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An *In Vitro* Comparative Evaluation of the Effect of Two Chelating Agents and Irrigation Techniques on the Push-Out Bond Strength of a Bioceramic-Based Sealer

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Research Article

Abstract

Objective: To compare the push-out bond strength (PBS) and failure modes of root canals obturated with gutta-percha and a bioceramic-based (BC) sealer after final rinsing with either 17% EDTA or 0.2% chitosan, using conventional syringe irrigation and passive ultrasonic irrigation (PUI).

Materials and Methods: Fifty-two extracted human mandibular premolars were selected and sectioned to obtain 16-mm root lengths. The canals were prepared using ProTaper Next rotary files up to size X3 and randomly divided into four groups (n = 13 per group) according to the final irrigant and irrigation method; Group 1: Syringe irrigation with 17% EDTA, Group 2: PUI with 17% EDTA, Group 3: Syringe irrigation with 0.2% Chitosan and Group 4: PUI with 0.2% Chitosan. The root canals were filled with gutta-percha cone size X3 and BC sealer (CeraSeal) using matched single cone technique. After being kept at 37°C and 100% humidity for 2 weeks to allow setting of sealer, the samples were cut at 5 mm from root apex to achieve 2 mm in thickness of dentin disc as the representative of middle 1/3 of root canal. Mechanical dislodgement resistance was examined using a universal testing machine and PBS was calculated. Specimens were examined under stereomicroscope at 40× magnification to define the bond failure mode. One-way analysis of variance (ANOVA), followed by Bonferroni's post hoc tests, was performed to evaluate the differences in PBS among the groups. Mode of failure was analyzed by Fisher exact test. The level of significance was set at p=0.05.

Results: There were no significant differences in the mean PBS values or failure modes among the experimental groups. Mean PBS values were 1.67±0.76 MPa, 1.88±0.75 MPa, 1.55±0.80 MPa, and 2.29±1.32 MPa for Groups 1–4, respectively. No significant differences were observed among the groups (p=0.202). Likewise, failure mode distributions were not significantly different among the experimental groups (p=0.209).

Conclusion: No significant differences in PBS or failure mode were observed between root canals irrigated with 17% EDTA and those irrigated with 0.2% chitosan.

Keywords: Bioceramic-based sealer/ Chitosan/ Passive ultrasonic irrigation / Push-out bond strength

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Introduction

Three-dimensional (3D) root canal obturation is one of the important factors for the success of root canal treatment.[1] Because gutta-percha lacks the ability to bond to root canal walls, the use of root canal sealer is critical for filling the space between the prepared dentin wall and the core filling material.[2] Various types of root canal sealers have been used in endodontics. Bioceramic-based (BC) sealers are the most recently introduced. They include alumina, zirconia, bioactive glass, glass ceramics, hydroxyapatite, and calcium phosphates.[3] The major advantage associated with the use of bioceramic materials as root canal sealer is that they contain calcium phosphate which enhances the setting properties of bioceramics and results in a chemical composition and crystalline structure similar to tooth and bone apatite[4], thereby improving sealer-to-root dentin bonding. During root canal preparation, a layer of organic and inorganic material called the "smear layer" is produced. The smear layer may clog the dentinal tubules and interfere with sealer adaptation to root canal walls[5]. Therefore, the removal of the smear layer before root canal obturation is recommended.

Ethylenediamine tetra-acetic acid (EDTA), a type of chelating agent, is the most widely used agent for removing the smear layer.[6] It reacts with the calcium ions in dentin and forms soluble calcium chelates and promotes decalcification of dentin. The use of EDTA for extended periods may lower the modulus of elasticity and flexural strength of dentin, which increase the risk of root fracture.[7] Moreover, a previous study showed that using EDTA for smear layer removal is detrimental to the bond strength between calcium silicate cements and dentin.[8]

Chitosan is a natural polysaccharide that has attracted attention in dental research. It has desirable properties such as biocompatibility, biodegradability and lack of toxicity.[9] It also has a chelating property similar to EDTA.[7] However, limited information is available regarding the effect of chitosan on the bonding performance of BC sealers. Because chitosan and EDTA may differently influence dentin surface chemistry and calcium availability, their effects on the bonding mechanism of BC sealers warrant further investigation.

