



Effect of laser-assisted retrograde cavity preparation on push-out-bond-strength of mineral trioxide aggregate and calcium-enriched-mixture-cement

Efeito de preparo cavitário retrógrado assistido por laser na força de união de cimento de agregado trióxido mineral e cimento enriquecido com cálcio

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ABSTRACT

Objective: This study aimed to determine the push-out-bond-strength(PBS) of mineral trioxide aggregate (BIOMTA+) and calcium-enriched-mixture-cement (CEM) in retrograde cavities prepared using Er: YAG laser and stainless-steel bur. **Material and Methods:** The root canals of 60 extracted single-rooted human teeth were prepared, filled and their apical portion of 3-mm were resected using a diamond bur and randomly divided into four groups according to technique of retrograde preparation and filling material as follows(n=15): Group1: bur/ BIOMTA+ ,Group2: bur/ CEM, Group3: Er:YAG laser/ BIOMTA+, Group4: Er:YAG laser/ CEM. PBS test were performed to specimens and failure modes were evaluated. The data were statistically analyzed with ANOVA, Post-Hoc Tukey and *t* tests ($p < 0.05$). **Results:** CEM was exhibited higher than bond strength compared to BIOMTA+ in retrograde cavity prepared using laser ($p = 0.021$) and BIOMTA+ in retrograde cavity prepared using bur was exhibited higher than bond strength compared to in retrograde cavities prepared using laser ($p = 0.024$). Failure modes were dominantly cohesive in all groups tested and one representative specimen each failure mode was examined in SEM and the general characteristics of the failure modes were confirmed. **Conclusion:** With in the limitations of the present study, when used CEM, Er: YAG laser-assisted retrograde cavity preparation positively affected the bond strength values compared to BIO MTA+. Considering its optimal adhesion, the calcium-enriched-mixture-cement (CEM) might be a good option as a filling material in retrograde cavities in clinical use.

KEYWORDS

Calcium-Enriched-Mixture-Cement (CEM); Er: YAG laser; Mineral trioxide aggregate; Retrograde cavity preparation.

RESUMO

Objetivo: O objetivo deste estudo foi determinar a força de união (PBS) de cimento de agregado trióxido mineral (BIO MTA+) e cimento enriquecido com cálcio (CEM) em preparos cavitários retrógrados realizados com: Laser Er-YAG e brocas de aço inoxidável. **Material e Métodos:** Canais radiculares de 60 dentes unirradiculares extraídos foram preparados, preenchidos e 3 mm de suas porções apicais foram ressecadas usando uma broca diamantada e divididos randomicamente em quatro grupos de acordo com a técnica de preparação retrógrada e o material de preenchimento (n=15): Grupo 1: Broca/BIO MTA+, Grupo 2: Broca/CEM, Grupo 3: Laser Er-YAG/BIO MTA+, Grupo 4: Laser Er-YAG/CEM. O teste de PBS foi realizado para as amostras e os modelos de falha foram avaliados. Os dados foram analisados estatisticamente pelos testes de ANOVA, Post-Hoc Tukey e testes *t* ($p < 0.05$). **Resultados:** CEM apresentou maior força de união que BIO MTA+ em cavidades retrógradas preparadas com laser ($p = 0.021$) e BIO MTA+ em cavidades retrógradas preparadas com brocas apresentou

maior força de união quando comparado à cavidades retrógradas preparadas com laser ($p= 0.024$). Os modelos de falha foram predominantemente coesos em todos os grupos testados e um espécime representativo de cada modelo de falha foi examinado em MEV e as características gerais dos modelos de falha foram confirmadas. **Conclusão:** Com as limitações do presente estudo, quando usou-se CEM, o preparo de cavidades retrógradas através de Laser Er-YAG afetou positivamente os valores da força de união quando comparados com BIO MTA+. Considerando sua ótima adesão, o cimento enriquecido com cálcio (CEM) pode ser uma boa opção como um material de preenchimento em cavidades retrógradas no uso clínico.

