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BASIC RESEARCH:

Intratubular Penetration Depth of Ultrasound Activated Bioceramic Sealant Cements at the Dentin-Radicular Cement Interface

Profundidad de penetración intratubular de cementos sellantes biocerámicos activados por ultrasonidos en la interfase dentina-cemento radicular

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ABSTRACT: To evaluate the intratubular penetration depth of ultrasound-activated bioceramic sealant cements into root dentin, specifically at the dentin-cement interface. This in vitro experimental study was conducted with samples that were randomly divided based on the type of sealer used and whether ultrasound activation was applied prior to obturation. This resulted in four experimental groups (n=40). Groups A1 and B1 utilized the Eighteeth Ultra X ultrasonic activator with a blue tip #20 featuring a 2% taper at low power for activation, while groups A2 and B2 did not employ any form of activation. The symbol (+) denotes groups with ultrasonic activation, while (-) represents groups without ultrasonic activation. In the middle third, it was observed that BioC Sealer (+) had a mean sealing value of 0.66±0.17, while MTA Fillapex (+) showed a mean value of 0.38±0.07. It was found that BioC Sealer (-) had a mean sealing value of 0.24±0.04 in the middle third, while MTA Fillapex (-) showed a mean value of 0.16±0.04. These differences were not statistically significant (p=0.569). In the apical third, the BioC Sealer (-) presented a mean sealing value of 0.44±0.17, while the MTA Fillapex (-) obtained a mean value of 0.30±0.04. However, this difference did not reach statistical significance (p=0.0745), suggesting that both materials can provide a similar seal in the apical third. The results suggest that both BioC Sealer and MTA Fillapex provide a comparable seal in the middle and apical thirds of the root canal system, however, this was not statistically significant. This indicates their potential efficacy in both endodontic sealing procedures.

KEYWORDS: Intratubular penetration; Ultrasound; Bioceramic sealant.

RESUMEN: Evaluar la profundidad de penetración intratubular de los cementos selladores biocerámicos activados por ultrasonidos en la dentina radicular, específicamente en la interfase dentina-cemento. Este estudio experimental in vitro se realizó con muestras que se dividieron aleatoriamente en función del tipo de sellador utilizado y de si la activación por ultrasonidos se aplicó antes de la obturación. El resultado fueron cuatro grupos experimentales (n=40). Los grupos A1 y B1 utilizaron el activador ultrasónico Eighteeth Ultra X con una punta azul nº 20 con una conicidad del 2% a baja potencia para la activación, mientras que los grupos A2 y B2 no emplearon ninguna forma de activación. El símbolo (+) denota los grupos con activación ultrasónica, mientras que (-) representa los grupos sin activación ultrasónica. En el tercio medio, se observó que BioC Sealer (+) tenía un valor medio de sellado de 0,66±0,17, mientras que MTA Fillapex (+) mostraba un valor medio de 0,38±0,07. Se observó que BioC Sealer (-) tenía un valor medio de sellado de 0,24±0,04 en el tercio medio, mientras que MTA Fillapex (-) mostraba un valor medio de 0,16±0,04. Estas diferencias no fueron estadísticamente significativas (0,5±0,05). Estas diferencias no fueron estadísticamente significativas (p=0,569). En el tercio apical, el BioC Sealer (-) presentó un valor medio de sellado de 0,44±0,17, mientras que el MTA Fillapex (-) obtuvo un valor medio de 0,30±0,04. Sin embargo, esta diferencia no alcanzó significación estadística (p=0,0745), lo que sugiere que ambos materiales pueden proporcionar un sellado similar en el tercio apical. Los resultados sugieren que tanto BioC Sealer como MTA Fillapex proporcionan un sellado comparable en los tercios medio y apical del sistema de conductos radiculares, sin embargo, esto no fue estadísticamente significativo. Esto indica su eficacia potencial en ambos procedimientos de sellado endodóntico.

PALABRAS CLAVE: Penetración intratubular; Ultrasonido; Sellante biocerámico.

INTRODUCTION

Root canal obturation seeks to achieve a three-dimensional seal to obtain a hermetic closure of the root canal system. Sealing cements play a fundamental role due to their physicochemical properties such as: fluidity, setting time, pH, volumetric changes (1) ability to penetrate dentinal tubules due to their small particle size, as well as the sealing of irregularities. Leveraging these properties, it can facilitate the creation of a physical barrier at the interface between the filling material and dentin (2). This not only enhances the sealing capability but also establishes an interface at the junction of cement and dentin. Furthermore, it aids in the retention and isolation of potential residual organisms within the dentinal tubules, thereby mitigating the risk of potential reinfection (3).