Besides the type of irrigant, the irrigation methods seem to influence smear layer removal. Passive ultrasonic irrigation (PUI) is more effective for bacterial elimination and smear layer removal than conventional syringe irrigation due to the acoustic streaming and cavitation mechanism.[10, 11]

This study aims to compare the push-out bond strength (PBS) and mode of failure of root canal filled with gutta-percha and BC sealer after a final rinse with either 17% EDTA and 0.2% chitosan using conventional syringe irrigation and PUI methods. The null hypothesis tested was that neither the type of chelating agent (17% EDTA or 0.2% chitosan) nor the irrigation technique (conventional syringe irrigation or PUI) would significantly affect the PBS or failure mode of the BC sealer.

Materials and Methods

The sample size was calculated using G*Power software. The calculation was based on the study by Bogari et al. (2022), which evaluated the effect of irrigation protocols on the bond strength of bioceramic sealers. Assuming an effect size of 0.55, an alpha level of 0.05, and a statistical power of 80%, the minimum required sample size was determined to be 13 specimens per group.

Fifty-two extracted human mandibular premolars with fully developed roots were selected for this study. Only teeth with straight roots and free from caries, cracks or fractures were included. All

teeth were kept in 0.1% thymol solution until use. Each tooth was radiographically examined to confirm the presence of a single canal. The crowns were removed using micro cutting instrument (Acutom-50; Struers, Copenhagen, Denmark). The roots were prepared and polished until approximately 16 mm length from the sectioned coronal surface to the apex of the root was obtained. A #10 K-file was inserted through the apical foramen and the working length was obtained by subtracting 1 mm from this measurement. A glide path was established with a #15 K-file at the working length. Root canal preparation was performed using nickel–titanium rotary files (ProTaper Next, Dentsply Maillefer, Switzerland) from X1 to X3 as the master apical file (MAF). The endodontic motor (X-Smart Plus; Dentsply Sirona, Switzerland) was set to 250 rpm and 2.5 N·cm torque. After each instrument, 5 mL of 2.5% NaOCl was used as the irrigant.

Following canal preparation, all specimens were randomly allocated into four experimental groups using a computer-generated randomization sequence, as follows:

Group 1: Syringe irrigation with 17% EDTA

Group 2: PUI with 17% EDTA

Group 3: Syringe irrigation with 0.2% Chitosan

Group 4: PUI with 0.2% Chitosan

All specimens received a final irrigation protocol consisting of 3 mL of either 17% EDTA or 0.2% chitosan. The irrigant was allowed to remain in the canal for a total contact time of 60 seconds. Subsequently, the canals were flushed with 10 mL distilled water to terminate the chelating action and remove residual irrigant before obturation. For Groups 1 and 3, irrigation was performed using a 30-gauge side-vented needle positioned 1 mm short of the working length and moved in a gentle up-and-down motion for 60 seconds. For Groups 2 and 4, passive ultrasonic irrigation (PUI) was performed using an Irrisafe tip size 25 (Acteon, Switzerland) attached to a piezoelectric ultrasonic unit operating at medium power setting according to the manufacturer's recommendations. The activation was performed in two cycles of 30 seconds each, with fresh irrigant replenished before the second cycle. The ultrasonic tip was positioned 1 mm short of the working length without contacting the canal walls.