PALAVRAS-CHAVE

Cimento Enriquecido com Cálcio (CEM); Lasers de estado sólido; Agregado de trióxido mineral; Preparo de cavidade retrógrada.

INTRODUCTION

In endodontic therapy, when a root canal treatment results in a periapical lesion or when the retreatment did not obtain satisfactory results endodontic surgery can be required. This clinical approach involves root-end resection and preparation, as well as sealing the cavity with biocompatible root-end filling material. During these procedures, different conventional burs, ultrasonic retro tips and lasers can be used to improve the quality of treatment. But due to anatomical reasons and limited access to the root apices, some conventional methods present difficulties when performing retrograde root-end cavity preparations [1]. Furthermore, it has been reported that fractures, cracks or chipping can occur while preparing the root-end cavity using those techniques [2].

The special retrograde filling material is required for a successful surgical endodontic treatment. The material should be compatible with periapical tissues, provide a sufficient sealing, induce osteogenic activity, and be nontoxic and adaptable to the root canal walls [3]. Although mineral trioxide aggregate (MTA), a biocompatible material with good sealing abilities, was accepted as the gold standard, its long setting time and difficult manipulation properties were disadvantages [4]. BIO-MTA+ is a newly introduced bio-ceramic material that contains the following components: calcium oxide, hydroxyapatite, oxides of silicon, sodium, potassium, bismuth, magnesium, aluminum, iron and zirconium, and calcium phosphate. The liquid of the material contains a purified water and a calcium catalyst. BIO MTA+ is recommended in root canal wall perforation, intracanal resorption, filling of the root apex, direct capping of the pulp, pulp amputation and treatment of teeth with interrupted root development [5]. In 2006,

the endodontic filling material calcium-enriched mixture (CEM) was introduced. The structure of CEM contains different calcium compounds, such as calcium carbonate, calcium phosphate, calcium silicate, calcium sulfate, calcium oxide, calcium hydroxide and calcium chloride. CEM cement has similar clinical properties to MTA, however, CEM has a shorter setting time (less than 1h) and a simpler application process [6].

Root-end filling material should provide good apical sealing and demonstrate a sufficient adhesion to root dentin. In the dental literature, tensile, shear and push-out tests were generally preferred to determine the adhesion of root-end filling materials. The push-out test is performed in sections parallel to the interfacial area of dentin-material interface and is considered a better option to evaluate bond strength [7].

Considering the significance of the bonding of calcium-containing cements to dentin, this study aimed to investigate the effect of Er: YAG laser preparation technique of root dentin cavities on the push-out bond strength of BIO MTA+ and CEM cement. The null hypothesis tested was that the preparation technique of root-end cavities did not affect the push-out-bond-strength (PBS) values of BIO MTA and CEM.

MATERIAL AND METHODS

Ethical approval (2019.10.12 2019/17) was granted for this study from the Kırıkkale University Ethics Committee. The required minimum sample size was calculated using G*Power v.3.1 software (Heinrich Heine, University of Dusseldorf, Dusseldorf, Germany). An alpha of 0.05 (type I error), effect size of 0.45 and a beta power of 0.80" (1 - type II error) were specified, and for each group the minimal estimated sample size was found to be 15. Sixty intact human single- circular-rooted incisors and

canines, extracted due to periodontal reasons were obtained and stored in saline solution until being used. The absence of one canal and the selection of circular root canals were verified radiographically. The extrusion criteria were the presence of calcification, external resorption, root fracture, presence of dentinal defects on the external surface or previous endodontic treatment.

Specimen preparation

Using a water-cooling low-speed cutting device (Microcut, Metkon, Bursa, Turkey), coronal parts of the teeth were removed, with a root length of 14 mm as standard. The root canals of all teeth were enlarged using the crown-down technique employing to #F4 Protaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) rotary file properly [1]. The 2.5% NaOCl and 5% EDTA solutions were used for irrigating the specimens, and drying of the root canals was performed using paper points. The root canals were filled with .06 tapered #F4 gutta-percha (Dentsply Maillefer, Ballaigues, Switzerland) and an epoxy matrix resin-based root canal sealer (TG Adseal, Technical & General Ltd, London, England). The roots were then stored at 37°C in a 100% moist environment to leave the canal sealer material to set.

