, may be of potential benefit to reduce the pre-operative anxiety.

Table 1: Demographics of Study Participants

Characteristics	Category	SurPT	LAPT	P value
Gender	Male	8 (53.3%)	8 (53.3%)	1.000
	Female	7 (46.7%)	7 (46.7%)	
	< 35yrs	4 (26.7%)	5 (33.3%)	
Age	35-60yrs	6 (40%)	5 (33.3%)	0.904
	>60yrs	5 (33.3%)	5 (33.3%)	

Table 2: Comparison of Vitals at Different Time Intervals within SurPT group

Parameters	Time intervals	MEAN	SD	P VALUE
SBP	T1	129.6000	7.56684	0.001*
	T2	131.4667	8.12287	
	T3	129.6000	7.56684	
	T4	129.6000	7.56684	
DBP	T1	80.5333	2.55976	0.234
	T2	80.9333	2.81493	
	T3	80.4000	2.29285	
	T4	80.2667	2.12020	
HR	T1	80.1333	3.44065	0.000*
	T2	81.4000	3.22490	
	T3	79.1333	3.56304	
	T4	78.0667	3.32666	
SPO ₂	T1	97.4000	1.24212	0.059
	T2	96.8667	1.59762	
	T3	97.4000	1.35225	
	T4	97.4000	1.35225	

*- $p \leq 0.05$ considered statistically significant

Table 3: Comparison of Vitals at Different Time Intervals Within LAPT

Parameters	Time intervals	MEAN	SD	P VALUE
SBP	T1	124.5333	5.57887	0.000*
	T2	126.2667	5.84889	
	T3	124.2667	4.89120	
	T4	122.6667	5.05211	
DBP	T1	79.0000	2.13809	0.243
	T2	79.7333	2.25093	
	T3	79.7333	2.25093	
	T4	79.7333	2.25093	
HR	T1	77.9333	2.25093	0.000*
	T2	79.0000	2.13809	
	T3	76.9333	2.34419	
	T4	76.0000	1.60357	
SPO2	T1	97.2000	1.14642	0.002*
	T2	96.3333	1.11270	
	T3	97.1333	1.30201	
	T4	97.4000	1.24212	

*- p≤0.05 considered statistically significant

Table 4: Comparison of vitals between the groups at different time intervals

Parameters	Time intervals	Groups	MEAN	SD	P VALUE
SBP	T1	SurPT	129.6000	7.56684	.046*
		LAPT	124.5333	5.57887	
	T2	SurPT	131.4667	8.12287	.054
		LAPT	126.2667	5.84889	
	T3	SurPT	129.6000	7.56684	.030*
		LAPT	124.2667	4.89120	
	T4	SurPT	129.6000	7.56684	.006*
		LAPT	122.6667	5.05211	
DBP	T1	SurPT	80.5333	2.55976	.371
		LAPT	79.7333	2.25093	
	T2	SurPT	80.9333	2.81493	.208
		LAPT	79.7333	2.25093	
	T3	SurPT	80.4000	2.29285	.428
		LAPT	79.7333	2.25093	
	T4	SurPT	80.2667	2.12020	.510
		LAPT	79.7333	2.25093	
HR	T1	SurPT	80.1333	3.44065	.048*
		LAPT	77.9333	2.25093	
	T2	SurPT	81.4000	3.22490	.023*
		LAPT	79.0000	2.13809	
	T3	SurPT	79.1333	3.56304	.056
		LAPT	76.9333	2.34419	
	T4	SurPT	78.0667	3.32666	.039*
		LAPT	76.0000	1.60357	
SPO2	T1	SurPT	97.4000	1.24212	.650
		LAPT	97.2000	1.14642	
	T2	SurPT	96.8667	1.59762	.298
		LAPT	96.3333	1.11270	
	T3	SurPT	97.4000	1.35225	.587
		LAPT	97.1333	1.30201	
	T4	SurPT	97.4000	1.35225	1.000
		LAPT	97.4000	1.24212	
ANXIETY	Anxiety scale	SurPT	6.8667	2.13363	.017*
		LAPT	5.0667	1.70992	
	Info scale	SurPT	5.5333	1.50555	.100
		LAPT	4.6000	1.50238	
	Overall	SurPT	12.4000	3.35517	.027*
		LAPT	9.6667	3.03942	

*- p≤0.05 considered statistically significant

Future prospects

A methodology to assure safe periodontal practice may require further research in patients with compromised medical situations such hypertension and pre-existing cardiovascular disorders/abnormalities.

Conclusions

The hemodynamic alterations including SBP and HR were considerably greater during surgical periodontal therapy and in particular after injection of LA compared those induced by laser assisted periodontal therapy. The fluctuations in BP and HR, however, were normal ranges. Greater pre-operative anxiety brought on by surgical periodontal therapy may be a potential source of hemodynamic changes.

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Ethical Statement

The institutional ethics committee (ref.No. IECVDC/15/F01/PI/IVV/47) accepted the trial protocol. Before the trial began, all patients were told about the study's design and methodology, and signed informed permission was acquired.

Author contributions

NVSG, RSVK, carried out the research and collected the data. NVSG and KSVR designed and supervised the study, visualized and validated the data, acquired funding, and reviewed draft material. The data were organized, analyzed, and interpreted by NVSG, MSN, and GP. NVSG, RSVK and GSP reviewed the article. All authors were responsible for the content and similarity of the manuscript index and who have reviewed and critically approved the final draft.

Conflict of Interest

"This research did not receive any specific grant from funding agencies in the public, commercial, or notfor-profit sectors"

Informed Consent

All the participants were informed and a written consent was taken before their participation in the study.

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Evaluating the Knowledge, Awareness and Exposure of Malaysian Dentists in Conscious Sedation for Dentistry in Relation to their Current Practice and Future Expectations

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ABSTRACT

Introduction: Conscious sedation (CS) in dentistry becoming more popular in Malaysia nowadays. However, the knowledge, exposure and practice of CS in dental setting has rarely been explored. Therefore, this study aimed to evaluate the knowledge awareness and exposure of Malaysian dentists about CS for dentistry and their association with sociodemographic profile.

Materials and Methods: A cross-sectional study using an online questionnaire (Qualtrics@ Software) was conducted among Malaysian registered dentists. Questions on sociodemographic profile, knowledge, awareness, exposure and practice on CS were collected. Chi square test was used to analyse the associated factors for knowledge, exposure and practice.

Results: A total of 166 responses received, resulting in response rate of 43.1%. Majority agreed that CS is beneficial in allaying dental anxiety and knows at least 3 types of CS. More than half had been exposed in inhalation sedation (IS) with less than half had CS exposure during undergraduate (UG). However, only a quarter practicing CS in their dental practice.

Discussion: Malaysian dentists were familiar with the indications of CS in dentistry which corroborates with other studies. Meanwhile, the exposure to CS during undergraduate study were dependant on the availability and the program structure.

Conclusions: Majority of respondents know, but only some are practicing oral and inhalation sedation. Only a few of respondents know about other types of CS. Thus, there is a need for more exposure and training of CS during undergraduate to cater the need of patients with dental anxiety.

Key words: Conscious sedation, dentistry, knowledge.

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Introduction

Conscious sedation (CS) is a technique in which the use of a drug or drugs produce a state of depression of the central nervous system enabling treatment to be carried out, but verbal contact with the patient is maintained throughout the period of sedation.¹ Conscious sedation (CS) in dentistry is the pharmacological approach usually offered after non-pharmacological behavioural modification techniques proof to be no avail. This method is found to be successful in managing patients with dental anxiety or patients with challenging behaviour in order to deliver dental treatment safely.^{2,3}

CS in dentistry is still new for Malaysian dentists. The technique of CS that is widely used among dentists in Malaysia is inhalation sedation using mixture of nitrous oxide and oxygen. It was first introduced by a company named Tiensen& Tiensen Sdn. Bhd about fifteen years ago in Malaysia to manage children with dental anxiety. However, the usage of nitrous oxide among adults with

special needs was recently introduced after Special Care/Needs Dentistry service was established in 2011 in the Ministry of Health, Malaysia.⁴

Using sedation agents other than nitrous oxide such as midazolam in dental clinics has always been a controversial issue due to the cross-intervention between medical and dental fraternities.^{3,5} Even though, techniques of CS such as inhalation and oral sedation have been widely used among children in the paediatric dental clinic by the specialists, many clinicians are sceptical about performing intravenous sedation (IVS) in dental clinics.¹⁴ Lack of knowledge, exposures and experience could be the contributing factors to this 'fear'.¹⁴ However, little is known about the knowledge and exposure that the Malaysian dentists have in regards to CS. To date, there is no study exploring this option among the Malaysian dentists. Lack of knowledge and exposure may pose some stigma in performing the procedure. The results of the

study will indicate the readiness and acceptance of CS to be practiced in dental setting in Malaysia. The need for training in CS can also be justified to be incorporated in undergraduate and postgraduate curriculum in the future.

In the Western countries such as the UK and USA, CS can be provided at the primary and secondary dental care settings.^{1,3,4} However, in the Ministry of Health Malaysia facilities, CS only provided in the hospital-based dentistry services such as the Oral and Maxillofacial, Paediatric Dentistry and Special Care Dentistry services.⁶ As stated by several health departments around the world, the drugs and techniques used to provide CS for dental treatment should carry a margin of safety wide enough to render loss of consciousness unlikely (minimal to moderate level of sedation).^{1,3,4,6} To that extent, the Ministry of Health Malaysia refers to the guideline proposed by the College of Anaesthesiologist Academy of Medicine Malaysia for the continuum of sedation depth as in Figure 1.⁵

The recommendation of the settings for CS in dentistry in Malaysia based on the guideline of CS for adult patients which was just recently published in April 2021.⁶ This is very much similar to the recommendations set by several guidelines produced in the United Kingdom.^{1,4}

CS is also offered by private dental clinics or general dental practice (GDP) for patients with dental anxiety. Most of them have undergone training in inhalation sedation with nitrous oxide organized by the Tiensen & Tiensen company. Those who offer intravenous sedation (IVS) have medical practitioner in the practice who act as the sedationist, whilst the dentist focuses on dental treatments as recommended by the local guideline.⁵

Thus, this study aimed to explore the knowledge, awareness and exposure of Malaysian dentists about CS for dentistry and how this is practised in Malaysian dental setting while identifying their expectations on this aspect in the future.

Research Methodology

Research ethical approval

This project was registered and approved by the National Malaysian Medical Research Registry (NMRR) in compliance with current Ministry of Health, National Institute of Health (MOH NIH) research guideline and Helsinki Declaration of 1975. The approval number is NMRR-20-799-54299 (IIR).

Inclusion and exclusion criteria

This was a cross sectional, online survey conducted among 11,607 registered Malaysian dentists who registered with Malaysian Dental Council (MDC) in 2020. The inclusion criteria were dental practitioners who held current Annual Practising Certificate (APC) in Malaysia. Those who had less than 1 year of working experience and did not hold current APC were excluded in this study.

The power sample calculated for this study was 385 using Kisch formula with confidence rate was set at 95%. A total of 166 responses received, resulting in the response rate of 43.1

Research tools

This study involved the distribution of survey questionnaires online using Qualtrics@software through their emails and social media. The survey consists of four sections whereby the first section covers the sociodemographic information about the participants. The second section concentrates on the awareness and knowledge that the participants have in CS. The third section focuses on the exposure and practice of CS based on their experience. The online survey took about 5 -8 minutes to complete. Face and content validation of the questionnaires were done with two experts in the related field and 27 dentists who attended a course entitled "Awareness in Conscious Sedation for Dentistry" in 2019. Some minor amendments made based on the responses given by the participants.

Data entry and analysis

The data collected from Qualtrics@software application was entered and analyzed using SPSS version 26.0 IBM Corp with a 5% level of significance. Years of service was categorized into two categories within 10 years of service and more than 10 years. Place of practice also were further dichotomized into government and private practice.⁷

Results

Table 1 illustrates the sociodemographic profile of the respondents. Out of 166 responses, 2 respondents did not answer the questions on gender and work place. Majority of respondents were female dentists (n=129, 78.7%). Only n=35, 21.3% were male dentists. The median age of the respondents were 33 years old. Junior dentists with less than 5 years of service were the highest group of respondents (n=56, 34.3%). In contrast, senior dentists with more than 20 years of service comprised the least number of respondents (n=31, 18.7%). The rest represented the group of 5 to 20 years of service. About 90 percent (n= 149) of them work in the government sector which includes Ministry of Health (MOH), Ministry of Higher Education (MOHE) and Ministry of Defence (MOD).

Table 2 shows majority (n=147, 88.6%) of respondents were aware of CS in dentistry. Among 130 respondents who answered the question on the source of exposure, more than one third (n= 60, 46.1%) had their CS exposure during undergraduate. For the third and fourth questions in this section, participants were allowed to give more than one response. Majority out of 140 participants responded to the former question agreed that the purpose of performing CS was to alleviate anxiety (n=113, 80.7%) with only minority (n=57, 40.7%) said CS could reduce gagging reflex. From 141 respondents who claimed that they were familiar with several types of CS (fourth question), majority knew about 3 techniques of CS which could be performed in dental settings, which was inhalation (n=128, 90.8%), oral (n=113, 80.1%) and intravenous (n=108, 76.6%) being the most common ones.

More than 80% from 139 respondents claimed that they knew how to manage medical emergency related to CS in dental settings. Lastly, more than half (n=81, 57.9%) of the respondents out of 140 responses were aware of the legislation code for CS that is available in Malaysia.

Table 3 illustrates the exposure received by the respondents on CS. More than half (n=73, 52.5%) of 139 respondents had been exposed to inhalation sedation (IHS) with n=112 agreed that CS was safe to be performed in dental settings. More than half of the respondents received undergraduate exposure on CS (n= 57, 85.1) in the form of theory and clinical observation whilst on the other hand, half of the respondents (50.7%) received postgraduate exposure on CS in the form of theory, observation and practical. Majority (n=128, 92.1%) agreed that more exposure on CS should be given during their undergraduate study. Apart from that, 123 participants claimed that they underwent training on Basic Life Support (BLS).

Table 4 illustrates that less than one third of respondents (n=39, 28.9%) offered CS in their practices with two third provided inhalation sedation (IHS) (76.9%) followed by oral sedation (66.7%). Majority (n= 132, 98.5%) agreed that CS is helpful for carrying out dental treatments and perceived the needs for CS (n=107, 82.9%) for their patients. Only a small number of respondents (n=10, 7.7%) had encountered emergency events while performing CS.

Table 5 shows the prospective of CS in Malaysia where majority (n=127, 92.0%) showed interest to have training in CS and n=108 (77.7%) respondents perceived that CS is needed for their dental patients. Most of the respondents (n=130, 93.5%) also would like to have CS available in their practice.

Discussion

Knowledge and awareness in this study were evaluated in three subjects, how familiar the participants with the CS, knowing how to react to medical emergency in dental settings and awareness on the legislation and code for CS. In a study done among dentists in Brazil, about 75% reported to be familiar about CS with 70% of the dentists claimed they knew how to react in the event of emergency in dental settings.⁸ Meanwhile in this study, 88.6% of the respondents agreed on having some knowledge and awareness on CS and 82.7% knew how to react during emergency events. However, this study did not explore on the confidence level of the respondents when it comes to emergency events. Our study also corroborates with the findings in the previous studies done in Brazil and in the UK in which mentioned that patients with dental anxiety were more likely to be referred for sedation.^{9,10}

Figure 1. Continuum of Depth of Sedation

	Minimal Sedation (Anxiolysis)	Moderate Sedation/ Analgesia (Conscious Sedation)	Deep Sedation/ Analgesia	General Anaesthesia
Responsiveness	Normal response to verbal stimulation	Purposeful response to verbal or tactile stimulation	Purposeful response after repeated or painful stimulation	Unarousable even with painful stimulation
Airway	Unaffected	No intervention required	Intervention may be required	Intervention often required
Spontaneous Ventilation	Unaffected	Adequate	May be inadequate	Frequently inadequate
Cardiovascular Function	Unaffected	Usually maintained	Usually maintained	May be impaired

Table 1. Sociodemographic profile of the respondents

	N (%)
Gender(n=164)	
Male	35 (21.3)
Female	129 (78.7)
Age (median (IQR))	33 (13)
Years of service (n=166)	
<5	57 (34.3)
5-10	40 (24.1)
11-20	38 (22.9)
>20	31 (18.7)
Current practice place (n=164)	
Stand-alone dental clinic MOH/MOHE	11 (6.7)
Dental clinic in health clinic MOH/MOHE	77 (47.0)
Hospital based dental clinic MOH/MOHE	60 (36.6)
Stand-alone private dental practice	9 (5.5)
Dental clinic in private health clinic	3 (1.8)
Private dental hospital	3 (1.8)
Dental specialist polyclinic/clinic in MOD	1 (0.6)

MOH – Ministry of Health, MOHE – Ministry of Higher Education, MOD- Ministry of Defence

Table 2. Knowledge and awareness on conscious sedation

Familiar with conscious sedation (CS) (n=166)	N (%)
Yes	147(88.6)
No	19 (11.4)
Source of information on CS (n=130)	
Undergraduate	60(46.1)
Postgraduate	30(23.1)
Continuous Dental Education (CDE)	11(8.5)
Hospital training	17(13.1)
Complimentary reading	12(9.2)
Purpose of CS (n=140) *	
Alleviate anxiety	113(80.7)
To reduce gag reflex	57 (40.7)
As alternative for GA	68(48.6)
Part of behaviour guidance	80(57.1)
To help dentist deliver treatment easier	87(62.1)
Types of CS that you know (n=141)*	
Oral	113(80.1)
Inhalation (IHS)	128(90.8)
Intravenous (IVS)	108(76.6)
Intranasal (INS)	49(34.8)
Intramuscular (IM)	38(27.0)
General anaesthesia (GA)	13(9.2)
Know how to react to medical emergency in dental settings? (n=139)	
Yes	115(82.7)
No	24(17.3)
Aware of the legislation and code for CS?(n=140)	
Yes	81(57.9)
No	59(42.1)

*Respondents can answer more than one

Table 3. Exposure or training on conscious sedation

The most common type of CS you have seen (n=139)	N (%)
Oral	32(23.0)
Inhalation (IHS)	73(52.5)
Intravenous (IVS)	28(20.1)
Intranasal (INS)	2(1.4)
Intramuscular (IM)	1(0.7)
General anaesthesia (GA)	3(2.2)
Safe to perform CS in dental settings (n=134)	
Yes	112(83.6)
No	22(16.4)
Last training on BLS/ALS/ACLS (n=123)	
Within 5 years ago	91(74.0)
More than 5 years ago	32(26.0)
Exposure on CS:	
Exposure during undergraduate (n=141)	
Yes	67(46.8)
Theory only	19(28.4)
Theory and observation	38(56.7)
Theory, observation and practical	10(14.9)
No	74(52.9)
Exposure during postgraduate (n=115)	
Yes	59(51.3)
Theory only	10(16.9)
Theory and observation	19(32.2)
Theory, observation and practical	30(50.8)
No	56(49.7)
Should you get more exposure during UG? (n=139)	
Yes	128(92.1)
No	11(7.9)

Table 4. Practice in conscious sedation

Offer CS in your practice(n=135)	N (%)
Yes	39(28.9)
No	96(71.1)
Types of CS offered in your practice	
Oral (n=39)	26(66.7)
Inhalation (IHS) (n=39)	30(76.9)
Intravenous (IVS) (n=39)	13(33.3)
Intranasal (INS) (n=39)	5(12.8)
Intramuscular (IM) (n=39)	3(7.7)
General anaesthesia (GA) (n=39)	8(20.5)
Helpful to carry out dental treatment under CS?(n=134)	
Yes	132(98.5)
No	2(1.5)
Perceived need for your patients to CS?(n=129)	
Yes	107(82.9)
No	22(17.1)
Encountered emergency events during CS for your patients?(n=130)	
Yes	10(7.7)
No	120(92.3)

Table 5: Conscious sedation prospective

Interest in training in CS if it is available in Malaysia?(n=138)	N(%)
Yes	127(92.0)
No	11(8.0)
Perceived need of CS for dental patients in Malaysia(n=139)	
Needed	108(77.7)
Maybe	30(21.6)
Not needed	1(0.7)
Would like to have CS service(s) available in your practice(n=139)	
Yes	130(93.5)
No	9(6.5)

At the time when the questionnaire was distributed, there was no specific guideline with regards to CS in dentistry in Malaysia. However, more than half of the respondents reported that they were aware of the legislation and regulation pertaining to CS that was available in Malaysia. Presumably, they were referring to one and only document, Recommendations for Sedation And Analgesia By Non-Anaesthesiologists proposed by the College of Anaesthesiologist Academy of Medicine Malaysia in 2012.⁵ However, this recommendation is not specific for dentistry and the information related to dental sedation is very limited. Nevertheless, in April 2021, the Oral Health Programme, Ministry of Health Malaysia had published a guideline on Conscious Sedation in Dentistry for Adult Patients which was more specific and relevant to the practice of dentistry.⁶ Adherence and compliance to the local guidelines and regulations pertaining to CS in dental service are crucial to ensure patients' safety and to protect the dental professionals. Every country, state, municipal or even department may have different rules and concerns.^{1,4,6} Awareness on the existence of the local code of practice in CS in dentistry will prevent the dental practitioners from practicing techniques that are against the local laws.

In the UK, there is a requirement for undergraduate dental students to have exposure in CS for at least 1 performed or assisted CS case during their undergraduate

studies.¹¹ However, there is no requirement for exposure in CS for dental undergraduate in Malaysia.¹² Despite of no requirement for CS, some universities in Malaysia have given some exposure on CS to their undergraduates. Although less than 50% had exposure on CS during their undergraduate studies, it is a good indicator that majority of our dental undergraduates have had at least theoretical and observation on CS. It is crucial for dentists to undergo a proper post graduate training for performing CS to dental patients.^{14,15} In the present study, 60% of specialists had exposure on CS during their postgraduate studies with more than 50% had their practical training. This could be due to the specialisation area whether CS is required as part of their postgraduate studies.^{13,14} In Malaysia, only three specialisation areas that required for the postgraduates to have practical exposure on CS, which are; Special Care Dentistry, Paediatric Dentistry and Oral and Maxillofacial Surgery.¹³

Inhalation sedation using mixture of nitrous oxide and oxygen is the commonest type of CS being offered by the dentists in Malaysia. This is due to the properties of nitrous oxide which make it the safest form of CS to be delivered on dental chair.^{16,17} Oral sedation is the second commonest method of CS offered by the participants perhaps because of its methods of delivery which is less invasive compared to intravenous sedation despite of its disadvantages which are delayed onset and prolonged

recovery.¹⁸ Intravenous sedation was found to be helpful, cost effective, and less trauma to the patients as reported by previous researchers.¹⁹⁻²¹

Advantages and limitations

This particular study was able to capture the data on knowledge, awareness, practice and exposure of CS among Malaysian dentists. Since, there are no other local studies addressing these issues so far, the data collected acts as a baseline reference for future research in similar field. At the same time, it also creates awareness among the Malaysian dentists about the availability of CS in dental practice in Malaysia and how this may lead to curiosity and further research especially amongst those who have never encountered such services before. On the other hand, the study did not assess the level of competencies of the participants to respond to emergency event. The responses captured only based on their self-perception of their ability and the related theoretical knowledge they might have.

Conclusion

Majority of the Malaysian dentists were aware about CS in dentistry regardless of their background of training, age, length of experience as well as the place of practice. Unsurprisingly, postgraduate students in certain disciplines such as Oral Maxillofacial Surgery, Paediatric Dentistry and Special Care Dentistry had more exposure in CS. Although the training module may vary from one university to another, having such an exposure will certainly increase the confidence of the dental specialist to offer or at least suggest the service whenever it is indicated for the patients. It is motivating to conclude that majority of the respondents are interested to receive further training in CS if it is made available and expecting to offer the service in their practice in the future.

Recommendations

This study has suggested that training in CS in dentistry in Malaysia should be strengthened in the future either during undergraduate or postgraduate studies as well as in service training. The dental faculty of the local universities should be well prepared to include CS in various techniques as part of the training module in undergraduate or postgraduate curriculum. Therefore, a proper in-service training module should be developed, perhaps, in collaboration with the anaesthetic team at the initial stage to train a team in this field which may include specialists, general dental practitioners, dental nurses/therapists or dental surgery assistants instead of a training that focus on an individual desire. Performing CS requires a team to manage the patient either pre-operatively, during the operation or post-operatively. This will encourage more interests among the Malaysian general dental practitioners in offering the service to the public. On the other hand, the future research should explore on the demands of such

services among the Malaysian population to support the needs of the service provision.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Evaluation of the Effect of Different Irrigation Systems on the Removal of Intracanal Medicaments

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ABSTRACT

Objectives: This study aimed to evaluate the effects of different irrigation systems on the removal of intracanal medicament used in regenerative endodontic treatment.

Materials and Methods: Following standardized preparation to obtain an immature tooth model, modified triple antibiotic paste (mTAP) consisting of metronidazole, ciprofloxacin and clindamycin was applied to the root canals of single-rooted human maxillary teeth and waited for three weeks. A total of 50 teeth with standardized preparation and intracanal medicament were randomly divided into five groups according to the irrigation method to be applied (n=10); Group 1: Conventional Syringe Irrigation (CSI), Group 2: Passive Ultrasonic Irrigation (PUI), Group 3: EndoActivator (EA), Group 4: EndoVac (EV) Group 5: Nd:YAG laser (LSR). Following the removal of the mTAP with different irrigation systems, the teeth were divided along their bucco-lingual axis. The impact roots were examined under the stereomicroscope with the 4-grade scoring scale for the presence of residual intracanal medicaments. Scores for coronal, middle and apical regions of each root were recorded.

Results: In terms of total scores, PUI group showed higher intracanal medicament removal efficacy compared to the other groups (p<0.05). In the intra-group comparison of the root canal third, less intracanal medicament was found in the apical third (p <0.05). The highest intracanal medicament removal efficacy was found in PUI and LSR groups in the coronal third, PUI group in the middle third, and PUI and EV groups in the apical third (p<0.05).

Conclusions: Intracanal medicament removal scores were significantly associated with irrigation system. PUI showed statistically significantly higher intracanal medicament removal efficiency than other irrigation methods. While there was no difference between the coronal and middle thirds, statistically significantly less intracanal medicament removal was found in the apical third compared to the other regions.

Keywords: Bond strength, biodentine, irrigation, intracanal medicament, regenerative endodontic treatment.

Farklı İrrigasyon Sistemlerinin Kanal İçi İlaçların Uzaklaştırılmasına Etkisinin İncelenmesi

Süreç

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ÖZ

Amaç: Bu çalışmanın amacı farklı irrigasyon sistemlerinin rejeneratif endodonti tedavisinde kullanılan kanal içi ilaçların uzaklaştırılması üzerindeki etkilerini değerlendirmektir.

Gereç ve Yöntem: İmmatür diş modeli elde etmek için standardize edilmiş preparasyonun ardından, tek köklü üst insan dişlerinin kök kanallarına metronidazol, siprofloksasin ve klindamisininden oluşan modifiye üçlü antibiyotik pat (mTAP) uygulanmış ve üç hafta beklendi. Standart preparasyon ve kanal içi ilaç uygulanan toplam 50 diş, uygulanacak irrigasyon yöntemine göre (n=10) rastgele beş gruba ayrıldı; Grup 1: Gelenekel Şırınga İrrigasyonu (GSI), Grup 2: Pasif Ultrasonik İrrigasyon (PUI), Grup 3: EndoActivator (EA), Grup 4: EndoVac (EV) Grup 5: Nd:YAG lazer (LZR). Farklı irrigasyon sistemleri ile mTAP'ın uzaklaştırılmasını takiben dişler bukkal-lingual ekseninde boyunca ayrıldı. Darbe kökleri, artık intrakanal ilaç varlığı için 4 dereceli puanlama skalası ile stereomikroskop altında incelendi. Her kökün koronal, orta ve apikal bölgeleri için skorlar kaydedildi.

Bulgular: Toplam puanlar açısından, PUI grubu diğer gruplara göre daha yüksek kanal içi ilaç uzaklaştırma etkinliği gösterdi (p<0.05). Kök kanal üçlünün grup içi karşılaştırmasında, apikal üçlüde daha az kanal içi ilaç bulundu (p<0.05). En yüksek kanal içi ilaç uzaklaştırma etkinliği PUI ve LZR gruplarında koronal üçlüde, PUI grubunda orta üçlüde ve PUI ve EV gruplarında apikal üçlüde bulundu (p<0,05).

Sonuçlar: Kanal içi ilaç uzaklaştırma skorları, irrigasyon sistemi ile istatistiksel olarak anlamlı şekilde ilişkiliydi. PUI, diğer irrigasyon yöntemlerine göre istatistiksel olarak anlamlı derecede daha yüksek kanal içi ilaç uzaklaştırma etkinliği gösterdi. Koronal ve orta üçlü arasında fark yokken, apikal üçlüde diğer bölgelere göre istatistiksel olarak anlamlı derecede daha az kanal içi ilaç uzaklaştırma saptandı.

Anahtar kelimeler: Bağlanma dayanımı, Biodentine, irrigasyon, kanal içi ilaç, rejeneratif endodontik tedavi.

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Introduction

The pulp of immature permanent teeth is at risk for necrosis due to trauma, dental anomalies or caries. Although apexification procedures were routinely used to treat apical periodontitis in immature necrotic young permanent teeth¹, they cannot provide ongoing root development as in the regenerative endodontic treatment (RET) procedure.²

RET is defined as biological applications that replace damaged tissues, including dentin and root structure, in addition to the cells in the pulp-dentin complex.³ The advantage of RET over other existing treatment options is leading to increase in root length and the thickness of the root canal walls.⁴ The basis of RET is the effective disinfection of the root canal. Calcium hydroxide (Ca(OH)₂) and antibiotic pastes are often preferred for disinfection. However, antibiotic pastes have negative effects on the bond strength of calcium silicate-based cements.⁵ Bond strength and marginal adaptation of endodontic material to dentin to prevent apical or coronal leakage is an important factor for the successful implementation of various endodontic treatment procedures.⁶ Triple antibiotic paste (TAP) is acidic and, if not completely removed from the root canal, will cause insufficient bonding of calcium silicate cement to root dentin.⁷

Intracanal medicaments used in RET cannot be completely removed from the root canal with current irrigation methods and solutions.^{5, 8-10} A wide variety of techniques and irrigation solutions have been used to remove antibiotic paste from mature permanent tooth root canals.^{11, 12} However, there has been limited research attention on the removal of triple antibiotic paste from the root canal of immature teeth with different irrigation systems.

Calcium silicate-based cements are used as pulp barrier in RET to provide coronal sealing and to create resistance against incoming forces.^{13, 14} In order to increase the bond strength of cements to dentin, intracanal medicaments must be removed from the root canal.¹⁵⁻¹⁸ Therefore, evaluating the effects of irrigation methods on the bond strength of calcium silicate-based cements to dentin is important for clinical success in RET.

The aim of this study was to examine the effects of different irrigation systems on the removal of the mTAP used as intracanal medicament in RET. The null hypothesis was that here is no effect of irrigation system on intracanal medicament removal efficiency.

Materials and Methods

Ethics approval was obtained from the ethics committee with the decision dated 27.12.2016 and numbered 2016-12/08 from the Clinical Research and Ethics Committee of Sivas Cumhuriyet University. 50 single-rooted maxillary permanent incisors extracted for caries or orthodontically etc. reasons were examined under a stereomicroscope and with multi-angle preoperative radiographs (65 kVp, 8 mA, 0.1 sec, Novelix, Trophy). The inclusion criteria for the study were determined as follows;

(a) single root and canal, (b) no previous root canal treatment, (c) canal angle less than 20 degrees. The exclusion criteria for teeth were determined as follows; (a) presence of caries, fractures, cracks, internal and external resorption on the root surface, (b) canal angle greater than 20 degrees, (c) extensive coronal destruction, and (d) calcification and obliteration of root canals.

In order to obtain a standard working size, the crowns of 50 single-rooted teeth were cut from the enamel-cementum junction with a steel separator with a root length of 15±1 mm. The mechanical root canal preparation was applied with TF Adaptive (SybronEndo, Glendora, CA, USA) rotary system files, ML1-ML2, respectively, and finished apically in ML3 (050.04 taper) size. Root canals were washed with 2 ml of 2.5% NaOCl at each rotary file change. In order to provide a standard 1.5 mm in-canal diameter and an immature tooth model, 1 mm protrusion from the apical was studied with Peezo reamers, starting from 1 and using reamer number 6 last, and a standard root canal diameter was achieved. The prepared teeth were irrigated with 5 ml of 2.5% NaOCl and then with 5 ml of 17% EDTA (Canal + Septodont, France). Final irrigation was performed with 10 ml of distilled water to remove the effects of the irrigation solutions used in the canal. Root canals were dried with sterile paper cones (Aceonedent, Geonggi-Do, Korea).

Modified triple antibiotic paste (mTAP, Metronidazole +Ciprofloxacin+Clindamycin) was prepared in accordance with the described procedure by Hoshino et al.¹⁹ The procedure for intracanal medicament preparation was as follows; the coatings of Nidazole 500 mg film tablet (İ. E. Ulagay, Istanbul, Turkey) and Cipronatin 250 mg film tablet (Atabay, Istanbul, Turkey) were scraped with a surgical scalpel. Each antibiotic tablet was crushed separately in sterile porcelain mortars until powdery form. The powdered antibiotics were mixed on a clean glass at a ratio of 1:1. Then, 150 mg of Clindane capsules (Bilim, Istanbul, Turkey) were added to the resulting mixture, mixed homogeneously and a mixture was obtained at a ratio of 1:1:1. A homogeneous carrier was obtained by mixing macrol and propylene glycol at a ratio of 1:1 on a separate clean glass surface. Then, modified triple antibiotic paste (mTAP) was obtained by mixing 1 scoop of carrier and 5 scoops of antibiotic powder.

The prepared mTAP and distilled water were mixed with an antibiotic paste/sterile water ratio of 0.9 gr/ml. The paste was inserted to the root canals with a #40 lentulo file. Following placing cotton in the canals of 100 teeth, the teeth were closed with temporary filling material (Cavit; 3M ESPE, Seefeld, Germany). All samples were kept in an oven at 37°C in a 100% humidity environment for 3 weeks.

A total of 50 teeth with standardized preparation and intracanal medicament were randomly divided into five main groups according to the test method to be applied (n=10 per each group): Group 1: Conventional Syringe Irrigation (CSI), Group 2: Passive Ultrasonic Irrigation (PUI), Group 3: EndoActivator (EA), Group 4: EndoVac (EV), Group 5: Nd:YAG Laser (LSR).

Standardized irrigation protocol was applied in all experimental groups. Intracanal medicament removal was performed on the teeth of each experimental group with the relevant irrigation method. 2.5% NaOCl, 17% EDTA and distilled water were used as irrigation solution. The irrigation solution flow rate and irrigation time were determined to be 5 ml/min.

Group 1: Conventional Syringe Irrigation (CSI)

An area where the 27 gauge injector cannula could advance was created by advancing from the coronal to the apical direction with the #30 K file by 1 mm shorter than the working length. The injector cannula was positioned 1 mm coronal from the working length and irrigated with 5 ml of 2.5% NaOCl for 1 minute, then 5 ml of 17% EDTA (Nazar Kimya Ltd., Istanbul) was applied to the canal for 1 minute. Finally, the canals were irrigated with 10 ml of distilled water.

Group 2: Passive Ultrasonic Irrigation (PUI)

An area where the ultrasonic tip can be placed was created by advancing from the coronal to the apical direction with the #30 K file by 1 mm shorter than the working length. The irrigation tip of the VDW ULTRA ultrasonic device (VDW, Munich, Germany) was positioned 1 mm shorter than the working length with an application power of 25 (irrigation mode) in accordance with the manufacturer's instructions. Irrigation was performed in three rounds of twenty seconds each. After the root canal was filled with 2.5% NaOCl, the tip of the ultrasonic device was activated for 10 seconds, 2 mm up-down movement was made, and then the irrigation solution was renewed for 10 seconds and waited. In this way, a total of 3 rounds of irrigation were performed. Then, irrigation was done in a similar way for 1 min with 5 ml of 17% EDTA. Finally, the canals were irrigated with 10 ml of distilled water.

Group 3: EndoActivator (EA)

An area where the polymer tip can be placed was created by advancing from the coronal to the apical direction with the #30 K file in the root canal by 1 mm shorter than the working length. The power of the EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) sonic irrigation device was adjusted to 10000 cpm by attaching the irrigation tip number 25 and positioning it 2 mm shorter than the working length. Irrigation was performed in three rounds of twenty seconds each. After the root canal was filled with 2.5% NaOCl, activation was performed with 3 mm slight back and forth movements for 10 seconds, and then the irrigation solution was renewed for 10 seconds and waited. In this way, a total of 3 rounds of irrigation were performed. Then, irrigation was done in a similar way for 1 min with 5 ml of 17% EDTA. Finally, the canals were irrigated with 10 ml of distilled water.

Group 4: EndoVac

Final irrigation procedure was performed according to the manufacturer's instructions. After active irrigation with 2.5% NaOCl for 30 seconds with the macrocannula, the canals were filled with NaOCl and a total of 30 seconds of

irrigation was performed with the microcannula in 6 seconds working size, 2 mm shorter than 6 seconds working size, and 6 seconds working size, respectively. Then, after 30 seconds of active irrigation with 17% EDTA, the root canals were filled with EDTA and a total of 30 seconds of EDTA irrigation was performed with a microcannula in 6 seconds working size, 2 mm shorter than 6 seconds working size, and 6 seconds working size, respectively. Finally, the canals were irrigated with 10 ml of distilled water.

Group 5: Nd: YAG Laser (LSR)

An area where the laser tip can be placed was created by advancing from the coronal to the apical direction with the #30 K file by 1 mm shorter than the working length. Nd:YAG (Fidelis, Fotona, Ljubljana-Slovenia) laser with a wavelength of 1064 nm adjusted to 1.5 W energy, 100 mJ pulse rate and 15 Hz frequency as recommended²⁰, was placed in the 200 µm fiber optic end channel attached to the laser. Irrigation was performed in three rounds of twenty seconds each. After the root canal was filled with 2.5% NaOCl, activation was performed with an optical fiber tip for 10 seconds, and the irrigation solution was renewed for 10 seconds and waited. During the irrigation activation, the optic tip was applied by moving it from the apical foramen to the canal mouth with uninterrupted circular movements. Then, irrigation was performed in a similar way for 1 min with 5 ml of 17% EDTA. Finally, the canals were irrigated with 10 ml of distilled water.

The root canals of a total of 50 teeth with standardized irrigation protocol for each irrigation method group were dried with sterile paper cones (Aceonedent, Geonggi-Do, Korea) by advancing to the working length.

Grooves of sufficient depth were created on the mesial and distal surfaces of the roots along the long axis, taking care not to penetrate the root canals with a diamond cutting separator. Afterwards, the roots were divided into two with the help of a strong and sharp hand tool. The intact part of the roots obtained along the long axis was selected for stereomicroscopic evaluation in terms of the amount of residual intracanal antibiotic paste.

Stereomicroscopic Analysis

A stereomicroscope (Zeiss, Oberkochen, Germany) was used to examine the coronal, middle and apical third regions of the prepared root canal walls for residual intracanal medicament presence. All samples were examined under 25X magnification with a stereomicroscope and images were recorded with a camera (Canon EOS 1000D, Japan) connected to the stereomicroscope.

A 4-point scoring scale defined by Van der Sluis *et al.*²¹ was used for evaluation.

- Score 0: The root canal is completely clean, there is no residual medicament in the canal.
- Score 1: Less than 50% of the root canal has residual intracanal medicament.
- Score 2: More than 50% of the root canal has residual intracanal medicament.
- Score 3: There is residual intracanal medicament in almost the entire root canal.

Scoring of the images was performed by two researchers (B.B and C.H). Each researcher evaluated each image twice, three weeks apart, independently of each other, on different dates and without knowing which group the samples belonged to. In the final scoring, both researchers examined the images with disagreement together and reached a final consensus.