In recent years, bioceramic cements such as Bio C Sealer and Fillapex MTA have been introduced, which are ideal materials for achieving an airtight seal. The cements are highly radiopaque, non-deformable, insoluble, and hydrophilic, and possess antimicrobial and antifungal properties. They are also available in premixed and injectable forms (4). Bioceramic cements could bond to root dentin to create a tight seal due to their excellent adhesive properties on root dentin. The reason for this is the tubular diffusion of their particles, the mineral content of the root cement, and the reaction of the calcium silicates with the wetting of the dentin. Hydroxyapatite formation occurs in the area where the minerals are introduced (5-10).

MTA Fillapex facilitates the formation of hydroxyapatite, thereby initiating the healing process at the apical level. It is biocompatible, antimicrobial, and dimensionally stable, and notably, it does not contain resin in its composition. On the other hand, Bio-C Sealer, with its short setting time, favorable alkaline pH, high flow rate, and radiopacity (10), proves to be highly effective. It is also biocompatible, making it suitable for use near periapical tissue. This interaction triggers a mild inflammatory response, which aids in tissue repair (7). Bio-C Sealer is conveniently available in a premixed syringe form and is manufactured by Angelus (8). This enhances its practicality in endodontic procedures.

The ability of a sealer to penetrate root dentin and provide a tight marginal seal are crucial factors for successful intracanal treatment (9-10). A sealer with low surface tension and good wettability is ideal for achieving better penetration into irregularities and a tight seal. A root canal sealer with excellent penetration and adaptability can enhance obturation by increasing the surface contact area between the obturation material and root canal dentin, thus improving its sealing ability (11).

Therefore, the aim of this research was to analyze the intratubular penetration depth into the root canal dentin, specifically at the dentin-cement interface, of ultrasound-activated bioceramic sealer cements.

MATERIALS AND METHODS

STUDY DESIGN

An experimental *in vitro* study was carried out, which was written according to the CRIS Guidelines (Checklist for Reporting *In-vitro* Studies) (12).

SAMPLING METHOD AND SELECTION CRITERIA

The sample size was determined using a mean comparison formula, with an alpha of 0.5 and a beta of 0.8, through the application of Stata 16.0 statistical software (College Station, Texas 77845 USA). The samples were then randomly allocated, considering the type of sealer used and whether ultrasound activation was applied prior to obturation. This resulted in the formation of four distinct experimental groups, each consisting of 40 samples. This method ensures a balanced and unbiased distribution of samples across the different conditions being studied.

For this study, a specific set of criteria was established to ensure the selection of suitable samples, thereby enhancing the reliability of the results. The inclusion criteria comprised of single-rooted teeth with permeable and straight canals, fully formed apices, and no signs of internal or external resorption. Conversely, the exclusion criteria ruled out teeth with open-apex canals and teeth with calcified canals. Adherence to these criteria ensured a robust and focused study, paving the way for meaningful and applicable findings.

ALLOCATION

Group 1: Groups A1 and B1 were activated by Ultra X ultrasonic activator with blue tip #20 tapered to 2% at low power.

Group 2: Groups A2 and B2 were not activated.

SPECIMEN PREPARATION

Forty single-rooted teeth with a single straight root canal and fully formed apices were selected. The teeth were stored in 0.9% saline solution at a temperature of 4°C until they were ready for further processing. The specimens were then decoronated with the help of a diamond disk, while being constantly irrigated, and the roots were trimmed to a standard length of 16mm. A #10 C-Pilot K-file (VDW, Munich, Germany) was used to establish canal patency.

ROOT CANAL TREATMENT

The working length was established 1mm below the apical foramen. The Reciproc R25.08 system was used to perform root canal preparation (VDW-Zipperer), powered by the VDW Silver Reciproc motor + Sirona 6:1 contra-angle handpiece. Irrigation was performed with 20mL of 2.5% sodium hypochlorite (NaOCl), followed by 5mL of 17% ethylenediaminetetraacetic acid (EDTA, Prevest Dent Pro, India) for 5 minutes for smear layer removal. Gutta-percha main cones (25.08) were then inserted into the canals and radiographs were taken to verify that they reached the established working length and fit in the apical third. Rhodamine-B (Thermo Scientific Chemicals) was weighed on a laboratory microbalance (XPR2U/M) and added to BIO C Sealer (Angelus-Brasil) and Fillapex MTA sealer cement, Lot 912 (Angelus-Brasil) at a concentration of 0.1% to allow visualization under a stereo microscope (13). Finally, all root canals were performed by the same operator.