Root-end cavity preparation

The specimens were placed in acrylic resin (Imicryl Ltd., Konya, Turkey), and after the polymerization process apical resections were performed at an angle of 90° to the long axis of the root, removing 3 mm from the apex using a low-speed cutting device under continuous water cooling (Microcut, Metkon, Bursa, Turkey).

The resected specimens were randomly divided into 4 groups, with 15 specimens in each group. Similar to a previous study [8], the total area of resected root-end surface and the root canal space area were measured with Image J (NIH, Bethesda, MD, USA) software program. The groups were as follows:

Bur/BIO MTA+ Group: The root-end cavities were formed with a round stainless-steel bur (0.9 mm in diameter) (KAVO do Brasil Ind. Com., Joinville, SC, Brazil) to a depth of 3-mm under distilled water at a speed of 2000 rpm. An up-and-down motion was applied on the gutta-percha with slight

pressure and all cavities were controlled with a periodontal probe.

After preparing the root-end cavity, BIO MTA+ (Cerkamed, Stalowa Wola, Poland) was prepared by mixing according to the manufacturer's recommendations (Table I) and placed in the cavities.

Bur/CEM Group: The root-end cavities were prepared similar to the Bur/BIO MTA+ group. After preparation, the CEM cement (BioniqueDent, Tehran, Iran) was prepared by mixing according to the manufacturer's recommendations (Table I) and placed in the cavities.

Er: YAG laser/BIO MTA+ Group: The root-end cavities were formed 3-mm deep with an Er: YAG laser using a 16-mm long and 0.6-mm wide conical fiber tip (Fotona, Ljubljana, Slovenia) with the following parameters: quantum square pulse (QSP) mode [In the QSP mode, a standard laser pulse of 600 μ s was chopped into a series of five short pulses of 50 μ s separated by 85 μ s.] [4], contact, 200 mJ, 10 Hz, 2.00 W, water spray on level 3 and air on level 5 according to the user manual. After preparing the root-end cavity, the BIO MTA+ (Cerkamed, Stalowa Wola, Poland) was prepared by mixing according to the manufacturer's recommendations (Table I) and placed in the cavities.

Er: YAG laser/CEM Group: The root-end cavities were prepared similar to the Er: YAG laser/BIO MTA+ group. After preparation, the CEM cement (BioniqueDent, Tehran, Iran) was prepared by mixing according to the manufacturer's recommendations (Table I) and placed in the cavities.

All restorative procedures were performed by one operator of the research team.

Next, all the specimens were rolled into a moist sponge immersed in distilled water and incubated at 37 °C for 72 h at 100% relative humidity to complete the setting reaction. After the setting time, the excess cement was cleaned with a spatula and fine grain sandpaper until the entire cement/dentin interface appeared. All specimens were then stored in a phosphate buffered saline (PBS) solution for 3 months [9].

A total of 60 slices with equal numbers from all groups were made at 1 ± 0.2 mm thickness using a water-cooling low-speed cutting device

(Microcut, Metkon, Bursa, Turkey). The area of the bonding surface was calculated using the following conical frustum formula[4]:

$$Area = \pi(R1 + R2) \sqrt{(R1 - R2)^2 + h^2} \quad (1)$$

R1: apical surface radius as a larger radius, R2: coronal surface radius as a smaller radius, and h as the thickness of the slice.