Statistical Evaluation

The data were analyzed using the SPSS (Version 22.0; SPSS Inc., Chicago, USA) program. Kappa statistics were used for the intra-observer and inter-observer agreement. Parametric test assumptions were tested with Kolmogorov-Smirnov test. Kruskal-Wallis and Mann-Whitney U tests were used for pairwise comparisons in case of significance, since parametric test assumptions were not fulfilled. Descriptive statistics including mean, standard deviation, median, minimum and maximum values were presented in the tables. The significance level was set at 0.05.

Results

The distribution of intra-canal medicament removal scores of the experimental groups and the comparison of the root regions in terms of the intra-group comparisons are shown in Table 1 and Figure 1. The findings showed that the different irrigation systems had a statistically significant effect on intracanal medicament removal ($p < 0.05$).

With regard to irrigation methods, the intracanal medicament removal scores of the PUI group were found to be statistically significantly lower than the scores of the LSR, EV, EA and CSI groups ($p < 0.05$). Although there was no

statistically significant difference between the LSR and EV groups ($p > 0.05$), the scores of the two groups were found to be statistically significantly lower than the scores of the EA group ($p < 0.05$). The scores of the EA group were statistically significantly lower than the CSI group ($p < 0.05$). The scores of the CSI group were statistically significantly higher than the scores of the PUI, LSR, EV and EA groups ($p < 0.05$).

With regard to root regions, although there was no statistically significant difference between the intracanal medicament removal scores of the coronal and middle third ($p > 0.05$), the scores of these two groups were found to be statistically significantly lower than the scores of the apical third. The intracanal medicament removal scores of the apical third were statistically significantly higher than the scores of the coronal and middle third.

There was no statistically significant difference between the scores of the coronal, middle and apical third of the PUI group ($p > 0.05$). In the EA group, although there was no statistically significant difference between the coronal and middle third ($p > 0.05$), higher intracanal medicament was removed with statistically significantly lower scores than the apical third region ($p < 0.05$). No statistically significant difference was found between the scores in the coronal, middle and apical thirds in the EV group ($p > 0.05$). In the LSR group, although the scores of the coronal triplet region were statistically significantly lower than the middle and apical third regions ($p < 0.05$), no statistically significant difference was found between the middle and apical third ($p > 0.05$). There was no statistically significant difference between the scores of the coronal, middle and apical third regions in the CSI group ($p > 0.05$).

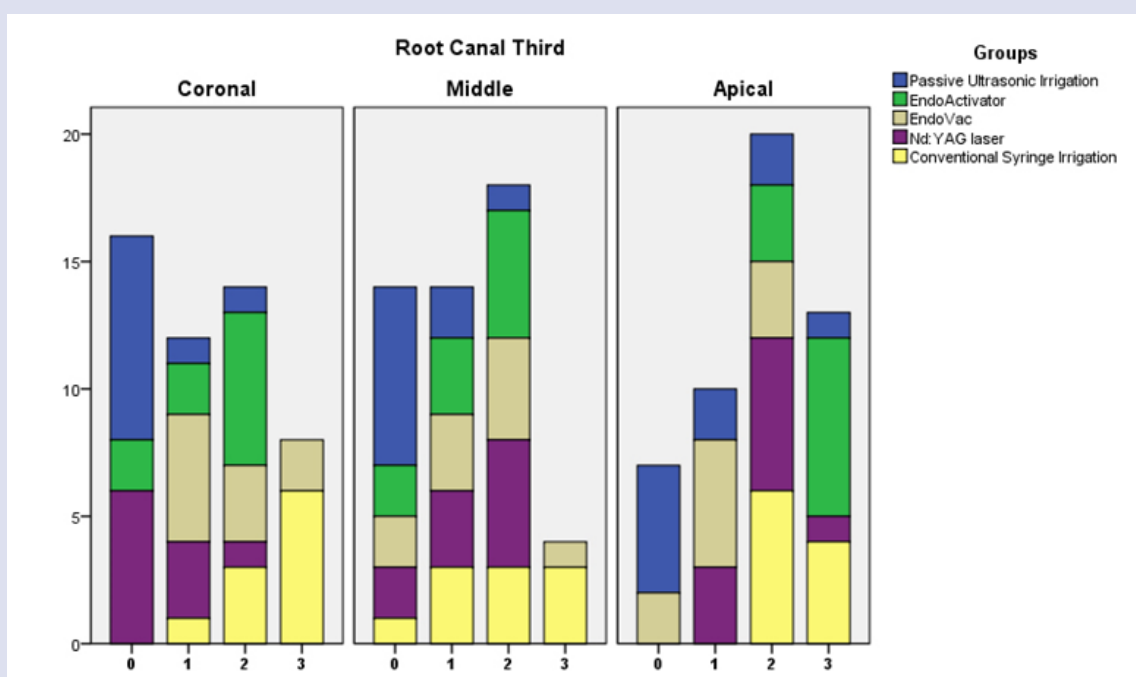


Figure 1. Distribution of intracanal medicament removal scores of the experimental groups according to root regions

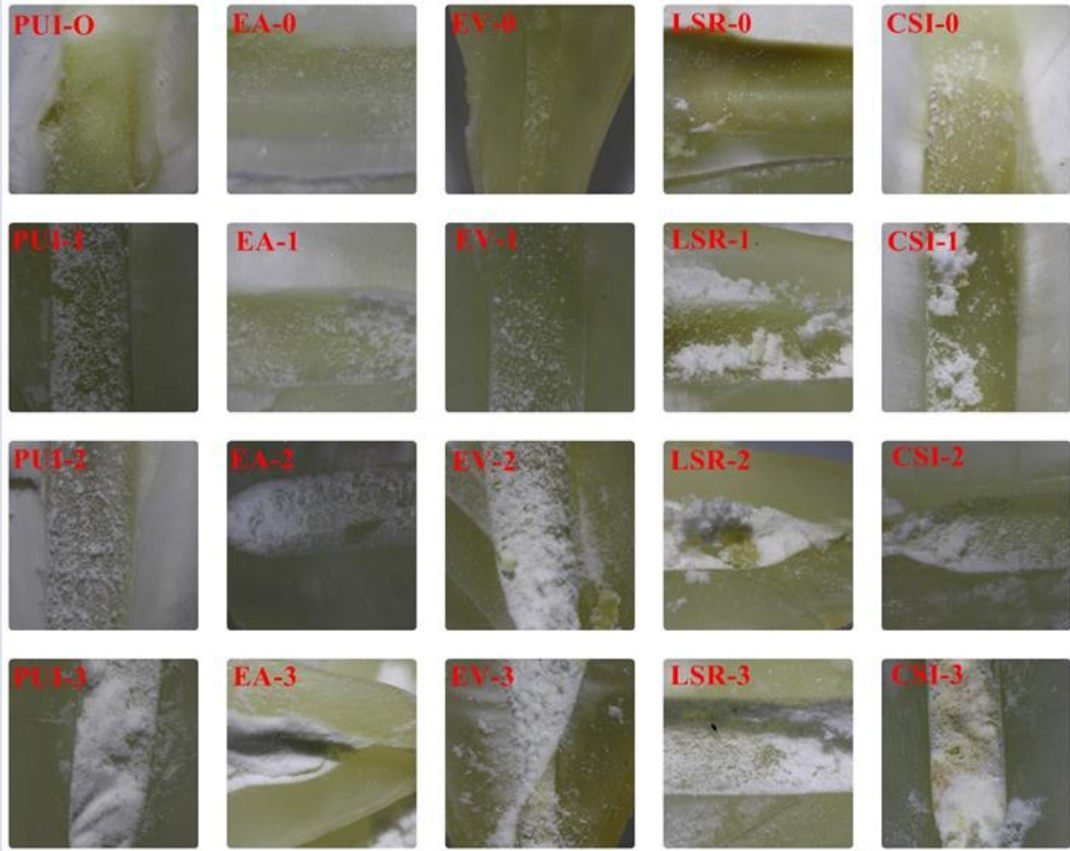


Figure 2. Representative intracanal residual medicament presence and scores of the sections taken from the samples belonging to each experimental group

Table 1. Intra-group comparison of the distribution of intracanal medicament removal scores of the experimental groups and root regions.

Scores	0	1	2	3	Median	Root region*
Passive Ultrasonic Irrigation						$p>0.05$
Coronal	8	1	1	-	0.0	
Middle	7	2	1	-	0.0	
Apical	5	2	2	1	0.5	
EndoActivator						
Coronal	2	2	6	-	2.0	A
Middle	2	3	5	-	1.5	A
Apical	-	-	3	7	3.0	B
EndoVac						$p>0.05$
Coronal	-	5	3	2	1.5	
Middle	2	3	4	1	1.5	
Apical	2	5	3	-	1.0	
Nd:YAG Laser						
Coronal	6	3	1	-	0.0	A
Middle	2	3	5	-	1.5	B
Apical	-	3	6	1	2.0	B
Conventional Syringe Irrigation						$p>0.05$
Coronal	-	1	3	6	3.0	
Middle	1	3	3	3	2.0	
Apical	-	-	6	4	2.0	

*Comparisons were performed within the group in terms of root regions of each group (Kruskal-Wallis and Mann-Whitney U tests. $p<0.05$). Different letters represent statistically significant difference.

Table 2. Pairwise comparisons and p values of the experimental groups in terms of each root region

Root Region	Groups	1	2	3	4	5
Coronal	1. Passive Ultrasonic Irrigation	-	.008	.001	.399	.000
	2. EndoActivator		-	.626	.024	.006
	3. EndoVac			-	.004	.037
	4. Nd:YAG Laser				-	.000
	5. Conventional Syringe Irrigation					-
Middle	1. Passive Ultrasonic Irrigation	-	.021	.019	.021	.004
	2. EndoActivator		-	.840	1.000	.266
	3. EndoVac			-	.840	.386
	4. Nd:YAG Laser				-	.266
	5. Conventional Syringe Irrigation					-
Apical	1. Passive Ultrasonic Irrigation	-	.001	.473	.043	.004
	2. EndoActivator		-	.000	.005	.189
	3. EndoVac			-	.044	.001
	4. Nd:YAG Laser				-	.038
	5. Conventional Syringe Irrigation					-

* Bold values represent statistically significant difference (Kruskal-Wallis and Mann-Whitney U tests. $p < 0.05$).

The p-values of pairwise comparisons between groups in terms of each root third and irrigation method are shown in Table 2. There were significant differences between the groups in terms of root regions of the tested irrigation systems ($p < 0.05$). Although there was no statistically significant difference between the PUI and LSR groups in the coronal third ($p > 0.05$), the scores of the two groups were found to be statistically significantly lower than the scores of the EV, EA and CSI groups ($p < 0.05$). Although there was no statistically significant difference between the EV and EA groups in the coronal third ($p > 0.05$), the scores of the two groups were found to be statistically significantly lower than the scores of the CSI group ($p < 0.05$). The CSI group, on the other hand, showed lower intracanal medicament removal efficiency with a statistically significant difference with all groups ($p < 0.05$).

In the middle third, the scores of the PUI group were found to be statistically significantly lower than the scores of the LSR, EV, EA and CSI groups ($p < 0.05$). Although there was no statistically significant difference between the LSR, EV, EA and CSI groups ($p > 0.05$), they showed the lowest intra-canal medicament removal efficiency, with a statistically significant difference ($p < 0.05$).

Although there was no statistically significant difference between the PUI and EV groups in the apical third ($p > 0.05$), the scores of the two groups were found to be statistically significantly lower than the scores of the LCR, EA and CSI groups ($p < 0.05$). LSR group scores were statistically significantly lower than EA and CSI groups ($p < 0.05$). Although there was no statistically significant difference between the EA and CSI groups ($p > 0.05$), it showed lower intracanal medicament removal efficiency with a statistically significant difference with all groups ($p < 0.05$).

Discussion

The first null hypothesis was rejected because different irrigation systems had a statistically significant effect on intracanal medicament removal. The second null hypothesis was rejected because there was statistically significant difference between root canal third on intracanal medicament removal scores. Findings of this study showed that irrigation system had effect both on intracanal medicament removal. As intracanal medicaments used in RET reduce the bond strength of materials to root canal dentin, they should be removed from root canals. Residual intracanal medicaments also cause tooth discoloration and damage the stem cells in the apical papilla.²²

This study was conducted with extracted human permanent tooth was used for our study. In in vitro studies, for the standardization of imitated immature tooth samples, the root tips of the teeth to be used in the studies were required to be closed, single rooted and single canal. Teeth that did not have these characteristics were not used in this study. It has been sought that there should not be any caries, crown or root fracture, crack or root resorption in the teeth that could change the results of the study.

Different methods have been used in different studies in order to provide the apical form of the immature tooth.^{5,23,24} Topcuoglu *et al.*²⁴ studied 1 mm protrusion from the apex with a #6 no Peeso reamer. Based on the methodology of these studies, the immature tooth model was obtained by working 1 mm protrusion from the apex with a #6 no Peeso reamer, and the root lengths of all teeth were fixed at a size of 15 ± 1 mm. All roots were prepared with the TF Adaptive rotary instrument system in accordance with the procedures and standardization of apical foramen diameters was achieved.

Concentrations of NaOCl ranging from 0.5 to 6% have been used in the majority of cases reported with regenerative/revascularization.^{25,26} NaOCl is cytotoxic to periodontal ligament cells and apical papilla stem cells (SCAPs), although at high concentrations (greater than 3%) it has a positive antimicrobial effect.²⁷ Therefore, it is recommended to use low concentrations of NaOCl. In the early days when RET procedures were introduced, some researchers used NaOCl and chlorhexidine together to perform irrigation. However, its use was abandoned because 2% chlorhexidine solution was found to have cytotoxic effects on stem cells.²⁸

EDTA solution releases various growth factors found in dentin, thereby promoting the differentiation of stem cells attached to dentin surfaces into odontoblast-like cells. Trevino *et al.*²⁸ reported that irrigation protocol containing 17% EDTA preserves the viability of SCAPs. Martin *et al.*²⁹ also reported that final irrigation with 17% EDTA partially reversed the deleterious effects of the NaOCl solution on the survival and differentiation of SCAPs.

This study followed a standardized irrigation protocol based on previous studies. Root canals were irrigated with 2.5% NaOCl at a flow rate of 5 ml/min for 1 min, and then irrigated with 17% EDTA at a flow rate of 5 ml/min for 1 min. During the change of irrigation solutions, while the root canals were filled with solution, the solution was activated with irrigation activation devices for 10 seconds. Activation was carried out for a total of 30 seconds. Removed the effects of irrigation solutions associated with distilled water between passes. The canals were finally irrigated with 10 ml of distilled water to remove the prolonged effects of EDTA and NaOCl.

There is no consensus on the removal or preservation of the smear layer formed during root canal treatment. It is reported that irrigation solutions, medicaments and root canal fillers prevent penetration into dentinal tubules, reduce their bond strength against micromechanical forces, and therefore argued that it should be removed. Contrary, it is also reported that smear layer reduces dentin permeability and acts as a barrier against bacterial products, so it should not be removed because it prevents bacterial invasion into the dentinal tubules.^{14,17} It is known that due to the acidic nature of EDTA, which is used to remove the smear layer, calcium silicate-based cements have a negative effect on their hydration properties. This prevents the cements from bonding to the root canal. When EDTA cannot be effectively removed from the root canal in the final wash, it chelates with calcium ions released from the cement during the hydration of calcium silicate-based cements, resulting in the formation of hydrated products and also reduces the hardness and bond strength of tricalcium silicate-based cements.^{30,31}

The findings of this study were found to be consistent with the results of previous studies.³² Jiang *et al.*³² compared different irrigation systems in removing dentin chips from the grooves artificially opened in the root canals and concluded that PUI group showed the highest removal scores, while the CSI group showed the lowest removal values. Arslan *et al.*³³ compared Qmix and different

irrigation systems in removing the smear layer and concluded that use of Er:YAG and fiber tips showed more effective removal of the smear layer than the EA and CSI groups, and no statistically significant difference was found between the EA and CSI groups. These findings are consistent with this study.

Various methods such as digital photographs, stereomicroscopes, scanning electron microscopes, microcomputed tomography imaging and spiral computed tomographic imaging are used to evaluate the amount of medicament remaining in the root canal walls. Stereomicroscope has been used as an evaluation device in many studies on intracanal medicament removal. Therefore, in this study, a stereomicroscope was used to evaluate mTAP residues in the root canal walls.

Similar to current study, Plotino *et al.*³⁴ compared sonic and ultrasonic systems in intracanal medicament removal and reported that ultrasonic systems are more effective than sonic systems. In this study, with regard to root canal third, PUI had significantly higher intracanal medicament removal scores than EA, which is a sonic system. Typically, a sonic device operates at 1-8 kHz and an ultrasonic device operates at 25-40 kHz. This may be possible reason for the statistically significant results is the difference in operating speed between the devices.

Similar to previous studies³⁵, this study revealed that PUI removed TAP from the canal walls more effectively than CSI. However, no statistically significant difference was found between PUI, EV and EA groups. Akman *et al.*³⁵ also concluded that no significant difference was found between regions in CSI group. In that study, similar to this study, the CSI method did not show a statistically significant difference in the coronal, middle and apical thirds. In the PUI group, statistically significantly higher mTAP removal scores were observed in the apical and coronal triads. In this study, however, the PUI group did not show a statistically significant difference in the coronal, middle and apical third. This may be due to the decrease in the effectiveness of the PUI tip by contacting the canal wall as a result of the use of a tooth with complete root development in the study by Akman *et al.*³⁵. Finally, EV and EA showed significantly higher mTAP removal scores in the apical triad than in the middle and coronal third. In this study, while EA showed significantly lower scores in the apical triad compared to the coronal and middle triad, the EV did not show a significant difference in the coronal, middle and apical triad. This difference may have arisen due to the differences in the effectiveness of irrigation activation methods.

Berkhoff *et al.*³⁶ reported that TAP in the root canal could not be removed using EDTA and different irrigation activation methods. They also showed that more than 85% of the TAP remained in the dentin, and the majority of the TAP was found at depths greater than 350 µm. Contrary to these results, the use of different irrigation techniques in this study increased the removal of TAP from the root canals. However, in our study, mTAP was not labeled as radioactive and evaluation was made on photographs taken under 25x magnification under a stereomicroscope. This can be considered as a limitation of our study.

Conclusions

Intracanal medicament removal scores were significantly associated with irrigation system. PUI showed statistically significantly higher intracanal medicament removal efficiency than other irrigation methods. The least intracanal drug removal efficiency was seen with conventional syringe irrigation. While there was no difference between the coronal and middle thirds, statistically significantly less intracanal medicament removal was found in the apical third compared to the other regions. While adhesive fracture was the most common type of fracture in all groups, mixed fracture was the least common type of fracture.

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The Effect of Aging and Different Surface Treatments on Temporary Cement Bonding of Temporary Crown Materials

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ABSTRACT

Objectives: The purpose of this research was to determine the effect of different surface treatments and aging on the bond strength of different temporary restorative materials with temporary cements.

Methods: 252 temporary crown materials 2 mm thick with a 10 mm diameter were prepared. No surface treatment was administered to the control group. 4% hydrofluoric acid gel was administered to one of the other groups and sandblasted to the other. Two types of temporary cement were used. After a 5000-cycle thermal cycle was administered to half of the materials, cement bond strengths were measured.

Results: It was found that the material used, the type of cement, the aging treatment, the material*aging treatment, and the material-cement interaction ($p<0.001$) were statistically very significant, the material*cement*aging treatment interaction ($p<0.05$) were significant, and the other interactions were insignificant ($p>0.05$).

Conclusion: Within the limitations of this in vitro study, it was found that the tested cements and surface treatments could not be implemented for all materials tested. It was found that the cement bond strength increased significantly in the temporary crowns administered by sandblasting. In the case of long-term use of the temporary restoration tested by evaluating the simulation of the oral environment, the use of a sandblasting surface treatment may be appropriate. It may be said that polymethylmethacrylate temporary crowns obtained by the conventional method have better bonding with eugenol-containing cements.

Keywords: Temporary dental restoration, luting agents, dental bonding, surface properties, thermocycling.

Farklı Yüzey İşlemlerinin ve Yaşlandırmanın Geçici Kuron Malzemelerinin Geçici Siman ile Yapıştırılmasına Etkisi

Süreç

Geliş: 02/01/2023

Kabul: 24/04/2023

Öz

Amaç: Bu araştırmanın amacı, farklı yüzey işlemlerinin ve yaşlandırma işleminin, farklı geçici restoratif materyallerin geçici simanlarla bağlanma dayanımına etkisini belirlemektir.

Yöntemler: 2 mm kalınlığında 10 mm çapında 252 adet geçici kuron materyali hazırlandı. Kontrol grubuna herhangi bir yüzey işlemi uygulanmadı. Diğer gruplardan birine %4'lük hidroflorik asit jeli, diğer gruba kumlama uygulandı. İki tür geçici siman kullanıldı. Malzemelerin yarısına 5000 döngü (6 ay) termal siklus uygulandıktan sonra makaslama bağlanma dayanımları ölçüldü.

Bulgular: Kullanılan materyal, siman tipi, yaşlandırma işlemi, malzeme*yaşlandırma işlemi ve malzeme-siman etkileşimi ($p<0.001$) istatistiksel olarak çok anlamlı, malzeme*siman*yaşlandırma işlemi etkileşimi ($p<0.05$) anlamlı ve diğer etkileşimler anlamsız bulundu ($p>0.05$).

Sonuç: Bu in vitro çalışmanın sınırlamaları dahilinde, test edilen simanların ve yüzey işlemlerinin test edilen tüm materyaller için uygun olmadığı bulunmuştur. Kumlama uygulanan geçici kronlarda makaslama bağlanım dayanımının önemli ölçüde arttığı bulundu. Ağız içinin simülasyonu değerlendirilerek test edilen geçici restorasyonun uzun süreli kullanımının söz konusu olduğu durumunda, kumlama yüzey işlemi uygun olabilir. Konvansiyonel yöntemle elde edilen polimetilmetakrilat geçici kronların öjenol içeren simanlarla daha iyi bağlandığı söylenebilir.

Anahtar Kelimeler: Geçici restoratif materyaller, geçici simanlar, yüzey işlemleri, yaşlandırma.

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Introduction

The use of temporary restorative materials is of great importance in the success of fixed prosthetic restorations until the completion of the final restoration. An ideal temporary restoration guides the healing of soft tissues and is also of the essence in maintaining pulp and gingival health, preventing elongation of abutment teeth, and providing aesthetics.¹

These crown materials can be prepared by direct and indirect techniques. Temporary restorative materials were designed using CAD/CAM technology; thus, it was produced with the use of digital dentistry in many areas.²

It has been reported that bis-glycidyl methacrylate-based and bis-acryl-based materials showed lower polymerization shrinkage and have better mechanical properties compared to acrylic resin-based materials.³ However, the prolongation of the time spent at the chairside with direct technique has increased the popularity of temporary restorative materials produced by digital methods.⁴

Long-term use of temporary restorations may be required in implant applications and occlusal reconstruction situations.⁵ The stability of physical and mechanical properties, which increase in importance in long-term use, has been argued to be sufficient in temporary crown materials produced with CAD/CAM.² It has been argued that the blocks are more homogeneous due to the pre-polymerization and that there is no polymerization shrinkage, compared to the polymethylmethacrylate (PMMA) blocks prepared by the traditional method in the temporary crown materials produced with CAD/CAM.^{2,6} All restorative materials used in the oral environment must be resistant to masticatory forces.³

Bonding cements have a supporting role between the fixed prosthesis and the cut tooth.⁷ Bonding cements prevent the movement of the restoration against the masticatory forces and prevents the teeth from interacting with the external environment. They not only bond the restoration with the tooth but also fills the gap between the two structures and provide resistance against vertical and lateral forces.⁷ The strength of the cement to which the restorative material is adhered to the tooth and its bonding to the tooth and restorative material are also important in the success of fixed prosthetic restorations. By bonding the restorations with ideally prepared cements, the health of the surrounding tissues of the teeth and abutments will also be preserved. Adhesive agents and mechanical and antibacterial properties should be good.⁸

The marginal adaptation of cementation techniques in temporary crowns was investigated⁷, and the effect of different surface treatments on bonding in the repair of temporary crown materials was evaluated⁹; however, no study has been found which examined the bond strength of temporary restorative materials with temporary cement and the way in which this strength is affected by different surface treatments and thermal cycles.

The aim of this study was to investigate the effect of aging treatment and surface treatments on the bond strength of temporary restorative materials prepared by

the conventional method and CAD/CAM with temporary cements with different contents.

In the study, the H0 hypothesis showed that surface treatment application would increase bond strength, and the H1 hypothesis showed that aging treatment would reduce bonding.

Material and Methods

This study was approved with the decision taken in Ataturk University Faculty of Dentistry (Date: 22.06.2021 Issue No: 43) meeting of the Ethics Committee.

The temporary restorative materials shown in Table 1 were used in this study. A total of 252 samples were prepared with a diameter of 10 mm and a thickness of 2 mm. In the main hypotheses of the research, the differences between the independent groups were planned to be investigated, and the sample size was calculated at a 95% confidence level using the G Power-3.1.9.2 program. According to the analysis result, the minimum sample size of 52 per group (180 for total sample size), based on a theoretical power of 0.80, α value of 0.05, and a standardized effect size of 0.25 (moderate).

A metal plate with circular cavities with a diameter of 10 mm and a thickness of 2 mm was used as a mold for the temporary crown materials (Imicryl Imident, Konya, Türkiye; 3M ESPE Protemp™ 4, 3M Deutschland GmbH Dental Products Carl-Schurz-Str.1 41453 Neuss-Germany) prepared by the conventional method. The materials prepared according to the manufacturer's instructions were placed in the mold, and slightly pressure was applied to the cement glass in order to obtain a smooth shape. Polymethylmethacrylate (PMMA) blocks (Tempo Cad., On-Dent Ltd, Izmir, Türkiye) were digitally designed and prepared and smoothed with a diamond burr. The samples were kept in 37°C distilled water for 24 hours.

Samples prepared with an autopolymerizing acrylic resin (Imicryl, SC, Konya, Türkiye) were placed in silicone molds with a diameter of 15 mm and a height of 20 mm obtained in order to fit the test device on which the experiment would be performed. The samples were washed in an ultrasonic cleaner for 15 minutes and prepared for surface treatments by air drying. Each material was randomly divided into three groups and surface treatments were applied. No surface treatment was applied to the samples in the first group. A thin layer of 4% hydrofluoric acid gel (Porcelain etchant, Bisco, Schaumburg, IL, 60193, USA) was applied for 120 seconds to samples in the second group. Then the samples were washed for 120 seconds and dried for 10 seconds.⁹ Sandblasting was performed on the samples in the third group by applying 50 μ m aluminum oxide powder (Korox, Bego, Canada) for 10 seconds from a distance of 10 mm with an air abrasion device.

A silicone mold with an internal clearance diameter of 5 mm and a height of 4 mm was prepared to apply the cements. Temporary cements which were mixed in accordance with the manufacturer's instructions containing

eugenol (Temp-Bond™, Kerr, Italy) and calcium hydroxide cement (Life Regular Set, Kerr, Italy) were placed one by one in the molds, covered with a cellulose tape and hardened by applying with finger pressure. The samples were cut from the silicone mold by means of lancet and removed slowly. Half of the samples (n=7) were kept in distilled water at 37 °C for 24 hours before measuring the cement bond strength.¹⁰ 5000 aging treatment were applied to the other half in the baths with 5 and 55 °C (Esetron Smart Robotechnologies, mod dental, 220 V AC – 50 Hz – 3500 W, Ankara, Türkiye) so that the transfer time between baths would be 60 sec.¹⁰

The samples were fixed to the metal test equipment, and the test was placed at one end of the device. The edge was placed in the form of a knife-edge and ended in accordance with the round section of the sample at the other end of the test device.⁹ Loading was done on a Universal test device (Instron, Model 2710-003, Instron Corp., USA) with a 0.5 mm/min head speed. The maximum load was recorded when the fracture occurred. The bond strength was calculated by using the following formula:⁹ $\sigma = F / A$ (σ , bond strength (MPa); F, load at fracture (N); and A area of cement (mm²).

Statistical Analysis

Statistical analysis was performed using the Statistics 20.00 (SPSS Inc., Chicago, IL., USA) computer program at 95% confidence interval and p=0.05 significance level. The conformity of the variables to the normal distribution was examined using the Shapiro Wilk test, which is one of the analytical methods, and it was found to be suitable. The data obtained in this study were evaluated with three-way analysis of variance multiple comparison (Tukey) test.

Results

As a result of the analysis of variance; it was found that the material used, the type of cement, the type of aging treatment, the material*aging treatment, and the material-cement interaction (p<0.001) were statistically very significant, the material*cement*aging treatment interaction (p0.05) were significant, and the other interactions were insignificant (p0.05). (Table 2)

The average bond strength values and standard deviations of all groups are shown in Table 3.

Table 1. Temporary restorative materials used in the study

Materials	Manufacturer	Feature
CAD/CAM	(Tempo Cad. PMMA Block)	On-Dent Ltd, Izmir, Türkiye
Conventional Method	Protemp	3M Deutschland GmbH Dental Products Carl-Schurz-Strabe 141453 lieuss-Germany
	Imicryl	Imicryl Imident; Konya, Türkiye
		polymethylmethacrylate Block
		nano-filled bis-akrilik composite
		Chemically polymerized cadmium-free polymethylmethacrylate

Table 2. Analysis of variance

	Sum of Squares	df	Mean Square	F	p
Materials (M)	11.80	8	1.47	12.27	< .001
Aging Treatment (AT)	3.06	1	3.06	25.47	< .001
Cements (C)	2.82	1	2.82	23.45	< .001
M * AT	4.34	8	0.54	4.52	< .001
M * C	4.29	8	0.54	4.46	< .001
AT * C	0.29	1	0.29	2.43	0.121
M * Yı * S	2.46	8	0.31	2.56	0.011
Total	25.95	216	0.12		

Table 3. The average bond strength values and standard deviations

MATERIALS	Surface Treatments	Aging Treatment							
		Without Aging Cements				With Aging Cements			
		Tempbond		Dycal		Tempbond		Dycal	
		Means	SD	Means	SD	Means	SD	Means	SD
CAD/CAM (polymethylmethacrylate block)	Control	0.23	0.13	0.37	0.26	0.14	0.10	0.44	0.25
	HF acid	0.40	0.16	0.27	0.12	0.29	0.23	0.18	0.12
	Sandblasting	0.63	0.18	0.64	0.40	0.56	0.17	0.65	0.44
Protemp (nano-filled bis-akrilik composite)	Control	0.10	0.20	0.38	0.16	0.30	0.88	0.46	0.17
	HF acid	0.11	0.14	0.59	0.14	0.30	0.27	0.46	0.29
	Sandblasting	0.52	0.20	0.67	0.23	0.41	0.26	1.49	0.72
Imicryl (cadmium-free polymethylmethacrylate)	Control	0.35	0.18	0.42	0.23	0.65	0.24	0.72	0.32
	HF acid	0.46	0.10	0.42	0.29	0.68	0.20	0.63	0.35
	Sandblasting	1.87	0.92	0.78	0.20	0.86	0.50	0.75	0.22

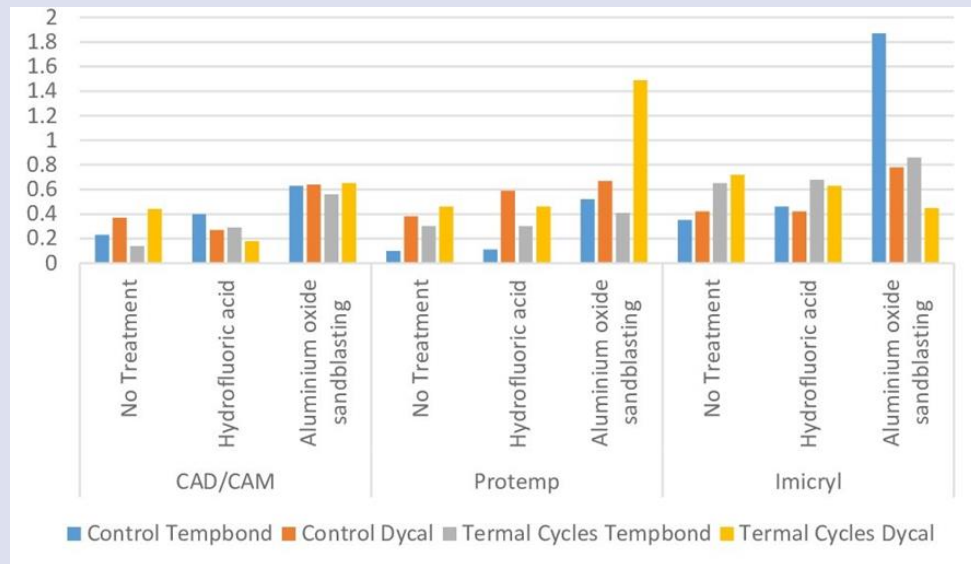


Figure 1. Distribution of bond strength of luting cements

It was found that the highest bond strength was in the (Imicryl) (1.87 MPa) samples without aging applied with eugenol-containing cement (Tempbond) applied with sandblasting, and the lowest bond strength was in the control group Protemp samples (0.10 MPa) bonded with untreated eugenol-containing cement (Tempbond).

Figure 1 shown distribution of bond strength of luting cements according to surface treatments.

As a result of the multiple comparison (Tukey) test of material* cement* aging treatment interaction:

In the samples which were sandblasted Protemp, treated with aging, and bonded with calcium hydroxide cements (Dycal),

A statistically significant difference was found at the $p < 0.001$ level in the samples that were sandblasted Imicryl, treated with aging, and bonded with calcium hydroxide cements (Dycal).

The bond strength values obtained with the acid-treated samples were similar to those of the untreated samples.

Discussion

The hypotheses of the study were accepted since it was found that the bond strength increased in the samples that were sandblasted, and the aging treatment reduced bond strength.

Bond strength of temporary cements to dentin, implant abutments, and restorative materials is particularly important if they are to be used long-term. It was emphasized that it may be beneficial to increase the surface roughness to improve bond strength and promote mechanical joints.¹¹ It was found that surface treatments showed different effects on different materials in a study in which temporary restorations were repaired with related material.⁹

It has been reported that the roughness on the surfaces of the sandblasted temporary crown materials increased

significantly; SEM images confirm the presence of micromechanical retention and show visible changes in the topographic model.¹²

To increase the surface roughness, in this study, mechanical methods including sandblasting with 50 μm Al_2O_3 particles and etching with 4% hydrofluoric acid were used.⁹ These methods are easily available in all dental clinics and are easy to apply.^{9,11} Standardization attempted by smoothing the surface of each material with a diamond burr. Some authors recommend the use of SiC paper with 220-grain for this purpose.¹³ However, it is not possible to sand the inside of the crown in the clinic. At the clinic, inside surface of the crown may gently be removed by a carbide or diamond burr in some cases.

In studies, it has been reported that sandblasting increases the bond strength by creating the micro-retaining areas on the surface of the composite resin material.^{9,11,14} It has been emphasized that micromechanical adhesion is very important in the repair processes of composite resin.^{15,16} In this study, the increase in the cement bond strength in all samples can be explained by an increase in micromechanical retention. Sandblasting is thought to be an effective surface treatment for cement bonding.

Hydrofluoric (HF) acid gel provides micromechanical adhesion by abrading the glass particles in the matrix to form porous surfaces and voids.¹⁷ In one study, it was reported that the application of HF acid did not affect the bond strength in polymethylmethacrylate or even reduce it, and it was stated that this may be due to the low amount of glass particles and high hydrophobic monomer content of polymethylmethacrylate.⁹ It was found to have a bond strength similar to the untreated surfaces on the acid-treated surfaces in this study. It has also been reported that the surface of polymethylmethacrylate softens and its roughness decreases when HF acid is applied.^{18,19} It was found that the shear bond strength increased with the application of HF acid in bis-acrylic resins such as Protemp.⁹ This was attributed to the resin containing more than 24%

filler by its volume rate.⁹ It has been stated that the erosivity of HF acid depends on the amount and type of filler.⁹ and that the use of HF acid might be inconvenient in cases where the resin content of the restorative material is unknown.¹⁷⁻¹⁹ In this study, similar bond strength values were obtained in all bis acrylic resin groups including the groups treated with HF acid and the groups without surface treatment. It would be more appropriate to choose calcium hydroxide cements in this type of temporary crown materials.

Shear tests are applied frequently on the grounds that results are more able to be estimated.²⁰ However, the distribution of the applied load is standardized.²¹ Teeth and restorative materials are exposed to complex forces in the oral environment.²² This is one of the limitations of the study.

It was found that the polymethyl methacrylate samples prepared with CAD/CAM were not superior to the polymethyl methacrylate produced by the conventional method in bond strength with eugenol-containing cement. It was found that the type of cement to be used in the bis-acrylic composite-based temporary restorative material that can be prepared directly at the chairside is important. In this sense, it was found that bonding bis-acrylic composite-based temporary restorations with calcium hydroxide cement and bonding polymethyl methacrylate temporary restorations obtained by conventional method with eugenol-containing cement were better. It was found that a sandblasting surface treatment can be applied in cases requiring long-term use.

The most important function of temporary cements is to seal, thus preventing marginal seepage and irritating the pulp.²³ Temporary cements should be easy to remove when necessary. However, they must be sufficiently retentive to perform their functions. Little is known about the retentive properties of temporary cements, but it has been stated that there is a strong relationship between the holding properties of temporary cements and their compressive strength.²⁴

It has been found that aging treatments reduce the time that temporary cements remain functional.²⁵ It is known that not only the retention properties of cements but also the shaping of the crown has a significant effect on retention.²⁶

It has been found that the stress causing dislocation movement is higher in polymethyl methacrylate crowns than in composite crowns for temporary cements. However, it has been found that there is a reverse situation when using eugenol-containing cement (Temp-Bond). In the same study, calcium hydroxide cements were found to be more resistant to dislocation forces than eugenol-containing cement (Temp-Bond) in polymethylmethacrylate temporary crowns.²⁷ For this reason, eugenol-containing cement and calcium hydroxide cement was used in the study. These are temporary cement materials frequently used, and in other respects, the temporary cements used in the present study were economical and easily accessible.

Being sufficient bonded with cement will increase the resistance of the restoration. Long-term use of temporary restorations may be possible with the selection of the appropriate material and surface treatment and the use of the appropriate cement. It was found that the cement bond strength of all the materials used in this study increased with sandblasting surface treatment. However, it was seen that not all types of cement can be used with all types of materials.

The thermal cycle is a method used to simulate the oral environment. As *in vivo*, no evidence of the number of possible cycles was found. However, it has been reported that 10,000 thermal cycles per year correspond to approximately one year of *in vivo* function.²⁸ The use of 5000 cycles in this study corresponds to a six-month retention time in the mouth. This period is likely to be applicable in the clinical setting when long-term temporary crown use is required.

The polymethylmethacrylate samples produced by CAD/CAM, which is one of the materials subjected to 5000 thermal cycles (5 C and 55 C) in a study examining the effect of thermal cycling on the properties of temporary restorative materials, showed better marginal accuracy than bis-acryl materials. It has been reported that CAD/CAM temporary crowns can be recommended for long-term treatments.²⁹ In this study, it was found that the sandblasting increased the cement bond in the polymethylmethacrylate samples produced with CAD/CAM. However, it was found that the polymethylmethacrylate samples produced by the conventional method were superior in the long term and when calcium hydroxide cement was used. In addition, it was found that the bond of nano-filled bis-acrylic composite-based temporary crown materials with eugenol-containing cements was very weak. It was seen that the bond values of the polymethylmethacrylate produced with CAD/CAM are similar with this type of cement. It is known that eugenol and resin polymers are incompatible.^{30,31} In this study, the bond of a eugenol-containing cement to the bisacryl composite-based temporary crown material and the polymethylmethacrylate produced by CAD/CAM was slightly increased by sandblasting.

Conclusions

Cement selection is important depending upon the material used in temporary crown materials. Sandblasting surface treatment increased bonding. The thermal cycle application had a negative effect on the cement bond. The reason for the decrease in cement bonding over time according to the time it remains in the mouth should be examined.

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Conflicts of Interest Statement

The authors have no conflicts of interest

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Effect of Repair Material Type and Thermocycling Aging on Repair Bond Strength to Various Artificial Teeth

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ABSTRACT

Objectives: To compare repair bond strength of acrylic and composite resin to different type of artificial teeth with and without thermocycling aging (TMC).