PROCESSING AND EVALUATION OF THE CLUSTERS

Groups A1 and B1 were activated using Eighteeth's Ultra X ultrasonic activator with a blue #20 tip tapered to 2% at low power. The spacer length was standardized to 2mm shorter than the working length. Since the ultrasound oscillates in only one plane, the file was activated for 20 seconds in the buccolingual direction and then for an additional 20 seconds in the mesiodistal direction of the root canal. Groups A2 and B2 were not activated.

The obturation technique used in both cases was the single cone technique. The cervical portion of the roots was sealed with Fuji II Mini Vitreous ionomer Lot, 2204191 (GC Corporation, Japan) and stored at 37°C and 100% humidity for one week to allow the sealer to set. Transverse cuts of the middle and apical thirds of the root were made 3 and 7mm from the apical foramen using a diamond disk at 200rpm with continuous water cooling to avoid frictional heat in all four groups. Dentin segments were 1.5 mm±0.1mm thick and were analyzed under a stereo microscope (M125, Leica, Germany). Images were captured at 10x magnification with a size of 36 pixels and a scale set at 1mm. The penetration depth was calibrated at standardized buccal, lingual, mesial, and distal points, with the root dentin wall serving as the reference position in each section up to the maximum extent of the penetrated staining area (Figure 1). To obtain a single mean value of penetration depth in each section, an average of the four measured readings (buccal, lingual, mesial, and distal) was taken to obtain a mean value for each section in mm. The penetration area of the sealer inside the dentinal tubules was measured using Image J software (14).

Figure 1. Penetration depth.

STATISTICAL ANALYSIS

The evaluation of the measures of central tendency and dispersion was performed by means of the mean and standard deviation. The Shapiro Wilk test and Levene's test were used to analyze the homogeneity of the data. Finally, for the bivariate analysis of the groups, the Kruskall Wallis test was used as appropriate.

RESULTS

The intratubular penetration depth in root dentin for the BioC Sealer (+) group at the middle level was on average 1.22±0.15mm and median 1.25mm, while at the apical level it was 0.66±0.17 and median 0.62mm, these differences being significant ($p<0.05$). For the BioC Sealer (-) group, in the middle zone it was 0.44±0.17mm with median 0.45mm and in the apical zone 0.24±0.04 mm with median 0.24mm, also being significant ($p=0.003$). For the MTA Fillapex (+) group the middle zone presented a mean of 1.04±0.23mm with median 1.02mm and in apical 0.38±0.07mm with median 0.36mm, with significant differences ($p<0.05$). For the non-activated group MTA Fillapex (-) at mid-level was 0.30±0.04mm with median 0.29mm and at apical 0.16±0.04mm and median 0.16mm, being also significant ($p<0.05$) (Table 1).

For the comparisons of ceramic cements with and without activation, at apical level BioC Sealer (+) was significantly higher than BioC Sealer (-) ($p<0.001$), MTA Fillapex (+) ($p=0.026$) and MTA Fillapex (-) ($p<0.01$). While the MTA Fillapex (+) group was significantly higher than MTA Fillapex (-) ($p<0.01$). In the mid-root zone, BioC Sealer was significantly higher BioC Sealer (-) ($p<0.01$) and MTA Fillapex (-) ($p<0.01$) but was not different from the MTA Fillapex (+) group ($p>0.05$). On the other hand, the MTA Fillapex (+) group was significantly higher than BioC Sealer (-) ($p<0.01$) and MTA Fillapex (-) ($p<0.01$) (Table 2).

Table 1. *In vitro* analyses of endodontic sealants.