Push-out bond strength test

A universal testing device (Lloyd LRX, Lloyd Instruments Ltd, Frahem, England) was used to determine the PBS values of the specimens. The dentin slices were placed on a metal slab having a central hole to provide easy movement of a stainless steel blunt-shaped probe with a 1.0-mm diameter and tested until failure occurred. The test was run on slices through the apical to coronal direction due to the reversed taper formed during the root-end cavity preparation. The compressive load was run to the material surface with a cross-head speed of 1mm/min [6]. Before starting the push-out procedure, it was ensured that the plunger would only contact the material surface. The maximum value causing dislodgement of the material was recorded in newtons (N). The results in Ns were divided by adhesion surface area in square millimeters (mm²) to obtain the data in megapascals (MPa).

After the test procedure, the slices were observed under 40× magnification using a dental microscope (Zeiss OPMI pico s100, Carl Zeiss, Germany). The failure modes were classified as follows:

Adhesive Failure: If the fracture was seen at the dentin-material interface

Cohesive Failure: If the fracture was seen within the material

Mixed Failure: If the fracture was seen both at the dentin-material interface and within the material.

One specimen for each failure mode was randomly selected, coated with palladium and evaluated under scanning electron microscopy (SEM; JEOL JSM-5600, Tokyo, Japan). For standardization, SEM images were taken at 500X magnification. The SEM evaluations were performed by two dental specialists.

Statistical analysis

The data were controlled with a Shapiro-Wilk test, and normal distribution was observed. Also, the data were controlled with a Levene's Test for equality of variances. One-way ANOVA, Tukey post-hoc and independent *t* tests were performed to analyze the data using SPSS 22.0 software (IBM, Chicago, IL) at *p*<0.05 significance level.

RESULTS

While the Er: YAG/BIO MTA+ group exhibited the lowest PBS (5.63±2.84 MPa), the Bur/CEM group exhibited the highest PBS (11.23±7.19 MPa). While, the Er: YAG/CEM group had statistically significant higher PBS values observed than the Er: YAG/BIO MTA+ group (*p*= 0.021), the Bur/BIO MTA+ group demonstrated significantly higher PBS values than the Er: YAG/BIO MTA+ group (*p*= 0.024) (Table II).

The comparison of failure modes observed in this study is shown in Figure 1. The dominant failure mode in the test groups was cohesive, followed by mixed and no adhesive bond failure modes in the CEM groups. Representative dental microscope and SEM images of each failure mode were shown in Figure 2.

Representative sample for adhesive failure mode was belong to Er: YAG laser / BIO MTA + group and the cement was completely removed from the dentin wall and remaining dentin tissue was observed in SEM image. However, for cohesive failure mode, sample of the Bur/ CEM group evaluated by SEM, only the cement was completely present on the outer wall of dentin. Besides, cement was found to be present in some parts of the dentin in the mixed failure mode in the example of the Er: YAG laser / CEM group.

DISCUSSION

In the present study, push-out bond strength was investigated in root-end cavities prepared using a bur and an Er: YAG laser and filled with BIO MTA+ and CEM. While there was no difference between the materials in the cavity technique prepared with a bur, a difference was found between the materials in the cavity technique prepared with the Er: YAG laser. Therefore, the null hypothesis was partially rejected.

Table I - Manufacturers and the Compositions of Materials Used

Material	MANUFACTURER	COMPOSITION	LOT NUMBER	application procedure
Calcium-enriched-mixture-cement*	BioniqueDent, Tehran, Iran	The main components of the powder are 51.75%, CaO9.53% SO ₃ , 8.49% P ₂ O ₅ , 6.32% SiO ₂ by weight and small components are Al ₂ O ₃ > Na ₂ O> MgO> Cl.	C160319	<p>CEM is mixed with powder-to-liquid ratio of 2:1.</p> <p>Gradually incorporate the liquid into the cement using appropriate spatula/mixing sticks.</p> <p>Mix the powder with liquid for 15-30 sec to ensure all powder particles are hydrated. The working time for the mixed material is approximately 5 min (though CEM cement sets fully approximately an hour). If more working time needed, cover the mixed material with wet gauze pad to prevent evaporation.</p> <p>Condense it into the cavity using a small plugger then use gentle movements of a dry a cotton pellet alternatively. Remove excess cement and clean the surface of the root with a moist piece of gauze.</p>
Mineral Trioxide Aggregate**	Cerkamed, Stalowa Wola, Poland	Bio MTA+ powder: calcium oxide, hydroxyapatite, oxides of: silicon, iron, aluminum, sodium, potassium, bismuth, magnesium, zirconium; calcium phosphate. Bio MTA+ liquid: Ph. Eur. purified water, calcium catalyst	2602181	Bio MTA + powder is mixed on the mixing plate with 1 to 2 drops of Bio MTA + liquid for 30 seconds until it becomes a paste. If the compound mix is too thick or too brittle, add one more drop of the Bio MTA+ liquid. When mixed with the Bio MTA+ liquid, the working life is 4 min, and the compound sets completely in 2 h. Place the compound at the target site with an applicator.