Materials and Methods: A total of 192 specimens were prepared using four different types of artificial teeth (n=48) (Group CA-Conventional polymethylmethacrylate, Group IS-Isosite, Group DCL-Double cross-linked acrylic, Group NC-Nanohybrid composite). All specimens were aged with TMC (5000 cycles, 5°C/55°C) and repaired with auto-polymerized acrylic or composite resin. Half of the repaired samples were subjected to the shear bond strength (SBS) test while the other half were subjected to TMC to simulate the aging of the repair material. Then, artificially aged specimens were also subjected to the SBS test. Data were statistically analyzed by three-way analysis of variance (ANOVA). Paired comparisons were made using one-way ANOVA, and multiple comparisons were made using the Tukey HSD test ($\alpha=0.05$).

Results: The bond strength of both non-aged and artificially aged composite resin and non-aged acrylic resin did not differ according to artificial teeth type ($p>0.05$). However, artificially aged acrylic resin showed higher bond strength for group CA (9.25 ± 2.96) than for group NC (5.01 ± 3.09) ($p<0.05$). The bond strength of composite resin was higher than acrylic resin regardless of artificial teeth type and TMC ($p<0.05$). TMC decreased the bond strength of both acrylic and composite repair materials to all types of artificial teeth ($p<0.05$).

Conclusions: New-generation artificial teeth used in removable dentures are costly. Therefore, repairing fractured teeth with composite resins instead of replacing them can be both time-efficient and economical.

Keywords: Artificial teeth, shear bond strength, autopolymerizing acrylic resin, composite resin.

Tamir Materyali Tipi ve Yaşlandırmanın Çeşitli Suni Dişlere olan Bağlanma Dayanımı Üzerine Etkisi

Süreç

Geliş: 02/01/2023

Kabul: 10/04/2023

Öz

Amaç: Akrilik ve kompozit rezinin farklı tipteki yapay dişlere termal yaşlandırma ile ve yaşlandırma olmadan tamir bağlantı dayanımını karşılaştırmaktır.

Gereç ve Yöntemler: Dört farklı yapay diş tipi (n=48) kullanılarak toplam 192 numune hazırlandı (Grup CA-Konvansiyonel polimetilmetakrilat, Grup IS-İzosite, Grup DCL-Çift çapraz bağlı akrilik, Grup NC-Nanohibrit kompozit). Tüm örnekler termal siklus ile yaşlandırıldı (TMC; 5000 döngü, 5°C/55°C) ve otopolimerize akrilik rezin veya kompozit rezin ile tamir edildi. Tamir edilen örneklerin yarı makaslama bağlanma dayanımı (SBS) testine, diğer yarı ise tamir materyalinin yaşlanmasını taklit etmek için TMC'ye tabi tutuldu. Daha sonra yaşlandırılmış örnekler de SBS testi uygulandı. Veriler, üç yönlü varyans analizi (ANOVA) ile istatistiksel olarak analiz edildi. İkili karşılaştırmalar tek yönlü ANOVA kullanılarak, çoklu karşılaştırmalar ise Tukey HSD testi kullanılarak yapıldı.

Bulgular: Hem yapay olarak yaşlandırılmamış hem de yaşlandırılmış kompozit rezinin ve yapay olarak yaşlandırılmış akrilik rezinin bağlanma dayanımı yapay diş tipine bağlı olarak değişiklik göstermedi ($p>0,05$). Öte yandan yapay olarak yaşlandırılmış akrilik rezin CA grubu ($9,25\pm2,96$) için NC grubuna ($5,01\pm3,09$) olandan daha yüksek bağlanma gücü gösterdi ($p<0,05$). Yapay diş çeşidi ve TMC den bağımsız olarak kompozitin bağlanma dayanımı akrilikten daha yüksek bulundu ($p<0,05$). TMC, hem akrilik hem de kompozit tamir materyalinin bağlanma gücünü tüm yapay diş tiplerinde azalttı ($p<0,05$).

Sonuçlar: Hareketli bölümlü protezlerde kullanılan yeni nesil suni dişler pahalı olduğu için kırılan dişleri değiştirmek yerine kompozit rezinlerle tamir etmek hem zamandan kazanç sağlar hem de daha ekonomik bir çözüm sunar.

Anahtar Kelimeler: Suni diş, makaslama bağlanma direnci, otopolimerize akrilik rezin, kompozit rezin.

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Introduction

Artificial teeth of complete and partial dentures restore chewing function, phonation, and aesthetics caused by teeth loss and adjacent structures.¹ Polymethyl methacrylate (PMMA) is the most frequently used artificial teeth type due to its advantages including good biocompatibility, dimensional stability, adhesion to denture base, and ease of manipulation.² However, PMMA artificial teeth have disadvantages such as low fracture resistance, susceptibility to wear, and color instability.²⁻⁴ To overcome these unsatisfactory features, new generation acrylic teeth with highly cross-linked isosit material, acrylic resin teeth with interpenetrating polymer mesh, and composite resin teeth with different filler size have been developed with the help of advancements in new technologies.⁵ As such, by the addition of crosslinking agents, interpenetrating polymer mesh, different monomers, and fillers of various sizes, conventional acrylic resin teeth have been modified to strengthen their physical and mechanical properties.^{6,7}

Fracture of artificial teeth after a certain time of clinical service or due to accidental denture dropping by patients who suffer from motor control impairments is a common problem.³ Denture tooth fracture and chipping may become a more significant clinical problem with the increased use of implants and the associated increase in forces applied to prosthetic components.⁸ The fact that these new generation acrylic artificial teeth are expensive makes it advantageous to repair the teeth instead of replacing them.⁹ Immediate in-office repair of fractured denture teeth with composite or auto-polymerized acrylic resins reduces the frequency of appointments and costs, causes minimal discomfort to patients, and eliminates the need for time-consuming procedures.^{9,10}

Previous studies mainly focused on the effect of surface treatments and bonding agents on debonding of artificial teeth from denture base caused by clinical service.^{8,9,11} However, studies evaluating the repair of new-generation artificial teeth with composite or acrylic resin are lacking in the literature. Therefore, this study aimed to compare the bond strength of auto-polymerized acrylic and composite resin before and after thermocycling aging (TMC) to repair newly developed artificial teeth. The null hypotheses of the study are: 1) The bond strength will not differ according to the repair material used, 2) The bond strength will not differ regarding the type of artificial teeth, 3) The bond strength will not change after TMC.

Materials and Methods

This study evaluated the effect of TMC on repair bond strength of auto-polymerized acrylic and composite resin to 4 different types (conventional polymethylmethacrylate, isosite, double cross-linked acrylic and nanohybrid composite) of artificial teeth (Table 1). The minimum sample size was calculated at 0.25 effect size, 85% power, and $\alpha=0.05$ error level as $n=12$ per group ($N=192$). The design of this research was approved by the Clinical

Research Ethics Committee of Lokman Hekim University Faculty of Dentistry, Turkey (2022/152; 2022/161). The workflow of the study is shown in Figure 1.

Specimen Preparation

Twenty-four mandibular first molar artificial teeth from each group were sliced vertically by a diamond saw (Microcut 201; Metkon, Turkey) to divide the teeth into two parts. A total of 48 specimens were prepared for each type of artificial teeth and wet-polished with 600- and 800-grit silicon carbide papers. Then, all specimens were artificially aged with TMC (THE-1100; SD Mechatronik, Feldkirchen-Westerham, Germany) in distilled water to simulate intraoral service (5000 cycles, 5°C/ 55°C thermal application, and dwell time of 30 s).

Half of the specimens were treated with acrylic resin and the other half was treated with composite resin to simulate repair. The surface of the specimens repaired with acrylic resin was wetted with methyl methacrylate (MMA) monomer (Meliodent; Heraeus Kulzer Ltd, Newbury, Berkshire, United Kingdom) for 3 minutes and air-dried. Then, auto polymerizing acrylic resin was mixed according to the manufacturer's directions and placed in a polytetrafluorethylene (PTFE) mold (2.5 mm diameter, 4 mm height) which was fixed on the surface of the specimen. After complete polymerization, the PTFE mold was cut and separated carefully from the specimen-resin assembly.

The surface of the specimens repaired with composite resin was treated with Scotchbond Universal Adhesive (3M ESPE, St. Paul, MN, USA) and polymerized with a light curing unit (Bluephase 20i-High power mode; Ivoclar Vivadent, Amherst, NY, USA) for 10 seconds. Then, light curing nanohybrid composite (i-Light N; i-dental, Šiauliai, Lithuania) was placed in the PTFE mold and polymerized with the same light-curing device for 20 seconds. Then, the mold was cut and separated. All repaired specimens were kept in an etuve at 37 °C for 48 hours.

Half of the repaired specimens in each group were immediately subjected to shear bond strength (SBS) test, while the other half were artificially aged with TMC in distilled water (5000 cycles, 5°C/ 55°C thermal application, and dwell time of 30 s) to simulate a period of intraoral service of repaired artificial tooth ($n=12$). Then, artificially aged specimens were also subjected to the SBS test.

Shear Bond Strength Test

The SBS test was performed using a computer-controlled universal testing machine (Lloyd Instruments Ltd.; Hampshire, United Kingdom) with 1 mm/min crosshead speed. The maximum load at failure was recorded in Newtons (N). This value was divided by the adhesive surface area (mm^2) to calculate the SBS value in megapascals (MPa) for each specimen.

Failure Mode Analysis

The surface of all debonded specimens was examined with a stereomicroscope (M3Z; Leica Microsystems,

Wetzlar, Germany) under 25x magnification to determine the failure modes. The failure modes were classified as follows: 1) adhesive failure between the repaired acrylic/composite resin and the artificial tooth interface, 2) cohesive failure either within the repaired acrylic/composite resin or the artificial tooth, 3) mixed failure with both adhesive and cohesive failures exhibited (Figure 2).

Statistical Analysis

The Shapiro-Wilk test was used to evaluate the normal distribution of the data. The effects of the artificial teeth (4 levels: CA, IS, DCL, NC), repair material (2 levels: auto-polymerized acrylic resin, composite resin) and TMC condition (2 levels: +, -) on SBS were statistically analyzed by three-way analysis of variance (ANOVA). As the binary and triple interactions were significant, comparisons were analyzed using one-way ANOVA. Multiple comparisons were performed using the post-hoc Tukey HSD test. The statistical significance level of the data was taken as $\alpha=0.05$. All statistical analyses were performed using IBM SPSS statistical software (v 27.0, Armonk, New York, USA)

Results

Descriptive statistical data are shown in Table 2. Mean bond strengths (MPa) ranged between 5.01 ± 3.09 (Groups NC-A, with TMC) and 21.34 ± 6.17 MPa (Group CA-C, without TMC).

SBS comparisons for different artificial teeth are shown in Figure 3. Intergroup comparisons revealed no difference in bond strength between different artificial tooth types for composite resin repair material regardless of artificial aging condition ($p > 0.05$). Likewise, the bond

strength of acrylic resin was similar for all groups without TMC ($p > 0.05$). However, artificially aged acrylic resin repair material showed higher bond strength for group CA (9.25 ± 2.96) than for group NC (5.01 ± 3.09) ($p < 0.05$) (Figure 3). The bond strength of artificially aged acrylic resin to groups IS (6.16 ± 3.75) and DCL (7.09 ± 2.72) were comparable to each other and the other 2 groups ($p > 0.05$). Considering intra-group comparisons, composite repair material showed better bond strength than acrylic resin for all groups regardless of aging condition ($p < 0.05$) (Table 2). Artificial aging decreased the bond strength of both acrylic and composite repair materials to all types of artificial teeth ($p < 0.05$) (Table 2).

All groups repaired with acrylic resin showed type 1 failure regardless of the artificial aging condition. Failure type distribution for the groups repaired with composite resin is shown in Figure 4. For DCL and CA groups, type 1 failure was dominant, while Type 2 failure mode was more common for groups NC and IS regardless of TMC. All artificially aged CA samples showed type 1 failure, while none of the samples of group NC showed Type 1 failure regardless of artificial aging.

Discussion

This study investigated the repair bond strength of auto-polymerized acrylic and composite resin to artificial teeth with different chemical properties. Also, the effect of artificial aging on bond strength was evaluated with thermal cycling. The bond strength differed according to the repair material used, the type of artificial teeth, and artificial aging condition. Therefore, all 3 null hypotheses were rejected

Table 1: Content details of artificial teeth evaluated

Material	Group abbreviations	Trade name	Manufacturer
Conventional polymethylmethacrylate	CA	Ivostar, Gnathostar	Ivoclar Vivodent AG, Italy
Isosite	IS	SR Orthosit PE	Ivoclar Vivodent AG, Italy
Double cross-linked acrylic	DCL	SR Vivodent DCL	Ivoclar Vivodent AG, Italy
Nanohybrid composite	NC	SR Phonares II	Ivoclar Vivodent AG, Italy

Table 2: Descriptive statistics of the study

Groups	Repair Material	Artificial Aging Condition	
		Without thermocycling (TMC -) Mean (MPa) ± SD**	With thermocycling (TMC+) Mean (MPa) ± SD
CA	A	13.01 ± 6.35	9.25 ± 2.96
	C*	21.34 ± 6.17	10.48 ± 4.97
IS	A	10.46 ± 4.39	6.16 ± 3.75
	C*	19.53 ± 7.45	12.71 ± 5.69
DCL	A	12.99 ± 4.48	7.09 ± 2.72
	C*	15.52 ± 4.77	12.46 ± 4.26
NC	A	8.31 ± 4.8	5.01 ± 3.09
	C*	15.48 ± 3.26	13.89 ± 2.82

† Conventional polymethylmethacrylate (CA), Isosite (IS), Double cross-linked acrylic (DCL), Nanohybrid composite (NC), Acrylic resin (A), Composite resin (C), standard deviation (SD), Thermal cycling (TMC)

* Composite repair material showed better bond strength than acrylic resin regardless of artificial teeth group and aging condition.

**Artificial aging decreased the bond strength of both repair materials for all groups.

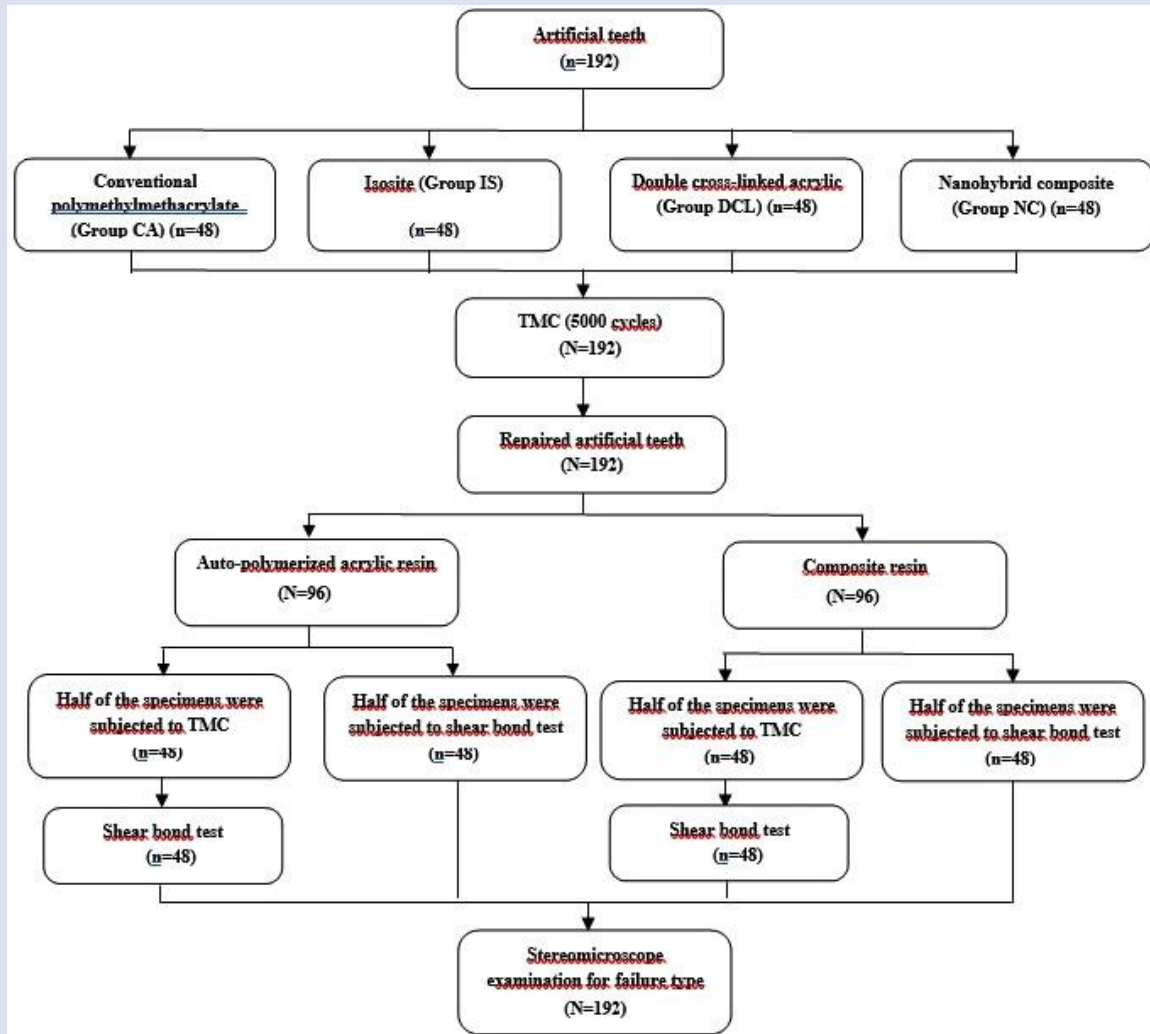


Figure 1. The schematization of all steps of the study

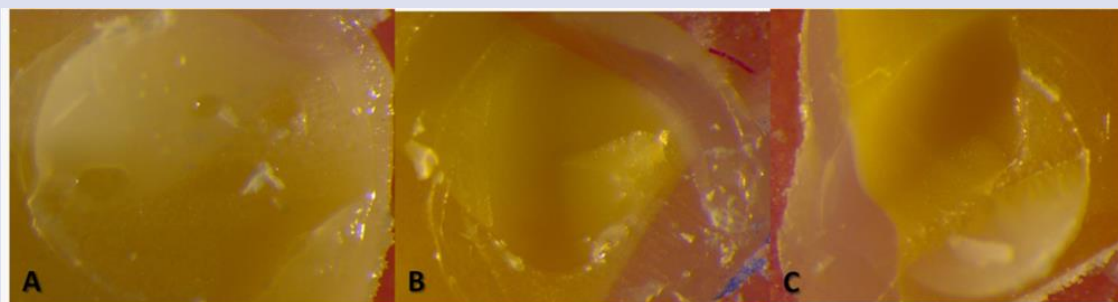


Figure 2. Demonstrative images of failure modes. A. Adhesive failure B. Cohesive failure C. Mixed failure

Conventional artificial teeth have a linear polymer structure and PMMA is the main structure.¹² However, mechanical and physical properties are improved with the help of technological advancements.⁶ As such, the addition of crosslinks to acrylic teeth increased the physical and mechanical properties.¹² On the other hand, composite artificial teeth contain methacrylate matrix, urethane dimethacrylate (UDMA) based crosslinking agent, and inorganic micro-fillers, which increases the hardness and rigidity.¹³ In the present study, the bond strength of acrylic

resin repair material was similar for all types of artificial teeth prior to artificial aging. The only significant difference in bond strength regarding different type of artificial teeth was found between conventional polymethylmethacrylate and nanohybrid composite artificial teeth when repaired with acrylic resin and subjected to artificial aging. This condition may have derived from the difference in chemical structures of artificial teeth and the bond of repair materials to them. Acrylic resin presented higher bond strength to conventional PMMA teeth than to nanohybrid

composite artificial teeth after TMC. Acrylic resin may have presented better bond strength to PMMA teeth with a similar chemical structure. As nanohybrid teeth consist mainly of composite material, the repair bond strength of acrylic resin to these teeth would have been affected by artificial aging and decreased. Based on this result, clinicians should be aware of the debonding risk in long term when nanohybrid composite artificial teeth are repaired with acrylic resin. On the other hand, this difference in repair bond strength was not present when the teeth were repaired with composite resin, as the bond strength was similar for all artificial tooth types and for both artificial aging conditions. Previous studies noted that the application of bonding agent prior to acrylic resin artificial teeth substantially increases the bond strength of composite resin due to the similarity between the molecules of reactive methacrylate groups and the similar

pattens of MMA and bisphenol A diglycidylether methacrylate (BIS GMA) polymerization process.^{14,15} According to these considerations, in the present study, a chemical bonding between acrylic resin teeth and composite repair material might have occurred.¹⁵ Papazoglou *et al.*¹⁶ stated that when MMA is applied to acrylic artificial teeth, a swelling effect causes micro irregularities on the surface of acrylic teeth in which composite material penetrates and enhances the mechanical bonding. As a result, a combination of chemical and mechanical bonding between composite and acrylic artificial teeth occurs.^{15,16} These reports may also explain the higher repair bond strength of composite than acrylic resin regardless of the artificial teeth type and aging condition. It can be considered that the bonding system applied prior to composite resin provided better wettability and enhanced bonding for all types of artificial teeth.

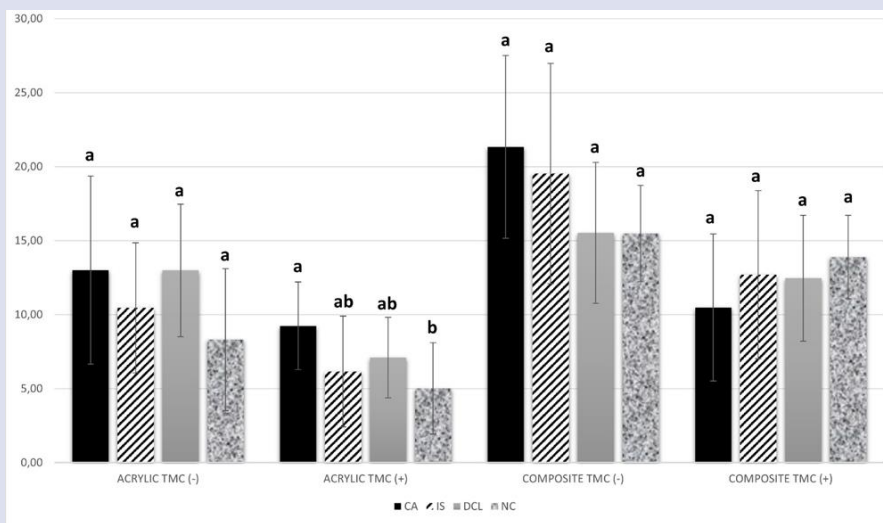


Figure 3. Statistical differences between the mean bond strengths of groups regarding repair material and TMC.

*Different superscript letters represent the statistically significant differences ($p < 0.05$).

† Conventional polymethylmethacrylate (CA), Isosite (IS), Double cross-linked acrylic (DCL), Nanohybrid composite (NC), Acrylic resin (A), Composite resin (C), standard deviation (SD), Thermal cycling (TMC)

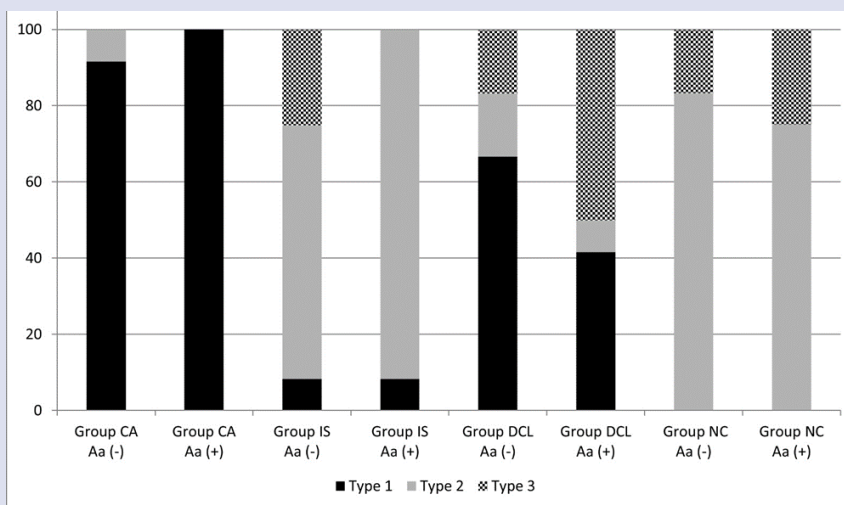


Figure 4. Distribution of failure modes for each group repaired with composite resin.

The stability of dental materials is of importance for the long-time service of restoration. The oral environment may cause deterioration in restorative materials, leading to early failures.¹⁷ TMC application is widely used to simulate dental materials' aging of and to evaluate the durability in in vitro studies. The International Organization for Standardization (ISO) recommends a thermal cycling regime between 5°C and 55°C to simulate aging.¹⁸ It was reported that 3000 cycles of thermal cycling application corresponds to 3 years of intraoral service, assuming that the patient consumes an average of 3 meals daily.¹⁹ Based on this information, in the present study, 5000 thermal cycles applied between 5°C and 55°C corresponds to 5 years of clinical service. To simulate five years of oral service, TMC was applied to all specimens and then reapplied to the experimental group of repaired artificial teeth to detect the effect of TMC on the bond strength of repair materials. Artificial aging resulted in a significant reduction in the bond strength of both acrylic and composite repair materials regardless of the artificial teeth type. This finding is in line with previous studies which stated that the bond strength of acrylic and composite resin to various materials decreases significantly by artificial aging.²⁰⁻²³

While adhesive failure in the bonding interface indicates poor bond strength, cohesive and mix failures are associated with a strong bond between two materials.²⁴ All samples repaired with acrylic resin repair material demonstrated lower bond strength than composite resin and showed adhesive-type failure. Therefore, this finding was supported by the analysis via a stereomicroscope. On the other hand, conventional acrylic resin teeth repaired with composite showed adhesive-type failure, yet no difference was found in bond strength between conventional acrylic and other artificial tooth types. This finding can be explained by the weak mechanical bonding between composite repair material and conventional acrylic teeth. However, the failure mode analysis was not in line with the findings of the bond strength test. Further studies are needed to investigate the bonding mechanism between the composite material and newly introduced artificial teeth.

Previous studies investigated the effect of roughening artificial teeth with various methods on the bond strength of acrylic or composite resin to acrylic artificial teeth.^{16,25} In the present study, standard methods recommended by the manufacturers were applied and different roughening methods were not evaluated. Future studies may focus on the effect of different surface treatments on the bond strength of acrylic and composite resin to various types of artificial teeth.

As in any in vitro study, the experimental design of this study may not be sufficient to simulate intraoral conditions. The results may vary in the dynamic oral environment. Therefore, the presented results should be supported by future in-vivo studies. Also, types of repair materials and artificial teeth were limited. Future in vitro studies may diversify these and compare different types of materials.

Conclusions

Within the limitations of this study, the following conclusions can be drawn;

- 1) Repair with composite resin showed similar bond strength for all types of artificial teeth regardless of artificial aging.
- 2) The bond strength of acrylic resin was similar for all artificial teeth before aging. However, after artificial aging, the bond strength of acrylic resin to conventional PMMA teeth was higher than that of nanohybrid composite teeth.
- 3) The application of artificial aging reduced the repair bond strength of both acrylic and composite repair materials.
- 4) Composite repair material exhibited higher bond strength to all artificial tooth types than acrylic resin, regardless of artificial aging condition.

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Conflicts of Interest

The authors of this study declare no conflict of interest.

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Effect of Repair and Thermal Cycling on The Flexural Strength of Denture Base Materials Fabricated from Different Methods

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Research Article

History

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ABSTRACT

Objectives: The aim of this study was to evaluate the flexural strength of the denture base materials produced by different methods after repair and was to evaluate effect of thermal aging.

Materials and Methods: A total of 120 specimens were fabricated by conventional, CAD/CAM milled, and 3D-printed denture base materials for this in vitro study. Specimens were divided into four groups; non-repaired groups (N), repaired groups (R), non-repaired-aged groups (N-aged), and repaired-aged (R-aged) groups. In N groups, specimens were stored in the water bath for 24 h before the flexural strength test. In N-aged groups, samples were subjected to thermal aging for 5000 cycles, then tested. For repaired R groups, samples were repaired with auto-polymerized acrylic resin and storage in the water bath for 24 h then tested. For the R-aged groups, samples were repaired with auto-polymerized acrylic resin, aged for 5000 cycles, then tested. Specimens were performed a three-point loading test using a universal testing machine. Data were analyzed using Wilcoxon and Kruskal-Wallis tests.

Results: When the groups were compared with each other, the difference between all groups was found to be statistically significant ($p < 0.05$). Regardless of the thermal cycling process, in N groups, the highest flexural strength was recorded in N-CAD group (92.53 ± 6.52 MPa), the lowest flexural strength was recorded in N-3D group (33.72 ± 2.74 MPa). Regardless of the thermal cycling process, in R groups, the highest flexural strength was recorded in R-C groups (31.80 ± 5.86 MPa) and the lowest flexural strength was recorded in R-3D groups (8.37 ± 1.20 MPa). Thermal cycling and repair process showed a decreasing effect on flexural strength in all groups.

Conclusions: The ideal flexural strength among denture base materials was found in CAD/CAM milled denture base materials.

Keywords: Denture base resin, CAD/CAM, 3D-printed, repair.

Tamir ve Termal Döngünün Farklı Yöntemlerle Üretilen Protez Kaide Materyallerinde Bükülme Dayanımına Etkisi

Süreç

Geliş: 18/04/2023

Kabul: 15/05/2023

Öz

Amaç: Bu çalışmanın amacı farklı yöntemlerle üretilen kaide materyallerinin tamir sonrası bükülme dayanımına ve termal yaşlandırmanın etkisini değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmada konvansiyonel, CAD/CAM kazıma ve 3D yazıcı ile toplamda 120 örnek üretildi. Örnekler dört gruba bölündü; Tamir edilmemiş grup (N), tamir edilmiş grup (R), tamir edilmemiş-yaşlandırılmış grup (N-aged), tamir edilmiş-yaşlandırılmış grup (R-aged). N gruptaki örnekler eğme testinden önce 24 saat su banyosunda bekletildi. N-aged gruptaki örnekler termal yaşlandırma için 5000 döngü yapıldı ve sonrasında test edildi. R gruptaki örnekler, otopolimerizan akrilik ile tamir edildi. Sonrasında 24 saat su banyosunda bekletildikten sonra test edildi. R-aged grupta ise örnekler otopolimerizan akrilik ile tamir edilip termal yaşlandırma için 5000 döngü yapıldıktan sonra test edildi. Örnekler Universal test cihazında üç nokta eğme testi yapıldı. Verilerin değerlendirilmesinde Wilcoxon ve Kruskal-Wallis testi kullanıldı.

Bulgular: Gruplar birbirleri ile karşılaştırıldığında tüm gruplar arası fark istatistiksel olarak anlamlı bulundu ($p < 0,05$). Termal siklus işlemine bakılmaksızın N grupları içerisinde en yüksek bükülme dayanımının N-CAD grubunda ($92,53 \pm 6,52$ MPa), en düşük bükülme dayanımının ise N-3D grupta ($33,72 \pm 2,74$ MPa) olduğu görüldü. Termal siklus işlemine bakılmaksızın R gruplarında en yüksek bükülme dayanımının R-C grupta ($31,80 \pm 5,86$ MPa) olduğu, en düşük bükülme dayanımının ise R-3D grupta ($8,37 \pm 1,20$ MPa) olduğu görüldü. Termal siklus ve tamir işlemi tüm gruplarda bükülme dayanımını azalttığı görüldü.

Sonuçlar: Protez kaide malzemeleri arasında ideal bükülme mukavemeti, CAD/CAM kazıma ile elde edilen protez kaide materyalinde bulunmuştur.

Anahtar Kelimeler: Protez kaide reçinesi, CAD/CAM, 3D yazıcı, tamir.

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Introduction

Conventional complete dentures and partial dentures are the treatment option frequently used in prosthodontics to replace missing teeth.¹ When fabrication of removable prostheses (complete or partial denture bases), a synthetic polymer, polymethyl methacrylate (PMMA) mostly preferred.^{1,2} Being cheap, biocompatible, easy to apply and repair, stable in the oral environment, satisfactory aesthetic, and mechanical properties are suitable and can be counted as advantages of PMMA.^{2,3,4} Besides these advantages, PMMA has low dimensional stability and low impact, and flexural strength. Low dimensional stability leads to crack propagation and the formation of microporosities which may cause prosthesis fracture in the oral environment under different thermal temperatures.^{1,3} These fractures can happen inside the patient's mouth fatigue failure in the course of service or outside the patient's mouth because of an accidental fall.^{1,4} Repeated denture flexure by occlusal forces causes stress accumulation and fatigue failure.^{1,4,5} Today, the most common reason for the failure of the removable prosthesis is fracturing of the denture bases.¹ Denture repair is preferred to new denture fabrication because the repair is less time-consuming and decreases the cost.⁴ Denture repair material should be the same color as the denture base, easy and quickly perform, and improves the strength to prevent further fracture.^{4,5,6} Auto-polymerized acrylic resin, visible light polymerized acrylic resin, heat polymerized acrylic resin, and microwave polymerized acrylic resins have been used to repair fractured denture bases.^{4,7} Auto-polymerized acrylic resin is used for denture base repairs commonly because of ease of manipulation, being less time-consuming and economic.^{1,4}

Recently, with improvements in digital technologies computer-aided design/computer-aided manufacturing (CAD/CAM) method and three-dimensional (3D) printed technologies started to use for denture base production.^{2,8,9} Digital methods have faster denture fabrication and decrease the number of clinical visits.^{2,9} Allowing visualization of residual ridge and dentures to be made from different aspects, permitting digital record keeping, and improving patient satisfaction by better speech and higher comfort are other advantages of the digital process.⁸

CAD/CAM milled resin blocs that are processed at high temperature and pressure, this minimizes shrinkage, voids, and residual monomer.⁹ As a result, one block denture base is produced by providing process control developed by clinicians and technicians.^{2,10} This technique improves denture strength and adaptation while reducing microbial adhesion on denture surfaces.^{2,9} 3D printing enables concurrent manufacturing of many products and economical than milling due to material and tool conservation.^{8,9} When comparing conventional denture base resin, CAD/CAM-milled resins have superior surface characteristics, flexural strength, elastic modulus, and 3D-printed materials have superior color stability and fracture toughness.¹¹

The thermal aging process is important to simulate intraoral conditions. While water absorption, water molecules interfere with polymeric chains and affect the physical properties of the denture base resin by causing excessive dimensional instability in the resulting polymer.¹²

The mechanical properties of conventionally polymerized denture base acrylics have been the subject of much research to date. But there is a lack of consensus on the comparison of mechanical properties between the fabrication of CAD-CAM milled, 3D-printed, and conventional denture base resins.^{8,13,14} The objective of this study was to evaluate the flexural strength of denture base materials fabricated by conventional method and digital technologies (CAD/CAM milled and 3D-printed) and to examine the effect of repair and thermal cycling. The null hypotheses of this study were that the thermal cycling process and repair would not affect the flexural strength of denture base materials produced by CAD/CAM milled, 3D-printed, and conventional methods.

Materials and Methods

Three different methods were used for fabricating the specimens; the conventional, CAD/CAM milled, and 3D-printed methods in this study (Table 1). A total of 120 specimens were tested for flexural strength and each group was divided into two subgroups (n=60); 1. No-repaired (N) group with no fracture 2. Repaired (R) group (Table 2). Half of each subgroup was subjected to thermal cycling, and the other half of the specimens were not subjected to thermal cycling.

Fabrication of Conventional Specimens

For conventional groups (C), a total of forty specimens were fabricated following the conventional method. Firstly, 65 mm x 10 mm x 2.5 mm stainless-steel molds were fabricated and wax samples were obtained from these molds (Figure 1). After these wax samples were muffled, acrylic samples were obtained. In the polymerization of acrylic, the muffle was placed in boiling water. The heat source was turned off and left for 15 minutes. It was then boiled for 20 minutes and allowed to cool slowly in a water bath.

After finishing the specimens with 600-grit silicon carbide paper, half of them were stored at 37°C temperature in water bath for 24 hours. Other half of the specimens were thermal aged before the flexural strength test.

For conventional repaired (R-C) groups, a vertical line was drawn with a marker pen to divide the prepared specimen into 2 equal parts. The prepared specimens were cut vertically with a gap of 3 mm between two parts by using a micromotor handpiece and fissure carbide. In order to hold two pieces of acrylic denture bases together, a stainless-steel mold was used. The consistent space of repair of 3 mm was sustained between segments, and repaired with auto-polymerized acrylic resin. The repaired

specimens were finished with 600-grit silicon carbide paper.

Half of the specimens were stored at 37°C temperature in a water bath for 24 hours. Other half of the specimens were thermal aged before the flexural strength test.

Fabrication of CAD/CAM Milled Specimens

A rectangular block with the dimensions of 65 mm x10 mm x 2.5 mm was designed using software (Solid Works 2022, Dassault Systems S.A, Service pack 5.0, France) and saved as a standard tessellation language (STL) file. For the fabrication of the designed specimens, the STL files were uploaded to the CAM program (Work, NC). Then a computer-aided machine (Redon, Istanbul, Turkey) milled the specimens from pre-polymerized CAD/CAM resin discs (Yamahachi Dental MFG, Aichi-Pref, Japan) using a subtractive technique.

For CAD/CAM milled no-repaired (N-CAD) groups, specimens from polymethyl methacrylate denture base resin, with the dimensions of 65 mm x 10 mm x 2.5 mm, were fabricated using CAD/CAM technology as mentioned above. For the CAD/CAM milled repaired group (R-CAD), specimens from polymethyl methacrylate denture base resin, with dimensions of 31 mm x10 mm x 2.5 mm, were fabricated using CAD/CAM technology. Then, the repairing process was done as mentioned in the R-C group.

Half of the specimens were stored at 37°C temperature in a water bath for 24 hours. Other half of the specimens were thermal aged before the flexural strength test.

Fabrication of 3D Printed Specimens

A rectangular block with the dimensions of 65 mm x10 mm x 2.5 mm was designed using software (AutoCAD 2018, Autodesk, California, United States) and saved as an STL file. The STL files of the designed specimen were exported to a 3D printer (Free Shape 120 Printer, Ackuretta, Chinese) with a UV light source. The specimens were printed using acrylate ester-based resin (MACK4D Resin, Dentona, Germany) with an additive technique. The printed specimens were rinsed with isopropyl alcohol (99% concentration) for 5 minutes to remove unpolymerized acrylic resin and then placed in an Ackuretta UV Box (Taiwan) for 3 minutes to complete post-polymerization.

For 3D-Printed no-repaired groups (N-3D), specimens from polymethyl methacrylate denture base resin, with 65 mm x10 mm x 2.5 mm dimensions, were printed as mentioned above using 3D printing technology. For 3D-Printed repaired groups (R-3D), specimens from polymethyl methacrylate denture base resin, with 31 mm x10 mm x 2.5 mm dimensions, were printed as mentioned above using 3D printing technology (Figure 2). Then, the repairing process was done as mentioned in the R-C group.

Half of the specimens were stored at 37°C temperature in a water bath for 48 hours. Another half of

the specimens were thermal aged before the flexural strength test.

Thermal Aging Process

Half of the specimens in all groups were thermocycled for 5000 cycles with a dwell time of 30 seconds in water at 5°C and 55°C.

Flexural Strength Test

The flexural strength was conducted using a universal testing machine (Lloyd LF Plus; Ametek Inc, Lloyd Instruments, Leicester, UK). Acrylic specimens were placed in a universal test machine. Three-point bending tests were performed on every specimen at a speed of 5 mm/min.

The maximum load at fracture was recorded for each specimen, and the flexural strength was calculated as follows:

$$FS = 3FL/2bd^2$$

FS = flexural strength in Mega Pascal (MPa), F = load at fracture in newtons (N), L = span between the supports (mm), and b and d = width and thickness of the specimen (mm).

Statistical Analysis

SPSS 22.0 program was used in this study. The parametric test assumptions could not be fulfilled in the evaluation of the data, so the Wilcoxon test was used. Kruskal-Wallis's test was used when comparing the measurements obtained from two independent groups. The Mann-Whitney U test was applied to find the groups that made a difference in the analysis result ($p = 0.05$).