After the final rinse, each sample was dried with three paper points. The root canals were then filled with gutta-percha cone size X3 (Dentsply Maillefer, Switzerland), and BC sealer (CeraSeal: Metabiomed, Korea) using the matched single-cone technique. CeraSeal was delivered into the canal using the manufacturer-provided intracanal tip. The tip was inserted to approximately the middle third of the canal and withdrawn slowly during sealer placement to promote uniform distribution. A standardized amount of sealer was applied in all specimens according to the manufacturer's instructions. Radiographs were taken in bucco-lingual and mesio-distal directions to verify the obturation quality. All samples were stored at 37°C and 100% humidity for 2 weeks to allow complete sealer setting.

Each sample was embedded in resin molds. After that, the specimens were sectioned 5 mm from the root apex to obtain 2-mm-thick dentin slices as representative specimens of the middle third of root canal. The root filling in each specimen was subjected to loading using a universal testing machine (Shimadzu, model AGS-X, Japan) with 0.5 mm cylindrical plunger (Figure 1). The loading force was applied in an apico-coronal direction to minimize the influence of root canal taper and prevent mechanical locking of the filling material within the canal. The loading speed was set at 1 mm/min until dislodgement of root filling material occurred. Then the force value at the time of dislodgement was recorded in Newtons (N). After that, the force was converted to push-out bond strength in megapascal (MPa) following this formula:

$$MPa = \frac{F}{SL}$$

Where SL (sealer adhesion area) = π(R+r)g

π = 3.14;

R = mean radius of coronal canal, in mm

r = mean radius of apical canal, in mm

g = height relative to tapered inverted cone, in mm[12]

The failure mode was examined under a stereomicroscope at 40× magnification and categorized based on a previous study, as: Adhesive failure at the dentin/sealer or sealer/gutta-percha interface, cohesive failure within the sealer, and mixed failure that includes both adhesive and cohesive failures.[13] Failure mode evaluation was performed independently by two calibrated examiners. Prior to evaluation, ten representative specimens were assessed jointly to establish classification criteria. In cases of disagreement, consensus was reached through discussion.

All statistical analyses were performed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Normality of the data distribution was assessed using the Shapiro–Wilk test, while homogeneity of variance was evaluated using Levene's test. Push-out bond strength values were analyzed using one-way analysis of variance (ANOVA), followed by Bonferroni post hoc comparisons. Failure mode distributions were analyzed using Fisher's exact test. The significance level was set at α = 0.05.



Figure 1 Push-out bond strength assessment using a universal testing machine

Results

Descriptive statistics of the PBS values are presented (Table 1) as mean±standard deviations. Shapiro–Wilk analysis demonstrated that PBS values were normally distributed in all experimental groups (p>0.05). Furthermore, Levene's test confirmed homogeneity of variance among groups (p>0.05), supporting the use of parametric statistical analysis. No statistically significant differences were observed among the four experimental groups (p=0.202). Although Groups 2 and 4 demonstrated numerically higher mean PBS values than Groups 1 and 3, the observed differences did not reach statistical significance.

Table 1 The mean and standard deviation (SD) of push-out bond strengths values (MPa) at middle 1/3 level

Group	Irrigation technique	Push-out bond strength (MPa)	P value
1	Syringe irrigation with 17% EDTA	1.67 ± 0.76	0.202
2	PUI with 17% EDTA	1.88 ± 0.75	
3	Syringe irrigation with 0.2% Chitosan	1.55 ± 0.80	
4	PUI with 0.2% Chitosan	2.29 ± 1.32	

PBS = push-out bond strength;
EDTA = ethylenediaminetetraacetic acid;
PUI = passive ultrasonic irrigation.
Data are presented as mean±standard deviation.
Statistical analysis was performed using one-way ANOVA followed by Bonferroni post hoc testing.
Statistical significance was established at p<0.05.

No statistically significant differences were observed in failure mode distribution among the experimental groups (p = 0.209). Adhesive failure was the predominant mode of failure in all groups, particularly in the conventional syringe irrigation with 0.2% chitosan group (Table 2).

Although the standard deviations represented a relatively large proportion of the corresponding mean values, all datasets satisfied the assumptions of normality and homogeneity of variance. Therefore, parametric statistical analysis was considered appropriate.

