EUROPEAN ENDODONTIC JOURNAL

ORIGINAL ARTICLE

Effect of Ultrasonic Activation on Dentinal Tubule Penetration of Bio-C Temp and Ultracal XS: A Comparative CLSM Assessment

Fernando PEÑA-BENGOA,[1] María Consuelo MAGASICH,[2] Delia BUSTAMANTE,[2] Catalina WASTAVINO,[2] Sven Eric NIKLANDER,[3] Carolina CÁCERES[1]

[1]Department of Endodontics, Universidad Andres Bello Faculty of Dentistry, Viña del Mar, Chile
[2]Private Practice, Viña del Mar, Chile
[3]Department of Oral Pathology and Medicine, Universidad Andres Bello Faculty of Dentistry, Viña del Mar, Chile

ABSTRACT

Objective: The aim of this study was to compare the effect of ultrasonic activation (UA) on tubular penetration between Bio-C Temp and Ultracal XS intracanal medicaments.

Methods: Forty single-rooted human premolars were endodontically prepared and divided into 4 experimental groups (n=10): Bio-C Temp, Bio-C Temp+UA, UltraCal XS and UltraCal XS+UA. All medicaments were previously mixed with a specific calcium marker (Fluo-3) and passively injected into the canals. The samples were incubated for 7 days. For each tooth, 1 mm thick sections were obtained from the middle and apical thirds of the canals. The samples were examined by confocal laser scanning microscopy (CLSM) and the depth and area of penetration were determined for each group. The Student t test was used to compare results between groups ($p<0.05$).

Results: UA increased the depth and penetration area of Bio-C Temp and Ultracal XS, showing significant differences in the penetration area of the apical third for Bio-C Temp ($p<0.0339$). Bio-C Temp presented greater tubular penetration than Ultracal XS, showing significant differences in the depth of penetration in the apical third ($p<0.0005$), and in the penetration area in the middle ($p<0.0016$) and apical third ($p<0.0339$) after UA.

Conclusion: UA increases tubular penetration (both depth and area) of Bio-C Temp at the apical third but has no significant effect on Ultracal XS. Bio-C Temp has a greater depth and tubular penetration area than Ultracal XS after UA.

Keywords: Calcium hydroxide, calcium silicate, confocal laser scanning microscopy, endodontics, ultrasonics

HIGHLIGHTS

- Bio-C Temp is one calcium silicate-based intracanal medicament which has been proposed as an alternative to calcium hydroxide.
- The comparison of these new medicaments with respect to those considered "Gold Stardard" is essential to evaluate their performance.
- The physical properties of Bio-C Temp associated with ultrasonic activation could present a more homogeneous distribution and greater dentine penetration.
- The results showed that Bio-C Temp has a greater depth and area of tubular penetration than Ultracal XS after ultrasonic activation.

Please cite this article as:
Peña-Bengoa F, Magasich MC, Bustamante D, Wastavino C, Niklander SE, Cáceres C. Effect of Ultrasonic Activation on Dentinal Tubule Penetration of Bio-C Temp and Ultracal XS: A Comparative CLSM Assessment. Eur Endod J 2023; 8: 268-73

Address for correspondence:
Fernando Peña-Bengoa
Department of Endodontics, Universidad Andres Bello Faculty of Dentistry, Viña del Mar, Chile
E-mail: fernandopenab@gmail.com

Received February 09, 2023,
Revised April 21, 2023,
Accepted May 25, 2023

Published online: July 12, 2023
DOI 10.14744/eej.2023.24196

INTRODUCTION

The main purpose of endodontic treatment in teeth with apical periodontitis, is to reduce bacterial load to levels compatible with periapical health, favouring tissue repair, avoiding bacterial recolonization (1). In necrotic teeth, the use of medicaments between sessions is a common practice used to maximize and potentiate the antibacterial effect of the chemo-mechanical preparation, with the aim of preventing residual microorganisms from reinfecting the treated canal, which would lead to endodontic failure (1, 2).

Calcium hydroxide (CH) is one of the most commonly used medicaments in endodontics (3). Its antibacterial properties (4), ability to induce hard tissue deposition (5) and tissue dissolution (6) (among other advantages), have make it the most used intracanal medication (7). CH is highly alkaline with slow-acting antiseptic properties causing irreversible alterations on the bacterial cell wall and protein structure (4). Its mechanism of action is based on the ionic dissociation of Ca^{2+} and OH^{-}. It acts on the dentine, spreading through the dentinal tubules if they are permeable enough (3). This is a relevant point, considering that 70% of the teeth with primary infections will present infected dentinal tubules (8). These may remain infected after the chemo-mechanical preparation of the canal, which can obliterate (partially or totally) the dentinal tubules, as a result of smear layer generation (9).