Results

When the groups were compared with each other, the difference between all groups was found to be statistically significant ($p < 0.05$). Descriptive statistics are presented in Table 3, 4, 5.

Regardless of the thermal cycling process, in no-repaired groups, the highest flexural strength was recorded in N-CAD groups (92.53 ± 6.52 MPa), the lowest flexural strength was recorded in N-3D groups (33.72 ± 2.74 MPa) (Table 3). Regardless of the thermal cycling process, in repaired groups, the highest flexural strength was recorded in R-C groups (31.80 ± 5.86 MPa) and the lowest flexural strength was recorded in R-3D groups (8.37 ± 1.20 MPa) (Table 4).

Thermal cycling showed a decreasing effect on flexural strength in all groups. A statistically significant difference emerged between N-C, N-CAD, N-3D, and R-C flexural strength values. There was no statistical difference in R-CAD and R-3D groups.

Flexural strength values were decreased after the repair process in all groups (Table 5). The repair process has the most decreasing effect on flexural strength values in CAD/CAM groups (before and after thermal cycling, $p < 0.05$).

Table 1. Composition and manufacturer of materials used in this study

Brand	Material Type	Composition	Manufacturer
Meliodent	Heat-cured, conventionally manufactured denture base resin	Polymethylmethacrylate, benzoylperoxide, methyl methacrylate, ethylene glycol dimethacrylate	Heraeus Kulzer, Hanau, Germany
Yamahachi	CAD/CAM Milling Block	Polymethylmethacrylate, ferric oxide, titanium dioxide	Yamahachi Dental MFG, Aichi-Pref, Japan
MACK 4D Denture Light Pink	Additive manufactured denture base resin	Urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA)	Dentona, Germany
Blaucryl	Auto-polymerizing acrylic resin as repair material	Polymethylmethacrylate, benzoylperoxide, methyl methacrylate, hydroquinone, dimethyl-p-toluidine	Blaudent, UK

Table 2. Study Groups

Groups	Technique	Abbreviation	n
Conventional	No-repaired	N-C	20
Conventional	Repaired	R-C	20
CAD/CAM Milled	No-repaired	N-CAD	20
CAD/CAM Milled	Repaired	R-CAD	20
3D Printed	No-repaired	N-3D	20
3D Printed	Repaired	R-3D	20

Table 3. Flexural strength values (MPa) of no-repaired groups of each denture base before and after thermocycling

No-repaired group	Before thermocycling	After thermocycling	p-value
Conventional	78.13 (3.95) ^{aA}	66.16 (8.66) ^{aB}	p=0.013*
CAD/CAM milled	92.53 (6.52) ^{bC}	85.98 (3.92) ^{bD}	p=0.047*
3D Printed	45.17 (6.54) ^{cE}	33.72 (2.74) ^{cF}	p=0.007*
	KW=25,36 P=0.001*	KW=24,87 P=0.001*	

* The different superscript lower-case letters per column indicate statistically significant ($p < 0.05$); Different superscript upper-case letters indicate the statistically significant between each group before and after thermocycling ($p < 0.05$).

Table 4. Flexural strength values (MPa) of repaired groups of each denture base before and after thermocycling

Repaired group	Before thermocycling	After thermocycling	p-value
Conventional	31.80 (5.86) ^{aA}	22.76 (5.20) ^{aB}	p=0.012*
CAD/CAM milled	25.28 (3.33) ^{bC}	24.87 (2.42) ^{bC}	p=0.721
3D Printed	9.42 (1.51) ^{cD}	8.37 (1.20) ^{cD}	p=0.333
	KW=22.59 P=0.001*	KW=19.77 P=0.001*	

*The different superscript lower-case letters per column indicate statistically significant ($p < 0.05$); Different superscript upper-case letters indicate the statistically significant between each group before and after thermocycling ($p < 0.05$).

Table 5. Comparison of flexural strength of each base material

Groups		Before Thermocycling			After Thermocycling		
		Mean (MPa)	SD	p value	Mean (MPa)	SD	p value
Conventional	(No-repaired)	78.13	3.95	P=0.005*	66.16	8.66	P=0.004*
	(Repaired)	31.80	5.86		22.76	5.20	
CAD/CAM milled	(No-repaired)	92.53	6.52	P=0.004*	85.98	3.92	P=0.005*
	(Repaired)	25.28	2.42		24.87	3.33	
3D Printed	(No-repaired)	45.17	6.54	P=0.002*	33.72	2.74	P=0.003*
	(Repaired)	9.42	1.51		8.37	1.20	

*Statistically significant difference ($p < 0.05$).



Figure 1. Stainless steel mold used to repair specimens.



Figure 2. (a) Specimens fabricated from different techniques, (b) Specimens fabricated for repair

Discussion

This in vitro study investigated the repair and thermal cycling effect on the flexure strength of denture base materials fabricated with CAD/CAM milling and 3D-printed, compared with the conventional method. Statistical analysis indicated significant differences between groups for flexural strength thus the null hypotheses were rejected.

A complete denture is often the preferred treatment option for edentulous patients³. Complete and removable partial dentures and overdentures are most commonly made from PMMA.³ In prosthetic dentistry, fractures of PMMA denture base resin are common problems.¹ In many cases, repairing the denture base is a viable solution.¹ For repairing fractured denture bases, auto-polymerizing acrylic resin is the most common method.⁴ So, in this in vitro study, the auto-polymerized acrylic resin was used for repairing specimens. The repair area dimension was 3 mm for reducing the repair material and consequently, minimizing the polymerization shrinkage according to AlQahtani *et al.* and Deb *et al.*^{1,15} Flexural strength values decreased after the repairing process in all groups in this study. According to the author's current knowledge, there is no evidence for flexural strength values after repairing denture base materials fabricated with digital technologies. Current studies in the literature evaluated the flexural strength with surface treatment modification on the repaired areas.^{13,16}

The flexure strength is a material property that is defined as the amount of stress that exists in the material

just before it gives in a flexure test.^{2,8} This test simulates stress forces that are typically applied during the masticatory function to dentures, which can result with fracture.¹⁷

Two digital technologies, namely the CAD/CAM milled and 3D-printed methods, are frequently used today.¹³ There is no consensus on the flexural strength of CAD/CAM materials.² Freitas *et al.*¹⁷ evaluated the flexure strength of three different denture base materials and concluded CAD/CAM showed the highest flexural strength values while 3D-Printed lowest flexural strength.¹⁷ According to Ayman¹⁸ and Pacquet *et al.*¹⁹, heat-polymerized PMMA had higher flexural strength values than CAD/CAM milled denture bases. Ayman¹⁸, Pacquet *et al.*¹⁹, and Steinmassl *et al.*²⁰, results were in contrast to this in vitro study. Hence, CAD/CAM materials showed higher flexural strength values than conventionally fabricated denture base materials, in line with Aguirre *et al.* and Freitas *et al.*^{21,17} Differences between the flexural strength values of CAD/CAM and heat-polymerized denture base materials can explain by the use of different manufacturers and materials in the studies mention above.^{2,9,14,17}

The physical properties produced with 3D printers depend on the rate of monomer conversion formed by liquid photopolymer resins and light-induced polymerization.¹⁷ In this process, carbon double bonds are converted into single bonds.¹⁷ The higher the double bond conversion, the higher the polymer.¹⁷ If an unreacted monomer is present, the mechanical strength may decrease and cause soft tissue irritation.¹⁷ According to

ISO standards, a denture base resin must have a flexural strength value of 65 MPa.¹⁷ According to previous studies, 3D Printed denture base materials had lower flexural strength comparing CAD/CAM milled, conventionally fabricated, and polyamide denture bases.^{2,9} The results of this in vitro study corroborate Prpic and Zeiadan's results. The variation in the values reported can be attributed to the material's brand and manufacturer.⁸ Further studies are needed to determine the mechanical properties of 3D-printed denture bases.⁸

A thermocycling cycle of 5000 cycles corresponds to six months of clinical use.¹³ A thermal cycle simulates the thermal changes in the oral environment during long-term use of dental materials.^{13,15} It is consistent with earlier research that shows reduced flexural strength after thermocycling.¹ This in vitro study corroborates with AlQahtani *et al.*, as reduced flexural strength after thermocycling for conventionally produced denture base materials.¹ According to Çakmak *et al.*, the thermal cycling process has decreasing effect on 3D-printed denture base resin, these results also corroborate with this in vitro study's results.¹⁴ Water absorption, thermal stress, and the presence of porous structures may be the cause of the reduction of fracture strength values.¹

According to the literature, no studies evaluate the effect of repair on flexural strength after the thermal cycling process. Repair and thermal cycling processes together have on decreasing effect on flexural strength in this study. Biocompatibility, microbial adhesion, manufacturing trueness, clinical fit, and color stability of the denture base resin materials should be investigated in further studies. According to the results of this study, new digitally produced denture base materials can be considered as an alternative.

Conclusions

Within the limitations of this study, it was possible to conclude that:

- 1- No-repaired specimens showed higher flexural strength than the repaired specimens.
- 2- Both before and after thermal cycling, 3D-printed specimens showed the lowest flexural strength values.
- 3- Thermal cycling process was found to have decreasing effect on flexural strength in three different denture base resins.

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Effect of Self-Cured and Self-Etch Adhesive Systems on Shear Bond Strength After Photodynamic Therapy and Disinfection with Different Types of Lasers

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Research Article

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ABSTRACT

Objectives: The aim of this study was to investigate the strength of shear bonding of self-cured and self-etch adhesive systems to dentin after surface disinfection with Photodynamic Therapy (PDT), Er:YAG, Nd:YAG, and KTP laser.

Materials and Methods: In the study, 6 main-groups were formed as Control, Er:YAG Laser, Nd: YAG Laser, KTP Laser, KTP Laser activated PDT and Diode Laser activated PDT. Each group was divided into two subgroups; Self-cured (Tokuyama Universal) and self-etch (Scotchbond Universal) adhesive system(n=10). 120 molar teeth were prepared to expose dentin surfaces. After disinfection and adhesive application, nanohybrid composite resin with a diameter of 3 mm and a height of 2 mm was applied to the dentin surface with the help of transparent molds. All samples were thermally cycled 5.000 times at 5°C and 55°C. Shear bond strength (SBS) test was applied to the samples in a universal test device.

Results: The differences between the Er:YAG laser group and the control, KTP and Nd:YAG laser groups of the self-cured adhesive samples were statistically significant. While the differences between the Nd:YAG laser group and the PDT groups were statistically significant, the differences between all other groups were no significant. In the samples applied with self-etch adhesive, while the differences between the KTP and Nd:YAG laser groups and all other groups were statistically significant, the difference between them was not statistically significant. Self-etch adhesive applied samples showed higher bond strength values in all disinfection applications.

Conclusions: Er:YAG laser and PDT for surface disinfection applications increased the bond strength to dentin while Er:YAG laser showed the highest shear bond strength value. The application of Nd:YAG and KTP lasers adversely affected the bond strength. In cases of using surface disinfection applications with laser for self-curing adhesive systems the Er:YAG laser or laser-activated PDT procedures are recommended.

Keywords: Photodynamic therapy, self-cured adhesive, laser disinfection, bond strength.

Fotodinamik Tedavi ve Farklı Tip Lazerler ile Dezenfeksiyon Sonrası Self-Cured ve Self-Etch Sistemlerin Makaslama Bağlanma Dayanımına Etkisi

Süreç

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Öz

Amaç: Bu çalışmanın amacı Fotodinamik Tedavi (FDT), Er:YAG, Nd:YAG, ve KTP lazer ile yüzey dezenfeksiyonu uygulanması sonrası self-cured ve self-etch adeziv sistemlerin dentine makaslama bağlanma dayanımını araştırmaktır.

Gereç ve Yöntemler: Çalışmada Kontrol, Er:YAG Lazer, Nd:YAG Lazer, KTP Lazer, KTP Lazer aktivasyonlu FDT ve Diyet Lazer aktivasyonlu FDT olmak üzere 6 ana-grup oluşturuldu. Her grup iki alt gruba ayrıldı; Self-cured (Tokuyama Universal) ve self-etch (Scotchbond Universal)(n=10). 120 adet molar diş dentin yüzeyleri açığa çıkacak şekilde prepare edildi. Dezenfeksiyon ve adeziv uygulamasını takiben şeffaf kalıplar yardımıyla 3 mm çapında 2 mm yüksekliğinde nanohibrit kompozit rezin dentin yüzeyine uygulandı. Tüm örnekler 5°C ve 55°C'de 5.000 kez ısı döngü işlemi uygulandı. Örnekler universal test cihazında makaslama bağlanma dayanımı testine tabi tutuldu.

Bulgular: Self-cured adeziv uygulanan örneklerden Er:YAG lazer grubu ile kontrol, KTP ve Nd:YAG lazer grubu arasındaki farklar istatistiksel olarak önemli bulunmuştur (p<0,05). Nd:YAG lazer grubu ile FDT grupları arasındaki farklar istatistiksel olarak önemli iken (p<0,05), diğer tüm gruplar arasındaki farklar önemsiz bulunmuştur (p>0,05). Self-etch adeziv uygulanan örneklerde, KTP ve Nd:YAG lazer grupları ile diğer tüm gruplar arasındaki farklar istatistiksel olarak anlamlı bulunurken (p<0,05), kendi aralarındaki fark istatistiksel olarak önemsiz bulunmuştur (p>0,05). Self-etch adeziv uygulanan örnekler, tüm dezenfeksiyon uygulamalarında daha yüksek bağlanma dayanım değeri göstermiştir.

Sonuçlar: Yüzey dezenfeksiyon amacı ile Er:YAG lazer ve FDT uygulamaları dentine bağlanma dayanımını değerini arttırırken, Er:YAG lazer en iyi makaslama bağlanma dayanımı değerini göstermiştir. Nd:YAG ve KTP lazer uygulanması ise bağlanma dayanımını olumsuz etkilemiştir. Lazerler ile yüzey dezenfeksiyonu uygulamalarında self-cured adeziv sistemler kullanılması durumunda Er:YAG lazer ya da lazer aktivasyonlu FDT prosedürlerinin tercih edilmesi önerilmektedir.

Anahtar Kelimeler: Fotodinamik tedavi, self-cured adeziv, lazer dezenfeksiyon, bağlanma.

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Introduction

Generally, removal of bacteria after cavity preparation and prior to placement of the restoration is one of the most important factors influencing the success of the restoration.¹ The use of cavity disinfectants is recommended to eliminate bacteria and longevity of the restoration.² Chlorhexidine digluconate (CHX), sodium hypochlorite (NaOCl), hydrogen peroxide, potassium iodine, benzalkonium chloride, alcohol, propolis, ozone and laser are used for cavity disinfection.³ In addition, photodynamic therapy (PDT), which has come to the fore with the development of resistance to antibiotics in recent years, can be used as an alternative disinfection method.⁴

Erbium lasers used in dentistry show the bactericidal effect by ablating the tissue by increasing the temperature of the water in the cell to 100°C. It is used in cavity disinfection and root canal disinfection by utilizing the bactericidal effects of the laser beam.⁵ In addition, Erbium laser beams have advantages such as roughening the enamel and dentin surface, increasing the amount of Ca and P on the tooth surface, creating a more resistant structure against acid attacks.⁶ Nd:YAG lasers can be used in many clinical procedures such as reduction of dentin hypersensitivity, remineralization of initial caries, disinfection of cavity and root canals, gingivectomy, gingivoplasty, frenectomy, crown lengthening.⁷⁻⁸ The KTP laser is a type of Nd:YAG laser with a wavelength cut in half. KTP laser can be used both in the areas of use of Nd:YAG laser and in teeth whitening applications.⁹ Recently, Diode lasers have been used in root canal and cavity disinfection to take advantage of their antibacterial effects.¹⁰

Photodynamic therapy is based on the principle that target cells, microorganisms or molecules are stained with a photosensitizer and then activated by light of a specific wavelength.¹¹ PDT is used in periodontitis, periimplantitis¹², root canal disinfection and cavity disinfection.^{13,14} The PDT method is applied to deep dentin caries using light sensitive agents (toluidine blue, methylene blue, azure dyes, crystal violet, hematoporphyrins, aluminum disulfonated phthocyanine, chlorins). Takasaki *et al.*¹⁵, the photosensitive agents used in the studies were investigated and it was determined that the most commonly used photosensitive agents were methylene blue and toluidine blue.

In recent years, single-stage adhesive systems, also known as universal or multi-mode systems, have been introduced to the market. Universal adhesives have advantages such as ease of application for clinicians, wide usage areas, and being able to be used in etch-and-rinse, self-etch and selective-etch modes.¹⁶ Dimethacrylate monomers have been replaced by 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomers in universal adhesive systems. The 10-MDP monomer plays an important role in establishing a chemical bond between enamel and dentin, and in obtaining a stable and durable interface.¹⁷ One of the universal adhesive systems is a two-component, one-stage, self-curing adhesive system with its own polymerization initiator, which does not require light-curing.¹⁸ It has been stated that the 3D-SR (three dimensional self-reinforcing)

monomer in the adhesive can organize alone and the phosphate group it contains can interact with calcium to form ionic bonds.¹⁹

There are many studies investigating the bonding of adhesive systems after the application of laser cavity disinfection methods. While some of these studies, disinfection of the cavity adversely affected the bond strength^{20,21}, Kaptan *et al.*²² stated that it increased the bond strength.

The aim of this study is to investigate the shear bond strength of self-cured and self-etch adhesive systems to dentin after surface disinfection with Photodynamic Therapy, Er:YAG, Nd:YAG, and KTP laser and to examine the reasons for failure in SEM.

Materials and Methods

Teeth Samples

Ethics Committee approval dated 17.11.2021 and numbered 2021-11/23 was obtained by Sivas Cumhuriyet University Non-Interventional Clinical Research Ethics Committee to start the study. In the study, 120 permanent human molars were used and these teeth were procured from the teeth extracted for orthodontic or periodontal reasons in the last 6 months in Sivas Cumhuriyet University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery. No tooth extraction was performed for the study. Organic residues on the tooth surface were cleaned. During the storage of teeth, the teeth were kept in distilled water at room temperature and the storage fluid was renewed every week.

Preparation of Specimens

Before starting the test, 120 human molars were molded using self-curing acrylic to be subjected to shear bond strength testing. The teeth were sectioned to expose superficial dentin with a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff IL, USA) under water coolant. Then 600-800-1200 grit silicon carbide papers were applied to the surfaces to obtain a standard smear layer.

Experimental Groups

Teeth were randomly divided into 6 main groups with different disinfection treatments (n=20).

Control Group: No disinfection treatment was applied to the dentin surface.

Er:YAG laser Group: Er:YAG (Smarty 2940D, DEKA M.E.L.A. SRL, Italy) laser systems' parameters used were 100 mJ, 15 Hz, 1.5 W and was held up to 1 mm distance from the dentine surface. Er:YAG laser was held up to 1 mm distance from the dentin surface and irradiate for 30 seconds.

Nd:YAG laser Group: Nd:YAG (Smarty A10, DEKA M.E.L.A. SRL, Italy) laser systems' parameters used were 100 mJ, 15 Hz, and 1.5 W with repeated pulse mode. A 300 µm fiber optic energy delivery system was started with spiral movements applied for 30 seconds irradiation.

KTP laser Group: KTP (SmartLite D, DEKA M.E.L.A. SRL, Italy) laser systems' parameters used were 100 mJ, 15 Hz, and 1.5 W with repeated pulse mode. A 300 µm fiber optic

energy delivery system was started with spiral movements applied for 30 seconds irradiation.

KTP laser activated PDT Group: 0.1% methylene blue (Noratex) was applied to the tooth surface and the teeth were kept in the dark for 5 minutes. KTP laser systems' parameters used were 100 mJ, 15 Hz, and 1.5 W with repeated pulse mode. A 300 µm fiber optic energy delivery system was started with spiral movements applied for 30s irradiation. Then the dentin surface was washed with distilled water followed by drying for 5 s.

Diode laser activated PDT Group: 0.1% methylene blue (Noratex) was applied to the tooth surface and the teeth were kept in the dark for 5 minutes. Then the samples were exposed to a diode laser (BiOLASE Technology Inc, Irvine, CA, USA) for 30 s keeping at a 1 mm distance. Diode laser system was used 1.5W repeated pulse mode. The dentin surface was washed with distilled water followed by drying for 5 s.

The main groups that underwent different disinfection procedures were randomly divided into 2 subgroups according to the adhesive systems used (n=10). In our study, the materials tested and their composition information are given in Table 1.

Subgroup 1; Self-cured adhesive system: The self-cured Universal Bond (Tokuyama Dental Corp, Tsukuba, Japonya) A and B were mixed, the application was completed within 60 s and no light application was performed.

Subgroup 2; Self-etch adhesive system: Scotchbond Universal (3M ESPE, St. Paul MN, ABD) was applied to the surface using applicator, After waiting for 10 s, it was air dried and polymerized using a 10 s LED light device (Valo Cordless, Ultradent, USA).

After adhesive applications, composites (Filtek Z550, 3M ESPE, St. Paul MN, ABD) were placed using 3 mm diameter, 2 mm high transparent cylindrical mold and polymerized using a 20 s LED light device.

Thermal Aging of Samples

All prepared samples were stored in distilled water at 37°C for 24 hours. For termal ageing, the specimens underwent 5000 cycles in distilled water baths at 55°C and 5°C with transfer time of 5 s and waiting time of 60 s in a thermocycler (Gökçeler Machinery Trade. and Ind. Co. Ltd. Sivas/Turkiye).

Shear Bond Strength Test

The samples were subjected to shear bond strength test using the universal test machine (LLOYD Instruments, Ametek Inc. England). The crushing apparatus was placed

at an angle of 90° with the dentin-composite interface. A shear force was applied at a head speed of 1.0 mm/min until failure occurred. SBS was expressed in MPa.

Failure mode Analysis

After shear bond strength test, the fractured dentin surfaces were examined under SEM to determine the mode of failure at magnification of X25. Modes of failiure were reported as adhesive, cohesive and admixed.

SEM Analysis

After all samples were examined by stereomicroscope, SEM analyzes were performed to evaluate the fracture surfaces in detail. The samples were analyzed with SEM device.

Statistical analysis

Variation data of were analyzed using the SPSS statistical software program (22.0 version, SPSS Inc., Chicago, USA). The data were subjected to statistical analysis with using one-way analysis of variance and Tukey's post hoc test to examine pairwise difference. P<0.05 was accepted significant.

Results

The results, described in Table 2, show the mean bond values, standard deviations and statistical evaluation of the adhesive groups applied after disinfection. The differences between the Er:YAG laser group and the control, KTP and Nd:YAG laser groups of the self-cured adhesive samples were statistically significant. While the differences between the Nd:YAG laser group and the PDT groups were statistically significant (p<0.05), the differences between all other groups were not significant. In the samples applied with self-etch adhesive, while the differences between the KTP and Nd:YAG laser groups and all other groups were statistically significant (p<0.05), the difference between them was not statistically significant (p>0.05). When adhesive systems are compared; while the differences in the control and PDT groups were statistically significant (p<0.05), the differences between all other groups were not statistically significant (p>0.05). The highest SBS values was exhibited in the disinfection group with Er:YAG laser. The lowest SBS values was exhibited in the disinfection group with Nd:YAG laser. The predominant failure type in dentine disinfected with Er:YAG laser, Nd:YAG laser, KTP laser and PDT was adhesive (Table 3). SEM images of the groups are given in Figure 1-6.

Table 1: Materials tested and their composition

Material	Composition
Universal Bond (Tokuyama Dental Corp, Tsukuba, Japonya)	Phosphoric acid monomer (3D-SR monomer) MTU-6 HEMA Bis-GEMA, TEGDMA, Acetoney-MPTES Borate Peroxide Acetone, Isopropyl alcohol, water
Scotchbond Universal Adhesive (3M ESPE, St. Paul MN, ABD)	Dimethacrylate resin, MDP phosphate monomer, Vitrebond, HEMA, fillers, copolymer, silane
Filtek Z550 Nanohybrid Universal Restorative (3M ESPE, St. Paul MN, ABD)	Silanize seramik, silanize silika, BIGMA, UDMA, bisfenol polietilen, glikol dieter dimetakrilat, TEGDMA
Metilen blue (Norateks)	0.1% methylene blue, distilled water

Table 2: The mean bond values, standard deviations and statistical evaluation of the adhesive groups applied after disinfection in the study

Main Groups	Self-cure adhesive		Self-etch adhesive	
	Mean	SD	Mean	SD
Control	7.64 ^{A,a}	1.78	12.84 ^{A,f,g}	2.58
Er:YAG laser	11.39 ^{a,b,c}	1.69	13.94 ^{h,i}	2.33
Nd:YAG laser	6.27 ^{b,d,e}	1.48	7.48 ^{f,h,j,k}	1.04
KTP laser	7.17 ^c	1.51	8.93 ^{g,i,l,m}	0.91
KTP laser activated PDT	9.61 ^{B,d}	2.53	13.37 ^{B,j,l}	2.85
Diyot laser activated PDT	9.99 ^{C,e}	2.31	13.75 ^{C,k,m}	2.58

F=9,809 P=0.000 p<0.05

^{A,B,C} There is a statistical difference between the groups shown with the same uppercase letters in the rows

^{a,b,c,d,e,f,g,h,i,j,k,l,m} There is a statistical difference between the groups shown with the same lowercase letters in the columns

Table 3: Number of modes of failure in each group

Main Groups	Failure mode					
	Self-cure adhesive			Self-etch adhesive		
	Adhesive	Cohesive	Mix	Adhesive	Cohesive	Mix
Control	10	0	0	9	0	1
Er:YAG laser	9	1	0	10	0	0
Nd:YAG laser	10	0	0	10	0	0
KTP laser	10	0	0	10	0	0
KTP laser activated PDT	9	1	0	9	1	0
Diyot laser activated PDT	10	0	0	9	1	0

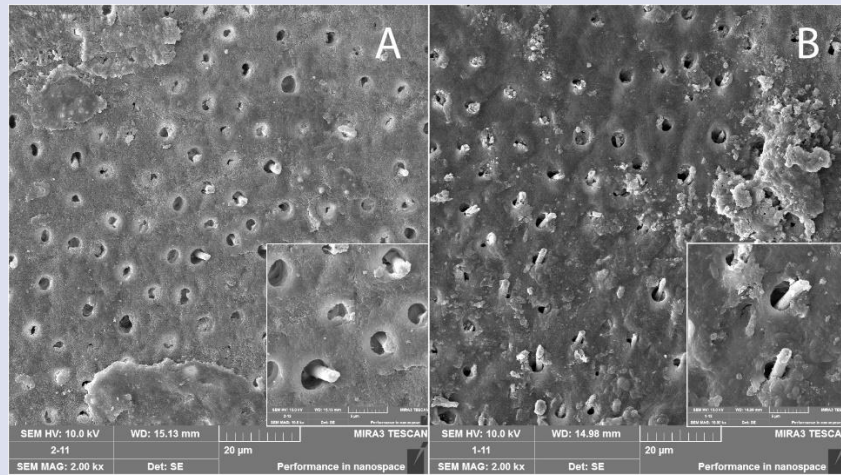


Figure 1. SEM image of control groups, A) Self-cured adhesive B) Self-etch adhesive.

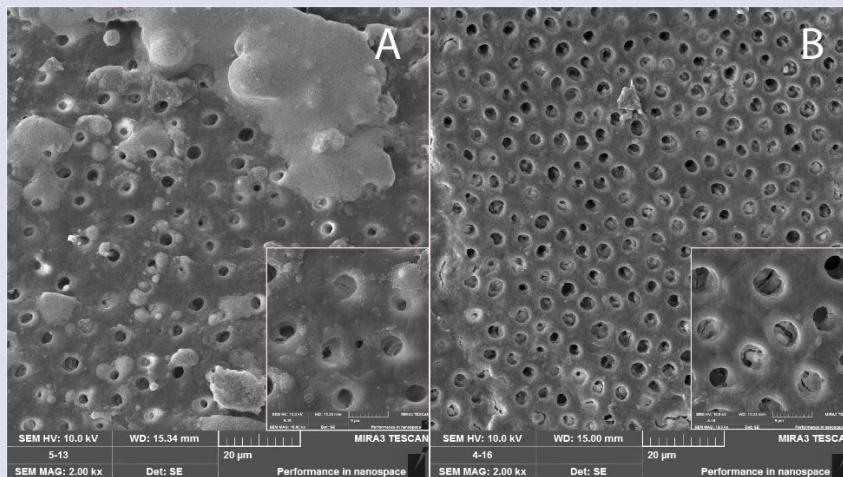


Figure 2. SEM image of dentin disinfected with Er:YAG laser groups A) Self-cured adhesive B) Self-etch adhesive

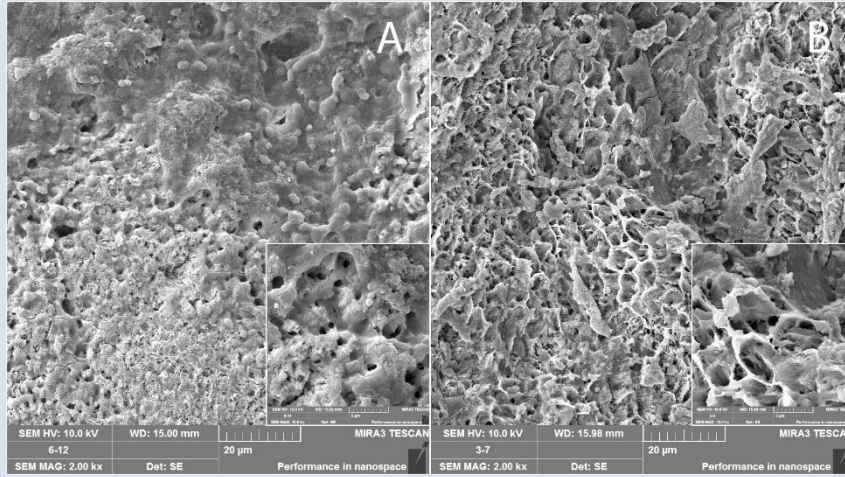


Figure 3. SEM image of dentin disinfected with Nd:YAG laser groups
A) Self-cured adhesive B) Self-etch adhesive.

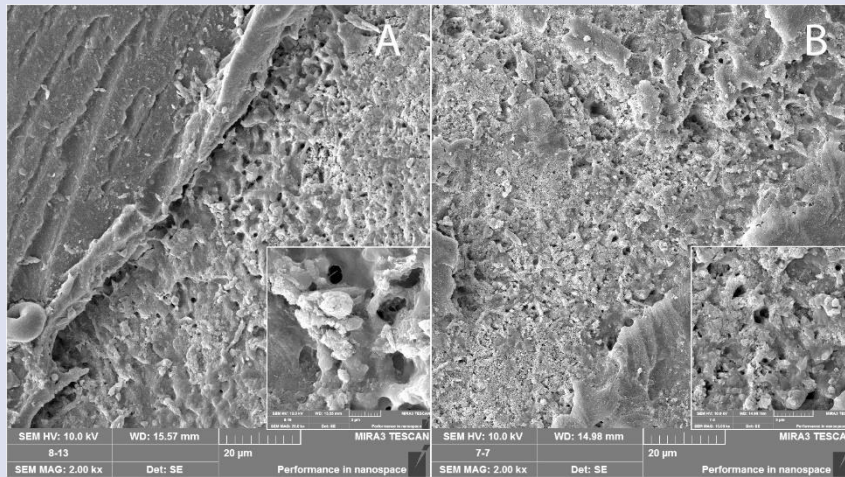


Figure 4. SEM image of dentin disinfected with KTP laser groups
A) Self-cured adhesive B) Self-etch adhesive.

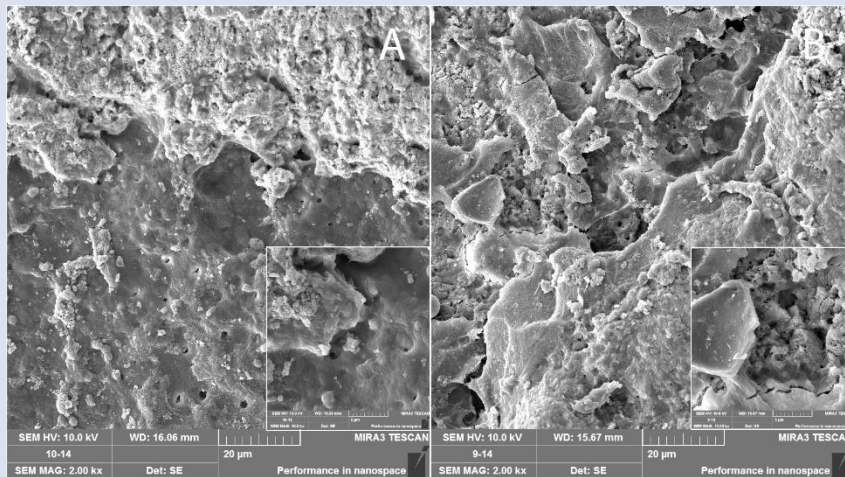


Figure 5. SEM image of dentin disinfected with KTP laser activated PDT groups
A) Self-cured adhesive B) Self-etch adhesive.

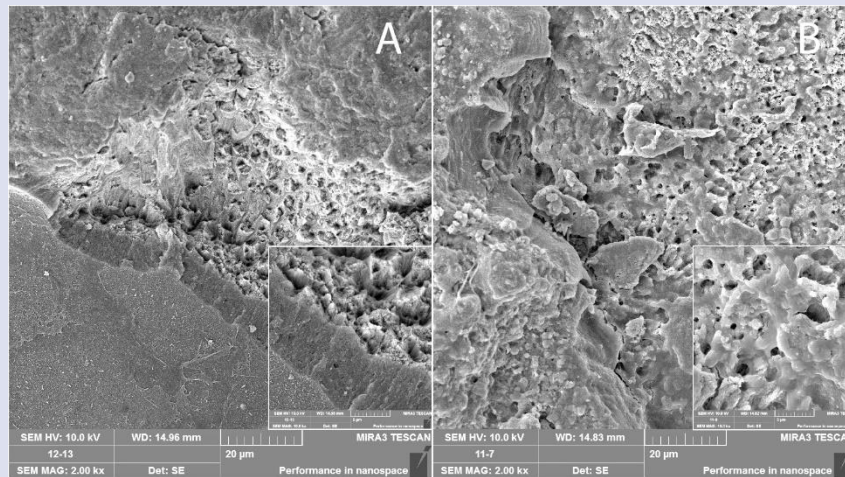


Figure 6. SEM image of dentin disinfected with Diode laser activated PDT groups
A) Self-cured adhesive B) Self-etch adhesive.

Discussion

Resin-containing restorative materials have started to be preferred more and thus, adhesive system technology has gained importance with the development of minimally invasive dentistry.²³ Although the bonding of resin-containing materials to the tooth has shown successful results with the development of adhesive systems, there are still some failures. The reasons for failure include bacteria remaining in the cavity after preparation, secondary caries formation, pulp inflammations and postoperative sensitivity.²⁴

Residual bacteria remaining in the cavity during cavity preparation can multiply within the smear layer, even when the cavity margins of the restoration are isolated from the oral cavity.¹ The use of cavity disinfectants is recommended to eliminate bacteria and increase the longevity of the restoration.² There are different studies on the effect of disinfection procedures on bonding. Tulunoglu *et al.*⁴ reported that the effects of disinfectant solutions on bonding are related to the adhesive system used. There is no definite information in the literature about the effect of cavity disinfection methods on bond strength. Therefore, in this study were investigated the effects of Er:YAG laser, Nd:YAG laser, KTP laser and photodynamic therapy and disinfection methods on the shear bond strength of two different adhesive systems (self-cured, self-etch).

Celik *et al.*²⁵ evaluated the effect of chlorhexidine gluconate-based cavity disinfectant and Er,Cr:YSGG laser irradiation on bond strength of etch-and-rinse and self-etch adhesive systems. In self-etch adhesives, Er,Cr:YSGG laser increased the bond strength, while CHX-based cavity disinfection solution decreased the bond strength. Kaptan *et al.*²² investigated the effects of Er:YAG and Er,Cr:YSGG laser irradiation and adhesive systems on the microtensile bond strength of self-adhering composite. They reported that both adhesives used in combination with Er:YAG and Er,Cr:YSSG laser applications increased the bond strength. Yazici *et al.*²⁶ in their study examining the effect of Er:YAG

laser on the bond strength of conventional and self-adhesive flowable composite to dentin, they stated that Er:YAG laser application increased the bond strength of both flowable composites. However, in the study of Ramos *et al.*²¹ in which they examined the effect of Er:YAG laser application on the bond strength of three different adhesive systems to dentin, they reported that laser application reduced the bond strength of adhesives. It has been stated that the reason for this negative effect may be related to the formation of subsurface cracks in the dentin. In this study, the highest bond strength value was observed in the Er:YAG laser group in both adhesive system applications. Celik *et al.*²⁵, Kaptan *et al.*²² and Yazici *et al.*²⁶ in their study on hard tissue lasers, this study supports our results in terms of obtaining high bonding values. Contrary to this study results, Ramos *et al.*²¹ reported that Er:YAG laser application reduces the bond strength to dentin. The reason for this difference may be due to the different adhesive systems, laser devices and application parameters used in the studies. In this study, when the SEM images of the Er:YAG laser applied samples are examined, it is seen that the smear layer is completely removed, the dentinal tubules are opened and there is a high rate of adhesive infiltration into these opened tubules (SEM).

Nisar *et al.*²⁷ investigated the effects of KTP laser, Er:YAG laser, ozonated water, CHX, and Rose Bengal on the bond strength of the adhesive applied in self-etch mode to the caries-affected dentine. They emphasized that high shear bond strength was demonstrated in ozonated water followed by Er:YAG laser and KTP laser. Gurgan *et al.*²⁸ evaluated the effect of self-etch adhesive systems on the shear bond strength of dentin after Nd:YAG laser and ozone application. Although the difference between the Nd:YAG laser and the control group was no statistically significant, it was stated that both adhesive systems reduced the shear bond strength value. Tarcin *et al.*²⁰ evaluated the effects of Nd:YAG laser, Er,Cr:YSGG laser and 37% orthophosphoric acid gel on the tensile bond strength of two different adhesive systems to

dentin. While Nd:YAG laser application showed the lowest bond strength values statistically, acid gel application showed the highest bond strength. Castro *et al.*²⁹ investigated the effect of Nd:YAG laser application in different parameters and the etch and rinse adhesive system on the microtensile bond strength to dentin. It was stated that the application of Nd:YAG laser on the non-acid applied dentin surface increased the bond strength value in both parameters. Kobayashi *et al.*³⁰ investigated the effect of applying Nd:YAG laser in different parameters on shear bond strength of glass ionomer luting cement to dentin surface. The reason why the laser application process increased the bond strength value was attributed to the increase in calcium in dentin and the strengthening of the bonds between the carboxyl group of polyalkenoic acid and calcium.

In this study among the disinfection procedures, the lowest bond strength values were observed in the Nd:YAG laser group, followed by the KTP laser group. Tarcin *et al.*²⁰ Gurgan *et al.*²⁸ and Nisar *et al.*²⁷'s laser application results show parallelism with the results of this study. In contrast to this study, Castro *et al.*²⁹ and Kobayashi *et al.*³⁰ They reported that Nd:YAG laser application increased the bond strength to dentin. The reason for this difference may be shown the different laser devices, parameters and application times. In this study, when the SEM images of the Nd:YAG and KTP laser applied samples were examined, it was observed that the surface properties changed due to the rapid melting and freezing of the dentin surface. The decrease in bond strength in Nd:YAG and KTP laser applied samples may be due to the reduction of the adhesion surface as a result of occlusion or narrowing of the dentinal tubules.

Alonaizan *et al.*³¹ investigated the effects of PDT, Er,Cr:YSGG laser and conventional disinfection application on the pushing bond strength of fiber post to root dentin. While PDT showed the highest bond strength value, they stated that there was no significant difference between PDT and Er,Cr:YSGG laser. Keskin *et al.*³² evaluated the effect of PDT, Er,Cr:YSGG laser, CHX and NaOCl on the microtensile bond strength of caries-affected dentin. Although Er,Cr:YSGG laser showed higher bond strength value than PDT, it was stated that the difference between them was no significant. Vellappally *et al.*³³ investigated the effects of Er,Cr:YSGG laser, PDT, CHX and NaOCl self-etch adhesive on the microtensile bond strength of caries-affected dentin. Although Er,Cr:YSGG laser and PDT showed higher bonding values compared to other disinfection methods, they reported that the difference between them was no significant.