Table 2 The frequency of the mode of failure

Experimental groups	Mode of failure			P value
	Adhesive failure	Cohesive failure	Mixed failure	
Syringe irrigation with 17% EDTA	5 (38.4)	2 (15.4)	6 (46.2)	0.209
PUI with 17% EDTA	6 (46.2)	1 (7.7)	6 (46.2)	
Syringe irrigation with 0.2% Chitosan	11 (84.6)	1 (7.7)	1 (7.7)	
PUI with 0.2% Chitosan	8 (61.5)	1 (7.7)	4 (30.8)	

Values are presented as frequency (percentage).
Statistical analysis was performed using Fisher's exact test.
Statistical significance was established at $p<0.05$.

Discussion

The PBS test is widely used to evaluate the dislocation resistance and interfacial adhesion of root filling materials to dentin. Although PBS does not directly measure sealing ability or resistance to microleakage, improved interfacial adaptation may reduce gap formation and contribute to the long-term stability of root canal obturation. Therefore, PBS remains a useful laboratory method for assessing the bonding performance of endodontic sealers.

However, this test model cannot reproduce the exact clinical conditions because root dentin is not uniform and the surface of the canal walls prepared during the chemo-mechanical instrumentation varies considerably.[14,15] The thickness of dentin slices used in previous PBS studies has also varied, ranging from 0.6 mm to 7.0 mm.[15,16] However, the thickness of approximately 1 mm is generally preferred. In the present study, 2-mm-thick slices were used to prevent premature debonding which is in accordance with Kreimer et al.[17]

The obturation technique also influences PBS results. Although the warm vertical compaction technique is considered superior to single-cone technique, it is not recommended for use with BC sealers because the heat applied during warm vertical compaction can alter the material's properties, including adhesion.[18] Therefore, the present study used the matched single-cone technique for root canal obturation.

BC sealers exhibit a unique bonding mechanism compared with conventional resin-based sealers. In addition to micromechanical interlocking, calcium silicate-based sealers release calcium ions that interact with phosphate-containing fluids to form hydroxyapatite deposits at the dentin–sealer interface. This biomineralization process may enhance interfacial adaptation and contribute to bond development over time. Consequently, alterations in dentin surface chemistry caused by chelating agents may influence the bonding performance of bioceramic sealers.

The present study demonstrated no significant differences in PBS between specimens irrigated with 17% EDTA and those irrigated with 0.2% chitosan. Several explanations may account for this finding. Both solutions possess chelating properties and can remove the smear layer, thereby exposing dentinal tubules and facilitating sealer adaptation. Furthermore, because calcium silicate-based sealers rely partially on biomineralization rather than solely on micromechanical retention, minor differences in dentin surface characteristics produced by the two chelating agents may not substantially influence bond strength. In addition, the relatively short contact time of the chelating solutions may have limited their ability to produce measurable differences in dentin substrate characteristics.

Although previous studies have performed the PBS test at different levels of root (coronal, middle and apical), the cross-sectional shape of root canal is typically oval. Consequently, the hydraulic pressure generated by the matched single-cone technique may not be sufficient to force sealer penetration into dentinal tubules, particularly at the coronal 1/3, which may influence PBS values. Moreover, the diameter and number of dentinal tubules decrease from the coronal to the apical regions, making smear layer removal less effective apically.[19] To minimize such confounding factors, this study selected the root slices from the middle 1/3 level as the representative specimens for PBS testing.

In this study, PUI was used as an adjunct to irrigation because several reports have shown that ultrasonic activation enhanced the penetration of irrigating solutions.[20, 21] These findings align with the present study, in which EDTA and chitosan irrigation with PUI produced higher PBS values than conventional syringe irrigation. Although the PUI groups demonstrated numerically higher PBS values than the conventional syringe irrigation groups, the differences were not statistically significant. Therefore, the present findings do not support a definitive influence of irrigation activation on bond strength under the conditions tested. The absence of statistical significance may be explained by the relatively high variability observed within the experimental groups, the modest sample size, and the evaluation being restricted to the middle third of the root canal. Moreover, because calcium silicate-based sealers are less dependent on micromechanical retention than resin-based sealers, enhanced smear layer removal achieved by PUI may not necessarily translate into significantly greater bond strength.