Today, there is a wide variety of commercial CH medicaments, which differ in their composition and CH concentration (10). One of the most commonly used CH is UltraCal XS (Ultradent Products, South Jordan, UT, USA), which is composed of CH, barium sulphate and methylcellulose, in an aqueous solution (11). According to Komabayashi et al. (12), Ultracal XS presents smaller and more regular particles of CH than CH in its research-grade, which improves its wetting abilities on the dentinal surface, favouring tubular penetration. On the other hand, the vehicle in which CH is immersed plays an important role, determining the velocity of ionic dissociation and the ability to be reabsorbed (13). An aqueous vehicle allows a high ionic dissociation and a higher rate of reabsorption, while viscous vehicles prolong the action of the paste, keeping it in the desired area for longer, delivering Ca^{2+} and OH^{-} ions at a lower velocity (14).

The increasing development and promising results shown by bioceramic materials have led researchers to seek new applications for these types of materials. Bio-C Temp (Angelus, Paraná, Brazil) is the first calcium-silicate based medicament for use between sessions. It was presented as a new alternative to CH, indicated for medication between sessions in necrotic teeth or retreatments, management of internal and/or external root resorption, and for the treatment of immature teeth (15). According to the manufacturer, Bio-C Temp is a highly alkaline material that has micronized particles (<2 µm), which improves flow, penetration into dentinal tubules, and material reactivity, favouring the release of Ca^{2+} and OH^{-} ions (16).

Ultrasound is a versatile tool that can be used in all stages of the endodontic treatment (17). One of its most widely used applications is for the ultrasonic activation (UA) of irrigant solutions, a widely documented procedure that has been shown to increase the cleaning and decontamination of the canals, allowing solutions to penetrate the dentinal tubules and anatomical complexities (18). Its mechanism of action is based on acoustic transmission, where activation of an ultrasonic tip within a shaped root canal produces a small and intense circular fluid movement around the tip (6). The UA in a shaped canal, allows the ultrasonic tips to vibrate freely, transferring energy to irrigant solutions inside the canal (6). UA has also been used successfully to obtain a greater projection and tubular penetration of medicaments and sealers, increasing the antimicrobial action of CH (17, 19) and reducing medication time between sessions (20), as well as preventing reinfection over time (21–24).

Although the UA of CH has shown favourable results regarding tubular penetration (3, 17, 19), the effect of UA on tubular penetration of calcium-silicate based medicaments has not been tested. For this reason, the aim of this study was to analyze and compare, using confocal laser scanning microscopy (CLSM), the effect of UA on the tubular penetration of Bio-C Temp and UltraCal XS.

MATERIALS AND METHODS

This study was approved by the Ethical-Scientific Committee of the local institution (Approval number 95-22).

For this experimental study, human maxillary and mandibular premolars with completely formed root apices extracted for orthodontic indications (patients between 15–25 age) were used. All teeth were preserved in a 0.9% saline solution at 4°C immediately after extraction, for a period of no more than 3 months. A non-probabilistic sample, based on studies with a similar methodology (3, 21), was used to select 40 healthy single-rooted premolars with oval and straight canals. Oval canal was considered when the buccolingual to the mesiodistal ratio of 2 or higher at 5 mm distance from the apex.These parameters were confirmed by a cone-beam tomography (CB-500, Gendex, Pennsylvania, USA) using a 0.2 mm voxel size, 160–80 mm FOV, 120 kV, 5 mA, and 14.7 second exposure. Premolars with previous endodontic treatment, signs of fracture, resorption, and medium or severe curvatures (>10°) according to the Schneider´s classification were extruded.

Sample Preparation

All samples were cleaned with P1 ultrasonic periodontal tips (Woodpecker, Guangxi, China) and prophylaxis brushes to remove hard and/or soft tissue traces. The crowns were sectioned with a 911 HK.104.220 diamond disc (Komet, Lemgo, Germany) to achieve a standardized root length of 14 mm. Root canals were prepared at a working length (WL) of 13 mm with the WaveOne Gold system (Dentsply Sirona, Ballaigues, Switzerland), using an X-SMART Plus motor (Dentsply Sirona) with the reciprocating program for the system. After verifying the patency of the canal with a K #10 instrument, chemo-mechanical preparation began with a Primary file (25/.07), using 3-mm-deep in and out movements. Once WL was reached, the patency of the foramen was checked and the canals were irrigated with copious amounts of 2.5% sodium hypochlorite (NaOCl) using Navitip 30-G irrigation needles (Ultradent, South Jordan, UT, USA). The final shaping of the canals was performed with a Medium file (35/.06). During each canal preparation, 20 mL of 2.5% NaOCl were used. Final irrigation was performed