In this study, the differences between the PDT groups (Diode laser and KTP laser activated PDT) and the Er:YAG laser group were statistically no significant. When the SEM images are examined, the formation of recessed, irregular and gapless areas in PDT may have affected the bond strength positively by increasing the micromechanical locking between dentin and adhesive. Vellappally *et al.*³³, Alonaizan *et al.*³¹, Keskin *et al.*³² supports this study in terms of obtaining high bonding values in PDT.

Saito *et al.*³⁴ investigated the effects of universal adhesives applied in different modes and times (immediate time, prolonged time) on shear bond strength to dentine. Scotchbond Universal demonstrated the highest bond strength when prolonged application time and applied according to the manufacturer's instructions. Karadas *et al.*³⁵ evaluated the effect of universal adhesives applied in different modes on microshear bond strength to enamel after 2 years of immersion in water. Although Scotchbond Universal Adhesive showed the highest bond strength to enamel in self-etch mode, they reported that the difference with Tokuyama Universal Bond was no significant. Madrigal *et al.*³⁶ investigated the effects of two different universal adhesives (Scotchbond Universal Adhesive, Tokuyama Universal Bond) and two luting cements on the tensile bond strength to enamel, dentin and different restorative materials. Although the bond strength of Scotchbond Universal to dentine was higher than that of Tokuyama Universal Bond, the difference between them was no significant.

In this study, the Scotchbond Universal Adhesive system showed higher shear bond strength than the Tokuyama Universal adhesive system. This study supports the evidence from the above studies.³⁴⁻³⁶

Scotchbond Universal contains 10-methacryloxy generation dihydrogen phosphate monomer (10-MDP). This monomer exhibits acidic characteristic and has an abrasive effect on the dentin surface. In addition, the 10-MDP monomer bonds the nanolayers and prevents hydrolytic degradation of the hybrid layer, thanks to its strong hydrophobic characteristic.^{23,37} Scotchbond Universal contains a polyalkenoic acid copolymer (Vitrebond copolymer) chemically bonding to calcium in hydroxyapatite.³⁸ More than 50% of the carboxyl groups in the polyalkenoic acid copolymer can be connected to hydroxyapatite. Carboxylic groups form ionic bonds with calcium, replacing the phosphate ions on the substrate.³⁹ Polyalkenoic acid copolymer provides high bond stability between dentine and adhesive during 6 months of aging. The polyalkenoic acid copolymer supports the bonding of Scotchbond Universal to dentine.⁴⁰ Moreover, Scotchbond Universal contains silica fillers (5-15%) containing silane, which can increase the mechanical properties of the adhesive layer and reduce water absorption.⁴¹ The irregular structure and residual solvents at the adhesive interface can affect the bond strength with time.⁴² Due to the high HEMA (10-30%) and acetone (30-60%) content of Tokuyama Universal Bond, it causes thinning of the adhesive layer. The high HEMA content of the adhesive results in increased water absorption and therefore a decrease in bond strength.⁴³ Hubbezoglu *et al.*⁴⁴ reported that when the fracture surface of Tokuyama Universal adhesive was examined in SEM, a 0.5-1 µm line-shaped space was observed along the hybrid layer, which negatively affected the bonding.

Conclusions

For the purpose of surface disinfection, Er:YAG laser and Photodynamic treatment applications increased the bond strength to dentine while Nd:YAG and KTP laser applications negatively affected the shear bond strength to dentine. KTP laser activated Photodynamic therapy application showed higher bond strength than KTP laser application alone. Self-etch adhesive system showed higher bonding value than self-cured adhesive system in all disinfection applications. In cases of using surface disinfection applications with laser for self-curing adhesive systems the Er:YAG laser or laser-activated PDT procedures are recommended.

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Conflict of Interest

None.

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Effectiveness of an Educational Intervention in Promoting Antibiotic Awareness Among 8th to 10th Grade Students in Kerala, India

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ABSTRACT

Aim: To assess the effectiveness of a training program on the knowledge, attitude, and behavior of antimicrobial resistance determinants among high school students in Kerala, India.

Methods: This quasi-experimental study was conducted among 8th – 10th grade students for six months in a high school in Kerala, India. This study consisted of a baseline assessment of consenting participants, educational intervention using PowerPoint slides, and post-intervention assessment. Assessments were done using a validated Knowledge, Attitude, and Behavior questionnaire.

Results: Among the 223 eligible students in the selected school, 159 (71.3%) participated in the study. The follow-up rate was 93.08%. Considering a p-value of less than 0.05 to be statistically significant, there was a statistically significant difference in the knowledge (pre-intervention mean score = 6.26 ± 2.06, post-intervention mean score = 7.44 ± 1.81), attitude (pre-intervention mean score = 2.51 ± 1.13, post-intervention mean score = 3.29 ± 1.19), and behavior (pre-intervention score = 4.27 ± 1.40, post-intervention score = 4.74 ± 1.38) of study participants.

Conclusions: Educational intervention based on improving the knowledge, attitude, and behavior of school children is an effective strategy to address the growing threat of antimicrobial resistance.

Key words: Antimicrobial resistance, school, behavior, knowledge, attitude, antibiotics.

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Background

The discovery of antibiotics is an important landmark in man's continuing combat against infectious diseases. Often called miracle drugs or magic bullets, these drugs were instrumental in saving millions of lives.¹ However, indiscriminate prescription and misuse of these medicines have resulted in the development of antimicrobial resistance (AMR). AMR is the "phenomenon where infection-causing microorganisms, such as bacteria, can survive exposure to medicine which would normally inhibit their growth or kill them".² This irony, wherein life-saving drugs become life-threatening, is a mounting global public health threat. The World Health Organization has highlighted the magnitude of AMR through its inclusion in the list of ten major global health threats in 2019.³

This is a caveat of an unprecedented apocalypse both in terms of mortality and economic burden. Approximately 2.4 million lives in Australia, North America, and Europe are estimated to be claimed by this 'superbug crisis' soon.⁴ Literature has estimated a mammoth cost of \$55 billion in the US within the context of antibiotic resistance.⁵ This is the cumulative cost of the impact of AMR concerning health service and loss of productivity. This economic burden encompasses

prolonged hospital admissions due to delayed recovery, rapid spread of infections, development and use of alternative treatment approaches, and undue stress on the healthcare system.⁴⁻⁶

Due to the weak AMR surveillance system, the Southeast Asia region is the focal point of AMR.⁷ India is one of the leading producers and consumers of antibiotics, and ipso facto accounts for a quarter of the incidence of multidrug-resistant tuberculosis.⁸ The "Chennai Declaration" (2012) that resulted from the first-ever consortium of medical societies was an initiative to address the alarming rate of AMR emergence and spread. It was a demand for a national policy for regulating antibiotic prescription, dispensing, and consumption. However, India continues to have high-level mortality related to drug-resistant pathogens.^{9,10}

Though global awareness has been awakened about this critical issue, attempts to resolve it are still preliminary. This can be attributed to the complexity of factors causing AMR's unavoidable consequences, like compromising our ability to treat common infections, resulting in disease prolongation, disability, and death.^{2,11} Extension of this health problem beyond the confines of

the hospital can be attributed to a lack of strict legislation, incorrect use of antibiotics, inappropriate knowledge of health professionals and beliefs, behaviors, and ignorance of patients.^{2,6,11,12} Moreover, patients' misconception of antibiotics as 'harmless panacea' to infections often influences treatment decisions related to antibiotics prescription and consequent indiscriminate drug use.^{13,14}

Being the most widely used medicine worldwide, both for prophylactic and therapeutic purposes, the rate of antibiotic use among children is exceptionally high.¹⁵ Injudicious use of antibiotics comprising over-dosages for symptomatic relief, over-the-counter purchases, and incomplete drug courses can lead to drug resistance in children. This may result in life-threatening conditions and disease burdens to health systems. Moreover, unreasonable expectations of treatment outcomes coupled with parental influences also contribute to unnecessary antibiotic prescriptions for children.¹⁶

In its technical note on antimicrobial resistance 2019, the United Nations Children's Fund (UNICEF) has emphasized that "AMR is perhaps the greatest threat to child survival and health of this generation".¹⁷ This reflects the need for immediate global actions focused on children to control the results of AMR, ranging from mortality and morbidity at the individual level to the burden of health expenditure at the community level.

An insight into the drivers of antibiotic resistance shows that an individual's health-related attitude and behavior is the cumulative effect of observations, perceptions, and interpretations of one's family, traditions, and environment, including media.¹⁸ As a complex issue, addressing antimicrobial resistance needs a One-health approach that warrants integration at all educational levels, especially schools.¹⁹ Using medicines for treating an illness is an essential health-related behavior. Therefore, a health-promoting educational intervention implemented among school-aged children will enable them to adopt healthy behaviors.¹³

Global efforts towards the reduction of antimicrobial misuse among children include the e-Bugs project in European countries¹³, the Bug Investigators school resource pack in the United Kingdom¹⁴, "Do Bugs Need Drugs?" in Canada, and CDC's "Get smart about antibiotics".²⁰ These interventions focused on knowledge assessment and awareness generation regarding immunity, hand hygiene, and the use of antibiotics because health-related practices like the usage of drugs are caused by factors like beliefs, attitudes, and expectations of treatment outcomes. As school-aged children are ideal for developing healthy attitudes and modifying existing beliefs, an educational intervention program focused on this group will be effective. Although the current school curriculum provides basic knowledge regarding health and hygiene, topics like antibiotic use and adverse reactions to drug use are not given due importance.

Existing literature on interventions among school-going children in India shows limited evidence on school-based educational interventions to create awareness

about antibiotic use and hygienic behaviors. Therefore, the present study was designed to address this gap in literature. The objective of this study was to assess the effectiveness of a training program on the knowledge, attitude, and behavior of AMR determinants among high school students in Kerala, India.

Methods

This quasi-experimental study was conducted in a high school in the southern state of Kerala, India. The school included was a government-aided high school located in a rural area. Due to then existing COVID-19 scenario, school was selected based on convenience. The institutional ethics committee approved the study (ECASM-AIMS-2021-356), and the study protocol was registered in the Clinical Trial Registry of India CTRI/2022/01/039628, registered 1 January 2022, (<http://ctri.nic.in/Clinicaltrials/showallp.php?mid1=64546&EncHid=&userName=Venkitachalam>). Permission from the management of the selected school was obtained. This was a census type of study wherein all students enrolled in the chosen school from grades 8 to 10 were invited to participate in the study. A participant information sheet and certificate of consent in the local language (Malayalam) were given to all eligible participants. A consent signature was obtained from both parents (or guardians) and participants. These were intended for perusal by them as well as by their parents or guardians. Non-consenting students and students whose parents did not permit them to participate in the study were excluded. The report was prepared using TREND guidelines.

The study lasted six months, from December 2021 to June 2022. It consisted of a baseline assessment of (pre-testing) the included participants, providing instructions (education module) using PowerPoint slides, and post-intervention assessment. Pre and post-intervention assessments were done using a validated Knowledge, Attitude and Behavior (KAB) questionnaires adapted from previous studies.^{15,21-23}

The e-Bug educational module customized to Indian school children was used for the education intervention. E-Bug is a project by the European Union to improve knowledge of infections, immunity, and antibiotic use among school children through interactive classes, quizzes, and hands-on activities.²² The educational module (duration – 45 minutes) was designed to promote judicious antibiotic use and healthy behavior, covering the following topics (i) Introduction to the educational module (ii) Microbes – meaning, types of microbes, useful and harmful microbes (iii) Body's defense mechanisms (iv) Infections (v) Healthy practices – hand hygiene, oral hygiene, healthy behaviors (vi) Antibiotics and (vii) Antibiotic resistance.

The educational project named "Students training on Antimicrobial Resistance (STAR)" was delivered by the investigator in the Malayalam language for better comprehension by the study participants. Pre and post-test questionnaires were also administered in Malayalam.

Powerpoint presentations, hands-on demonstrations of tooth brushing, and handwashing and instruction pamphlets were part of the module. A health kit comprising of face mask, hand sanitizer, instruction pamphlets, and a health calendar was also provided to the study participants. A reinforcement stage was also included in this intervention. It involved peer education and training the teachers. Student representatives and teachers were trained to disseminate the educational module at fixed intervals – three months after the first class and reinforcement after one month. This enabled the study participants to attend three sessions of the educational module within the duration of the study (six months). The study flowchart is given in Figure 1.

Data were analyzed using IBM Statistical Package for Social Sciences (Version 20). Descriptive statistics were expressed as mean and standard deviation for continuous data and frequency and percentages for categorical data. To test the statistical significance of the change in the mean score of knowledge, attitude, and behavior of antimicrobial resistance determinants among high school students before and after the training program, a paired t-test was applied. A p-value of less than 0.05 was considered statistically significant.

Results

This quasi-experimental study was conducted in a high school in Kerala. Among the 223 eligible students in the selected school, 159 (71.3%) participated in the study. A total of 148 students participated in the post-test, resulting in a follow-up rate of 93.08%. Among the 148 students, 70% of them were males and belonged to the age group of 12 to 16 years. (Table 1)

The knowledge, attitude, and behavior sections had 11, 6, and 5 items, respectively. A score of 1 for each correct response and 0 for each wrong response was allotted, yielding the maximum possible scores for knowledge, attitude, and behavior as 11, 6, and 5. It was observed that the mean knowledge score before the intervention was 6.26 ± 2.06 ; after the intervention, the score improved to 7.44 ± 1.81 . Similarly, the mean attitude at baseline assessment was 2.51 ± 1.13 and improved to 3.29 ± 1.19 after the intervention. A similar improvement in behavior score was noticed when the mean score for behavior changed from 4.27 ± 1.40 to 4.74 ± 1.38 . (Table 2) (Figure 2)

Considering a p-value of less than 0.05 to be statistically significant, the study analysis results show a statistically significant difference in the knowledge, attitude, and behavior of study participants following the educational intervention. Within the context of knowledge, items like “Are there bacteria in our body which are good for our health?” showed an improved percentage of correct responses from 58.1% to 93.9%. While the baseline assessment of study participants indicated that 41.2% “have heard about Antibiotic resistance” on post-test assessment yielded a positive response rate of 83.1% (Table 3.).

Consequent to the intervention, the number of participants who felt that “antibiotics are being presently misused” improved from 28.4% to 56.8%. Regarding attitude, while 56.1% believed they had to complete the antibiotic course despite relief from their disease, the post-test assessment showed an additional 16.9% increase in the correct response. (Table 3) However, some items like “Can antibiotics cure viral infections?” and “Is washing hands with soap and water more effective in removing microbes than washing with water alone?” showed a decrease in the percentage of correct responses in the post-test. (Table 3)

Discussion

The global threat posed by antimicrobial resistance has resulted in many interventions to combat it. Of significant importance are behavioral modification interventions that focus on redefining an individual's perceptions about health, healthcare-seeking behavior, and use of medications.⁶ Evidence shows that these behavioral interventions result in desirable changes^{12,20,24}, thereby proving to be a cost-effective strategy to address the issue of AMR.⁶ The present study was an educational intervention to improve the knowledge, attitude, and behavior of high school children regarding antibiotic use. We followed a quasi-experimental study design where the effectiveness of the educational intervention was assessed based on the change in the knowledge, attitude, and behavior of study participants.

Health-related practices like the usage of drugs are caused by factors like beliefs, attitudes, and expectations of treatment outcomes. Studies show a significant relationship between antibiotic consumption and antibiotic resistance in the community.²⁵ This is further aggravated by behaviors like the inappropriate use of antibiotics without physician consultation and non-completion of prescribed dosages of medicine, particularly on early relief from the health condition has been estimated as significant drivers of antibiotic resistance development.^{6,26,27} These detrimental habits can be attributed to ignorance, long waiting hours in hospitals, inaccessibility to the healthcare provider, over-expectations on the effectiveness of antibiotics, and ease of over-the-counter access to medications.⁶

The e-Bug project, an educational project by the European Union, has proven to improve children's knowledge regarding hygiene and antimicrobial use in multiple countries.²⁸ A study that aimed to assess the possibilities of global implementation of e-Bug project and the possible challenges to its broader implementation was conducted in 14 countries. It was found that most of these countries favored its endorsement through their ministries.²⁹ Upgradation of knowledge about personal hygiene and disease prevention is a recognized solution to the growing AMR.³⁰ This evidence strengthened our selection of the educational resource. The present study's e-Bug educational module was customized for Indian school children.

Child health research has highlighted that an individual's health beliefs and practices during childhood are a crucial determinant of one's health status in adulthood.³¹ Resonating with this finding, we focused our educational intervention on school children. Human behaviors are affected by complex factors like attitudes and beliefs that are susceptible to changes over time.²¹ In the present study, a baseline assessment using a Knowledge, Attitude, and Behavior questionnaire was done, which served as a reference to the evaluation of the effectiveness of the intervention.³²

Our study focus on schoolchildren from grades 8 to 10 because they are in the transition phase to adulthood. The World Health Organization has highlighted that adolescence is the window of opportunity for developing good health.³³ In the context of health beliefs and use of antibiotics, high school students are the ideal population as they are in the formative stages of critical thinking and independent decision making. Moreover, this phase is highly influenced by peer pressure. By targeting high school students, we can leverage their social networks and encourage the adoption of responsible antibiotic use practices. They can serve as agents of change, spreading awareness and correct information about antibiotic use among their peers and within their communities.

To ensure sustainability, this study incorporated a reinforcement strategy involving peer training and teacher training. Peer education is effective in behavior modification interventions like smoking³⁴, diet, and physical activity.^{35,36} Since the 'educator' is a person from the participant group, they will have enhanced trust, rapport, and interest in the program.¹⁵ Besides being mutually beneficial to the 'student-educator' and the 'taught,' it improved students' confidence and communication skills. A feasibility study on the effectiveness of peer education in delivering e-Bug modules showed that high school students revealed that it was an effective method for improving knowledge regarding antibiotic use.³⁶

Similarly, the "training the teachers" strategy was also adopted to ensure the educational module's sustainability beyond the study's duration. A schoolteacher who volunteered for the same was trained to impart the educational module. Children are accustomed to their teachers in academics, which favors their compliance if teachers are stakeholders in the reinforcement of the educational intervention. Public Health England undertook similar training to improve students' awareness regarding antibiotic use.¹⁵

Another unique feature of this study was the emphasis on the "prevention of diseases," for which we included hand washing and oral hygiene lessons. Oral diseases are one of the chronic diseases of childhood, often resulting in loss of school days and antibiotic use.³⁷ Similarly, the Centers for Disease Control and Prevention (CDC) has recognized hand hygiene – either by handwashing or using an alcohol-based hand sanitizer as an important method to keep school children healthy. Consequently, the CDC

has recommended teachers reinforce these habits through Early Care and Education programs.³⁸ Such healthy behaviors would lessen the risk of infections among children, indirectly reducing the need for antibiotics.

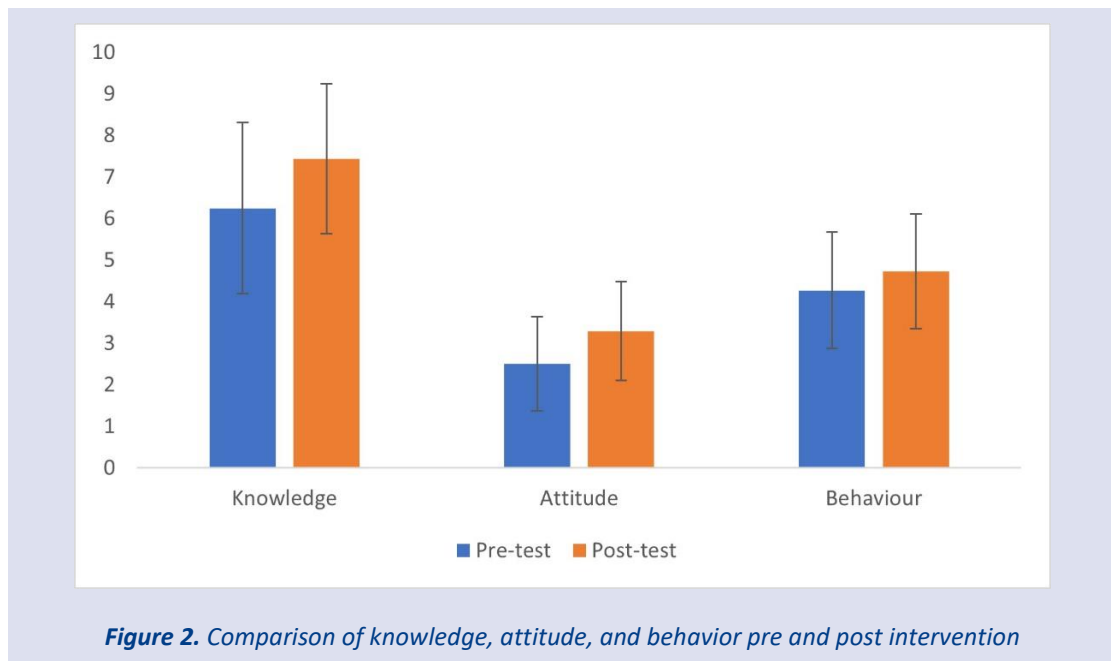
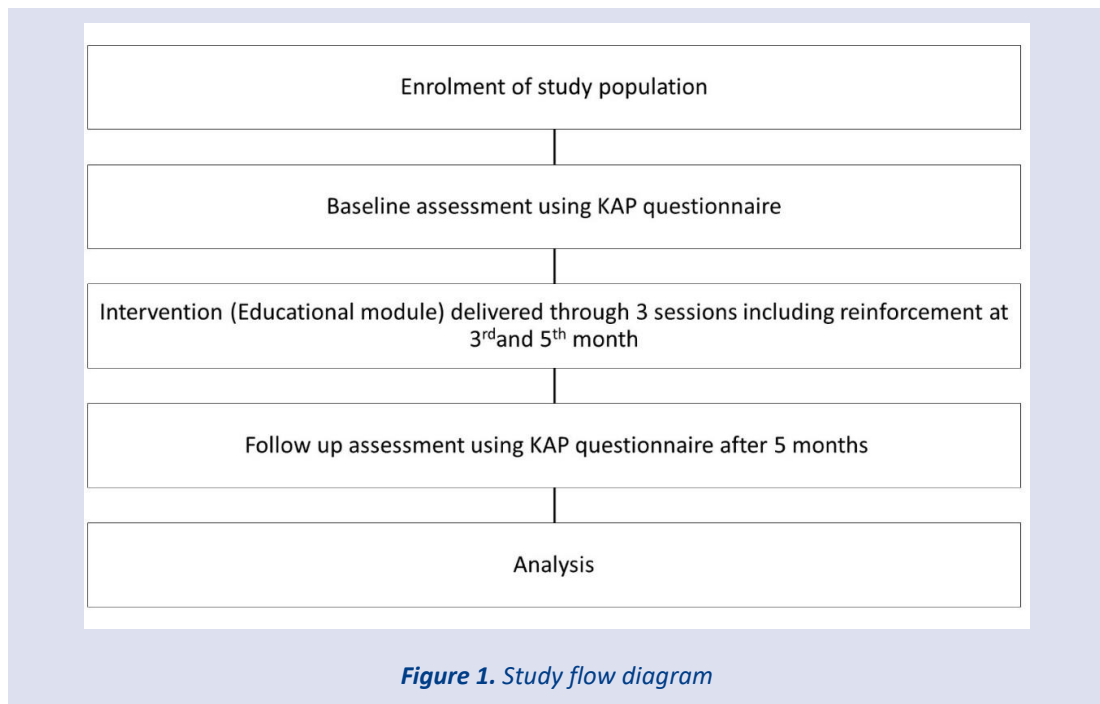
In terms of the effectiveness of the educational module, an improvement in knowledge, attitude, and behavior were observed. School-based health interventions have had mixed results in different settings. This result is in accordance with the study conducted in the United States of America, which showed positive changes in students' knowledge of microbes and microbial transmission.³⁹ Similarly, Fonseca *et al.*²⁴ showed that education activities improve awareness of antibiotic resistance and the importance of cautious antibiotic use.

The school-based educational intervention had a positive effect on knowledge^{13,28,40,41}, attitude^{13,42}, and behavior.^{42,43} Knowledge of the correct use of antibiotics for bacterial diseases among school children improved from 43% to 73% after the computerized slide show presentation-based intervention.¹³ The junior e-Bug teaching pack²⁸ and picture drawing intervention⁴² significantly improved students' knowledge. Education intervention by student volunteers trained as peer leaders delivered to their classmates had reported antibiotic use for colds and flu in a positive direction.⁴³

However, Farrell *et al.*⁴⁴ demonstrated no significant improvement in knowledge and attitude after the online e-Bug junior game-based intervention focused on the basic principles of hand and respiratory hygiene and antibiotic resistance in 9-12-year-old children. The authors attribute this observation to a high drop-out rate or a higher number of participants answering the pre-and post-game questions correctly, thereby not reflecting as an improvement.

Our study was the first quasi-experimental study among schoolchildren in the state of Kerala focused on improving health literacy regarding antibiotic use and antimicrobial resistance. This study's strengths are regular follow-up, high response rate, reinforcement sessions through peer education and teacher training, and the emphasis on "disease prevention" through hand washing and oral hygiene lessons. This broad coverage of the education module amplifies our study's public health relevance, particularly during the ongoing COVID-19 conditions. The study was not without its limitations. Due to the existing Covid-19 pandemic situation during the time of conduct of the study, only one school selected by convenience was included. Interactive teaching methods like group activities and demonstrations had to be restricted to adhere to social distancing norms.

While this study showed favorable and encouraging outcomes, replication and scaling up of such educational intervention for improving health, like that of antimicrobial resistance, is required to ensure sustainability.

**Table 1.** Descriptive characteristics of study participants (n=148)

	Variable	Number
Gender	Males	103 (70%)
	Females	45 (30%)
Class	8 th	30 (20.3%)
	9 th	60 (40.5%)
	10 th	58 (39.2%)

Table 2. Comparison of pre ad post-intervention knowledge, attitude, and behavior scores

Domains	Variables	Comparison	N	Mean	SD	p-value
Knowledge		Pre - intervention	143	6.25	2.06	0.001*
		Post - intervention	143	7.44	1.81	
Attitude		Pre - intervention	145	2.51	1.13	0.001*
		Post - intervention	145	3.29	1.19	
Behavior		Pre - intervention	147	4.27	1.40	0.002*
		Post - intervention	147	4.74	1.38	

Table 3. Comparison of pre and post-intervention correct responses (%)

Item	Correct response	
	Pre-test (%)	Post-test(%)
K1. Are microbes found everywhere?	69.6	82.3
K2. Are there bacteria in our body which are good for our health?	58.1	93.9
K3. Can antibiotics cure bacterial infections?	45.3	71.4
K4. Can antibiotics cure viral infections?	28.4	22.4
K5. Have you heard of 'Antibiotic Resistance'?	41.2	83.1
K6. Can frequent antibiotic use decrease its effect?	26.4	54.7
K7. Can the effect of antibiotics increase, if it is newer and more costly?	41.2	32.7
K8. Can unclean hands cause diseases?	87.2	87.8
K9. Is washing hands with soap and water more effective in removing microbes than washing with water alone?	95.3	75.7
K10. Can microbes spread by sneezing or coughing?	91.2	93.9
K11. Are good bacteria killed by antibiotics?	29.1	44.2
A1. Do you think that antibiotics are being presently misused?	28.4	56.8
A2. Is antibiotic resistance a problem in India?	14.9	21.6
A3. Does unnecessary antibiotic use increase the bacterial resistance towards them?	20.9	45.3
A4. Can antibiotic resistance affect you and your family's health?	19.6	35.6
A5. Is it necessary to get more education about antibiotics?	79.7	83.7
A6. Do you prefer getting antibiotics from friends or relatives than seeing a doctor?	87.2	85.8
B1. Do you use antibiotics without doctor's instructions?	75.7	87.8
B2. When you feel better, do you stop taking antibiotics without completing the full course?	56.1	73.0
B3. Do you store antibiotics at home for future needs?	66.9	81.8
B4. Do you always (or often) use antibiotics for common cold?	74.3	55.4
B5. Do you wash your hands before meals?	87.2	89.2
B6. Do you brush your teeth twice daily (at morning and before bedtime) with toothpaste?	68.2	84.4

Conclusions

This quasi-experimental study showed increased knowledge, attitude, and behavior scores following a structured educational module on antimicrobial resistance among 8th to 10th-grade children.

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Conflict of Interest

The authors declare no conflict of interest

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Evaluation of Surface Roughness and Microhardness of New Generation Bulk-Fill Composites

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ABSTRACT

Objectives: The main objective of the study was to evaluate and to compare the surface roughness and microhardness of three bulk-fill (ACTIVA Bioactive Restorative, SonicFill 2 Single Fill and SDR Flow Plus) and one conventional (G-aenial Posterior) composite resin at different depths.

Materials and Methods: Eighty disc-shaped composite resins (6 mm diameter, 2 mm height) were polymerized as recommended and then subjected to the appropriate finishing and polishing procedure. Transparent strips were placed between the samples before polymerization to evaluate the hardness at different depths (0-2mm and 2-4mm) of the bulk-fill composites. Microhardness was evaluated with a Vickers hardness tester and roughness was evaluated with an optical profilometer. One way ANOVA and Tukey multiple comparisons were performed for the statistical evaluation of microhardness and one way ANOVA was performed for roughness.

Results: No statistically significant difference was determined between the composite materials in respect of surface roughness ($p=0.336$). It was generally observed that as the layer thickness increased in all materials, the hardness values decreased ($p<0.0001$). SF was determined to have higher microhardness values in all the layers compared to the other samples ($p<0.001$).

Conclusions: While SF had the highest microhardness values in different layers, SDR was of equivalent value to a conventional composite. However, the hardness values of ACT in all layers were statistically significantly lower than conventional and other bulk-fillers.

Clinical Relevance: Within the limitation of this study, it may be recommended to use ACTIVA Restorative bulk-fill composite as dentin replacement in 2 mm layers and to cover the top surface with a composite.

Keywords: Bulk-fill composite, roughness, microhardness, profilometer, vickers.

Yeni Nesil Bulk-Fill Kompozitlerin Yüzey Pürüzlülüğü ve Mikro Sertliğinin Değerlendirilmesi

Süreç

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Öz

Amaç: Çalışmanın temel amacı, üç bulk-fill (ACTIVA Bioactive Restorative, SonicFill 2 SingleFill ve SDR Flow Plus) ve bir konvansiyonel (G-aenial Posterior) kompozit reçinenin yüzey pürüzlülüğünü ve farklı derinliklerdeki mikrosertliğini değerlendirmek ve karşılaştırmaktır.

Materyal ve Metot: Seksen adet disk şeklindeki (6 mm çapında, 2 mm yüksekliğinde) kompozit reçine önerilen şekilde polimerize edildikten sonra uygun bitirme ve cilalama prosedürüne tabi tutuldu. Bulk-fill kompozitlerin farklı derinliklerdeki (0-2 mm ve 2-4 mm) sertliği değerlendirmek için polimerizasyondan önce numunelerin arasına şeffaf bantlar yerleştirildi. Mikrosertlik bir Vickers sertlik test cihazı ile ve pürüzlülük bir optik profilometre ile değerlendirildi. Mikrosertliğin istatistiksel değerlendirilmesinde one way ANOVA ve Tukey çoklu karşılaştırmaları, pürüzlülükte ise one way ANOVA uygulandı.

Bulgular: Çalışmada kullanılan kompozit malzemeler arasında yüzey pürüzlülüğü açısından istatistiksel olarak anlamlı fark saptanmadı ($p=0,336$). Genel olarak tüm kompozit reçinelerde tabaka kalınlığı arttıkça sertlik değerlerinin azaldığı gözlemlendi ($p<0,0001$). SF'nin diğer örneklerle göre tüm katmanlarda daha yüksek mikrosertlik değerlerine sahip olduğu belirlendi ($p<0,001$).

Sonuç: SF farklı katmanlarda en yüksek mikrosertlik değerlerine sahipken, SDR sertlik açısından geleneksel bir kompozit ile eşdeğerdi. Bununla birlikte, ACT'nin tüm katmanlarındaki sertlik değerleri geleneksel ve diğer bulk-filllerden istatistiksel olarak anlamlı derecede düşüktü.

Klinik Çıkarım: Bu çalışmanın sınırları dahilinde, ACTIVA Restorative bulk-fill kompozitin dentin replasmanı olarak 2 mm'lik katmanlar halinde uygulanması ve üst yüzeyin bir kompozit ile kaplanması önerilebilir.

Anahtar Kelimeler: Bulk-fill kompozit, pürüzlülük, mikrosertlik, profilometre, vickers.

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Introduction

In recent years, almost only resin-based composite fillings have been used for restoration purposes in Restorative Dentistry. The clinical placement of these composites requires an incremental layering technique, but this is time-consuming and may lead to gaps between layers.¹ Technological developments have led to the production of bulk-fill resin-based composite materials which can fill cavities up to 4-6 mm in one application.²

The most important key to the success of bulk-fill resin-based composite materials is optimum polymerization, because polymerization to the full depth provides proper mechanical and physical properties. The polymerization of the restoration is directly related to the organic and inorganic composition of the material as well as the type and morphology of the filler contents.³

SDR Flow Plus is a new generation product of SDR, which has been used successfully since 2009. SDR technology has a patented structure of urethane dimethacrylate (UDMA), which results in less polymerization shrinkage and stretch, but it is recommended that the occlusal part is covered with conventional composite as SDR has low abrasion resistance.⁴

SonicFill is the only bulk-fill composite system with sonic activation, thereby allowing the possibility of placement in the cavity at low viscosity and modeling at a higher viscosity. SonicFill 2 SingleFill is a new product of the SonicFill family. SonicFill 2 has achieved significant improvements in polishability, overall aesthetics, wear resistance, and uptime using a new nanoscale zirconium oxide filler system. It can also be used without the need to coat the occlusal surface with a conventional composite.⁵

Activa, defined as a self-adhesive, dual-cure resin modified glass ionomer, is a mixture of modified polyacrylic acid, diurethane and other methacrylates, and contains 55.4% by weight bioactive glass and sodium fluoride. It is claimed by the manufacturer that Activa is durable, resistant to abrasion and breakage, and does not require an occlusal composite coating.⁶

The aim of this study was to evaluate the surface roughness and microhardness at different depths of Activa Bioactive Restorative, SonicFill 2 SingleFill and SDR Flow Plus, which are new generation bulk-fill composites, compared to G-aenial Posterior as a posterior conventional composite. The null hypothesis of the study was that there would be no difference between the bulk-fill composites compared to a conventional composite in respect of surface roughness and microhardness. It was also hypothesized that there would be no difference in the microhardness of different layer thicknesses of bulk-fill composites.

Materials and Methods

Sample preparation

In the present study, three bulk-fill (Activa Bioactive Restorative, SonicFill 2 SingleFill and SDR Flow Plus) and a conventional (G-aenial Posterior) composite resin materials were used. The properties of the composite

resins used are given in Table 1. (Table 1) Composite samples were prepared by titanium molds and transparent polyester strips as described below:

- Disc I (6 mm Ø x 2 mm) top surface cured directly (Disc I: 0 mm – Disc I: 2 mm)
- Disc II (6 mm Ø x 2 mm) has a bottom surface, cured by LED from the top surface of disc I and the distance traveled by the light reaching the bottom surface is 4 mm in total (Disc II: 2mm – Disc II: 4mm).

Polymerization was performed with an LED light source (FreeLight 2, Elipar, 3M ESPE) for the times mentioned as in Table 1. (Table 1) After demoulding, a point was placed on the side edge of the bottom surface with an acetate pen to identify the top and bottom surfaces. The samples were stored dry in the dark for 24 hours before analysis. All the samples were prepared by a single researcher in the same environment at the same time of day.

The composite samples were finished and polished dry and unidirectionally using Sof-Lex (3M ESPE) Al₂O₃ polishing discs for 20 s at 15,000 RPM. Each disc has been used once and were changed according to their grain thickness: Coarse 100 µm, Medium 29 µm, Fine 14 µm, and Super Fine 8 µm, respectively. After each disc, the samples were washed with distilled water and dried with air for 5 s.⁷

Microhardness Measurement

Microhardness measurements were made for different depths of bulk-fill composites. Conventional composites were obtained as 2 mm, and bulk-fill composites were obtained as 4 mm before separating them with transparent strips. The discs produced at 4 mm were divided into 2 mm. Thus, 4 measurements were obtained from a bulk-fill composite sample, 0 - 2mm top and bottom from Disc I, and top and bottom from 2 - 4mm Disc II. A total of 140 measurements were obtained from 70 disc-shaped composite resin samples.

Surface Vickers hardness (VH) was determined using a Vickers hardness tester (Micro Hardness Tester, Wilson, Buehler, USA) and a 100 g load (0.981 N) applied for 15 seconds, slope: 10 gf/s. Three indentations were recorded for each sample at different points of the irradiated top and non-irradiated bottom surfaces. VH values were expressed as N/mm² (MPa).

Roughness Measurements

Roughness measurements were made only for the top surface of the composite resins. A total of 40 disc-shaped composite resin samples were obtained at 2 mm for conventional and bulk-fill composites. The surface roughness of each specimen was measured using an optical profilometer (Filmetrics, Profilm3D, USA) with white light interferometry. Two- and three-dimensional profiles were obtained from the surfaces with a measurement 250x250 µm field of view and 0.44 µm spatial sampling at x4 magnification. The roughness values (Ra) of each specimen were recorded as µm. Three profiles were obtained from each specimen, and the arithmetic average of them was calculated. The profilometer was calibrated against a

reference block before each measurement. Surface images were obtained with AFM (Atomic Force Microscopes) for each composite sample.

Topographic imaging with Atomic Force Microscopy

Three-dimensional (3D) topographic images of 10 x 10 μm^2 sample surfaces were acquired with an atomic force microscope (AFM; Park Nx10, Suwon, Republic of Korea). The silicon probe tip was used with a scanning rate of 1 Hz and a resolution of 256 x 256 pixels.

Statistical analysis

The study data were analyzed statistically using SPSS Statistics 21 software (IBM Corp., Armonk, NY, USA). One-Way Variance analysis (ANOVA) and Tukey multi-comparisons were applied to evaluate microhardness, and One Way ANOVA for the analysis of roughness.

Table 1: Properties of the dental composites used.

Product name	Type	Shade and cure time	Composition	Filler size and content	Manufacturer
G-aenial Posterior	Micro-hybrid composite	A2, 2mm for 20s	UDMA, Silicon dioxide, Fluoro alumino-silicate glass, Composite filler, Pigment, Photo initiator.	>100 nm; Fluoroaluminosilicate, Inorganic filler <100 nm; Fumed silica, Pre-polymerized fillers 16-17 μm ; Strontium and lanthanoid fluoride wt/vol %: 77/65	GC, Japan.
ACTIVA BioActive-Restorative	Flowable bulk-fill	A2, 4mm for 20s	Blend of diurethane and other methacrylates with modified polyacrylic acid (44.6%), 1,4-Butanediol dimethacrylate, UDMA, Bis-GMA.	Reactive ionomer glass fillers (Amorphous silica 6.7 %) (Sodium fluoride 0.75 %) 55.4 wt % of bioactive glass and sodium fluoride.	Pulpdent, USA.
SonicFill 2 SingleFill	Sonic-activated flowable and sculpable bulk-fill	A2, 5mm for 20s	Bis-GMA, Bis-EMA, TEGDMA	Ba-B-Al-Si-glass, SiO ₂ wt/vol %= 83.5/66	Kavo Kerr, USA.
SDR Plus	Flowable bulk-fill base	A2, 4mm for 40s	Modified UDMA, BisEMA, TEGDMA.	Ba-Al-F-B-Si-glass, Sr-Al-F-Si-glass 0.02–10.0 μm (mean 4.2) wt/vol %= 68/45	Dentsply Sirona, USA.

*UDMA: Urethane dimethacrylate / Bis-GMA: Bisphenol A-glycidyl methacrylate / Bis-EMA: bisphenol A diglycidyl methacrylate ethoxylated / TEGDMA: triethylene glycol dimethacrylate

Results

No statistically significant difference was determined between the materials in respect of top surface roughness ($p=0.336$). (Table 2) AFM images of the samples are given in Figure 1. (Figure 1)

The hardness values of different composite resins were compared at the same thickness: (Figure 2) The top and bottom surfaces of the samples at 0-2 mm thickness were evaluated, SF>GC>SDR>ACT was determined. SF was statistically different from the other materials ($p<0.001$), and the difference between GC and ACT was statistically significant ($p<0.001$).