*CEM. **BIOMTA+.

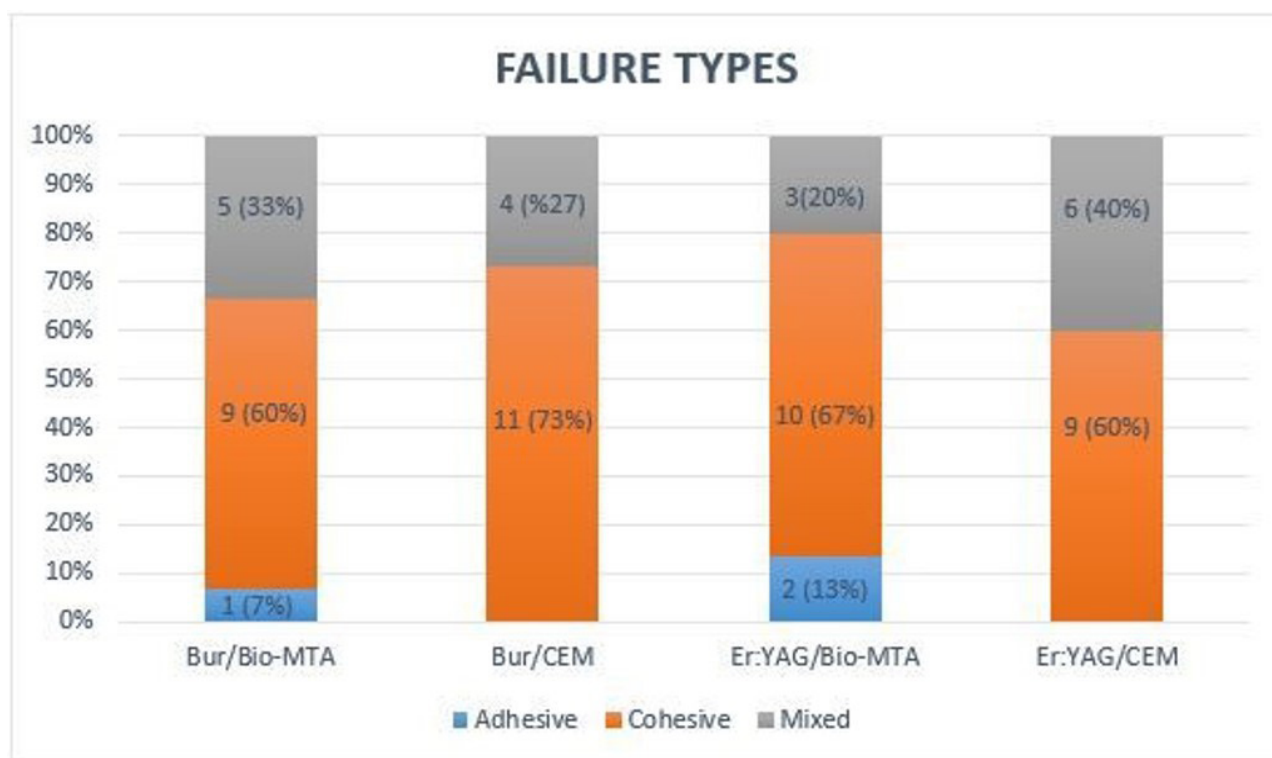


Figure 1 - Failure types (%) in each tested group after the push-out test.