	Mean ± SD	Median ± IQR	Min	Max	p**
BioC Sealer (+) Middle third	1.22±0.15	1.25±0.07	0.89	1.39	<0.001***
BioC Sealer (+) Apical third	0.66±0.17	0.62±0.13	0.52	1.09	
BioC Sealer (-) Middle third	0.44±0.17	0.45±0.32	0.22	0.68	0.003***
BioC Sealer (-) Apical third	0.24±0.04	0.24±0.06	0.18	0.29	
MTA Fillapex (+) Middle third	1.04±0.23	1.02±0.39	0.72	1.35	<0.001***
MTA Fillapex (+) Apical third	0.38±0.07	0.36±0.10	0.28	0.50	
MTA Fillapex (-) Middle third	0.30±0.04	0.29±0.06	0.22	0.37	<0.001***
MTA Fillapex (-) Apical third	0.16±0.04	0.16±0.07	0.11	0.22	

(+) With ultrasonic activation
(-) Without ultrasonic activation
** Wilcoxon rank sum test (***significance differences ($p<0.05$)
IQR: Interquartile range

Table 2. Multiple comparisons for intratubular penetration depth.

Zona	Grupos	BioC Sealer (+)	BioC Sealer (-)	MTA Fillapex (+)
Apical third	BioC Sealer (-)	<0.0001*		
	MTA Fillapex (+)	0.0255*	0.0255*	
	MTA Fillapex (-)	<0.0001*	0.0426*	0.0001*
Middle third	BioC Sealer (-)	0.0001*		
	MTA Fillapex (+)	0.2109	0.0016*	
	MTA Fillapex (-)	<0.0001*	0.1694	<0.0001*

Based on Dunn's multiple comparisons post estimation test for Kruskall Wallis for independent groups.
(+) With ultrasonic activation (-) Without ultrasonic activation.
*Significant differences ($p<0.05$)

DISCUSSION

This study stands out and is warranted due to several factors. Primarily, it emphasizes the significance of intratubular penetration depth, as this can influence adhesion, sealing, and the capacity to avert reinfection. Additionally, the application of ultrasound activation could potentially enhance the penetration and adjustment of these cements, although this area necessitates further exploration. Consequently, this research could yield insightful data that could augment the effectiveness of endodontic treatment.

In endodontic practice, new materials and instruments are continually being incorporated, resulting in the adoption of new root canal filling protocols and techniques. Recently, Arslan *et al*. (15) have proposed ultrasonic and sonic activation of root canal sealers to increase their penetration into the dentinal tubules and enhance adaptation between the material and root dentin, with promising results. The objective of this study was to evaluate *in vitro* the effect of ultrasonic activation on the intratubular penetration depth in root dentin of Bio-C sealer and MTA-Fillapex bioceramic sealer cements.

The application of ultrasonic activation to Bio-C sealer cement has been observed to enhance its penetration capabilities within the intratubular root dentin. This enhancement was evident in both the middle and apical thirds, where the penetration was significantly greater in specimens that underwent ultrasonic activation compared to those that did not. This difference was statistically significant ($p<0.05$). These findings align with the results obtained by Pérez-Alfayate *et al*., (16) further corroborating the effectiveness of ultrasonic activation with this type of cement.

The use of ultrasonic activation with MTA-Fillapex cement has been found to enhance its penetration capabilities within the intratubular root dentin. This enhancement was observed in both the middle and apical thirds, where the penetration was significantly greater in specimens that underwent ultrasonic activation compared to those that did not. This difference was statistically significant ($p<0.05$). These findings are in line with the research conducted by Bem IAD *et al.*, (17) further substantiating the effectiveness of ultrasonic activation with this type of cement.

With respect to the objective of comparing the effect of ultrasonic activation in both bioceramic cements, we found that the intratubular penetration depth in root dentin of Bio-C sealer cement with ultrasonic activation was greater than in MTA-Fillapex cement both in the middle third and in the apical third, and this difference was statistically significant ($p<0.05$) being consistent with the study of Makan, K. *et al*. (18) who investigated *in vitro* the effect of ultrasonic activation on the depth of penetration in root dentin of bioceramic and resinous root canal sealers, using confocal laser scanning microscopy, finding in their results that Bio C sealer had a greater depth of intratubular penetration than MTA-Fillapex cement.

The results related to Bio C sealer (Angelus) cement could be explained by the particle size of this sealer which is <2μm, providing it with a higher flow rate, and better runoff, an essential property to penetrate the dentinal tubules (18). In contrast, MTA Fillapex (Angelus) salicylate resin-based bioceramic cement contains MTA in its formulation, due to the small amount of resin matrix in its composition, influences a lower effectiveness of ultrasonic action (19).