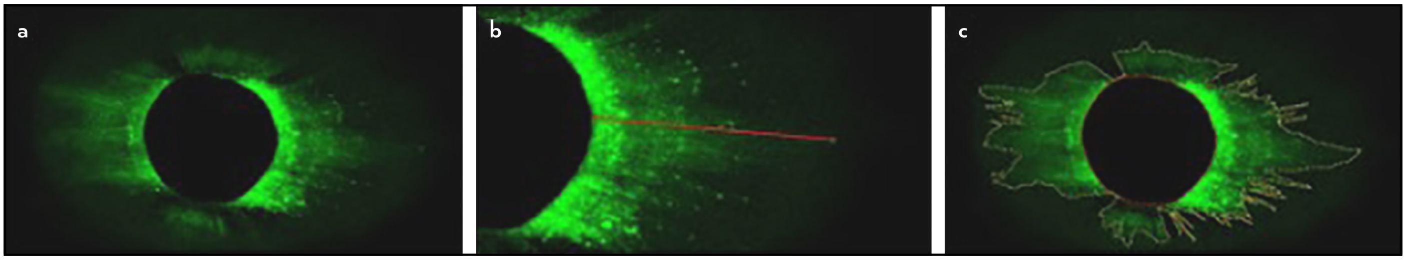

Figure 1. Representative images of the measurement stage. (a) Initial image. (b) Maximum tubular penetration: Straight red line from the contour of the shaped canal to the maximum penetration. (c) Total area of penetration: Delimitation of the cross-sectional area of penetration

with 5 mL of EDTA 17% for 3 minutes, followed by 5 mL of 2.5% NaOCl. The canals were dried with #35 paper points.

The teeth were randomly divided with an Excel spreadsheet (Microsoft Office, Washington, USA), into 4 groups (n=10): Bio-C Temp; Bio-C Temp+AU; UltraCal XS; UltraCal XS+UA.

Sample Medication

All experimental procedures were developed by a single operator. A specific fluorescent calcium indicator (Fluo-3, Thermo Fisher Scientific, Massachusetts, USA), at a rate of 0.1%, was added to Bio-C Temp and UltraCal XS. The medicaments were applied following the instructions of each manufacturer. For those groups in which AU was applied, an E1 Irrisonic ultrasonic tip (Helse Ultrasonics, Santa Rosa de Viterbo, Brazil) calibrated 2 mm short to WL was used. The E1 Irrisonic tip was used in a Varios 2 device (NSK Brasil Ltda, São Paulo, Brazil) with a power of 10% for 30 seconds at the mesial-distal direction and another 30 seconds at the buccal-lingual direction reaching 1 minute of total UA for each sample. The samples were sealed at the canal entrance with glass ionomer (Ketac Molar, 3M ESPE, Neuss, Germany), individually mounted in transparent acrylic cubes (Marché, Santiago, Chile) and were incubated for 7 days at 37°C and 100% humidity (Amilab, Santiago, Chile). After the incubation period, the roots were transversely sectioned at 3 mm and 6 mm from the apex using an IsoMet 1000 metallographic cutter (Buehler, Illinois, USA), obtaining 2 samples of 1 mm thickness, corresponding to the apical and middle third of the root canal.

Confocal Laser Scanning Microscopy Evaluation

The samples were analyzed by a confocal laser scanning microscope (D-eclipse C1, Nikon, Tokyo, Japan) using 10x magnification and a scale set to 100 µm. The images were analyzed with Image J (Rasband, W.S., U.S. NIH, Maryland, USA), and calibrated (Fig. 1a). To determine the maximum penetration depth, a straight line was drawn between the point of maximum penetration and the wall of the shaped canal (Fig.1b). The total area of penetration was determined by marking the total area of the dentine penetrated by the drug minus the area of the shaped canal (Fig. 1c).

Statistical Analysis

Statistical analysis was done using GraphPad Prism (GraphPad Software, version 9.4.1, California, USA). For determining statistical differences between groups, the t Student's test was applied. Statistically significant differences were considered with $p<0.05$.