The PBS values obtained in the present study were lower than those reported in some previous investigations evaluating BC sealers. Differences in experimental methodology may explain these discrepancies. Variations in root canal anatomy, dentin slice thickness, obturation technique, type of BC sealer, irrigation protocol, and testing methodology can substantially influence PBS values. Furthermore, the relatively large standard deviations observed in the present study indicate considerable biological variability among human teeth. Nevertheless, all datasets satisfied the assumptions of normality and homogeneity of variance, supporting the validity of the statistical analyses performed.

Adhesive failure was the predominant mode of failure observed in the present study. This finding suggests that the dentin–sealer interface represented the weakest component of the bonded complex. Although BC sealers have the potential to form hydroxyapatite and establish a mineral infiltration zone, the quality and extent of this interaction may vary depending on dentin characteristics, sealer composition, and experimental conditions. The predominance of adhesive failure observed in this study differs from some previous reports in which cohesive failure was more frequently observed. Such discrepancies may be related to differences in sealer formulation, irrigation protocols, specimen preparation, or failure mode classification criteria. The relatively low incidence of cohesive failure suggests that the internal strength of the sealer material exceeded its bond strength to dentin under the present experimental conditions.

Following chelator application, distilled water rather than NaOCl was used as the final rinse. This protocol was selected to minimize potential chemical interactions between residual NaOCl and the BC sealer. Because all experimental groups received the same final irrigation sequence, any influence of this protocol would be expected to affect all groups equally and would therefore be unlikely to alter the comparative findings of the study.

Several limitations of this study should be acknowledged. First, the investigation was conducted

under in vitro conditions and therefore may not fully replicate the clinical environment. Second, only mandibular premolars with relatively straight canals were evaluated. Third, PBS testing was performed only at the middle third of the root canal. Fourth, smear layer removal was not verified using scanning electron microscopy. Finally, only one BC sealer formulation was investigated. Future studies should evaluate different BC sealers, incorporate long-term aging protocols, and assess multiple root levels to improve clinical relevance.

Conclusion

Within the limitations of this in vitro study, no significant differences in push-out bond strength or failure mode were observed between root canals irrigated with 17% EDTA and those irrigated with 0.2% chitosan, regardless of whether conventional syringe irrigation or passive ultrasonic irrigation was used. These findings suggest that 0.2% chitosan may be considered a potential alternative chelating agent for final irrigation when used in conjunction with a BC sealer.

Ethics

This study was approved by the Human Research Ethics Committee (COE NO. 016/2566)

Conflict of Interest

The authors declare no conflict of interest.

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การเปรียบเทียบผลของการใช้สารคีเลต 2 ชนิด และวิธีการล้างคลองรากฟันต่อความแข็งแรงพันธะผลักออกระหว่างไบโอเซรามิกซีลเลอร์กับผนังคลองรากฟัน

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บทความวิจัย

บทคัดย่อ

วัตถุประสงค์: เพื่อเปรียบเทียบความแข็งแรงพันธะผลักออกและรูปแบบการหลุดของคลองรากฟันที่อุดด้วยไบโอเซรามิกซีลเลอร์ภายหลังการล้างคลองรากฟันครั้งสุดท้ายด้วยกรดเอทิลีนไดเอมีนเตตระอะซิติกความเข้มข้น 17% (17% EDTA) และสารไคโตซานความเข้มข้น 0.2% (0.2% chitosan) ด้วยวิธีการใช้เข็มล้างและการล้างด้วยระบบอัลตราโซนิกส์แบบไร้แรง