Calcium silicate-based materials might precipitate carbonated apatite in the presence of PBS, after which the formation of an interfacial layer and tag-like structures occur in the

dentin and may produce hydroxyapatite [4]. Sarkar et al. showed that a hydroxyapatite layer at the MTA-dentin interface was formed in MTA-filled teeth, which were stored in PBS

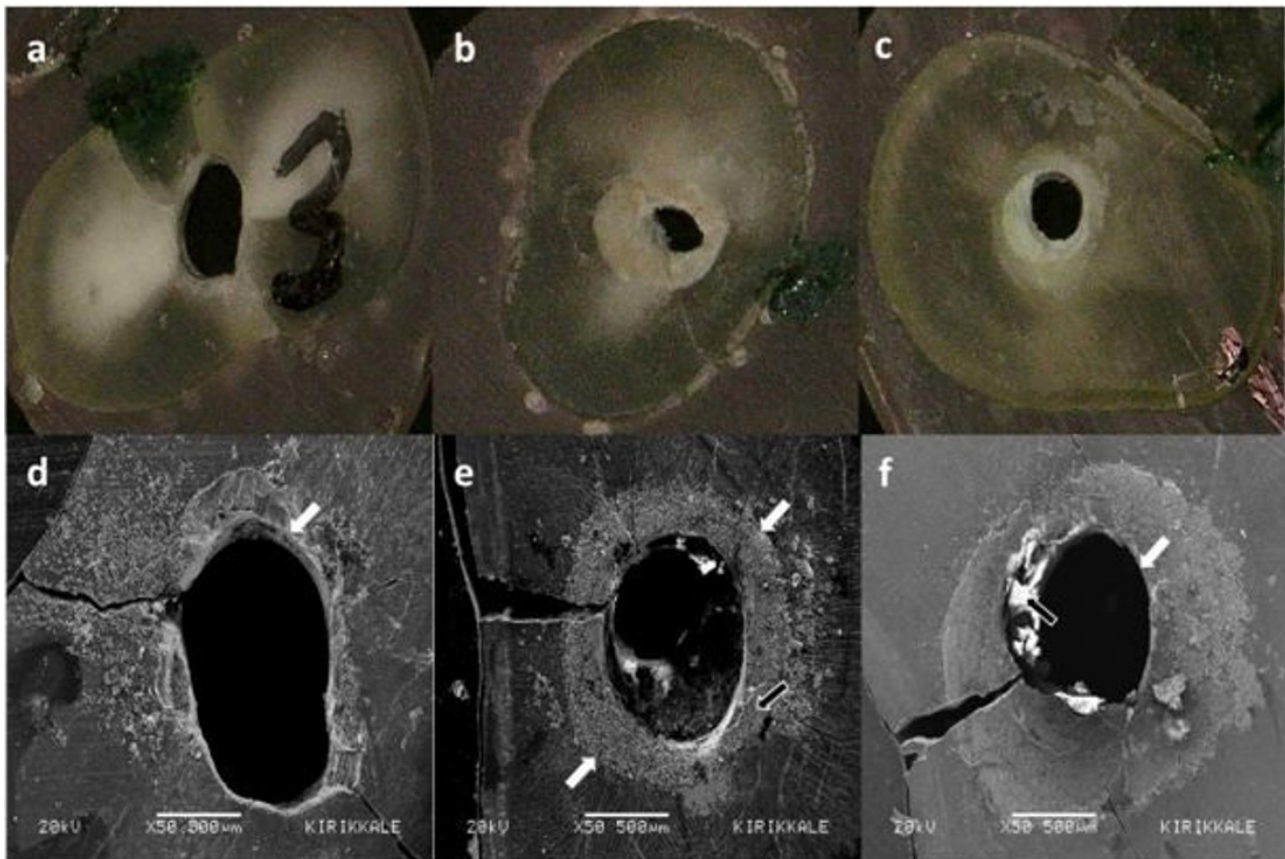


Figure 2 - Representative failure modes photographs and SEM microphotographs obtained from various specimens. **a, d.** adhesive failure (an example of the Er: YAG laser / BIO MTA + group) **b, e.** cohesive failure (an example of the Bur/ CEM group) **c, f.** mix failure (an example of the Er: YAG laser / CEM group). (White arrows show root dentin wall, black arrows show remain material in the root canal space after push-out test.

Table II - The mean and standard deviations the push-out bond strength values of the groups tested (in MPa)

	BIO MTA+		CEM		p
	n	Mean± SD	n	Mean± SD	
BUR	15	9.25±5.81*	15	11.23±7.19	0.363
ER YAG LASER	15	5.63±2.84	15	8.63±4.42*	0.021*
p	0.024*		0.193		

SD: Standard Deviation. *The same superscript lowercase letters in row and column are statistically significant ($p < 0.05$).

for 2 months [10]. Another study found that the bonding strength of calcium silicate cement to dentine increased at 3 months after obturation when compared with 2 weeks [11]. Therefore, in this study, all specimens were stored in PBS for 3 months before testing was conducted.

Although, retrograde cavity preparation with burs is a conventional approach, it has some disadvantages, such as the risk of perforation due to the formation of micro-cracks and the unsuitable bur angle for adequate preparation [4]. It was indicated that a dentine surface prepared using a bur was covered with a smear layer blocking the orifice of tubules [4]. Er: YAG laser irradiation causes structural changes on the

dentin surface, such as the removal of a smear layer and debris and open dentinal tubules that are irregular and rough [6]. Additionally, the properties of the root-end filling material, such as particle size, composition and setting time might affect its adhesion to the cavity walls. Kadić et al. [4] evaluated the adhesion of a calcium silicate cement, TotalFill RRM, to radicular dentin. Root-end cavities irradiated with the Er: YAG laser showed significantly higher bond strength values compared to bur-prepared ones. They concluded that the bio-ceramic content of TotalFill RRM or a differentiation in laser parameters might have affected their results. However, they demonstrated similar bond strength values between Er,Cr:YSGG laser-