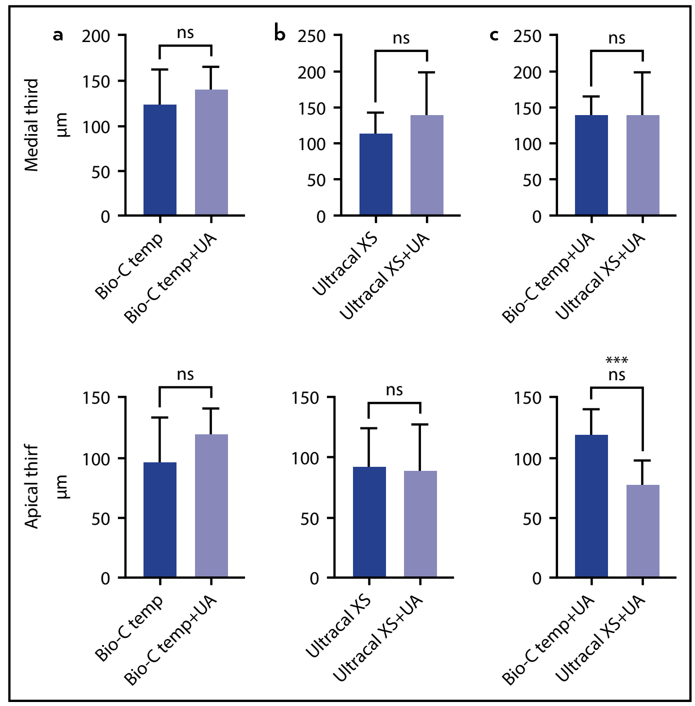

Figure 2. Maximum penetration depth (µm) achieved by: (a) Bio-C Temp v/s Bio-C Temp+UA; (b) UltraCal XS v/s UltraCal XS+UA; (c) Bio-C Temp+UA v/s UltraCal XS+UA

***: p=0.0005. ns: No significant differences, UA: Ultrasonic activation

RESULTS

There was a slight increase in the maximum depth of penetration after applying ultrasonic activation in all groups (apart from Ultracal XS at the apical third), but for neither of the medicaments the changes reached statistical significance ($p>0.05$), (Fig. 2a, b). Nevertheless, when comparing both medicaments at the apical third after UA, Bio-C Temp+UA had a statistically significant higher maximum depth of penetration than UltraCal XS+UA ($p=0.0005$) (Fig. 2c and Fig. 3). No differences were found when comparing both medicaments without ultrasonic activation, neither at the medial or apical third ($p>0.05$).

Ultrasonic activation increased the area of penetration of Bio-C Temp both at the medial and apical thirds, but the increase was statistically significant only at the apical third ($p=0.0016$) (Fig. 4a). UA slightly increased the area of penetration of UltraCal XS in both medial and apical thirds, but the changes were not statistically significant ($p>0.05$) (Fig. 4b). The area of

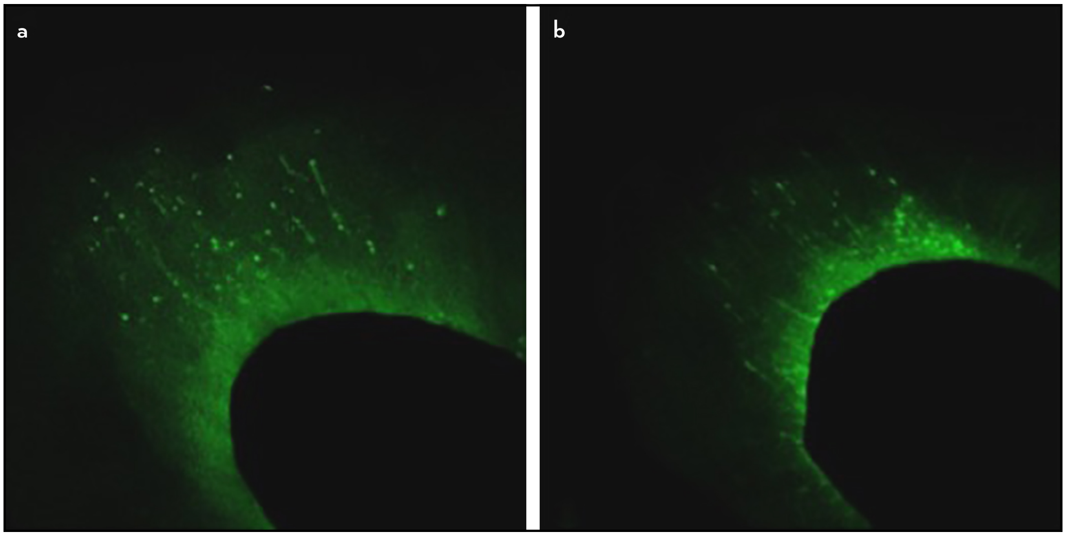

Figure 3. Image of the apical third showing the maximum penetration depth achieved by (a) Bio-C Temp+UA and (b) UltraCal XS+UA

UA: Ultrasonic activation

penetration of Bio-C Temp+UA was significantly higher than the one from UltraCal XS+UA, at both medial ($p=0.0339$) and apical ($p=0.0075$) thirds (Fig. 4c and Fig. 5).