In the evaluation of the top surface of the samples at 2-4 mm thickness, SF>ACT>SDR was observed, and SF was determined to be statistically significantly different from the other materials ($p<0.001$).

In the evaluation of the bottom surface of the samples at 2-4 mm thickness, SF>SDR>ACT was observed, and SF was determined to be statistically significantly different from the other materials ($p<0.001$).

The hardness values of the same material at different layer thicknesses: (Figure 2)

In all the materials, a decrease was seen in the hardness values as the layer thickness increased. A statistically significant increase was seen in hardness between the bottom surface of SF 0-2mm thickness and the top surface of SF 2-4mm ($p<0.0001$).

When the difference in hardness was evaluated between the layer thicknesses of SF, there was determined to be a statistically significant difference between SF 0-2mm thickness top surface and 2-4mm thickness bottom surface ($p<0.0001$). There was determined to be a statistically significant difference between SF 0-2 mm thickness bottom surface and 2-4mm thickness top and bottom surfaces ($p<0.0001$, $p=0.019$, respectively). The difference between the top and bottom surfaces of SF 2-4 mm thickness was statistically significant ($p<0.0001$).

When the difference in hardness was evaluated between the layer thicknesses of ACT, there was determined to be a statistically significant difference between the top and bottom surface ACT 0-2 mm thickness and the bottom surface 2-4 mm thickness ($p=0.006$, $p<0.0001$, respectively). The difference between the top and bottom surface of ACT 2-4 mm thickness was statistically significant ($p<0.0001$).

When the difference in hardness was evaluated between the layer thicknesses of SDR, there was determined to be a statistically significant difference between the top surface of SDR 0-2mm thickness and the top and bottom surfaces of 2-4mm thickness ($p=0.002$,

$p<0.0001$, respectively). The difference between the bottom surface of SDR 0-2 mm thickness and the bottom surface of 2-4 mm thickness was statistically significant ($p=0.013$).

Table 2: Surface roughness (Ra) of the materials.

	n	Mean	SD
GC	10	.1131	.14182
ACT	10	.1046	.05837
SF	10	.1007	.06138
SDR	10	.0827	.03273
Total	40	.1003	.08168

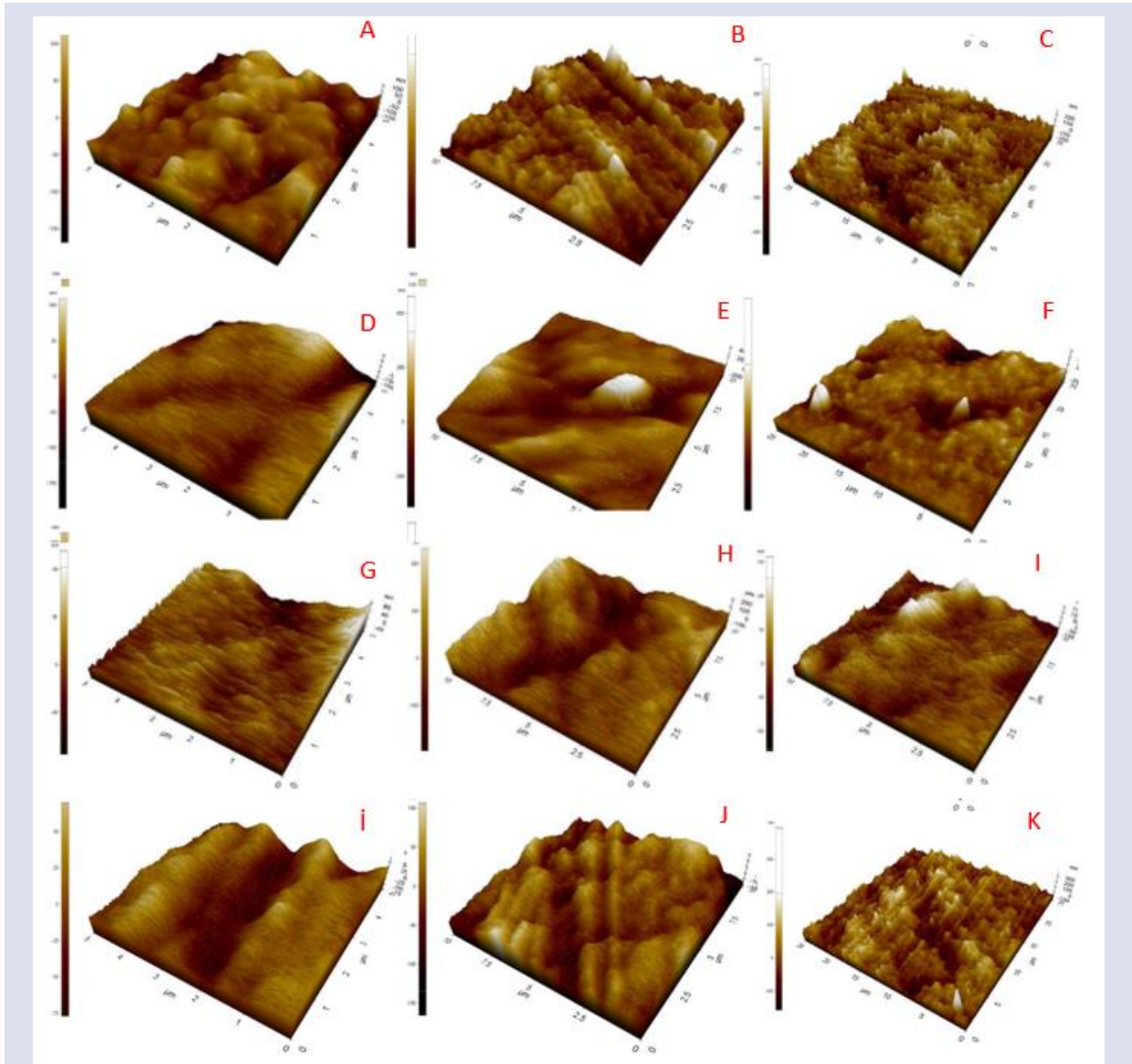


Figure 1. 3D AFM images of (A) GC Top surface 5µm (B) GC Top surface 10µm (C) GC Top surface 25µm (D) ACT Top surface 5µm (E) ACT Top surface 10µm (F) ACT Top surface 25µm (G) SF Top surface 5µm (H) SF Top surface 10µm (I) SF Top surface 25µm (J) SDR Top surface 5µm (K) SDR Top surface 10µm (L) SDR Top surface 25µm.

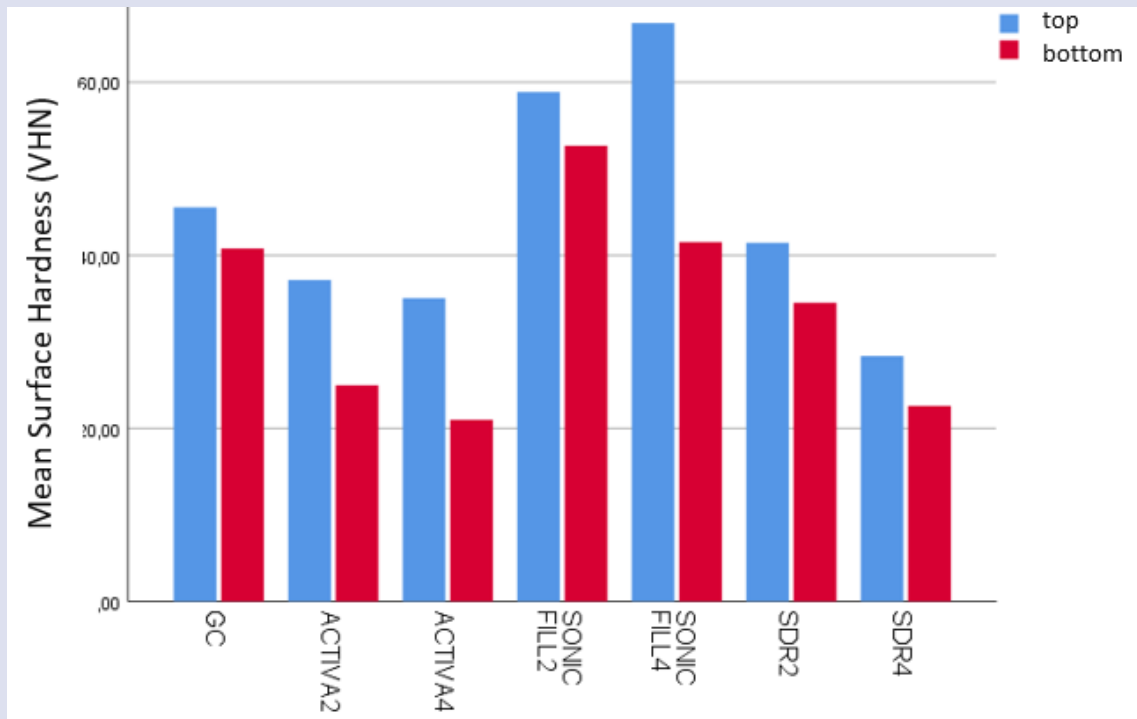


Figure 2. Top and bottom surface hardness of the materials. VHN – Vickers hardness number.

Discussion

The use of bulk-fill composite resins, which can be applied as a large mass with low technique sensitivity, has become more widespread among dental practitioners, especially for large cavities, because of advantages such as the reduced clinical working time. However, the surface hardness and roughness of bulk-fill composites applied to posterior teeth must be within acceptable clinical limits.⁸ According to the results of this study, which aimed to investigate this situation, the surface roughness values of bulk-fill and conventional resins were similar, and SF was seen to have higher microhardness values at different layer thicknesses than the other bulk-fill and conventional composites. Therefore, the null hypothesis of the study was partially rejected.

The Vickers microhardness (VH) test device was selected for use in this study as it requires a minimum area on the sample surface for the test and the method is simple and rapid results are provided.⁹⁻¹¹ The size of the Vickers hardness indentation is larger than the size of the filler particles in the material complex, so in the calculation the VH includes not only the filler component but also the surrounding softer resin matrix. In this context, VH indirectly considers cross-linking of the entire matrix network.^{12,13}

Profilometers and AFM have become the most preferred methods for the measurement of surface roughness of dental materials as they do not cause deformation of the sample surface and the results are highly accurate.¹⁴ In the literature related to the measurement of the surface roughness of bulk-fill composites, there has been reported to be no difference

between SDR and Sonic Fill in non-polished samples of different bulk-fill composites, and they have shown the lowest mean surface roughness compared to other bulk fillers.¹⁵ In a review evaluating surface roughness, 32 of 43 studies compared microhybrid/nano hybrid composites with other composite types such as suprananofill, nanofill, microfill, bulk-fill, and flow. In 14 of the 32 studies, the smoothest or at least one smooth surface was obtained in the microhybrid/nano hybrid samples of all the tested materials.¹⁶ In this study, no difference was found between GC posterior, a microhybrid composite, and all the other materials in respect of surface roughness following a polishing procedure.

In studies that have applied bulk-fill and conventional composites to cavities 4mm in depth, higher bonding strength and microhardness results have been obtained with bulk-fill composites.^{17,18} The reasons for this include the better light permeability due to the higher translucency of bulk-fill composites, and the variations in filler particles in the organic and inorganic matrixes such as increased molecular weight of the monomers, the addition of new stress-relieving monomers, the addition of pre-polymer particles and fiberglass rod segments, and the presence of plasticizing monomers, alternative and more reactive photo initiators, and polymerization modulators.^{17,19,20} It has been suggested that due to all these modifications, they have less polymerization shrinkage and a higher degree of conversion.²¹ According to the results of the current study, Sonic Fill had a statistically significantly higher level of microhardness than conventional G-aenial posterior composite. Sonic technology is recommended to facilitate the bulk

placement of the resin up to 5mm and the better spread to the cavity by increasing the flow of the resin.^{22, 23}

In a study that compared the effect on microhardness of sonic and incremental composite placement, the bond strength and microhardness values obtained from SonicFill resin were higher than from the other bulk-fill resins and showed similar results to the conventional resins placed with the incremental technique. Sonic Fill flow is optimized and can penetrate the cavity better because of the converters reacting to sonic energy and the barium glass and silicone dioxide in the inorganic particles.^{22, 23} Sonic energies have been shown to reduce the viscosity of the material by up to 87% with the technology. This means that by using SF in clinical practice, the time to construct a restoration is shortened by up to 30%.²⁴ The amount of filler particles of this material is said to be 83.5% by the manufacturer. This high rate could be responsible for minimizing the stress caused by polymerization shrinkage of the material.²⁵ All these above-mentioned modifications explain the higher hardness value obtained in the SonicFill measurements in all the layers compared to the other composites in the current study.

Although clinical procedures are simplified and working time is shortened by materials which allow single increment application, the problem of low microhardness may be encountered.²⁴ There are studies in literature showing that lower microhardness values have been obtained with bulk-fill resins compared to conventional resins applied with an incremental technique.^{20, 26} Similarly in another study, the microhardness of bulk-fill filling materials with high viscosity was found to be lower than the microhardness of conventional composites. The surface microhardness of the experimental groups of low viscosity materials was found to be even lower.²⁷ In the current study, when the hardness values of the top surface were evaluated, the conventional G-aenial posterior composite was seen to have higher microhardness values than SDR and ACT. That this result is expected from conventional composite can be attributed to the increasing polymerization associated with increased light dispersion of the greater number of particle/resin matrix interfaces because of the high filler content because of 2mm application as recommended by the manufacturer.²⁸

SDR is a fluid material with modified monomers and a relatively low amount of filler content (68% by weight). According to the manufacturer's information, as the low inorganic particle content decreases the organic matrix-filler particle interface contact, the depth of polymerization is increased by absorbing lighter during polymerization of the material. Moreover, low viscosity bulk-fill SDR includes a photoactive group embedded in urethane-based methacrylate monomers, which can enter a reaction with camphorquinone. The manufacturer claims that such an interaction helps to modulate the hardening reaction, and because of the higher flexibility of urethane groups, polymerization is reduced, and a more homogenous polymer structure is formed.^{29,30} The results

of the current study are consistent with this view that these modifications of SDR have surface roughness and hardness which may not show a statistically significant difference from those of conventional composites.

When the microhardness of the top layer was evaluated in the current study, ACT was determined to have the lowest microhardness. Low viscosity SDR, which currently has an indication for use as a base liner, was seen to have a higher hardness value than ACT, even on the lowest surface. Despite being a bioactive bulk-fill composite, ACT is not a recommended material for any additional occlusal coverage, and the low filler ratio content (56% by weight) can cause low hardness values. In a study which examined the Knoop Hardness values of bulk-fill composites with 5 different properties, the bioactive flowable composite ACT was seen to have the lowest hardness values. In the same study, no statistical difference was determined between the hardness values in different layers of ACT placed in bulk. It can be seen that the material not being affected by the increased thickness has undergone a chemical polymerization reaction caused by the glass ionomer components.³¹ In another study which compared bulk-fill resins, conventional glass ionomer, and ACT before and after thermocycles, ACT showed mechanical properties (diametral tensile strength, flexural strength) comparable to those of bulk-fill resin composites, and lower hardness values.³² The hardness of a material with a high blend of diurethane and other methacrylate with modified polyacrylic acid (44.6%) content and a low inorganic particle amount may be reduced compared to conventional resin composites. From these results, which are consistent with the findings of the current study, ACT, which is a low viscosity material, can be recommended as a liner for clinical dentin replacement.

To be able to accept that a composite resin has been sufficiently cured, generally the arbitrary minimum value of the bottom-to-top surface hardness ratio should be in the range of 0.8-0.85. At 0-2mm thickness, other than ACT, the bottom-to-top surface hardness ratio of the other composites was above this value and at 0-4mm thickness, it was below this value for all the bulk-fill composites.³³ However, in both conditions, ACT had the lowest cure depth values.

In a study which compared the microhardness and degree of conversion of bulk-fill composites, SDR, which was within the bulk-fill composites, was seen to have uniform conversion values throughout the 4 mm depth of the restorations.²⁴ The microhardness of high and low viscosity bulk-fill and conventional composites tested at 4 mm showed no different from the top surface values in another study.²⁷ In the results of the current study, there was seen to be a significant decrease in microhardness between the top and bottom layers in all the bulk-fill composites other than SF. Composite type and differences in cure depth, and even the general degree of conversion may be due to the viscosity of composites in uncured form. Composite viscosity is affected by the monomer component and filler particle content, and the

reaction kinetics and ultimately the degree of conversion is an important parameter as this parameter is affected by the activity of reactive species.³

In vitro situations and no aging process can be evaluated as a limitation of this study. In addition, that the method of obtaining different layer thicknesses was the application of a metal mold allowing the placement of a second 2 mm layer by separating with a clear band after the placement of the first 2 mm layer could also constitute a limitation of the study. Even if it is attempted to perform the application in the shortest possible time, the interface common to the 0-2mm and 2-4mm layers could be affected by the light. That the 2-4mm top surface was harder than the 0-2mm bottom surface in the SF group could have been a result of this.

Conclusions

The results of this study evaluating the surface properties of bulk-fill composites compared with a conventional composite demonstrated no difference between the materials in respect of roughness. When the hardness values were examined, SonicFill had the highest values in all different layers. SDR showed similar results to a conventional composite and ACT had the lowest microhardness values. Moreover, ACT did not have a sufficient depth of cure at both 0-2mm and 2-4mm thicknesses.

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Evaluation of Risk Factors Associated with Bruxism in Adult Turkish Population

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ABSTRACT

Objectives: The aim of the study is to evaluate various risk factors thought to be associated with the etiology of bruxism in adults in Turkey.

Materials and Methods: 400 adults aged 17-60 years were examined in terms of probable bruxism using the bruxism survey and clinical examination findings. The survey method was used to evaluate the correlation of bruxism with gastroesophageal reflux, gastric ulcer, gastritis, migraine, antidepressant use, cigarette consumption, alcohol consumption, caffeine consumption, and marital status between bruxist and control groups.

Results: It was found that gastroesophageal reflux, gastric ulcer, migraine, antidepressant use, caffeine consumption, and cigarette consumption were significantly higher in bruxists ($P=.000$, $P=.004$, $P=.000$, $P=.000$, $P=.000$, and $P=.008$, respectively). No significant difference was observed between bruxist and control groups in terms of the presence of gastritis, alcohol consumption, and marital status ($P=.163$, $P=.221$, and $P=.913$, respectively). While presence of migraine ($P=.041$) was higher in bruxist females, cigarette and alcohol consumption was higher in bruxist males ($P=.000$ and $P=.001$, respectively).

Conclusions: In conclusion, it was determined that medical disorders such as gastroesophageal reflux, gastric ulcer, and migraine as well as antidepressant use and cigarette consumption and caffeine consumption were risk factors associated with bruxism. If dentists are aware that these risk factors may cause or exacerbate bruxism, this may be beneficial in preventing damage to the mastication system before it occurs, and in planning treatment correctly.

Keywords: Bruxism, risk factor, etiology, probable bruxism, adults.

Yetişkin Türk Popülasyonda Bruksizmle İlişkili Risk Faktörlerinin Değerlendirilmesi

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ÖZ

Amaç: Türkiye’de, yetişkin bireylerde bruksizm etiyolojisi ile ilişkili olabileceği düşünülen çeşitli risk faktörlerini değerlendirmek amaçlanmıştır.

Materyal ve Metod: 17-60 yaş aralığındaki 400 yetişkin birey bruksizme yönelik anket ve klinik muayene bulguları ile muhtemel bruksizm açısından incelendi. Çalışmaya alınan bruksist ve kontrol gruplarında gastroözofajial reflü, gastrik ülser, gastrit, migren varlığı, antidepresan kullanımı, sigara, alkol, kafein tüketimi ve medeni durumun bruksizm ile ilişkisi anket yöntemiyle değerlendirildi.

Bulgular: Bruksistlerde gastroözofajial reflü, ülser, migren varlığı, antidepresan kullanımı, kafein ve sigara tüketimi anlamlı oranda daha yüksek bulundu ($P=.000$, $P=.004$, $P=.000$, $P=.000$, $P=.000$, $P=.008$ sırasıyla). Bruksist ve kontrol grubundaki bireyler arasında gastrit varlığı, alkol tüketimi ve medeni hal arasında anlamlı bir farklılık gözlenmedi ($P=.163$, $P=.221$, $P=.913$ sırasıyla). Bruksist kadınlarda migren varlığı ($P=.041$), bruksist erkeklerde ise sigara ve alkol tüketimi daha yüksek izlendi ($P=.000$, $P=.001$ sırasıyla).

Sonuç: Sonuç olarak gastroözofajial reflü, gastrik ülser, migren gibi tıbbi rahatsızlıklar, antidepresan kullanımı ve sigara, kafein tüketimi bruksizmle ilişkili risk faktörleridir. Diş hekimlerinin bu risk faktörlerinin bruksizme neden olabileceğinin ya da bruksizmi şiddetlendirebileceğinin farkında olması, çiğneme sistemi üzerinde oluşabilecek hasarların ortaya çıkmadan önce önlenbilmesinde ve tedavi planlamasının doğru yapılmasında fayda sağlayabilir.

Keywords: Bruksizm, risk faktörü, etiyoloji, muhtemel bruksizm, yetişkinler.

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Introduction

Bruxism is a non-physiological masticatory muscle activity characterized by clenching or grinding of the teeth whilst asleep (rhythmically or non-rhythmically) or awake (repetitive or continuous).¹

“Diurnal bruxism” refers to involuntary clenching and/or grinding of the teeth during the day while “nocturnal bruxism” refers to clenching and/or grinding activities during sleep at night.² While the prevalence of diurnal bruxism among adults ranges from 22% to 30%, the prevalence of nocturnal bruxism ranges from 1% to 15%.³

There are three clinical definitions for bruxism: ‘possible bruxism’ is defined as bruxism diagnosed based on patient report and/or the anamnesis-based part of the clinical examination; ‘probable bruxism’ is defined as bruxism diagnosed by patient report and clinical examination based on inspection; ‘definitive bruxism’ is defined as bruxism confirmed by polysomnographic or electromyographic recordings in addition to patient report and clinical examination.⁴

The lack of consensus on the definition and diagnostic methods of bruxism makes it difficult to explain the etiology of bruxism. The well accepted view is that multiple risk factors are effective in the pathogenesis of bruxism, the etiology of which cannot be exactly determined.^{1,4}

A risk factor indicates a high risk of disease without causing a causal effect on the development of the disease, while a causal risk factor contributes to increase the risk of disease etiologically.⁵ Determination of risk and etiological factors of bruxism and the physician's awareness of these factors can be guiding during the anamnesis and treatment of patients complaining of bruxism. It has been stated that systemic and psychological factors play a role in the pathogenesis of bruxism, but it is controversial which of these factors is more effective.⁶ Numerous studies have reported that psychosocial factors such as stress, anxiety, and depression and exogenous factors such as antidepressant use, cigarette consumption, alcohol consumption, and caffeine consumption⁴, as well as diseases such as migraine and gastroesophageal reflux may be among the major risk factors associated with bruxism.^{3,7-11}

This study aimed to evaluate various risk factors thought to be associated with the etiology of bruxism in adults and to examine the impact degrees of these risk factors. Two null hypotheses were established:

There is no correlation between bruxism and gastroesophageal reflux, gastric ulcer, gastritis, migraine, antidepressant use, cigarette consumption, alcohol consumption, caffeine consumption and marital status.

There is no correlation between these factors and gender in bruxists.

Materials and Methods

Patient selection

This study was conducted in accordance with the principles of the Declaration of Helsinki, and upon approval of the X University Non-Invasive Clinical Trials Ethics Committee with the decision dated 19.04.2023 and numbered 2023-04/13. The study was carried out on 200 bruxists and 200 controls who applied to the Department of Oral and Maxillofacial Radiology, Faculty of Dentistry of X University. All the participants aged 17-60 years who were voluntary to participate in the study signed the informed consent form.

Clinical examination

Survey recommended by Pintado *et al.*¹², and clinical selection criteria recommended by Rompre *et al.*¹³, were used to select the bruxist patients in this study.

The survey questions suggested by Pintado *et al.*¹², are as follows:

1. Has anyone ever told you that you grind your teeth at night?
2. Have you ever had a feeling of tiredness in your jaw when you woke up in the morning?
3. Do you feel pain in your teeth and gums when you wake up in the morning?
4. Have you ever had a headache when you woke up in the morning?
5. Have you noticed that you grind your teeth during the day before?
6. Have you noticed that you've clenched your teeth during the day before?

Clinical diagnostic criteria for bruxism recommended by Rompre *et al.*¹³, are as follows:

1. Presence of teeth clenching / grinding sound for at least 6 months, more than 3 nights a week in the patient
2. Tooth wear in accordance with the movements of the jaw in the normal or eccentric position
3. Presence of hypertrophy in the masseter muscle in voluntary contraction
4. Presence of feeling of discomfort, tiredness or stiffness in the masticatory muscles in the morning.

Individuals who answered positively to at least two of the survey questions recommended by Pintado *et al.*¹², and those who met all of clinical diagnostic criteria for bruxism recommended by Rompre *et al.*¹³, were accepted as probable bruxist.

Survey and clinical findings were assessed by an oral and maxillofacial radiologist having a 12 years of clinical experience including 6 years of maxillofacial radiology.

Survey Assessment

In the questions asked to individuals in the bruxist and control groups, the patient's socio-demographic information, systemic disorders and drug use, if any, were recorded. The presence of gastroesophageal reflux, gastric ulcer, gastritis, migraine, which are thought to be associated with bruxism, was questioned in the individuals included in the study, and information was obtained about the antidepressant use and consumption of cigarette,

alcohol, and caffeine. In addition, it was determined whether or not the individuals included in the bruxist group had diurnal and nocturnal bruxism and how individuals with nocturnal bruxism had awareness.

In this study, the calculation formula of sample with finite population was used to calculate the sample size ($n = Nt^2PQ / (N-1) d^2 + t^2PQ$). According to this formula, the precision was taken as 5%, the population size (population) was 6920, and the prevalence was taken as 15%. The sample size was determined as $n=200$ at margin of error of 5% and confidence interval of 95%.

Statistical analysis

The data of the study were analyzed using the SPSS Statistics 25.0 (Armonk, NY: IBM Corp) software. Whether or not the numerical data were normally distributed was checked by analyzing the skewness and kurtosis coefficients. Huck¹⁴, states that skewness and kurtosis values should range between -1 and +1 in order for the data to show normal distribution. The difference between age and groups was evaluated using the Mann Whitney U test. The mean, standard deviation, and frequency distributions of the data were analyzed. Chi-square test or Fisher's Exact Test was used to evaluate the correlation between bruxism and risk factors in individuals in the bruxist and control groups and to evaluate the correlation between gender and risk factors in bruxists. Significance level was taken as 0.05 and $P < 0.05$ was considered as statistically significant.

Results

It was found that the mean age of all the adults aged 17-60 years participating in the study was 26.56 ± 7.539 . The mean age of the bruxists was 27.17 ± 8.060 , and the mean age of the individuals in the control group was 25.94 ± 6.945 . The difference between the groups in terms of age was insignificant ($P = .109$).

The study was conducted on 400 individuals including 200 bruxists and 200 individuals in the control group, and the gender distribution was kept equal in each group.

When compared to the control group, gastroesophageal reflux, gastric ulcer, migraine, antidepressant use, cigarette consumption, and caffeine consumption were significantly higher in bruxists ($P = .000$, $P = .004$, $P = .000$, $P = .000$, $P = .008$, and $P = .000$, respectively); whereas, presence of gastritis and alcohol consumption did not show a significant difference between the groups ($P = .163$, $P = .221$) (Table 1).

Additionally, no significant difference was found between the bruxist and control groups in terms of marital status. ($P = .913$) (Table 1).

When examining according to gender, there were significant differences between the presence of migraine, cigarette consumption and alcohol consumption in bruxists. Accordingly, the presence of migraine was higher in women ($P = .041$) and cigarette and alcohol consumption was higher in men among bruxist individuals. ($P = .000$ and $P = .001$, respectively) (Table 2).

It was found that 84 (42%) of the bruxist individuals had only nocturnal bruxism and 116 (58%) had both nocturnal and diurnal bruxism.

Table 1. Distribution of Risk Factors among Bruxist and Control Group Individuals.

		Bruxist	Control	Total	Result
Gastroesophageal Reflux	Yes	29 (14.5%)	5 (2.5%)	34 (8.5%)	$X^2 = 18.515$
	No	171 (85.5%)	195 (97.5%)	366 (91.5%)	$P = .000^*$
Gastric Ulcer	Yes	9 (4.5%)	-	9 (2.25%)	$X^2 = 9.207$
	No	191 (95.5%)	200 (100%)	391 (97.75%)	$P = .004^a$
Gastritis	Yes	17 (8.5%)	10 (5%)	27 (6.75%)	$X^2 = 1.946$
	No	183 (91.5%)	190 (95%)	373 (93.25%)	$P = .163$
Migraine	Yes	44 (22%)	16 (8%)	60 (15%)	$X^2 = 15.373$
	No	156 (78%)	184 (92%)	340 (85%)	$P = .000^*$
Antidepressant use	Yes	19 (9.5%)	2 (1%)	21 (5.25%)	$X^2 = 14.524$
	No	181 (90.5%)	198 (99%)	379 (94.75%)	$P = .000^*$
Cigarette consumption	Yes	90 (45%)	64 (32%)	154 (38.5%)	$X^2 = 7.138$
	No	110 (55%)	136 (68%)	246 (61.5%)	$P = .008^*$
Caffeine consumption	Yes	82 (41%)	46 (23%)	128 (32%)	$X^2 = 14.133$
	No	118 (59%)	154 (77%)	272 (68%)	$P = .000^*$
Alcohol consumption	Yes	21 (10.5%)	14 (7%)	35 (8.75%)	$X^2 = 1.496$
	No	179 (89.5%)	186 (93%)	365 (91.25%)	$P = .221$
Marital status	Married	60 (30%)	61 (30.5%)	121 (30.25%)	$X^2 = 0.12$
	Single	140 (70%)	139 (69.5%)	279 (69.75%)	$P = .913$
Total		200 (100.0%)	200 (100.0%)	400 (100.0%)	

*Chi-square test, ^aFisher's exact test ($P < .05$ denotes significance)

Table 2. Distribution of Possible Risk Factors among Bruxists by Gender.

		Female	Male	Total (N%)	Result
Gastroesophageal Reflux	Yes	13 (13%)	16 (16%)	29 (14.5%)	X ² =0.363
	No	87 (87%)	84 (84%)	171 (85.5%)	P=.547
Gastric Ulcer	Yes	6 (6%)	3 (3%)	9 (4.5%)	X ² =1.047
	No	94 (94%)	97 (97%)	191 (95.5%)	P=.498
Gastritis	Yes	8 (8%)	9 (9%)	17 (8.5%)	X ² =0.064
	No	92 (92%)	91 (91%)	183 (91.5%)	P=.800
Migraine	Yes	28 (28%)	16 (16%)	44 (22%)	X ² =4.196
	No	72 (72%)	84 (84%)	156 (78%)	P=.041*
Antidepressant use	Yes	11 (11%)	8 (8%)	19 (9.5%)	X ² =0.523
	No	89 (89%)	92 (92%)	181 (90.5%)	P=.469
Cigarette consumption	Yes	31 (31%)	59 (59%)	90 (45%)	X ² =13.657
	No	69 (69%)	41 (41%)	110 (55%)	P=.000*
Caffeine consumption	Yes	36 (36%)	46 (46%)	82 (41%)	X ² =1.681
	No	64 (64%)	54 (54%)	118 (59%)	P=.195
Alcohol consumption	Yes	3 (3%)	18 (18%)	21 (10.5%)	X ² =11.971
	No	97 (97%)	82 (82%)	179 (89.5%)	P=.001*
Marital status	Married	26 (26%)	34 (34%)	60 (30%)	X ² =1.524
	Single	74 (74%)	66 (66%)	140 (70%)	P=.217
Total		100 (100.0%)	100 (100.0%)	200 (100.0%)	

* Chi-square test, ^aFisher's exact test (P<.05 denotes significance)

Discussion

It was determined that bruxism, which tended to decrease with increasing age, was most common in the age group of 20-40 years.¹⁵ The mean age of individuals aged 17-60 years included in this study was found to be 26.56±7.539 and these results were consistent with the view that bruxism is more effective on the young population.

Gastroesophageal reflux disease (GORD) is a condition that results from the reflux of stomach contents into the esophagus and causes disturbing symptoms and/or complications.¹⁶ Chemical wear is mostly seen in the palatal/lingual areas of the teeth in GORD and mechanical wear is seen on the contacting surfaces of the teeth in bruxism.^{11,13} Few studies have addressed and analyzed the possible relationship between GORD and bruxism.^{11,17} In the study conducted by Li *et al.*, in 2018^{11,17}, reported that GORD was associated with diurnal and nocturnal bruxism and GORD seen with bruxism was more common in women. Moreover, individuals with GORD having longer-lasting symptoms were more likely to develop bruxism.¹¹

In the present study, a significant correlation was found between bruxism and GORD, which was seen in 14.5% of the bruxists. In the light of these results, GORD appears to be a risk factor for bruxism. Bruxism should also be investigated in individuals with GORD, reflux symptoms and erosion-related tooth wear should be checked in bruxist individuals, and this disease should also be taken into account in planning of bruxism treatment. Differently from the study by Li *et al.*¹¹, no difference was observed between genders in terms of GORD in the present study, which may be caused by racial differences.

A review study mentioned that abdominal diseases such as gastric ulcer and gastritis may be included in the physiological factors in the etiology of bruxism.¹⁸ Hormones such as adrenaline and cortisol released under stress may contribute to the development of ulcers as a

result of increasing secretions such as acid that irritates the stomach.¹⁹ To the best of the author's knowledge, there is no human research on the correlation of bruxism with gastric ulcer and gastritis in the literature. In the present study, it was thought that there may be an indirect relationship between bruxism and these diseases, since bruxism, gastric ulcer and gastritis are associated with psychological factors such as stress. In this study, in which gastric ulcer was found as a risk factor for bruxism, there was no significant correlation between gastritis and bruxism. It is thought that future studies with a larger sample group consisting of bruxist individuals with ulcer will increase the reliability of the data. Furthermore, gastric ulcer and gastritis in bruxists did not differ between genders.

In their study, Fernandes *et al.*²⁰, reported that the prevalence of nocturnal bruxism was higher in individuals with headache and found a significant correlation between migraine and nocturnal bruxism in individuals who had chronic migraine and 74.6% of whom had nocturnal bruxism. In another systematic review, it was reported that migraine cannot be definitively considered as a potential risk factor due to the lack of a cause-effect relationship between bruxism and migraine.²¹ In the present study, in which a significant correlation was determined between migraine and bruxism, migraine appears to be a risk factor for bruxism, and further studies on etiopathogenesis are needed to evaluate whether or not migraine is an etiological factor for bruxism.

Bruxism may develop due to anxiety, stress and depression.²² Recently, there are studies reporting that the use of antidepressants increases the prevalence of bruxism.²³⁻²⁵ However, the mechanism of antidepressant-induced bruxism is unknown. It has been reported that various antidepressant drugs may inhibit dopamine release from the midbrain cortical pathway as a result of overstimulation of neurons by acting on the midbrain cortex in the ventral tegmental area, which may cause masticatory muscle dystonia and teeth grinding.^{26,27} Uca

*et al.*²⁴, evaluated the prevalence of bruxism on 807 patients (506 in the antidepressant group, 301 in the control group) and found that the frequency of bruxism was significantly higher in the antidepressant group (24.3%) than in the control group (15.3%). Likewise Melo *et al.*²⁵, reported that sleep bruxism increased with the use of antidepressants among adults. It was also reported that antidepressant-induced bruxism developed in pediatric and adult patients, mostly in female patients.²³ It was reported that teeth grinding symptoms may start after short and long-term use of antidepressants in patients and these symptoms may disappear within 3-4 weeks after discontinuing the drug or changing it with another drug.²³ The fact that antidepressant use was significantly higher in bruxists in the current study similar to the data in the literature supports the view that bruxism is affected by depression or antidepressant drug use.

Tobacco products, caffeine, and alcohol are legal psychoactive substances that can affect the central nervous system, behaviors, cognitive functions, and mood.²⁸ Numerous studies have reported that tobacco products containing nicotine, which is an acetylcholine agonist, stimulate and suppress the central nervous system²⁸, may cause sleep disorders²⁹ and also may be a predisposing factor for bruxism.^{8,9,30} In a systematic review, smokers were approximately 1.5 times more likely to have nocturnal bruxism than non-smokers.⁹ In a survey conducted by Alajbeg *et al.*³⁰, on 1092 Croatian navy employees, they found a 2.72 times higher risk of nocturnal bruxism in smokers. Again, studies conducted using polysomnographic evaluations revealed that smokers suffered from bruxism attacks with a higher number of arousal.³¹ As nicotine accumulating during wakefulness gradually decreases during sleep, oromotor activity may increase as a result of induction of glutamatergic synaptic transmission and increase in dopamine release.³² It is also thought that smoking affects sleep bruxism due to its proinflammatory effect on increased inflammation markers and cytokine levels in individuals.³¹

Contrary to these studies, Wincour *et al.*³³, conducted a study in a small group of Israeli adolescents and found no correlation between smoking and nocturnal bruxism.

The results of the current study showed that smoking is a risk factor for bruxism, especially in men, in parallel with most examples in the literature. The fact that smoking is more common in men may explain the different result between the genders.

Regarding the correlation between caffeine consumption and bruxism, Rintakoski *et al.*, conducted a cohort study of 12502 twins in 2013.¹⁰ In that study¹⁰ and also a systematic review⁹ caffeine consumption was significantly positively associated with bruxism, and the findings of the present study support these results. Positive correlations found between cigarette and caffeine consumption in the studies³⁴ may explain significant correlation of both risk factors with bruxism.

Alcohol contains ethanol, a central nervous system depressant²⁸ and alcohol consumption is known to have negative effects on health and sleep.^{35,36} While alcohol makes it easier to fall asleep, it also disrupts sleep balance.³⁶ There are studies reporting that alcohol consumption is positively correlated with bruxism^{9,10}; whereas, some other studies have also reported that alcohol do not have a significant effect on bruxism parameters.^{31,33} The lack of a significant correlation between alcohol consumption and bruxism in the present study may be due to the very low alcohol consumption in the study group (8.75%). Evaluation of bruxism in a larger sample group with alcohol consumption will give more meaningful and reliable results.

There are studies in the literature that marital status can affect the psychological life of the individual and indirectly trigger parafunctional behaviors such as bruxism.^{30,37} However, unlike the present study, the effect of marital status on bruxism was not observed. This result may have been caused by the low mean age in the sample group.

Diurnal bruxism is thought to have a pathophysiology different from nocturnal bruxism. While psychosocial factors (stress, anxiety) are especially effective in diurnal bruxism³⁸, alcohol, caffeine consumption, cigarette consumption, various psychotropic drugs, and gastroesophageal reflux have been associated with nocturnal bruxism.^{3,8,9} Although their etiopathogenesis is different, it has been observed that the causes of diurnal and nocturnal bruxism trigger each other.³⁹ According to the survey recommended by Pintado *et al.*¹², and the clinical examination findings recommended by Rompre *et al.*¹³, nocturnal bruxism was present in all bruxist individuals in this study, who were diagnosed with probable bruxism, and nocturnal bruxism and diurnal bruxism were seen together in 58% of the bruxist individuals. This may indicate that nocturnal and diurnal bruxism affected each other. However, the factors thought to be effective in nocturnal and diurnal bruxism could not be compared, since individuals with only diurnal bruxism were not found in the study.

Conclusions

Consequently, medical disorders such as gastroesophageal reflux, gastric ulcer, migraine, as well as antidepressant use, cigarette consumption, and caffeine consumption are associated with bruxism, and dentists should be aware that these risk factors may cause or exacerbate bruxism. The etiology and pathogenesis of bruxism will be elucidated by comprehensively evaluating these risk factors and investigating the causal relationships between them and bruxism. This may be beneficial in preventing the damage of bruxism on the mastication system before it occurs and in planning the bruxism treatment correctly.