irradiated and bur-prepared retrograde cavities. In another study, Mohammadian et al. [6] reported that an Er: YAG laser application only enhanced the bond strength of CEM material to dentin and had no significantly different effect on the bond strength of MTA. Shokouhinejad et al. [12] found significantly lower bond strength of MTA and CEM materials in root-end cavities prepared with an Er,Cr: YSGG laser compared to retrograde cavities prepared with ultrasonic. In the present study, CEM material was observed to have higher bond strength compared to MTA material in a cavity prepared with an Er: YAG laser. Furthermore, the MTA material showed higher bond strength in cavities prepared with a bur compared to cavities prepared with an Er: YAG laser. Although the results of these studies [4,12] are similar to those of our study, there have been controversial results regarding this issue in the dental literature, and while our study indicated no significant difference in push-out bond strength values between laser- and bur-prepared BIO MTA+, CEM indicated a significant difference.

The present study revealed that the bond strength of the CEM material was higher than the bond strength of BIO MTA+. MTA releases calcium hydroxide as well as has property the antibacterial and antifungal [13], and research has presented the creation of a tag-like construction spread between the dentinal tubules and intermediate layer [14]. This structural formation increases the mechanical retention of the material [15]; a previous study has shown that CEM releases hydroxyapatite endogenously and is biocompatible as well as when used as root-end filling material and the results are comparable with MTA. [16]. Therefore, elimination of the smear layer led to increased micro-mechanical interlocking. However, in this study, the minimum bond strength was shown in the Er: YAG/BIO MTA+ group, and the Er: YAG laser/BIO MTA+ group had a lower bond strength than the Bur/BIO MTA+ group. The explanation for these results could be the high viscosity of MTA, which rendered it unable to penetrate the irregular dentin surface and dentinal tubules after laser application [17]. Second, MTA cannot produce hydroxyapatite in the presence of water [14]. Winik et al. have shown interruptions and gaps in the interface of MTA-laser irradiated dentin [17]. During ablation, the mechanical shock occurring due to the evaporation of water