DISCUSSION

Dentinal tubular penetration of intracanal medicaments in teeth with apical periodontitis is a desirable property, considering that the persistence of microorganisms inside the tubules is associated with treatment reinfection (8). Microorganisms can penetrate up to 300 µm into the dentinal tubules (25), but their endotoxins can penetrate up to 500 µm (26). Therefore, the penetration properties of antiseptic agents are important aspects to consider when treating teeth with infected canals.

Ultrasonic activation of endodontic medicaments was introduced by Duarte et al. (17). The authors reported an increase in the release of Ca^{2+} ions and pH after UA of CH applied on the external surface of teeth with simulated root resorptions, attributing these results to greater tubular penetration (17). Although we did observe a tendency of UA to increase tubular penetration in both Bio-C Temp and Ultracal XS, the increase did not reach statistical significance when compared with the tubular penetration obtained when they were placed passively in the root canals according to the manufacturer's instructions. These results can be explained from an anatomical and mechanical point of view, considering the size of the canals and the dimensions of the ultrasonic tip. The samples used in this study corresponded to young premolars extracted for orthodontic indication with wide and oval canals. On the other hand, the E1 Irrisonic tip has a 20 size ISO tip and a 1% taper (27), dimensions that differ significantly from the size of these canals, which can cause that the acoustic transmission that reached the dentinal walls had a suboptimal intensity. Another factor to consider is the consistency of the medication pastes, however, the literature on this point is controversial. According to Arias et al. (19) UA on viscous substances such as medication pastes or root canal sealers, can be attenuated due to the different physical properties of these agents. On the other hand, Pereira et al. (28), reported that the use of CH using aqueous or viscous vehicles does not show significant differences with respect to the tubular penetration of the medication, however it is important to mention that these results were obtained without considering the use of UA.

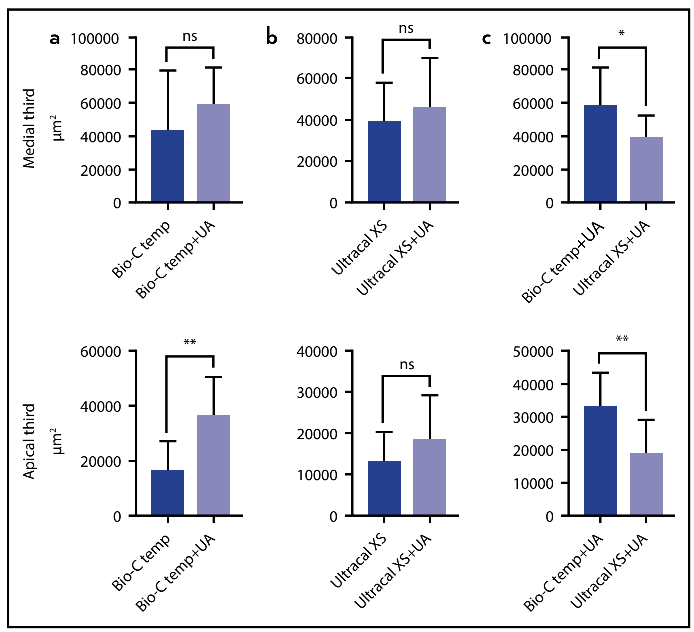

Figure 4. Penetration area (µm²) reached by: (a) Bio-C Temp v/s Bio-C Temp+UA; (b) UltraCal XS v/s UltraCal XS+UA; (c) Bio-C Temp+UA v/s UltraCal XS+UA

*: $p=0.0339$, **: $p<0.008$. ns: No significant differences, UA: Ultrasonic activation

The goal of using UA on medication is to improve mobilization of these substances into non-instrumented and/or difficult-to-access areas, favouring a deeper and more homogenous distribution through a micro-acoustic stream, increasing the total area of penetration (29). We observed this effect on Bio-C Temp, as UA significantly increased the penetration area in the apical thirds of

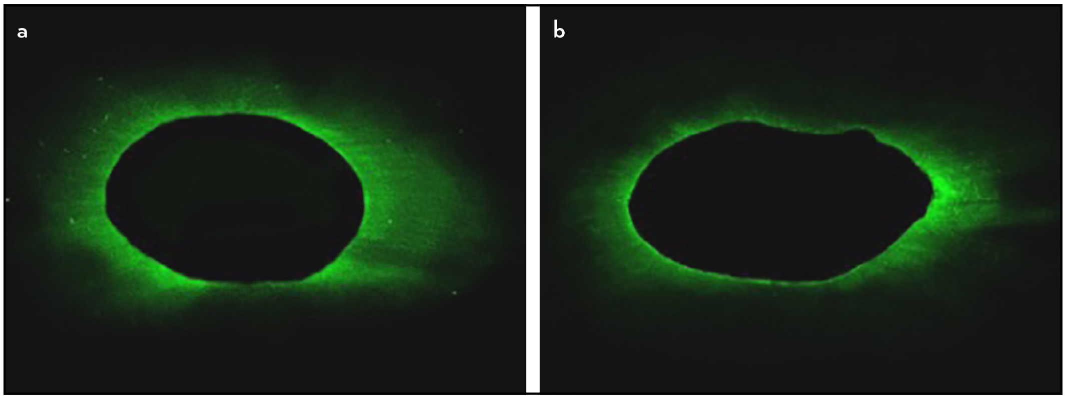

Figure 5. Image of the apical third showing the penetration area achieved by (a) Bio-C Temp+UA and (b) UltraCal XS+UA