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Platelet Concentrates – A Panacea for Periodontal Regeneration

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Review

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ABSTRACT

Periodontitis is an inflammatory condition of the periodontium leading to loss of supporting structures of the tooth. The regeneration of periodontium refers to the restoration of the lost tissues to their original form and function by reiterating the fundamental wound healing processes involved in their development. Research has led to the development of “Autologous biomaterials” provide signals for healing, repair, and regeneration. These autologous substances also fosters neo-angiogenesis and new bone formation and therefore have yielded encouraging results in the field of regenerative dentistry.

Platelet concentrates have higher concentration of growth factors which enhance periodontal regeneration. Blood derivatives have several advantages such as being autogenous, cost effective, less time consuming, simple to perform and prolonged release of growth factors. Since inception, many approaches have evolved also in-depth research has been done regarding its biological and clinical applications. Several modifications have been advocated in the conventional protocol like the advanced PRF, injectable PRF, PRF lysate and Titanium-prepared PRF.

This review paper addresses the evolution, applications of platelet concentrates for tissue engineering, recent advances, and novel protocols. Furthermore, several future perspectives of platelet concentrates, such as platelet concentrates as drug delivery agents, platelet dust and liposomes encapsulating platelet concentrates are also discussed.

Key words: Autologous biomaterials, periodontal regeneration, platelet concentrates, platelet rich fibrin.

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Introduction

Periodontitis is an inflammatory condition of the periodontium leading to loss of supporting structures of the tooth. A periodontal regeneration refers to the restoration of lost tissues to their original form and function by reiterating the fundamental wound healing processes involved in their development.¹ Research in the field has led to the development of “Autologous biomaterials” that are naturally occurring substances in the body, which provide signals for healing, repair, and regeneration. Furthermore, synthetically generated “alloplastic materials” have also produced encouraging results in the field of regenerative dentistry. However, these alloplastic materials are avascular and may trigger a foreign body reaction.² Therefore, to enhance the healing and regeneration, autologous substances are preferred as they have an added advantage of fostering neo-angiogenesis and new bone formation.³

The two autologous biomaterials- platelets and fibrin, are believed to play integral roles in wound regeneration and healing.⁴

Platelets are small irregularly shaped cells derived from precursor megakaryocytes. They are approximately 2–3 mm in diameter, and consists of granules, mitochondria and prominent membrane structures. The alpha granules and lysosomes present in platelets are

responsible for the expulsion of growth factors.⁵ The Alpha granules also contain biologic mediators in abundance. Platelets get activated during tissue injury and release growth factors such as platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor and epidermal growth factor.⁶ Due to their biological activity, platelets can create an accelerated chemo attractive gradient that recruits stem cells and facilitates regeneration. In recent years, blood proteins have led to the evolution of plasma concentrates enriched with growth factors like A-PRF, T-PRF, I-PRF, PRF lysates, and CGF and these autologous platelet concentrates offer hope for periodontal regeneration.

Evolution of Platelet Concentrates and Preparation Techniques⁷

The preparation of Fibrin Glue⁷, Platelet Rich Plasma^{7,8} and Platelet Rich Fibrin^{9,10,11} has been depicted in Figure-01.

Fibrin Glues, Fibrin Sealants or Fibrin Tissue Adhesives

They are derived from human plasma and are analogous to the final stages of blood coagulation in which a fibrin clot develops.

The fibrin sealants are of two types:

- Homologous
- Autologous.

Plasma cryoprecipitate was used to prepare homologous fibrinogen concentrates, which was formulated by mixing two components, thrombin containing calcium ions and fibrinogen containing factor XIII. Fibrin sealants were first prepared from autogenous whole plasma, but later bovine thrombin was used to polymerize them to reduce the risk of infection transmission.⁷

Platelet Rich Plasma^{7,8}

- CURASAN METHOD- Double centrifugation method (Figure-03)⁹
- The venous blood is drawn with an anticoagulant.
- The first centrifugation ("soft spin") allows the blood to be separated into three distinct layers. Using a sterile syringe, the practitioner aspirates platelet rich plasma (PRP), platelet poor plasma (PPP), and some red blood cells. Then the material is transferred to another tube.
- The second tube will undergo a second centrifugation, which will be substantially longer and faster (a "hard spin"). Thus, platelets can be concentrated at the bottom of the tube and subsequently separated into three layers.
- Using a syringe, the practitioner can discard most of the PPP, leaving just enough serum to suspend the concentrated platelets. cPRP (concentrated platelet-rich plasma) is then prepared by gently shaking the unit.
- Bovine thrombin and calcium chloride is then added to cPRP.
- Platelet concentrate gels very rapidly, and fibrinogen is also collected during cPRP preparation, resulting in a fibrin matrix with especially interesting hemostatic and adhesive properties.

Platelet Rich Fibrin^{10,11,12}

- Choukron's Protocol
- The research pioneer, Mr. Joseph Choukroun, has developed a 2nd generation platelet concentrate without the use of anticoagulants. After a single centrifugation cycle (3000 rpm, 10 minutes) it is possible to obtain (750 g) a platelet concentrate without coagulation factors from the superficial layer of centrifugation tubes¹⁰
- A fibrin matrix was formed after centrifugation of the formulation called Platelet rich fibrin. PRF is a concentrate of white blood cells, platelets, and fibrin.
- The initial PRF (also called **L-PRF**) was shown to contain 97% platelets and more than 50% leukocytes when compared with whole blood.
- The success of this technique depends solely on the speed at which the blood is collected and transferred to the centrifuge. To obtain a clinically useful clot from PRF, quick handling is absolutely necessary.

To address these limitations, several modifications are made and new forms of PRF were introduced.

The modifications are (Figure-02)¹²:

A-PRF

- The Low-Speed Centrifugation Concept was later developed to produce advanced PRF matrices in solid form.
- When compared to PRF, the A-PRF clot is more porous and contains more interfibrillar space.
- The sterile glass-based vacuum tubes were used for formation of A-PRF at slower speeds (1500 rpm) and for 14 minutes.¹³

A-PRF+

- In 2016, Kobayashi & co-workers suggested another modification of A-PRF by reducing the centrifugation time to 1300 rpm for 8 minutes.
- However, the PRF obtained in this manner is in the form of gel, which is not suitable for injection.
- To overcome this limitation, I-PRF was introduced.¹⁴

I-PRF

- Blood is drawn into tube without anticoagulants and centrifuged for three minutes at 700 RPM. In 2-4 minutes of centrifugation, the entire concentration of clotting factors and platelets reaches the top of the tube.
- Currently, it is mixed with bone grafts to form a gel-putty consistency with the particles incorporated into the bone graft.^{15,16}

T-PRF

By centrifuging blood at 2800 rpm for 12 minutes in Titanium tubes, Titanium-PRF is obtained. As titanium has better hemocompatibility than glass and possibly led to a more polymerized fibrin, T-PRF offer a tighter, thicker fibrin than L-PRF.¹⁷

Other modifications include:

Concentrated Growth Factors Preparation¹⁷

- 9ml of venous blood was drawn and collected in sterile Vacuette tubes without anticoagulant solutions.
- Thereafter, the tubes were immediately centrifuged with the following characteristics: 30 sec acceleration, 2 min at 2700 rpm, 4 min at 2400 rpm, 3 min at 3000 rpm, and 36 sec deceleration and stop.
- At the end of the process, three blood fractions were obtained:

(1) a superior phase represented by the serum (platelet-poor plasma, PPP)

(2) an interim phase represented by a very large and dense polymerized fibrin block containing the CGF, white blood cells and stem cells

(3) the lower red blood cell layer. The fibrin block with red interface was cut out as CGF

Autologous Fibrin Glue and Sticky Bone¹⁸

- To obtain Autologous protein glue (AFG), 20- 60CC of blood in non-coated tubes is centrifuged at 2400-2700 rpm for 2 minutes.
- Of the two layers obtained, the deeper layer contains RBCs, and the superficial layer contains AFGs.
- AFG is then extracted via syringe and mixed with bone powder and allowed to rest for 5-10 minutes for polymerisation, resulting in a very yellow coloured mass known as sticky bone.

Recent concepts-Albumin Gel-Platelet-Rich Fibrin Mixture (ALB-PRF)¹⁹

- 9 ml of blood was centrifuged at 700 g for 8 minutes.
- To produce albumin gel (denatured albumin), 2 ml of the uppermost layer (PPP layer) were collected and heated for 10 minutes at 75°C, followed by cooling for 10 minutes.
- Afterward, the liquid PRF, along with the residual cells and growth factors found within the buffy coat layer, was mixed with the albumin gel to produce **Alb-PRF**.
- Alb-PRF has higher growth factor content as compared to L-PRF. It contains PDGF-AA, PDGF-AB, PDGF-BB, TGF- β 1, VEGF, epidermal growth factor and insulin growth factor 1.
- In addition, it improves collagen synthesis, cell migration capacity, assays for proliferation.

BIO-PRF²⁰

- **The horizontal centrifugation concept** was found by Miron *et al.* (2019).
- Miron *et al.* had stated that horizontal action resulted in formulation consisting of higher levels of platelets and leukocytes.
- The horizontal centrifuge has the advantage of separating layers based on density due to its design.
- There were two protocols used during this study, a solid-PRF protocol of 700g for eight minutes and a liquid-PRF protocol of 200g for 8 minutes.

Clinical Applications of Platelet Concentrates⁷**Platelet Concentrates and Periodontal Regeneration Fibrin Sealants**

- Fibrin sealants have been used in surgical procedures since the late 1970s.
- "Fibrin sealants" are plasma-derived human plasma substances that mimic the process of blood coagulation, forming fibrin clots, and that may be used for topical haemostasis, tissue sealing, and melting agents for particulate bone substitutes.
- Due to the risk of cross infection associated with commercial adhesives, autologous fibrin sealants made from the patient's own plasma were developed. Despite this, they exhibited inadequate rheological properties.²¹
- **Limitations²²**
- Fibrin sealants have lower resistance to physical stress.

- Despite their documented benefits for soft tissues, it remain controversial when applied to periodontal surgery.
- Fibrin sealants may be associated with viral transmission risks.

Platelet-Rich Plasma (PRP)

- PRP mimics the clotting process at the end of the cascade of coagulation.
- Growth factors released through α -granules promote early wound healing via angiogenesis and cell proliferation.
- The three layers of PRP: - platelet rich plasma, platelet poor plasma, and red blood cells.²³
- PRP has been investigated for its efficacy in various periodontal regenerative procedures through various animal experiments, clinical studies, randomised trials, systematic reviews and meta-analyses which is listed in Table01.²⁴⁻²⁸

Advantages of PRP:

1. The growth factors and cytokines present in PRP elicit rapid regeneration
2. There is no risk of disease transmission
3. it is convenient and cost effective for patients

Disadvantages of PRP:

1. No standardisation of PRP preparation protocol
2. Bovine thrombin added to platelet concentrates has been reported to cause adverse reactions such as systemic lupus erythematosus
PRP has become less popular due to the disadvantages listed above and has prompted the advent of PRF.

Platelet Rich Fibrin (PRF)

- It is formed during centrifugation because of natural polymerization.
- It has the unique property of slow-releasing growth factors for 28 days, including transforming growth factor- β , platelet-derived growth factor, vascular endothelial growth factor, and matrix glycoproteins such as thrombospondin.³⁰
- It enhances regenerative and reparative processes. Moreover, it forms a trimolecular fibrin meshwork, facilitating cytokine function and cell migration.
- As PRF can lose structural integrity due to dehydration, it must be used immediately after preparation.
- PRF consists of three layers^{31,32}

Advantages of PRF: 29

1. Ease of preparation and use
2. cost effective
3. Reduces the incidence of adverse reactions as there is absence of bovine thrombin or anticoagulant.

Disadvantages of PRF:

1. samples obtained from PRF have a relatively low quantity

- Numerous studies have revealed PRF's regenerative potential when applied to various periodontal procedures and they are described in Table no-02.³⁴⁻³⁹

Leucocyte Platelet Rich Fibrin

A modified PRF clot or membrane encapsulates most of the platelets and leukocytes derived from the initial blood harvest in addition to platelet growth factors and stem cells. (Figure-3)⁴⁰

The use of L-PRF membrane for increasing the width of keratinized mucosa around implants has shown promising results in a randomized controlled trial by Temmerman *et al.*⁴¹

Advanced Platelet Rich Fibrin

- It is a modified form of pure PRF (Figure-4)⁴². The clot that results has an abundance of neutrophils and macrophages.
- These cells provide a matrix for cell migration into the defect and also contains biological factors that accelerate wound healing, such as platelet derived growth factor, transforming growth factor β , platelet factor 4, IL1, vascular endothelial growth factor, epidermal growth factor, endothelial cell growth factor, platelet-derived endothelial growth factor, insulin-like growth factor, osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin.⁴²
- Since it is a matrix obtained from the patient's own blood, foreign body reactions are eliminated.⁴²
- Both L-PRF and A-PRF induced periodontal ligament fibroblast proliferation in an in-vitro study. The use of A-PRF for gingival recession treatment has shown significant results.⁴³

Injectable Platelet-Rich Fibrin

- I-PRF is a liquid platelet concentrate that can be used alone or in combination with biomaterials. (Figure-5)⁴⁴
- As compared to other forms of PRF, it was found to contain more regenerative cells and growth factors.
- Furthermore, it clots and develops a gel after approximately 10-15 minutes, which provides sustained growth factor release in the tissue, and has the potential to induce transforming growth factor- β and collagen-1 mRNA expression.
- In a study by Ozsagir *et al.*, it has been observed that the application of I-PRF leads to increase gingival thickness in individuals with thin periodontal phenotypes.⁴⁴

Titanium Platelet Rich Fibrin

- T-PRF was introduced by Tunali *et al.*⁴⁵ (2014), who hypothesized that titanium might have a greater effect on activating platelets than silica, which was previously used to prepare other PRF concentrates. (Figure 6)
- The regenerative potential of T-PRF has recently been evaluated in several randomized controlled clinical trials.

- The effects of T-PRF on periodontal parameters and radiographic changes have been reported in a study by Chatterjee *et al.*⁴⁵
- When combined with open flap debridement, T-PRF membrane improved periodontal healing. 4 to 6 weeks after administration of T-PRF, it was concluded that the concentrations of growth factors were significantly higher and the RANKL/OPG ratio was significantly lower.⁴⁶

Concentrated Growth Factors

- The CGF preparation process yields a much denser and larger fibrin matrix rich in growth factors. (Figure-7)⁴⁷
- This matrix acts as an effective haemostatic agent.
- It accelerates osteogenesis and promotes wound healing.
- It improves wound stability by enhancing new connective tissue attachment.
- Moreover, it acts as a scaffold for cytokine attachment and cellular migration
- Researchers found an increase in gingival keratinized width and gingival thickness in response to CGF use in conjunction with coronally advanced flaps.⁴⁷
- CGF is also used to treat intra-bony defects. In conjunction with GTR, it acts as an efficient barrier for bone regeneration.⁴⁸

Autologous Fibrin Glue (AFG) And Sticky Bone

- It was introduced by Sohn (2010).
- Autologous fibrin glue was applied to bone graft which resulted in sticky bone, which was found to reduce post-operative complications and boost wound healing. (Figure-8)⁴⁹
- It has been determined that "sticky bone" preparation can be used for alveolar ridge augmentation, since bone graft gets trapped within cross-linked fibrin meshwork which minimizes undesirable movement during healing. This increased tissue healing by stabilizing the bone graft without the use of bone tacks or titanium mesh.^{49,50}

Future Perspectives

Platelets Concentrates as Drug Delivery Agents-

Platelets concentrates may serve as vehicles for loading drugs or biological therapies into specific targets. The ability of platelets to identify and interact with tumour cells has been demonstrated in studies. Therefore, developing new drug delivery systems and therapeutic strategies could be of use in the treatment of tumours since this might help to lessen the side effects of chemotherapy such as cytotoxicity.¹⁰

Application of Platelet Dust

Platelet microparticles (also known as "dust" or "PMPs") are among the most abundant cells-derived microparticles available. They are formed by the activation of platelets. These microparticles have drawn attention as potential diagnostic markers of various diseases. Research on these microparticles suggests they

can modulate immune systems and contribute to various diseases.⁵²

Liposomes Encapsulating Platelet Concentrates-

By using advanced delivery systems such as liposomes to encapsulate platelet concentrates, it is ensured that the growth factors will remain intact for a longer period. It will also help in selective targeted delivery. The encapsulation of PRP in biodegradable scaffolds such as calcium phosphates and polylactic-co-glycolic acid has demonstrated positive results for enhanced bone regeneration in vitro studies.⁵³

Conclusions

Using platelet concentrates as a regenerative procedure in periodontal surgery is a novel approach. Consequently, their ability to harbour growth factors makes them capable of accelerating periodontal tissue regeneration and enhancing wound healing. Considering it's an autologous, natural, and economic products, there is no risk of a reaction or disease transmission. The efficacy of treatment with platelet concentrates has been demonstrated in various studies, although large-scale studies are still needed to verify its efficacy.

Ethical approval letter and consent to participate

Not Applicable

Competing Interest

The authors declare that they have no competing interests in this section.

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None

Conflicts of Interest Statement

None.

Table-01. Studies of PRP

TRIALS	STUDIES
ANIMAL EXPERIMENTS	<ul style="list-style-type: none"> ➤ A study conducted by Suaid et al revealed that when PRP and subepithelial connective tissue graft were used for the treatment of gingival recessions, the treatment was very effective at promoting new cementum formation. ➤ A study by Marcelo Diniz Carvalho et al showed that, when combined with bovine glass, the use of PRP failed to significantly improve periodontal regeneration in dogs with three walled intrabony defects.
CLINICAL TRIALS	<ul style="list-style-type: none"> ➤ The results of a study conducted by Anitua showed improved soft tissue repair and bone regeneration after the application of PRP inside extraction sockets, indicating these sites could be prepared for implant placement in the future. An experimental study by Lekovic et al compared the effectiveness of bovine porous bone mineral combined with PRP and guided tissue regeneration in humans with intrabony defects. According to the study, PRP exhibited strong regenerative potential and resulted in reduced pocket depth and improved clinical attachment level.
RANDOMIZED CONTROLLED CLINICAL TRIALS	<ul style="list-style-type: none"> ➤ Using PRP and a cancellous allograft to augment ridges, the Eskan et al study found that PRP enhanced bone regeneration.
SYSTEMATIC REVIEW AND META-ANALYSIS	<ul style="list-style-type: none"> ➤ A systematic review by Panda et al evaluated the effectiveness of autologous platelet concentrates as well as other regenerative treatments in treating intrabony defects. The use of PRP in combination with grafting materials and guided tissue regeneration has been found to be effective but found to be ineffective when used alone.

Table-02. Studies of PRF

TRIALS	STUDIES
ANIMAL EXPERIMENTS	<ul style="list-style-type: none"> ➤ Duan et al concluded that PRF combined with rat periodontal ligament stem cells could be useful for periodontal tissue engineering. ➤ According to an animal study conducted by Wang et al, lyophilized PRF and mesenchymal stem cell sheet fragments from osteogenic bone marrow promote bone tissue regeneration.
CLINICAL TRIALS	<ul style="list-style-type: none"> ➤ In a study by Deepa and Jain (2015), PRF was shown to enhance healing of gingival fenestrations, which suggests its use as a membrane in aesthetically demanding areas.
RANDOMIZED CONTROLLED CLINICAL TRIALS	<ul style="list-style-type: none"> ➤ Clinical parameters improved when PRF was used to regenerate intrabony and furcation defects, according to Pradeep et al. ➤ As a result of PRF combined with freeze-dried bone and a decalcified allograft, Agarwal et al. found that clinical and radiographic parameters had improved significantly after 12 months.
SYSTEMATIC REVIEW AND META-ANALYSIS	<ul style="list-style-type: none"> ➤ The results of Strauss et al's systematic review suggest that PRF can increase implant stability and improve wound healing by reducing bone resorption after implant placement. ➤ In a meta-analysis by Li et al, it was determined that PRF and 1% alendronate were synergistically effective in regenerating periodontal tissues and bone.

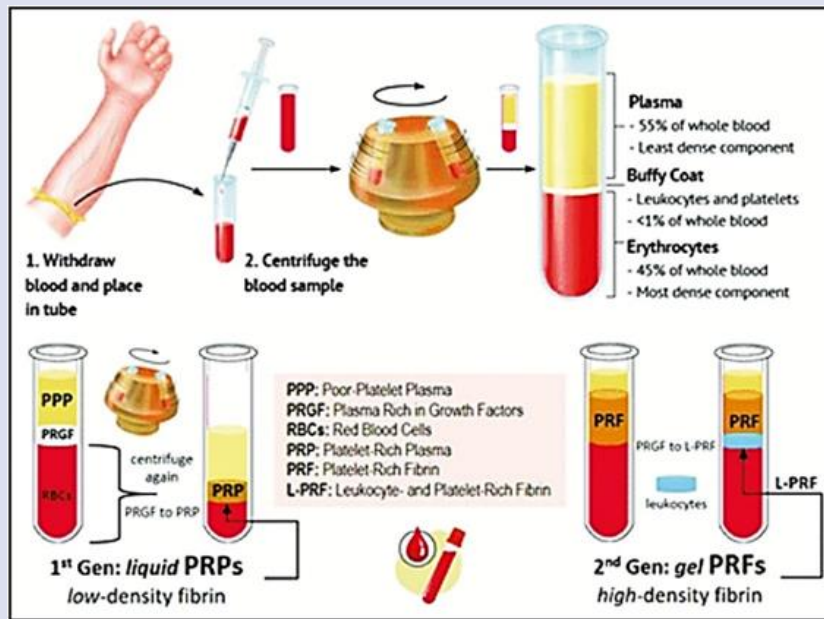


Figure 1. PRP and PRF

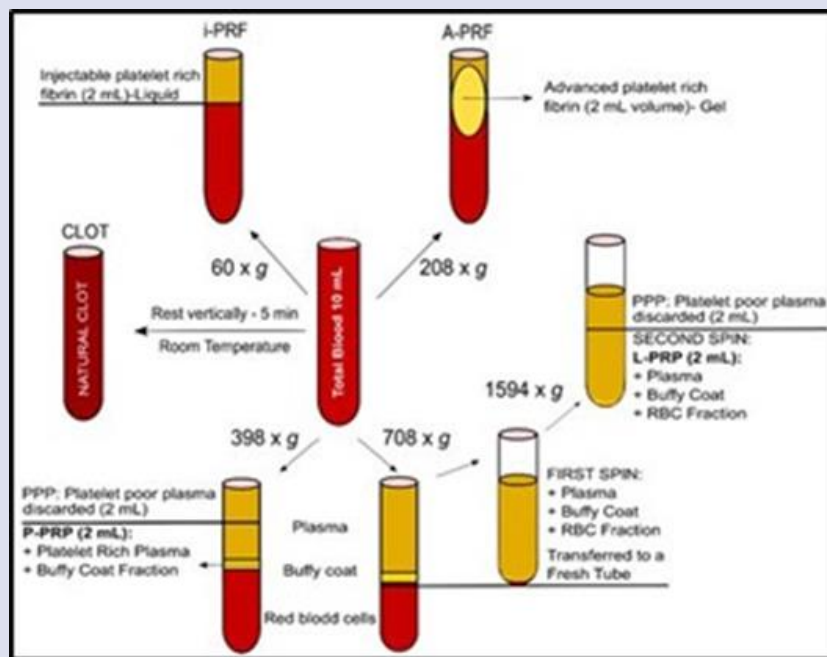


Figure 2. Modifications of PRF

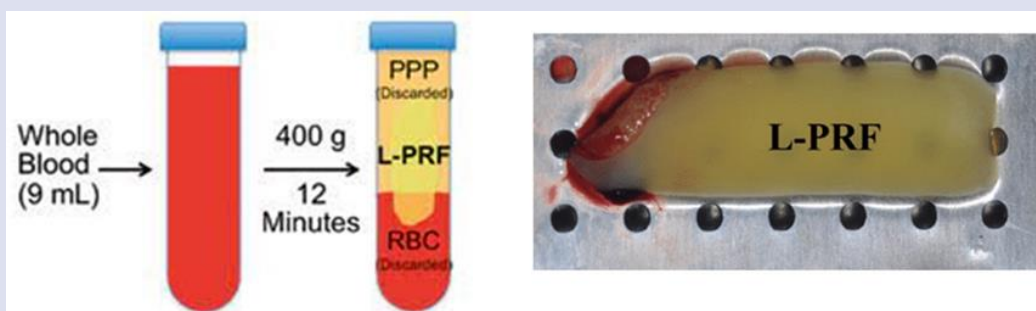


Figure 3. L PRF

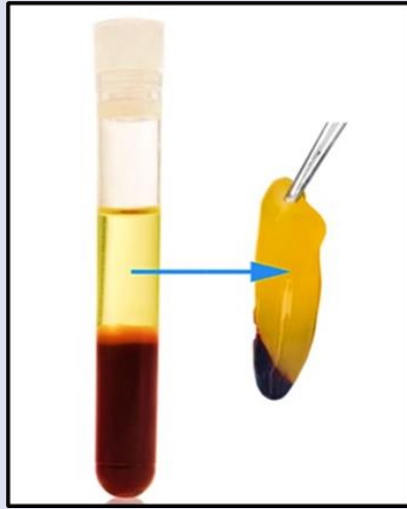


Figure 4. A PRF

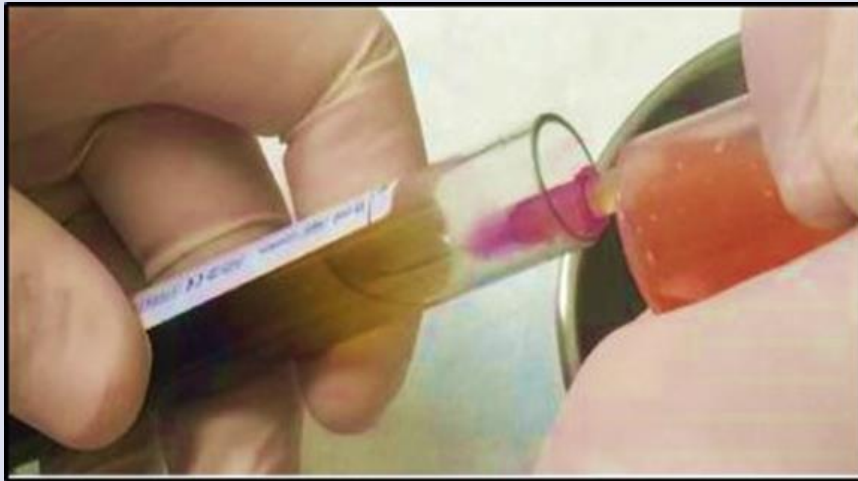


Figure 5. I PRF

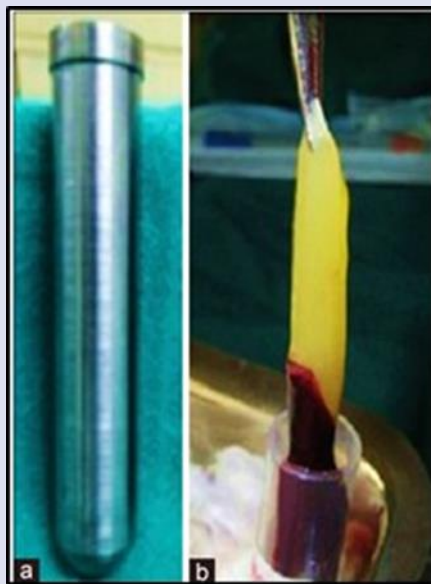


Figure 6. T PRF

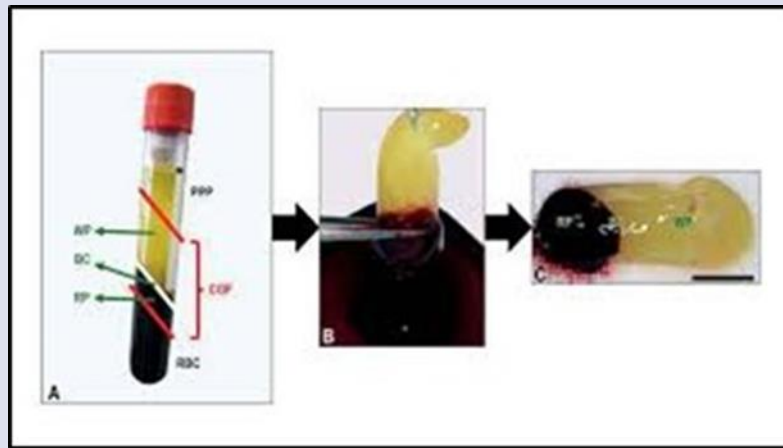


Figure 7. Concentrated Growth Factor



Figure 8. Sticky Bone

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Herbal Treatments Used as an Alternative in the Treatment of Periodontal Diseases

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Review

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ABSTRACT

Phytotherapy is the ancient practice of treating diseases with plant products. Herbal products were used in traditional medicine in both eastern and western medical ayurvedic traditions since ancient times. Periodontal diseases have been a major oral problem affecting humans for ages. The application of phytotherapy in treating periodontal diseases can help in solving many issues and answering many unanswered questions. This review on phytotherapy for periodontal diseases explains in detail about different plants and their products, which are used in various forms for treating bacterial, fungal, and viral infections causing periodontal diseases.

Key words: Ayurvedic, herbal, phytotherapy, periodontal disease.

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Introduction

Periodontal diseases have been a major oral problem affecting humans for ages. Epidemiological studies have shown that these periodontal diseases have also affected humans in ancient times. Treatment methodologies have been changed and are evolving for many decades. Periodontal disease management has been influenced by these predispositions in the period due to recent advances in management.¹

The word “phytotherapy” means treatment or management of disease with traditional plants that have pharmaceutical properties such as antioxidant, anti-inflammatory, and anti-microbial properties. Phytotherapy is a Latin term which is divided into the prefix “Phyto” which means plant, and “therapy” which means curing and healing.² Herbal products have been used in traditional medicine in eastern and western medical ayurvedic traditions since ancient times.

During the last 30 years, pharmaceutical industries have exponentially increased their research on converting herbal products to oral products as phototherapeutic agents with proven quality, safety and efficacy.³ The plants act as anti-inflammatory agents, antiseptic, antioxidant agents, antimicrobial agents, and anti-collagenase properties.⁴ In this study, the effect of plants or plant derivatives in the treatment of periodontal diseases is reviewed.

The main etiological factor for various gingival and periodontal diseases is microbial oral biofilm. *Aggregatibacter actinomycetemcomitans* (A. actinomycetemcomitans), *Prevotella intermedia* (P. intermedia), *Porphyromonas gingivalis* (P. gingivalis), *Treponema denticola* (T. denticola) are common periodontal pathogens which are found in microbial oral biofilm.⁵

Haffajee *et al.* have examined various plant products which had a benefit on the oral tissues. These plant products include anti-inflammatory agents such as aloe vera, calendula and anti-microbial agents such as grapefruit seeds, Golden Seal which were used as herbal mouth rinses. They reduced the inflammation of the gingiva and inhibited anaerobic and aerobic bacterial growth.⁶

Considering the above properties of various herbal products which had a beneficial effect on the oral tissues as well as periodontal complex, the present review aimed to investigate the role of few plant products in the management of periodontal disease and their efficacy.

Let us discuss a few plant products which are used as Phyto therapeutic agents:

Aloe vera (Aloe vera): It is recommended to add current studies examining the effect of aloe vera on periodontal disease.

Aloe vera is a succulent, cactus plant and belongs to the Liliaceae family. Aloe vera has more than 300 species, but only Aloe barbadense Miller and Aloe aborescens are used for their pharmaceutical properties.⁷

It contains vitamins, minerals, amino acids, salicylic acids, enzymes, sugars, and fatty acids. The central mucilaginous part of aloe vera acts as a gel and improves wound healing in various skin and mucosal lesions.⁷

Aloe vera's healing capabilities have been used in conditions such as after periodontal surgery, for damaged gingival sores caused by a toothbrush, and so on. It can be taken as a food supplement or administered topically as a gel. Aloe vera is a bactericide that is non-toxic and contains anti-fungal, anti-viral, analgesic, anti-inflammatory, and immune-stimulating characteristics. Aloe vera's anti-inflammatory properties hinder the cyclooxygenase pathway, lowering prostaglandin E2 synthesis.⁸

Researchers have investigated various forms of Aloe vera and the ones currently in use include: 1. Toothpaste 2. Mouthwash 3. Gel- protects the wound and promotes healing. It also keeps the wound moisturized. 4. Aloe activator topical spray - useful in throat infections, dental and joint pains 5. Aloe vera juice - useful as a strong detoxifying agent and an immune enhancer.⁹

Jing et al, (2022)¹⁰ in a systematic review and meta-analysis demonstrated that the adjunctive use of Aloe vera in SRP results in improved treatment outcomes compared to SRP alone or placebo.

Sharmasita vijay et al., (2022)¹¹ in a randomized controlled clinical trial investigated the role of aloe vera gel as an adjunct to scaling and root planning in chronic periodontitis patients. Results demonstrated that aloe vera gel an herbal product is proved to be advantageous as adjunctive use of aloe vera gel compared with placebo gel, which is associated with greater reduction in plaque index, bleeding index and probing depth as well as more clinical attachment gain.

Nasreen et al. (2021)¹² investigated the effect of using aloe vera mouthwash on improving periodontal health among secondary school children in her quasi-experimental study. The observed results revealed that use of aloe vera mouthwash can be used as an alternative product for the prevention and treatment of gingivitis.

Nazir et al. (2019)¹³ investigated the effect of aloe vera gel as an adjunctive to scaling and root planning among 40 patients and demonstrated that aloe vera gel shown significant decrease in periodontitis and improved the clinical parameters.

Penmetsa et al. (2019)¹⁴ in a randomized controlled comparative clinical study with double blind design with ocimum mouth wash, chlorhexidine mouthwash and aloe vera mouthwash. The obtained results signify that aloe vera and ocimum mouthwash proven to be effective as chlorhexidine mouthwash in its ability to reduce plaque index, gingival index and bleeding index scores by reducing plaque accumulation, gingival inflammation and bleeding when used in long-term.

Ahouri et al. (2017)⁸ in single blind clinical trial with split mouth design investigated the effect of local application of aloe vera gel as an adjunct to scaling and root planning. The observed results revealed that local application of aloe vera gel could be considered as an adjunctive to Scaling and root planning for chronic periodontitis.

Vangipuram et al. (2016)¹⁵ in a randomized controlled clinical study where they compared aloe vera and chlorhexidine mouthwash demonstrated that aloe vera being an herbal product has shown equal effectiveness as chlorhexidine. Hence aloe vera mouthwash can be used as an alternative product for curing and preventing gingivitis.

Bhat et al.⁷ confirmed that using an Aloe vera mouth rinse lowers gingival inflammation and bleeding. They also found out that when aloe vera gel was administered sub gingivally, it resulted in considerable pocket depth reduction and a relative drop in gingival and plaque indices in short-term research.

Improved healing properties were seen in areas that were traumatized by dentifrice abrasion, sharp foods, dental floss and after periodontal surgeries when aloe vera gel was applied. Aloe vera gel in toothpaste or mouthwashes at optimal quantities could help prevent periodontal disease and dental cavities.⁷

Sage (*Salvia officinalis*): There are recent studies investigating its effect on the treatment of periodontal disease. By adding these studies, the results can be discussed.

Sage is a member of the Lamiaceae family that can be found in fields and along roadsides. It is advised for the management of stomatitis, sore throat, gingivitis, and periodontitis and can be used as a mouth rinse. Sage essential oils offer anti-inflammatory characteristics that help with stomatitis and pharyngitis, as well as antibacterial, antifungal, and antiviral properties that can help with periodontitis progression.¹⁶

For Mouthwash preparation:¹⁷

1. As a mouth rinse: In 150 ml of boiling water, 3gms chopped leaf of sage is added and boiled for 10 minutes and used as mouthwash 3-4 times a day.

2. For gargling: In a half-liter water, chopped leaf of sage (two tablespoons) is immersed, and boiled. This boiled mixture is covered for 15 minutes. This is used for gargling 3-4 times a day for 5-10 minutes.

3. As a gel: One gram of Carbopol 940 was dissolved in 25 mL of distilled water to obtain 4% gel, 0.4 grams of *S. officinalis* alcoholic extract was dissolved in 10 mL of 5 mL of ethanol absolute, 3 mL of propylene glycol, and 2 mL of distilled water, to obtain 4% of active ingredients, after that 10 mL of the gel was added to 10 mL of active ingredients, triethanolamine was added to adjust the pH above 7. The gel was prepared with the final concentration of 2% for both of the gelling agent and the *S. officinalis* extract, and the gel set aside for 24 hours before loading in the syringes.¹⁸

Aljuboori et al. (2020)¹⁸ in randomized controlled clinical study investigated both clinical and immunological parameters depending on the application of gel

containing sage extract preceded by scaling and root planning procedure. Thus, it has been concluded that a significant difference between the control group (SRP alone) and test group (Sage extract gel).

Mendes *et al.* (2019)¹⁹ investigated the antibacterial activity of *salvia officinalis* against periodontal pathogens in an invitro study. It has been concluded that the extract has a moderate effect on periodontopathic bacteria and proved the activity against *S. Mutans* in the combination of dichloromethane – soluble fraction with chlorhexidine only.

Junger *et al.* (2020)²⁰ in a randomized placebo controlled double blind study did not confirm the beneficial effect of the mouthwash with *S. Officinalis* on inflammatory parameters and plaque indices compared with placebo after 6 weeks. On the other hand *S. officinalis* gel has a potential anti-inflammatory role in the management of chronic periodontitis.

Beheshti-Rouy *et al.* (2015)²¹ in a randomized clinical trial aimed to evaluate clinical effects of a mouthwash containing *salvia officinalis* extract on *S. Mutans* bacteria causing dental plaque demonstrated the effectiveness of sage mouthwash in reduction in the count of *S. Mutans* in dental plaque.

Pistorius *et al.* (2003)²² have reported that with daily use of mouth rinse, there was a significant improvement in gingival indices and concluded that it can be used every day as an additional therapy for reduction in inflammation of the gingiva.²¹

Salvia officinalis which is commonly used as mouth rinse has spasmolytic, aromatic, astringent, and antiseptic properties.¹⁷ *Salvia* treats gingivitis, throat infections, and mouth ulcers efficiently. Precautionary measures should be taken while using in pregnancy and lactation but gargling and mouthwash were allowed.²¹

Peppermint (*Menthapiperita*): There has been little mention of research on the effect of periodontal treatment. It is recommended to include studies investigating the effect of periodontal treatment.

Peppermint (Mint) is a member of the Lamiaceae family that helps to relieve inflammation. To relieve toothache, cotton balls soaked in peppermint oil and placed in the tooth cavity were used. Peppermint oil has analgesic properties when applied locally. Peppermint leaf pills and capsules, diluted to 3–6 g per day, are used as a mouth rinse following periodontal therapy to minimize gingival inflammation. Mouth rinses and gels containing peppermint leaves and essential oil as bases are used to combat periodontal germs (*streptococcus* species).^{23,24}

Flavonoids are the most essential phenolic compounds discovered in *Mentha* species, and they have a wide spectrum of pharmacological action, including anti-ulcer, antioxidant, cytoprotective, anti-inflammatory and chemo preventive properties. Peppermint tea is generally safe to drink on a regular basis. Peppermint oil can cause headaches.²⁵

It is well documented that the essential oil and extracts of *Mentha* species have antimicrobial, anti-inflammatory, and antioxidant properties with antimicrobial activity

against pathogens involved in the development of periodontal disease.²⁶

Essential oils of *Mentha piperita* showed good antibacterial and antibiofilm activities in the study conducted by Karicheri *et al.* concerning *Aggregatibacter actinomycetemcomitans*, a strain identified in periodontopathogenesis.²⁷

Peppermint oil is also used in novel formulations, for example as an anti-ulcer and anti-inflammatory agent in self-emulsifying drug delivery system for the improvement of meloxicam's solubility used in periodontal disease.²⁸

Chamomile (*Matricariachamomilla* or *Matricariarecutita*):

Studies comparing the effect of Chamomile and chemical antiseptic agents on periodontal treatment can be added. Thus, the advantages and disadvantages of using instead of chemical agents can be evaluated.