The rise in the intratubular canal penetration depth of the sealers can be attributed to the heat produced during ultrasonic activation, which lowers the viscosity of the sealer, leading to greater fluidity and better integration of filler particles into the organic matrix. This is linked to the high-frequency acoustic transmission generated by the ultrasonic tip, which propels the sealer into the dentinal tubules (17). Previous research has shown that the micro-flow acoustic energy transmission created by the ultrasonic tip decreases the number of voids, enhances interfacial adaptation, and improves intratubular penetration (20). In conjunction with the increased pressure of the sealer against the canal walls, this allows for more effective filling of irregularities and accessory canals, as well as greater penetration into dentinal tubules with the formation of numerous tags (20).

This study has an important impact on achieving a tight seal of the root canal system, which is complex due to its intricate anatomy, dimensional changes and lack of gutta-percha adhesion. In recent years, bioceramic cements such as BIO C Sealer and MTA Fillapex (Angelus) have been introduced, which are ideal materials with excellent radiopacity, no dimensional shrinkage, insoluble and hydrophilic, and antimicrobial action. In addition, they come in premixed and injectable presentations.

These bioceramic cements achieve a hermetic seal due to the intratubular penetration of their particles, the infiltration of the cement's mineral content, and the reaction of calcium silicates with dentin moisture. As a result, hydroxyapatite is formed along the mineral infiltration zone. Considering that it has been reported in the literature that *E. faecalis* and *Candida albicans* bacteria can penetrate dentinal tubules up to 244 and 184μm respectively, these cements are beneficial (15-20).

The observed variations in intratubular penetration at the middle and apical levels could be attributed to a multitude of factors. One plausible explanation could be the anatomical disparities between these two regions. The apical region of the root canal is typically narrower and more intricate than the middle region, which could potentially restrict the penetration of the sealing cements. Another contributing factor could be the efficacy of ultrasound activation, which was employed to augment the penetration of the sealing cements. The effectiveness of ultrasound activation can fluctuate based on several factors, including the power setting of the ultrasound device, the duration of activation, and the operator's technique (18-20).

As for the available methods to assess the penetration of the sealants, a variety of techniques can be utilized. These include Scanning Electron Microscopy (SEM), which offers high-resolution images of the sealant penetration, and Confocal Laser Scanning Microscopy (CLSM), which enables three-dimensional visualization of the sealant. Furthermore, Micro-Computed Tomography (micro-CT) can provide a non-destructive method for assessing the penetration depth and volume of the sealant (3, 4, 11). However, each of these methods has its own strengths and limitations, and the choice of method should be based on the specific needs of the study. Thus, continued research is necessary to better understand these differences and optimize the use of sealing cements in root canal treatment.

In the present study, there were some limitations. Firstly, the complex nature of root canal anatomy, which significantly varies among different teeth and individuals, could potentially impact the generalizability of the study results. The penetration depth of the sealant cements, a critical factor in this study, may be influenced by this anatomical variability. Secondly, the use of ultrasound activation, while a potent tool, presents its own set of challenges. The effectiveness of ultrasound activation is not a constant; it is a variable influenced by factors such as the power setting of the ultrasound device, the duration of activation, and even the operator's technique. This introduces a potential source of bias, subtly influencing the study's results. It is important to use irrigant activation techniques and to control the smear layer to achieve a correct obturation and unity between the obturation materials and the dental tissues. It would be useful to carry out comparative studies with other cements and to evaluate other means, such as bacterial filtration or scanning electron microscopy, to expand the information available and corroborate the data obtained, making them more reliable.

CONCLUSION

In summary, within the limitations of this *in vitro* study, it was concluded that BioC Sealer performs better in terms of root canal sealing compared to Fillapex MTA, especially in the middle third. These results support the preference for BioC Sealer as a root canal filling material in terms of its sealing ability under conditions like those evaluated in this study. However, additional research is necessary to fully comprehend the properties and clinical behavior of these materials. Ultrasonic activation enhances the projection of the sealer into areas of anatomical complexity, including deeper within the dentinal tubules where there are fewer spaces. This improves the quality of the obturation and yields promising results.

CONFLICT OF INTEREST

No conflict of interest.

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization and design: M.J.C., L.T.C., M.P., and D.S.M.
Literature review: M.J.C., L.T.C. and D.S.M.
Methodology and validation: F.M.T. and D.S.M.
Formal analysis: F.V.and F.M.T.
Investigation and data collection: M.J.C. and L.T.C.
Resources: M.J.C. and M.P.
Data analysis and interpretation: F.M.T. and F.V.
Writing-review and editing: M.P., D.S.M., M.J.C., F.V. and L.T.C.
Supervision: F.M.T.
Project administration: F.M.T.

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