UA: Ultrasonic activation

the canals. An increase in the middle third of the roots was also observed but did not reach statistical significance. These results can be explained because premolars, regardless of the diameter of the canal, present a significant decrease in their diameter towards the apical third (30), which would allow acoustic transmission to be generated closer to the canal walls, pushing the medicaments more intensely towards the walls. The results of UA on Bio-C Temp are promising, as the apical third of the root canal is considered a critical zone, and its decontamination is essential for the success of the endodontic treatment (31). The dentine of the apical third is characterized by having a smaller number and size of tubules (3), therefore its decontamination can be a challenge. The fact that UA increases the penetration area of Bio-C Temp in the apical third can be of clinical significance, but this must be evaluated in long-term clinical trials.

Bio-C Temp presented greater tubular penetration than Ultracal XS after UA, evidenced in greater depth and area of penetration (middle and apical third) highlighting the favorable physical properties that this new medication pastes presents compared to Ultracal XS. According to Komabayashi et al. (12), the ability of a material to penetrate the dentine depends on the diameter of the tubules and the size of the particles. UltraCal XS presents irregular particles and a size that can vary between 0.5–>2.5 µm (12), while Bio-C Temp presents regular particles with a diameter of less than 2.0 µm (16). Considering that the dentinal tubules have a diameter between 1–3 µm, particles smaller than these dimensions are required to achieve an effective tubule penetration, even more if they are placed passively (32). The vehicle of in which the medication is incorporated is another factor that can influence its penetration and diffusion into the dentinal tubules (2, 3, 28). The vehicle used by UltraCal XS is aqueous (11) while the one used by Bio-C Temp is viscous (16, 33), reducing Bio-C Temp surface tension facilitating a greater penetration into the dentinal tubules (20). Particle size, vehicle and anatomy of the canals are all factors that must be considered when analyzing dentinal tubular penetration of a root canal medication (28). The favorable physical properties of Bio-C Temp make it an interesting option as an alternative to Ultracal XS; however, future studies that evaluate other characteristics of this new medication are necessary to have a more complete view of the profile of Bio-C Temp.

One of the limitations of this study is the type of samples used. The use of paired teeth can be interesting to obtain more precise comparisons regarding the tubular penetration of the medication pastes. On the other hand, the incorporation of samples from the cervical area can provide a broader view of the results throughout the entire root canal. Although the cervical third is an area in which important dentine removal is performed during the root canal shaping which is also subjected to a significant volume and flow of irrigants, it is an area with a greater number and diameter of dentine tubules that potentially may be contaminated.

From a clinical point of view, a greater tubular penetration of an intracanal medicament is desirable, as this would favour a greater antibacterial action positively affecting the treatment prognosis (34). Nevertheless, root canal sealers can also penetrate the dentinal tubules and could interact with previously used medications, affecting the bonding and sealing ability of root canal sealers (35).Therefore, more research is needed to study possible interactions between materials. The literature regarding calcium-silicate based medicaments is still scarce. More studies are needed to analyze the effect of UA on the properties of this type of medicaments, in order to draw robust conclusions regarding its performance as medication between sessions.

CONCLUSION

Ultrasonic activation increases tubular penetration (both depth and area) of Bio-C Temp at the apical third but has no significant effect on Ultracal XS. Bio-C Temp has a greater depth and tubular penetration area than Ultracal XS after ultrasonic activation.

Disclosures

Conflict of interest: The authors deny any conflict of interest.

Ethics Committee Approval: This study was approved by The Andres Bello University Ethical-Scientific Committee (Date: 25/05/2022, Number: 95-22).

Peer-review: Externally peer-reviewed.

Financial Disclosure: This study did not receive any financial support.

Authorship contributions: Concept – F.P.B., M.C.M., C.C.; Design – F.P.B., M.C.M.; Supervision – F.P.B., C.C., S.E.N.; Funding - M.C.M., D.B., C.W., C.C.; Materials - M.C.M., D.B., C.W.; Data collection and/or processing – M.C.M., D.B., C.W.; Analysis and/or interpretation – F.P-B., M.C.M., C.C.; Literature search – F.P.B., M.C.M., D.B., C.W.; Writing – F.P-B., S.E.N.; Critical Review – F.P-B., S.E.N.