Chamomile is one of the most popular herbs and belongs to the Asteraceae family. Chamomile, has been shown to be anxiolytic, antistress and anti-allergic reactions. Chamomile flowers are used for herbal tea. It is integrated as one of the ingredients of mouth rinse and used in the prevention and management of periodontitis. They are given in the form of tinctures, tablets, and capsules.^{17,29}

Chamomile mouthwashes preparation: Dried Chamomile flowers were immersed in ethanol 55% at room temperature, and impurities were removed from the mixture. Ethanol was completely evaporated at a temperature of 79 °C by the rotary evaporator device. The pure Chamomile extract was obtained and then dissolved in distilled water, and spearmint flavour was added to the mixture.³³

McKay and Blumberg in an animal study demonstrated the anti-inflammatory activity of chamomile.¹⁷

Batista *et al.* (2014)³⁰ combined pomegranate and chamomile extracts which are used as mouthwash and is effective in inflammation reduction and bleeding of gingiva in periodontitis, suggesting that both extracts when combined or used individually had anti-inflammatory and anti-microbial actions similar to those of gold standard mouth wash chlorhexidine 0.12%. As a result, these can be employed as supplementary therapeutic agents to aid in the restoration and maintenance of periodontal health.

Lucena *et al.* (2009)³¹ found reduction in gingiva bleeding index, confirming that findings of this study, in which the mouthwash with *Matricaria recutita* extract also reduced the bleeding index, both in gingivitis and in chronic periodontitis, showing statistically significant results.

Agarwal *et al.* (2020)³² conducted comparative clinical and microbiological study with 1% *Matricaria chamomilla* mouth rinse on chronic periodontitis patients. The observed results signify that *Matricaria chamomilla* mouth rinse was found to improve the clinical and microbiological parameters. Its outcomes commensurate the gold standard mouthwash chlorhexidine. Therefore, chamomilla mouth rinse can be a potential therapeutic agent for chronic periodontitis patient.

Nemma *et al.* (2020)³³ compared the effect of chamomilla, aloe vera and chlorhexidine 0.12% mouthwash on gingival health among visually impaired persons and evaluated for parameters such as plaque index and gingival index. Results revealed that chamomilla mouthwash have a distinguished impact on gingival inflammation.

The comparative studies reported that chamomilla mouthwash is effective owing to its properties such as analgesic, anti-inflammatory, and antibacterial.³⁴

The only disadvantage of chamomile is that it is reported to have allergic reactions which were skin reactions on topical application followed by bronchitis leading to constriction on systemic administration.³⁰

Tea tree (*Melaleuca alternifolia*):

Melaleuca alternifolia, or tea tree, is a member of the Myrtaceae family. It can be immediately administered to swollen gums for immediate relief. Mouth rinse has been utilized in endodontic and irreversible pulpitis treatment to minimize inflammation. Tea tree has demonstrated good efficacy in microbial biofilm control, resulting in a considerable reduction in a gingival bleeding index.

Elgendy *et al.* (2013)³⁵ investigated the antimicrobial effects of tea tree essential oil and gel in preventing the formation of oral microbial biofilms and found it to be effective against bacteria.

Tea tree oil showed a significant reduction in the production of inflammatory cytokines *in vitro*, implying that it could be used to treat inflammatory disorders like a periodontal disease by modulating the host response.³⁶

Ripari *et al.* (2020)³⁷ evaluated the efficacy of tea tree oil for the treatment of gingivitis and compared with chlorhexidine mouthwash. The comparative results signify that tea tree oil offered a better improvement in the evaluation of plaque index, bleeding on probing and plaque index. Tea tree oil could be an effective non – toxic substitute for therapy of gingivitis.

Soulissa *et al.* (2020)³⁸ conducted an invitro study for evaluating the effect of tea tree oil in inhibiting the adhesion of pathogenic periodontal biofilms. The study reported that tea tree oil inhibits the adhesion of *P. Gingivalis* and *A. Actinomycetemcomitans* biofilms to enamel surfaces and may be useful as a treatment for oral diseases.

Singh *et al.* (2019)³⁹ evaluated the effectiveness of tea tree oil versus chlorhexidine in the treatment of periodontal diseases in a systematic review demonstrated that tea tree oil may be used as an alternative to chlorhexidine for reduction of gingival inflammation in conjunction with efficient plaque control measures. Tea tree oil found to be superior to chlorhexidine in reducing signs of gingival inflammation.

Echinacea (*Purple coneflower*):

Echinacea helps improving defects in immune system and belongs to an Asteraceae family herb. Its constituents work together to increase white blood cell (lymphocytes and macrophages) production and activity. Gingivitis and

periodontal disease are treated with a mouth rinse containing echinacea, mint oil, sage, and chamomile. Echinacea has anti-inflammatory and antibacterial properties, according to Kumar *et al.* Several studies have attested to its efficacy in the fastest and rapid management of flu like symptoms.^{40,41}

Safarabadi *et al.* (2017)⁴² in a randomized controlled comparative clinical study where they compared the efficacy of Echinacea versus chlorhexidine mouthwash on microbial flora of intubated patients in intensive care unit. The results demonstrated that Echinacea solution was more effective in decreasing the oral microbial flora of patients in intensive care unit owing to its phagocytic property and anti-inflammatory effect and immunomodulatory.

Kharaeva *et al.* (2020)⁴³ evaluated the antibacterial and anti-inflammatory effect of tooth paste for gingivitis and periodontitis patients demonstrated that antiseptic action and antibacterial action attributed for the safety of the usage of herbal product.

Rosemary (*Rosmarinus officinalis*):

The effect of Rosemary on Periodontal disease was not mentioned. It is recommended to add studies investigating the effect of periodontitis and gingivitis.

Rosemary is a volatile oil and a member of the Lamiaceae family and has various anti- bacterial and anti-fungal properties. It works well against chronic disseminated candidiasis. Oral administration of the concentrated volatile oil is not recommended. Other research on the antioxidant and antimicrobial properties of rosemary essential oil has been conducted. Santoyo *et al.* (2005)⁴⁴ investigated the antimicrobial activity of rosemary and determined that the following five essential oil components are to blame: 1,8-cineole, Pinene, camphor, borneol, and verbenone.

According to Lee *et al.* (2015)⁴⁵ rosmarinic acid significantly induced mineralization in osteoblasts and increased alkaline-phosphatase activity, suggesting that it can be used to arrest metabolic bone diseases.

Gunther *et al.* (2022)⁴⁶ evaluated the antimicrobial effect of *Rosmarinus officinalis* extracts on oral initial adhesion and demonstrated that the use of *R. Officinalis* extracts in biofilm control and thus in the treatment of caries and periodontitis as herbal adjuvant to synthetic substances.

Oliveria *et al.* (2017)⁴⁷ evaluated the effect of *Rosmarinus officinalis* on biofilms demonstrated that rosemary extract had a significant vitality reduction in the microflora such as *S. Aureus*, *S. Mutans* and *E. Faecalis*.

Valones *et al.* (2019)⁴⁸ assessed the effect of rosemary based tooth paste in clinical double blind randomized study which compared with conventional fluoridated tooth paste on clinical parameters such as plaque index and gingival bleeding scores. The observed results signify that rosemary based tooth paste effectively treated gingival bleeding and reduced bacterial plaque when compared with conventional tooth paste.

Azad *et al.* (2016)⁴⁹ evaluated the adjunctive use of mouthwash containing extract of *Rosmarinus officinalis* along with scaling and root planning. It had been concluded that the adjunctive use of a mouth rinse containing essential oils following scaling and root planning has a positive effect on clinical variables and on bacterial levels in the subgingival biofilm.

Wintergreen (*Gaultheria procumbens*):

Wintergreen is a member of the Ericaceae family. Its mouthwash is an antiseptic and also acts as an astringent. Wintergreen oil-soaked cotton ball is used as a remedy for sore throats and gum inflammation. Wintergreen essential oil has been shown to have antimicrobial activity against a wide range of wide-spectrum bacteria and fungi, as well as antioxidant potential, according to Nikolifietal⁵⁰

Michel *et al.*⁵¹ evaluated the effect of standardized leaf extract of *Gaultheria procumbens* on multiple oxidant, inflammation related enzymes and pro oxidants and pro-inflammatory functions of human neutrophils. The obtained results might partially explain the ethnomedicinal application of *G. Procumbens* leaves and support the usage of the standardized leaf extract in the adjuvant treatment of oxidative stress and inflammation-related chronic diseases.

Red clover (*Trifolium pretense*):

The Fabaceae family includes red clover. Gingivitis and periodontal disease have been treated with red clover mouthwash. After making the red clover tea, the flowers and leaves can be used to make an antibiotic gel. In their animal and human studies, Ramos *et al.*⁵² discovered that red clover dry extract has anti-inflammatory properties.

Barberry (*Berberis vulgaris*):

The Berberidaceae family includes barberry. Berberine, an alkaloid derived from *Berberis vulgaris*, has antimicrobial properties and has been used in toothpaste and mouthwashes. Barberry gel has been shown to be an effective adjuvant for oral biofilm control and gingival inflammation reduction in children. Barberry juice is rich in vitamin C, which boosts immunity and helps with the absorption of iron.⁵³

Alkaloids like berberine were more efficient against bacteria like *A. actinomycetemcomitans* and *P. gingivalis* than *Lactobacillus* and *Streptococcus*, according to Palombo El *et al.* Berberine also inhibited *A. actinomycetemcomitans* and *P. gingivalis* collagenase activity.⁵⁴

Berberine gel decreases gingival index by 33 % and oral biofilm by 56 %, according to Makarem *et al.*

According to various researchers, Berberine has a variety of pharmacological actions such as anti-cyclooxygenase, anti-inflammatory, and anti-inducible nitric oxide synthase properties. Berberine has been suggested to reduce periodontal tissue degradation by regulating matrix metalloproteinase during periodontal disease progression.⁵⁵

Periodontitis is the main etiological factor for bone loss and is considered a global public problem. Though conventional periodontal therapy like scaling and root planning and antibiotics were used as adjunctive, they have side effects such as gastrointestinal irritation, antibiotic resistance, and systemic alterations. Phytotherapy is an alternative therapy with beneficial effects and little or no side effects.

Gaetti-Jardim *et al.* tested the minimum inhibitory concentration and Minimum bactericidal concentration of aqueous and alcohol extracts of *Anacardiaceae*, *Cattleya guava* / *strawberry guava* / *cherry guava* and *Ficusenormis* against four strains: *A. actinomycetemcomitans*, *P. gingivalis*, *F. nucleatum*, and *P. intermedia*. MBC values were 2 to 16 times higher than MIC values.⁵⁶

Conclusions

Phytotherapy is the most ancient method of treating diseases and is still in demand today. It is believed that many plants with various benefits such as anti-inflammatory, healing, anti-bacterial, anti-viral and anti-fungal properties are yet to be discovered and used in medicine and in periodontal diseases. The major and primary concerns during the research of various new plants used in phytotherapy are quality, safety, toxicity and allergic reactions. Based on the investigations 'thus, concluded as 'the fact that medicinal plants have fewer side effects compared to conventional drugs, being affordable and accessible makes their use widespread in dental treatments as well'. However, more clinical research is needed to prevent unpredictable toxic effects and adverse drug interactions. A systematic approach that includes experimental and clinical validation of efficacy is required to introduce phytotherapy to modern medicine

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Immediate Oral Rehabilitation Using a Surgical Obturator with Buccal Extension After Partial Maxillectomy: A Case Report and Literature Review

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Case Report

History

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ABSTRACT

Maxillary defects resulting from surgical treatment of tumors, congenital malformations, and traumas might interrupt patients' speech, mastication, swallowing function, and physical appearance. The surgical obturator serves to restore the continuity of the palate as an immediate prosthesis after surgery. It provides an anatomically accurate, clean scaffold for surgical dressing while supporting and keeping the facial flap pressure. We report a case of temporary immediate oral rehabilitation after partial maxillectomy. A 39-year-old woman presented with a benign right maxillary tumor. Postoperatively, a surgical obturator with the addition of a buccal extension was placed. Buccal extension is a high buccal flange that supports the facial flap and, consequently, maintains the facial esthetics. This modified obturator also serves as a scaffold for surgical dressing and temporary defect closure between the nasal and oral cavities. We also outline a brief literature review of oral rehabilitation using a surgical obturator after maxillectomy. In the present case, follow-up evaluation showed that the obturator supported the upper lip, lifted the nasal lobe of the affected area, and provided a more symmetrical facial feature. Oral function improvements, such as swallowing, speech, and better facial profile, were also observed.

Key words: Partial maxillectomy, maxillary defect, surgical obturator.

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Introduction

The maxilla determines the shape of a person's face, particularly in the midfacial region. It plays a vital role in maintaining the airways and speech, chewing, swallowing, and esthetic functions.¹ Cavity opening between the antrum and the nasopharynx is the most common defect in the maxilla, causing difficulties in swallowing and speech and an unesthetic appearance.^{2,3} Maxillary defects can result from the surgical treatment of oral neoplasms, congenital disabilities, or high-impact injury.^{3,4}

The intraoral structures involved in maxillary defects may include the maxillary sinus cavity, palatal bone, and alveolar bone.³ The missing parts of the soft and hard palates alter the function because the structure is insufficient for the remaining tissues. A side effect of surgical resection of the maxilla or maxillectomy is anatomical changes because of secondary healing, tissue contractures, the underlying bone structure, and tissue edema. Rehabilitation after removal of the hard or soft palate includes a prosthesis to help the patient in deglutition and speech.² The prosthesis fabricated to repair the defect is known as a maxillary obturator.⁵

An obturator is made from natural or artificial materials, disc- or plate-shaped, and placed into the

maxillary defect after partial or total maxillectomy.^{4,5} The surgical obturator is a direct prosthesis rehabilitation tool for maxillary deformities placed during the surgical procedure. Surgical obturators serve to retain the surgical pack, reduce the risk of contamination of surgical wounds from the intraoral area, and enable speech and swallowing in the early postoperative period.⁶ Using prosthetic rehabilitation is affected by the location and size of the maxillary defect of the patient.⁷ Prosthetic rehabilitation after total and partial maxillectomy is aimed at creating an obturator that can separate the oral and nasal cavities and improve masticatory, speech, swallowing, and esthetic functions.^{3,4} The surgical obturator supports soft tissues and reduces scar contracture and damage, thereby postoperatively improving patient satisfaction.⁸

The surgical obturator varies widely in design and can be made from toothless acrylic resin prostheses, retention components using wire clasps, and acrylic resin prostheses to restore the dental arch and shape of the palate.^{8,9} Many techniques have been described for manufacturing immediate surgical obturators. These techniques have advantages and limitations, such as

multiple processing steps during fabrication.¹⁰ Here, we report a case in which a surgical obturator with buccal extension was used. Buccal extension is a high buccal flange that supports the facial flap and, consequently, maintains facial esthetics.

Case Report

A 39-year-old woman presented with a benign right maxillary tumor (Figure 1), referred from the General Surgery Department for immediate surgical obturator application. Radical maxillectomy was performed under general anesthesia in an operating room. Medical history of the patient revealed upper right cheekbone mass, since 7 years ago, that grew in the last 5 years, no history of medical compromise. Extraoral and intraoral examinations showed painless cystic mass in right maxilla, across the midline of palate. Particular attention was paid to the teeth with carious lesions, requirement of endodontic treatment, or mobility. Tooth surfaces were scaled to remove calculi and improve the patient's oral hygiene. The immediate surgical obturator design with buccal extension supported the facial flap and, consequently, maintained facial esthetics.

The study protocol was approved by the ethics committee of the Rumah Sakit Universitas Airlangga, Surabaya, Indonesia (no. UA-02-22119). The first step was to examine the oral lesion carefully before surgery and discuss the treatment plan with the oncology surgeon regarding the proposed incision line and extent of resection. A presurgical impression of the maxillary arch was taken with irreversible hydrocolloid and poured with gypsum material type II to obtain a working cast. The resection line was made on the maxillary working cast (Figure 2). The oncology surgeon reviewed the design to verify the extent of resection.

The cast of the lesion area was modified to acquire normal anatomical contours by comparing the patient's three-dimensional mouth model. It was intended for adaptation to the buccal extension design. Subsequently, a hard, round stainless-steel orthodontic wire was used to fabricate the C-clasp and Adam's clasp to form retentive areas of the remaining healthy teeth on the unresected side. The palatal plate was incorporated into the clasps with heat-polymerizing acrylic resin and finished and polished with stone polish.

The plate obturator was reinserted on the maxillary working cast to evaluate all surfaces of the defect side and retention on the unresected side. Finally, a surgical obturator with buccal extension was fabricated for the patient (Figure 3).

On the day of the operation, after the resection (Figure 4A), the palatal plate served as a temporary surgical obturator (Figure 4B). Plate reduction was applied during the operation for defect adaptation. The Adam's clasp and C-clasp were inserted into the teeth to increase retention. The surgical pack was placed inside the defect to fill the surgical void for surgical dressing. Subsequently, the

surgical obturator was fixed with #2.0 circular silk sutures between the remaining palatal or peri-palatal soft tissues.

In postoperative care, the patient was placed on a liquid diet for 3 days. The surgical dressing was removed on postoperative day 3. After discharge from the hospital, the patient was routinely monitored at the polyclinic. Follow-up evaluation included oral functions, such as swallowing and speech, and the facial profile (Figure 5). When the wound tissue had healed well, the patient was sent to a prosthodontist 1 month postoperatively to receive a temporary obturator.

Discussion

We searched the PubMed database for articles published in the last 10 years using the following keyword: "Surgical obturator for maxillectomy." We obtained 121 articles, including case reports, case series, clinical trials, and reviews, both narrative and systematic. We retrieved articles of clinical studies comparing flap reconstruction and surgical obturators for oral rehabilitation. A total of 143 (53%) patients underwent oral rehabilitation with flap reconstruction, including 59 (41%) men and 84 (59%) women, with a diagnosis of benign tumor in 25 (17%) patients and malignant tumor in 118 (83%) patients. A total of 126 (47%) patients underwent oral rehabilitation with a surgical obturator, including 63 (50%) men and 63 (69%) women, with a diagnosis of benign tumor in 12 (9%) patients and malignant tumor in 114 (91%) patients.

Oral rehabilitation of maxillary defects after maxillectomy can be carried out by using a material prosthesis or with surgical flap reconstruction. Rehabilitation using a prosthesis obturator is frequently performed by practitioners because it can reduce the risk of donor morbidity. The disadvantage of using an obturator is the gradual use process that is expensive and requires patient compliance to maintain the cleanliness of the obturator.¹¹

Oral rehabilitation using a prosthesis obturator is divided into three stages: immediate, temporary, and definitive.^{2,5,12} The immediate surgical obturator is the first temporary prosthesis rehabilitation to restore the palate and alveolar bone continuity after maxillectomy.^{2,7} In the present patient, this obturator was inserted at the time of surgery in the operating room.⁴ The primary function of the immediate surgical obturator was to separate the oral and nasal cavities and support the surgical dressing.^{10,13} It reduced the risk of infection from bacterial contamination from the oral cavity.⁴ The immediate surgical obturator is composed of an acrylic denture base without artificial teeth. It is usually used for a short duration, i.e., 7–14 days, up to 1 month.¹⁴ A transient/temporary obturator replaces the immediate surgical obturator and usually adds artificial teeth.² The temporary obturator is the second prosthesis used until healing is complete and meets the overall functional and esthetic needs. The last stage is using a definitive obturator after the tumor treatment and healing process

have been completed. The definitive obturator is usually used 6–12 months postoperatively.¹⁴

The surgical obturator design has been developed with various modifications, including a hollow obturator, U-loop modification, buccal flange, and labial flange. These modifications were made to improve the retention of the surgical obturator. Buccal flange modification serves to restore orofacial contours more normally and indirect retention to reduce the movement of the surgical obturator.¹⁵

Oral *et al.* (1979) used a surgical obturator with a different design for ten participants. Some participants used a surgical obturator with a buccal flange, while others used a hollow-bulb obturator. In live speech evaluation results, both groups showed good sound production, but the buccal flange obturator showed a better retention rate than the surgical hollow-bulb obturator.¹⁶

The maxilla is a facial structure with support and esthetic functions together with the orbital bones, nasal bone, zygomaticomaxillary complex, and stomatognathic bones. It is responsible for projecting the nose, cheeks, and upper lip.¹⁴ Maxillectomy creates a maxillary defect that causes a connection between the oral and nasal cavities. The maxillary defect causes the patient to be unable to speak or swallow and affects the physical appearance.¹² Maxillary defects require comprehensive treatment and complex processes to restore missing skeletal and soft tissues and reconstruct the dental arch.¹⁴

Maxillary defects can be rehabilitated using a device prosthesis and surgical flap reconstruction.^{5,11} The advantages of using the surgical obturator are shortening the operation time and no requirement of additional surgery.^{11,12} The immediate surgical obturator was fabricated preoperatively and adjusted to the defects at the time of surgery.¹² The general principles of denture construction may be addressed for maxillofacial prostheses, including the surgical obturator. Obturators should be qualified for retention and stability requirement of the prosthesis.¹⁷

Retention of the surgical obturator is influenced by the remaining bone, teeth and soft tissues, and defect size.¹⁷ The surgical obturator design with the maximum extension of the denture base and utilization of the Adam's clasp and C-clasp provides increased retention of the surgical obturator.⁹ Retention can be improved by suturing the remaining soft tissue.¹⁷ The degree of surgical obturator movement is influenced by the size of the defect, residual height of the alveolar bone, contour of the remaining bone and soft tissue, and presence of undercuts.^{9,17} The surgical obturator with a large maxillary defect involving the palatal defect requires a design mostly extended vertically and horizontally to improve retention and stability.¹² The periodontal status of the abutment teeth for the Adam's clasp and C-clasp should be in good condition because it affects the absorption of functional forces and retention and stability of the surgical obturator.⁹ When the surgical obturator shows adequate retention, it is stable as well.¹⁷

In the present case, the surgical obturator did not include artificial teeth. The difference in this case design was that the surgical obturator used a high buccal extension to support the facial flap and support the projections of the nose, cheeks, and upper lip.¹⁸ A modification of buccal flange extension was used to restore speech and improve the orofacial contour of the patient.¹⁵ Construction of the obturator without teeth minimized the weight of the obturator by reducing the amount of compound used to restore the facial contours.¹⁸

The surgical obturator with buccal flange extension provided direct retention of the obturator prosthesis. Rotation and movement of the surgical obturator could be minimized by these modifications.¹⁵ The modification also provided cross-arch stability of the jaw wherein the extension acrylic of the buccal flange prevented rotation of the prosthesis. Additional retention with buccal flange extension did not increase the difficulty of fitting the prosthesis and was more comfortable for the patient.¹⁹

The first aim of using the immediate surgical obturator was to sustain the surgical dressing placed over the defect and close the open link between the oral and nasal cavities, which helped the patient with speech and swallowing after partial maxillectomy.¹² The surgical obturator restores loss of the maxillofacial contour, oral structures, and esthetics. It improves the psychosocial status of the patient. Patients with immediate surgical obturators have advantages of speech, convenience, and comfort in social interactions because the design with buccal extension can improve the facial profile, unlike patients who do not use obturators during the healing process.^{12,20} The present patient's condition was evaluated with a questionnaire regarding the quality of life using the surgical obturator adapted from Ali *et al.*'s study (2018).²¹ The patient showed improved speech and mastication. The patient also felt that her face was more symmetrical when using the surgical obturator.

Conclusions

The surgical obturator is the main treatment option for patients after maxillectomy because it is more effective and carries minimal risks. An immediate surgical obturator is required for mastication and swallowing functions, speech and swallowing corrections, and esthetic improvement after maxillectomy. The modified buccal flange extension provides direct retention and improves the orofacial contour of the patient with easy placement of the surgical obturator prosthesis. The main purpose of therapy in the patient is to eradicate the disease and provide a near-normal life postoperatively.

Conflict of Interest

None



Figure 1. Photographs on the first visit. (A) Anterior view. (B) Bottom view.



Figure 2. Maxillary working cast with a line of resection marked. (A) Anterior view. (B) Lateral view. (C) Occlusal view.

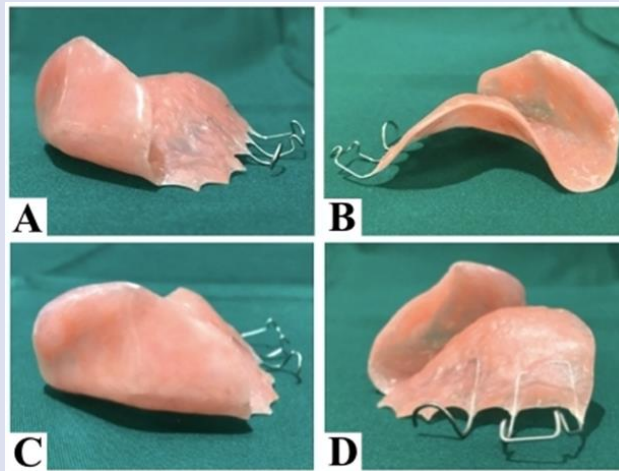


Figure 3. Surgical obturator with buccal extension. (A) Anterior view. (B) Posterior view. (C) Right-side view. (D) Left-side view.

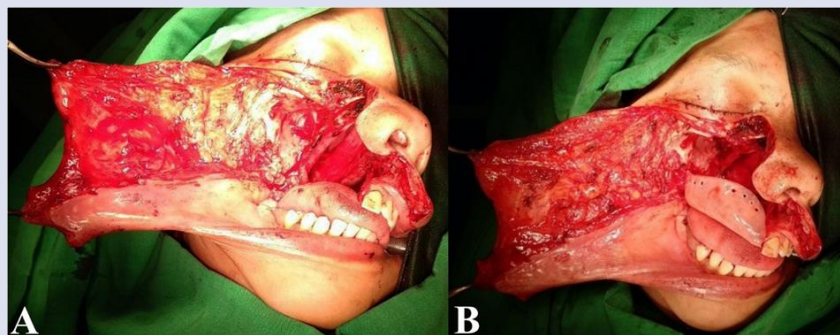


Figure 4. (A) Defect after resection. (B) Insertion of the temporary surgical obturator.

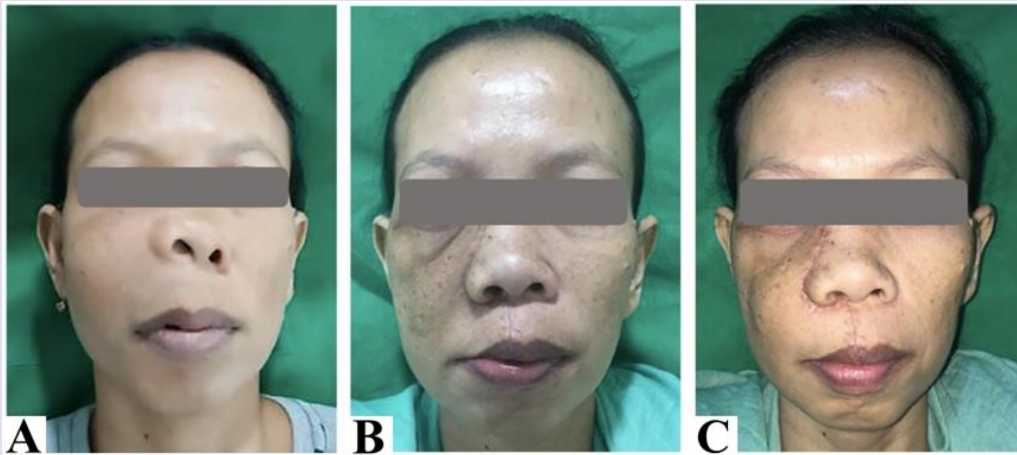


Figure 5. Facial profile. (A) Before surgery; (B) One-month postoperatively without a surgical obturator. (C) One-month postoperatively with a surgical obturator.

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Aggressive Osteoblastoma of the Mandible- A Journey of 2 Rare Cases

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ABSTRACT

Bone tumours are a distinct entity among which osteoblastoma is a rare variant constituting less than 1% of all bone tumours. The bones most commonly involved include the backbone, sacrum, calvaria, long bones and the small bones of the hand and feet. Osteoblastomas have only 15 % occurrence in the head and neck region with a higher incidence in the mandible. Osteoblastomas are known to have a benign or conventional form and the aggressive form which exhibits locally aggressive behaviour and a higher proneness for recurrence. We have reported the journey of two rare cases of aggressive osteoblastoma in the mandible. The treatment was planned considering the aggressiveness of the lesion and the reconstruction was carried out with free fibula graft to maintain the aesthetics, function, and to achieve a good quality of life for the patients.

Key words: Fibula, mandibular prosthesis şplantation, osteoblastoma.

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Introduction

Lichtenstein and Jaffe first described osteoblastoma as a distinct neoplasm and uncommon primary bone tumour in 1956.¹ Osteoblastoma constitutes less than 1% of all the osseous tumours and most commonly involves the long bones, sacrum, calvaria, spinal column, and small bones of the hand and feet.² In the head and neck area it has an incidence of 10-15% and higher propensity to occur in the mandible.² Patients in their first three decades of life are usually affected, with male to female ratio of 2:1.³ The two major clinicopathological forms of osteoblastoma are the conventional or benign form and the aggressive form which exhibits locally aggressive behaviour with a tendency to recur.⁴ We have reported 2 rare cases of aggressive osteoblastoma of the mandible which was managed aggressively and was functionally and cosmetically rehabilitated.

Case Report-1

23-year-old male reported to our department with the chief complaint of pain and swelling in the left lower 3rd of the face. On extraoral examination, a well-defined solitary swelling in the left lower third of the face was

noted which was bony hard, and tender on palpation (Figure 1a). Orthopantomogram (OPG) showed a well-defined osteolytic lesion with areas of radiopacities within the lesion extending from left premolar to 3rd molar region (Figure 1b). Contrast-enhanced Computerized tomography (CECT) showed a fairly well-defined large expansile lytic lesion, with thin internal septations and few areas of ill-defined cortex noted representing cortical break involving the mandible on the left side measuring approximately 5.5(AP) *4.4(CC)*3.5(ML) (Figure 1c).

Incisional biopsy of the lesion was done which was suggestive of aggressive osteoblastoma. The patient underwent wide excision of the lesion and segmental mandibulectomy and the defect was reconstructed with a free fibula graft (Figure 1d). The final histopathology report showed proliferative epithelioid osteoblasts arranged in sheets and overlying broad bony trabeculae rimmed by these osteoblasts with many osteoclastic giant cells which confirmed the diagnosis of aggressive osteoblastoma (Figure 1e). The patient had an uneventful postoperative course and is on regular follow-up with no evidence of recurrence till to date (Figure 1f).

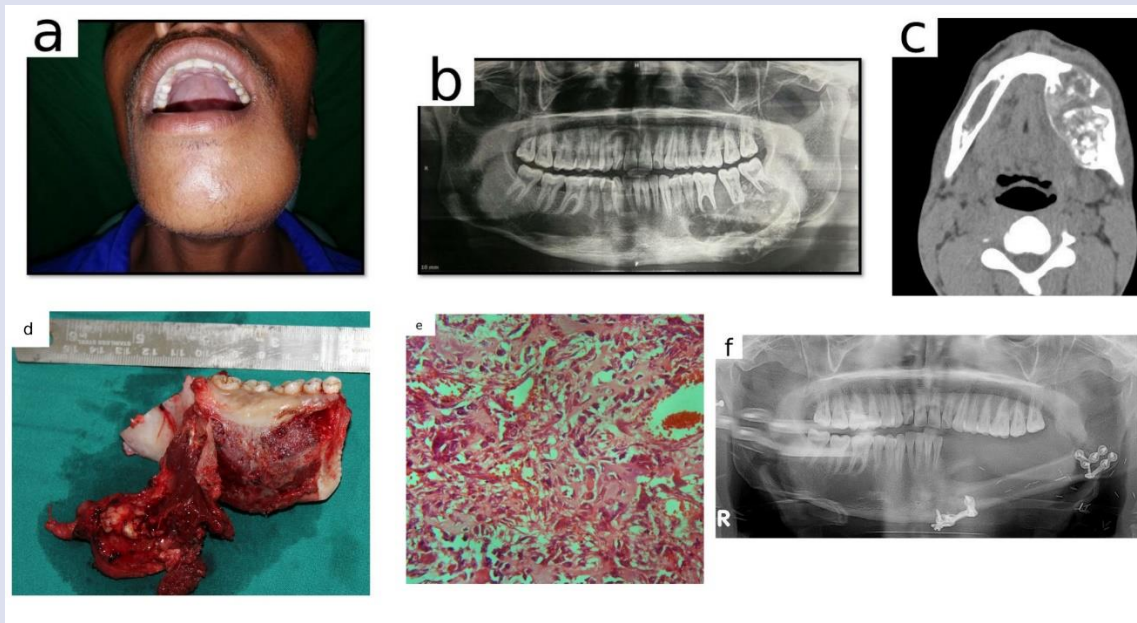


Figure 1. a) Extraoral clinical photograph shows facial asymmetry
 b) Orthopantomogram (OPG) showing ill-defined lesion with various radiopaque focal areas with disruption of inferior border of mandible
 c) Contrast enhanced Computed tomography (CECT) axial section shows expansile intramedullary lesion
 d) Resected portion of mandible showing the tumour
 e) Haematoxylin & Eosin-stained specimen shows highly cellular and vascular proliferative connective tissue consisting of epithelioid cells with abundant multinucleated osteoclast like giant cells and large areas of haemorrhage is evident throughout the lesion
 f) Post-operative OPG.

Case Report-2

35-year-old female reported to our department with swelling in the left side of the face for 4 months. On examination, there was restricted mouth opening and the swelling was firm to hard in consistency with bi cortical expansion extending from left mandibular canine to 2nd molar (Figure 2a). OPG revealed an ill-defined radiolucent lesion in the left body of the mandible with focal radiopaque areas with disruption of the inferior border of the mandible (Figure 2b). CECT revealed a large expansile intramedullary lesion with ground glass matrix, calcification noted involving the symphysis and body of the mandible on the left side associated with cortical expansion and significant thinning measuring 3.5cm x 5.0cm x 3.2cm (AP x ML x CC) (Figure 2c).

The patient underwent an incisional biopsy of the lesion which was suggestive of aggressive osteoblastoma. She underwent wide excision of the lesion with segmental mandibulectomy followed by reconstruction with free fibula graft and rehabilitation was done by immediate placement of implants (Figure 2d). The final histopathology report revealed the interconnecting bony trabeculae of coarsely woven bone rimmed by relatively plump osteoblasts to form an anastomosing net-like pattern. Many multinucleated osteoclast-like giant cells are also present along the bony trabeculae confirming the diagnosis of aggressive osteoblastoma (Figure 2e). The patient had an uneventful postoperative course and is on regular follow-up with no evidence of recurrence till to date (Figure 2f).

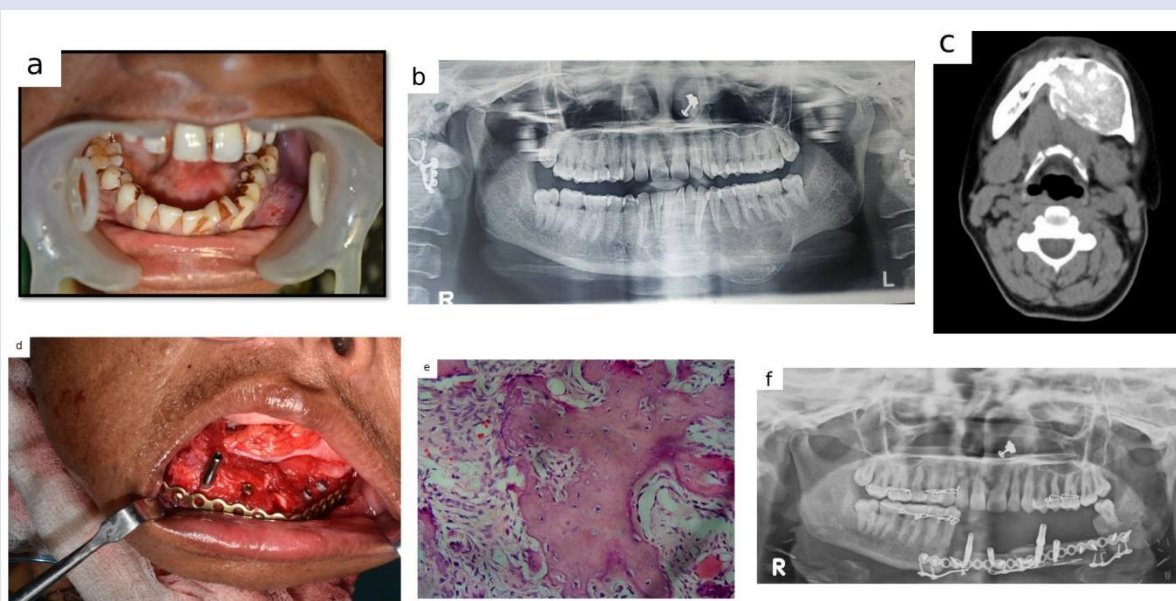


Figure 2. a) Intraoral examination shows swelling with bi-cortical expansion
 b) OPG showing radiolucent lesion extending from canine to molar with disruption of inferior border of mandible
 c) CECT scan (axial section) shows large intramedullary expansile lesion with calcification noted involving the symphysis and body of the mandible
 d) Reconstruction was done using free fibula flap followed by immediate implant placement
 e) Haematoxylin & eosin-stained final specimen shows interconnecting bony trabeculae of coarse woven bone rimmed by plump osteoblasts. Many multinucleated osteoclast-like giant cells present along the bony trabeculae. Connective tissue has multiple thin-walled blood capillaries
 f) Post-operative OPG.

Discussion

Jaffe and Lichtenstein in 1956 described osteoblastoma as a rare vascular osteoid and bone-forming benign tumour which is characterised by abundant osteoblasts.⁵ Even though osteoblastoma comprises less than 1% of all the osseous tumours, it has an incidence of 10-15% in head and neck region with increased chances of occurring in the mandible.^{2,5,6} Both our patients were in their first three decades of life and had lesions occurring in the posterior mandible.

The pathologic entity of 'aggressive' osteoblastoma was first proposed by DORFMAN in 1973.⁷ Osteoblastoma is classified into two major clinicopathological forms which includes the Conventional osteoblastomas (CO) and the Aggressive osteoblastoma (AO). CO grow slowly not exceeding 4 cm in diameter and have restricted growth potential.^{2,7} They have a well-defined sclerotic margin and are completely excised. Aggressive osteoblastoma (AO) usually exhibits locally aggressive behaviour and has a diameter of more than 4cm. Clinically they present mainly with pain, swelling, and expansion of the bony cortex.¹ Both our patients exhibited similar symptoms. Mobility of the teeth and pain are the early manifestations of these tumours, thus may be misdiagnosed as odontogenic infections or osteomyelitis.⁷ Radiographically AO present a variety of patterns. It may be characterised by an osteolytic lesion with focal radiopacities and cortical expansion, or as sclerotic masses with well or ill-defined

borders.^{7,8} Our patients also exhibited expansile osteolytic lesion with areas of radiopacities. AO may also be seen as a radiopaque nidus, within a radiolucent lesion, similar to osteoid osteoma.³ The histopathological pathognomonic feature of AO is plump epithelioid osteoblasts larger than CO, with prominent nuclei and nucleoli, abundant and slightly eosinophilic cytoplasm. They have increased multinucleated giant cells of osteoclast type with increased mitotic activity and non-trabecular sheets or lace-like areas of osteoid production.^{5,9}

AO has to be differentiated from conventional osteoblastoma and osteosarcoma to plan accurate treatment for the patient and to prevent recurrence.^{2,3} AO has locally aggressive behaviour and a propensity to recur, hence aggressive treatment is recommended including surgical resection with safety margins.² Such aggressive treatment could result in complex facial defects which requires reconstruction to achieve functional and aesthetic rehabilitation.² Hence, we have managed our patients too aggressively with segmental mandibular resection and functionally and cosmetically rehabilitated using free fibula graft.

Gordon et al emphasizes the importance of long-term follow-up as the recurrence rate for conventional osteoblastoma is 13.6%, whereas for aggressive osteoblastoma is 50%.^{3,10}

Conclusions

To summarize, we have described the journey of 2 rare cases of aggressive osteoblastoma of the mandible which was diagnosed accurately and managed aggressively. Both the patients were successfully rehabilitated functionally and cosmetically thereby improving their quality of life.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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