could cause sub-superficial cracks on dentin, decrease the mechanical quality of the dentin structure and make it difficult for the materials to adhere to the irradiated dentin [4,18]. There might also be some changes in the sub-superficial layer of laser-irradiated dentin that might affect adhesion.

In our study, the Er: YAG laser/CEM material exhibited higher bond strength than the Er: YAG laser/BIO MTA+ material. The difference between the bond strength of CEM cement and MTA might be attributed to their different compositions and particle sizes [7]. In a previous study [19], it was indicated that the particle size of a cement affects its mechanical properties. CEM cement has smaller particle size and a more homogeneous nature compared to MTA [7]. Mohammadian et al. [6] showed that CEM groups demonstrated higher bond strength due to their ability to penetrate into open dentinal tubules as the result of their smaller particle size compared to MTA. Moreover, it has been indicated that the growth of the hydroxyapatite layer is commensurate to the concentration of ions in the material. In the presence of the phosphate components of the CEM material, more phosphate groups react with the calcium ions to form a hydroxyapatite layer and tags. The tag formation could improve the retention of CEM cement [14].

Although the dominant failure mode was cohesive in all groups tested, adhesive and mixed failure modes were also demonstrated. Also, the general characteristics of the failure modes were confirmed by SEM examination. The results of the CEM cement groups were compatible with the results of previous studies [12,20,21]. However, the failure modes of the BIO MTA+ groups were not found, contrary to the results of Shokouhinejad et al. [12]. The difference of failure modes between MTA and CEM might be attributed to the materials' chemical compositions. CEM cement contains much more phosphorus compound than MTA, which results in more hydroxyapatite precipitation being observed in the CEM material when stored in normal saline and PBS solutions. This might reduce gaps and enhance the chemical adhesion between the CEM cement and the dentine wall without adhesive failures [12].

Although, push-out bond strength values indicate promising results, the use of only one laser

system parameter means the data obtained from the present study may not be valid for all laser systems, a limitation of this study. In a systematic review [5], it was concluded that, push out bond tests in endodontic research had to be standardized in terms of experimental protocols and it was emphasized that standardized control materials might allow calibration and comparability of techniques and materials used. Thus, further research with different methodologies, such as retrograde cavity preparation technique and standardize, material type, laser parameters and incubation time are needed to confirm these results. Also, it is important to influence of different teeth groups and their anatomical root canal variability in the type of root-end access cavities. One of the limitations of this study was that only representative samples of failure modes were examined in the SEM. It did not provide detailed information about the adhesion mechanism of the cements used and dentin characteristics. In vitro tests also present several drawbacks in simulating the clinical behavior of restorative materials; hence, our results might be better supported with long-term clinical trials.

CONCLUSION

Within the limitations of this study, it was concluded that: The push-out bond strength values resulting from the root-end cavity preparation techniques and calcium-enriched mixture cement (CEM) recommend these procedures and material for cavities prepared with Er: YAG lasers. Considering its optimal adhesion, the calcium-enriched mixture cement (CEM) might be a good option as a filling material in retrograde cavities in clinical use.

Author Contributions

HSB: The author effectively contributed to the study, being familiar with its contents. AT: The author effectively contributed to the study, being familiar with its contents. YB: The author effectively contributed to the study, being familiar with its contents. ÇÇ: The author effectively contributed to the study, being familiar with its contents.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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Regulatory Statement

This study was conducted after obtaining approval from ethics committee (2019.10.12 2019/17) in accordance with Helsinki declaration of human research.

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