REFERENCES

1. Vera J, Siqueira JF, Ricucci D, Loghin S, Fernández N, Flores B, et al. One-versus two-visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. J Endod 2012; 38(8):1040–52. [CrossRef]
2. Zancan RF, Vivan RR, Milanda Lopes MR, Weckwerth PH, de Andrade FB, Ponce JB, et al. Antimicrobial activity and physicochemical properties of calcium hydroxide pastes used as intracanal medication. J Endod 2016; 42(12):1822–8. [CrossRef]
3. de Almeida Barbosa M, de Oliveira KV, dos Santos VR, da Silva WJ, Tomazinho FSF, Baratto-Filho F, et al. Effect of vehicle and agitation methods on the penetration of calcium hydroxide paste in the dentinal tubules. J Endod 2020; 46(7):980–6. [CrossRef]
4. Siqueira JF, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999; 32(5):361–9. [CrossRef]
5. Mohammadi Z, Dummer PMH. Properties and applications of calcium hydroxide in endodontics and dental traumatology: calcium hydroxide in endodontics and dental traumatology. Int Endod J 2011; 44(8):697–730.
6. Wiseman A, Cox TC, Paranjpe A, Flake NM, Cohenca N, Johnson JD. Efficacy of sonic and ultrasonic activation for removal of calcium hydroxide from mesial canals of mandibular molars: a microtomographic study. J Endod 2011; 37(2):235–8. [CrossRef]
7. Martinho FC, Gomes CC, Nascimento GG, Gomes APM, Leite FRM. Clinical comparison of the effectiveness of 7- and 14-day intracanal medications in root canal disinfection and inflammatory cytokines. Clin Oral Investig 2018; 22(1):523–30. [CrossRef]
8. Matsuo T, Shirakami T, Ozaki K, Nakanishi T, Yumoto H, Ebisu S. An immunohistological study of the localization of bacteria invading root pulpal walls of teeth with periapical lesions. J Endod 2003; 29(3):194–200.
9. Morago A, Ruiz-Linares M, Ferrer-Luque CM, Baca P, Rodríguez Archilla A, Arias-Moliz MT. Dentine tubule disinfection by different irrigation protocols. Microsc Res Tech 2019; 82(5):558–63. [CrossRef]
10. Hawkins JJ, Torabinejad M, Li Y, Retamozo B. Effect of three calcium hydroxide formulations on fracture resistance of dentin over time. Dent Traumatol 2015; 31(5):380–4. [CrossRef]
11. Guerreiro JCM, Ochoa-Rodrígez VM, Rodrigues EM, Chavez-Andrade GM, Tanomaru-Filho M, Guerreiro-Tanomaru JM, et al. Antibacterial activity, cytocompatibility and effect of Bio-C Temp bioceramic intracanal medicament on osteoblast biology. Int Endod J 2021; 54(7):1155–65.
12. Komabayashi T, Ahn C, Spears R, Zhu Q. Comparison of particle morphology between commercial- and research-grade calcium hydroxide in endodontics. J Oral Sci 2014; 56(3):195–9. [CrossRef]
13. Ballal NV, Shavi GV, Kumar R, Kundabala M, Bhat KS. *In vitro* sustained release of calcium ions and ph maintenance from different vehicles containing calcium hydroxide. J Endod 2010; 36(5):862–6. [CrossRef]
14. Fava LRG, Saunders WP. Calcium hydroxide pastes: classification and clinical indications. Int Endod J 1999; 32(4):257–82. [CrossRef]
15. Villa N, Santos VVD, Costa UMD, Mendes AT, Duarte PHM, Rosa RAD, et al. A new calcium silicate-based root canal dressing: physical and chemical properties, cytotoxicity and dentinal tubule penetration. Braz Dent J 2020; 31(6):598–604. [CrossRef]
16. Angelus. Bio-C® Temp. Available at: https://angelus.ind.br/assets/uploads/2022/06/Folder-BIO-C%C2%AE-TEMP-Angelus-ES.pdf. Accessed Jun 22, 2023.
17. Duarte MAH, Balan NV, Zeferino MA, Vivan RR, Morais CAH, Tanomaru-Filho M, et al. Effect of ultrasonic activation on ph and calcium released by calcium hydroxide pastes in simulated external root resorption. J Endod 2012; 38(6):834–7. [CrossRef]
18. Plotino G, Cortese T, Grande NM, Leonardi DP, Di Giorgio G, Testarelli L, et al. New technologies to improve root canal disinfection. Braz Dent J 2016; 27(1):3–8. [CrossRef]
19. Arias MP, Maliza AG, Midena RZ, Graeff MS, Duarte MA, Andrade FB. Effect of ultrasonic streaming on intra-dentinal disinfection and penetration of calcium hydroxide paste in endodontic treatment. J Appl Oral Sci 2016; 24(6):575–81. [CrossRef]