วัสดุอุปกรณ์และวิธีการ: ฟันกรามน้อยขากรรไกรล่างของมนุษย์ที่ถูกถอนจำนวน 52 ซี่ ตัดส่วนตัวฟันออกให้เหลือความยาวประมาณ 16 มม. ทำการขยายคลองรากฟันด้วยนิกเกิลไทเทเนียมโรตารี่ Protaper Next จนถึงขนาด X3 จากนั้นสุ่มตัวอย่างกลุ่มละ 13 ชิ้น จำนวน 4 กลุ่ม ตามชนิดของน้ำยาและวิธีการล้าง ได้แก่ กลุ่มที่ 1: การล้างด้วยเข็มล้างร่วมกับ 17% EDTA กลุ่มที่ 2: การล้างด้วย PUI ร่วมกับ 17% EDTA กลุ่มที่ 3: การล้างด้วยเข็มล้างร่วมกับสารไคโตซาน 0.2% และกลุ่มที่ 4: การล้างด้วย PUI ร่วมกับสารไคโตซาน 0.2% ทำการอุดคลองรากฟันด้วยแท่งกัตตาเปอร์ชาเบอร์ X3 ร่วมกับไบโอเซรามิกด้วยเทคนิคกัตตาเปอร์ชาแท่งเดียวที่มีขนาดพอดีกับคลองรากฟัน เก็บตัวอย่างไว้ที่อุณหภูมิ 37°C และความชื้น 100% เป็นเวลา 2 สัปดาห์ เพื่อให้ซีลเลอร์แข็งตัว จากนั้นตัดตัวอย่างที่ตำแหน่ง 5 มม. จากปลายราก โดยให้ได้ชิ้นเนื้อฟันหนา 2 มม. ซึ่งใช้เป็นตัวแทนของคลองรากฟันส่วนกลาง ทดสอบความต้านทานต่อการหลุดของวัสดุอุดคลองรากฟันด้วยเครื่องทดสอบแรงสากล และคำนวณเป็นค่าความแข็งแรงพันธะผลักออก รูปแบบการหลุดของวัสดุอุดคลองรากฟันตรวจสอบด้วยกล้องสเตอริโอไมโครสโคปที่กำลังขยาย 40 เท่า เปรียบเทียบค่าความแข็งแรงพันธะผลักออกแต่ละกลุ่มด้วยการทดสอบอะโนวา และวิเคราะห์ความแตกต่างระหว่างกลุ่มด้วยการทดสอบบอนเฟอร์โรนี สำหรับรูปแบบการหลุดของวัสดุอุดคลองรากฟันทำการวิเคราะห์ด้วยวิธีฟิชเชอร์ กำหนดระดับนัยสำคัญที่ 0.05

ผล: ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่าเฉลี่ยแรงยึดติดแบบผลักออกหรือรูปแบบการหลุดระหว่างกลุ่มทดลอง ค่าเฉลี่ย PBS ของกลุ่มที่ 1, 2, 3 และ 4 เท่ากับ 1.67±0.76 MPa, 1.88±0.75 MPa, 1.55±0.80 MPa และ 2.29±1.32 MPa ตามลำดับ โดยไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่ม (p=0.202) นอกจากนี้ การกระจายตัวของรูปแบบการล้มเหลวยังไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่มทดลอง (p=0.209)

บทสรุป: ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของค่าแรงยึดติดแบบผลักออกหรือรูปแบบการหลุด ระหว่างคลองรากฟันที่ได้รับการล้างด้วยสารละลาย EDTA ความเข้มข้น 17% และคลองรากฟันที่ได้รับการล้างด้วยไคโตซานความเข้มข้น 0.2%

คำไขรหัส: ไบโอเซรามิกซีลเลอร์/ ไคโตซาน/ การล้างด้วยระบบอัลตราโซนิกส์แบบไร้แรง/ ความแข็งแรงพันธะผลักออก

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