20. de Andrade FB, da Silva Munhoz Vasconcelos LR, Pereira TC, Garcia RB, Bramante CM, Duarte MAH. Ultrasonic agitation reduces the time of calcium hydroxide antimicrobial effect and enhances its penetrability. J Mater Sci Mater Med 2021; 32(12):150. [CrossRef]
21. De Bem IA, de Oliveira RA, Weissheimer T, Bier CAS, Só MVR, Rosa RAD. Effect of ultrasonic activation of endodontic sealers on intratubular penetration and bond strength to root dentin. J Endod 2020; 46(9):1302–8.
22. Eymirli A, Sungur DD, Uyanik O, Purali N, Nagas E, Cehreli ZC. Dentinal tubule penetration and retreatability of a calcium silicate–based sealer tested in bulk or with different main core material. J Endod 2019; 45(8):1036–40. [CrossRef]
23. Yamini B, Gali PK, Nagesh B, Varri S, Garlapati R, Naik KMK. Effect of indirect ultrasonic activation of modified bioceramic materials on the bond strength and tubular penetration in root canals. Dent Res J (Isfahan) 2021; 18:45. [CrossRef]
24. Guimarães BM, Amoroso-Silva PA, Alcalde MP, Marciano MA, Bombarda de Andrade F, Hungaro Duarte MA. Influence of ultrasonic activation of 4 root canal sealers on the filling quality. J Endod 2014; 40(7):964–8.
25. Ando N, Hoshino E. Predominant obligate anaerobes invading the deep layers of root canal dentine. Int Endod J 1990; 23(1):20–7. [CrossRef]
26. Wong DTS, Cheung GSP. Extension of bactericidal effect of sodium hypochlorite into dentinal tubules. J Endod 2014; 40(6):825–9. [CrossRef]
27. Acris De Carvalho FM, Silva-Sousa YTC, Saraiva Miranda CE, Miller Calderon PH, Barbosa AFS, Domingues De Macedo LM, et al. Influence of ultrasonic activation on the physicochemical properties of calcium silicate-based cements. Int J Dent 2021; 2021:6697988. [CrossRef]
28. Pereira TC, da Silva Munhoz Vasconcelos LR, Graeff MSZ, Ribeiro MCM, Duarte MAH, de Andrade FB. Intratubular decontamination ability and physicochemical properties of calcium hydroxide pastes. Clin Oral Investig 2019; 23(3):1253–62. [CrossRef]
29. Salas H, Castrejon A, Fuentes D, Luque A, Luque E. Evaluation of the penetration of CHX 2% on dentinal tubules using Conventional Irrigation, Sonic Irrigation (EDDY) and Passive Ultrasonic Irrigation (PUI) techniques: an *in vitro* study. J Clin Exp Dent 2021; 13(1):e37–42. [CrossRef]
30. Bürklein S, Heck R, Schäfer E. Evaluation of the root canal anatomy of maxillary and mandibular premolars in a selected German population using cone-beam computed tomographic data. J Endod 2017; 43(9):1448–52.
31. Galler KM, Grubmüller V, Schlichting R, Widbiller M, Eidt A, Schuller C, et al. Penetration depth of irrigants into root dentine after sonic, ultrasonic and photoacoustic activation. Int Endod J 2019; 52(8): 1210–7. [CrossRef]
32. Donnermeyer D, Schmidt S, Rohrbach A, Berlandi J, Bürklein S, Schäfer E. Debunking the concept of dentinal tubule penetration of endodontic sealers: sealer staining with Rhodamine B fluorescent dye is an inadequate method. Materials (Basel) 2021; 14(12):3211. [CrossRef]
33. Oliveira LV, Silva GR, Souza GL, Magalhães TEA, Barbosa GLR, Turrioni AP, et al. A laboratory evaluation of cell viability, radiopacity and tooth discoloration induced by regenerative endodontic materials. Int Endod J 2020; 53(8):1140–52. [CrossRef]
34. Solakoğlu E, Topçuoğlu HS, Düzgün S. Effect of different final irrigation agitation techniques on root canal dentin tubule penetration of nanoparticle calcium hydroxide dressing. Aust Endod J 2023 May 5. doi: 10.1111/aej.12765. [Epub ahead of print]. [CrossRef]
35. Uzunoglu-Özyürek E, Erdoğan Ö, Aktemur Türker S. Effect of calcium hydroxide dressing on the dentinal tubule penetration of 2 different root canal sealers: a confocal laser scanning microscopic study. J Endod 2018; 44(6):1018–23. [CrossRef]