Original Article

Dentinal tubule penetration following ultrasonic, sonic, and single-cone technique of a biosealer: An *ex vivo* study

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Abstract

Background: The endodontic space is complex, and using a single-cone obturation technique fails to ensure a complete filling.

Introduction: This study aimed to investigate the effect of ultrasonic activation, sonic activation, and single-cone technique of a biosealer on its dentinal tubular penetration.

Materials and Methods: In the experiment, single-root mandibular premolars were randomly assigned to three groups ($n = 20$): group A, ultrasonic activation; Group B, sonic activation; and Group C, single-cone technique. Penetration of the fluorescently labeled biosealer was investigated using a confocal laser scanning microscope.

Results: The data were statistically analyzed using Kruskal–Wallis and Mann–Whitney tests ($P = 0.05$). The highest penetration of biosealer was observed in Group A, followed by Group B ($P < 0.05$).

Conclusions: Dentinal tubule penetration of biosealer was significantly improved by ultrasonic and sonic activation techniques.

Keywords: Biosealer; dentinal tubules; endodontic space; sonic; ultrasonic

INTRODUCTION

Certainly, the accurate performance of cleaning, shaping, and three-dimensional (3D) obturation of the root canal system is critical to the success of an endodontic treatment.[1] Significantly, the root canal filling phase, which aims to prevent bacteria and fluid entry from the oral cavity into the periapical tissues, traps bacteria that have resisted the intracanal instrumentation and irrigation and obstructs the entry of periradicular exudates.[2]

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Materials such as gutta-percha and root canal sealers have been advocated for the canal-sealing procedure. Specific physical, biological, and handling properties are required for obturation materials.[3] Moreover, the obturating material must form a bond with the dentin walls of the root canal and fill areas of the root canal anatomy inaccessible to shaping files to prevent fluid and bacterial leakage. The bond between the endodontic sealant and intracanal dentin is critical to adequately adapt the dentin–sealant interface when subjected to mechanical stresses during mastication or postplacement.

A dentinal smear layer develops during the shaping phase due to the cutting action of the rotary files.[4-6] The cleansing phase, performed using irrigants, is essential for

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removing the smear layer and disinfecting the endodontic space.[7] The complication of leaving the smear layer inhibits irrigant penetration inside the obliterated dentinal tubules, rendering the bacteria trapped inside unreachable. Accordingly, it is necessary to activate the irrigants by various techniques abundantly described in the literature to remove the abovementioned layer. It is a consequence of leaving the smear layer behind the endodontic sealer to penetrate the dentinal tubules, which, therefore, are not sealed.[4] It has been observed how micromechanical retention of endodontic sealant infiltrating the no longer obliterated dentinal tubules can improve the mechanical properties of the sealer–dentin interface in terms of resistance to dislocation.[4]

Several types of irrigants that can be used during the cleansing phase to remove the dentinal smear layer are documented in the literature. The most commonly used irrigants are ethylenediaminetetraacetic acid (EDTA) at 17%, followed by sodium hypochlorite (NaOCl) at 5.25%, which can remove the inorganic and organic components of the smear layer.[8] These irrigants have the potential to change dentin composition by influencing the interaction with root canal filling materials.[8] The possible effects of the action of irrigants on the bond strength between dentin and root canal sealants have been investigated in the literature.[8,9]

Once the shaping and cleansing steps are concluded, the obturation step follows, using gutta-percha, sealant, or biosealer.[10]

Biosealers were launched on the dental market with a recommendation to be used with the single-cone protocol. The assumption was that it is preferable not to bring the bioseal into contact with heat, which would lead to instantaneous hardening of the material.[11]

The most commonly used core material is gutta-percha, which does not adhere to the canal's dentinal walls.[12] As a result, a wide range of materials is available for this purpose, including sealers based on zinc oxide and eugenol, calcium hydroxide, glass ionomer, resin epoxy, silicone, and bioceramic-based sealers.[13,14]

The most typically used materials in endodontic treatments are epoxy resin-based root canal sealers and gutta-percha.[15] On the other hand, other techniques and materials with different physicochemical and biological properties have been developed.[11] Epoxy resin-based sealers have excellent physical properties such as slow setting reaction, low solubility, high flow rate, low volumetric polymerization contraction, and adaptation to the dentine walls of canals.[15]

Bioceramic materials, as previously mentioned, have emerged as a new option in dentistry.[16] Moreover, they offer advantages as endodontic sealers, including biocompatibility, osteoconductivity, satisfactory sealing capacity, adhesion, and radiopacity, in addition to containing calcium phosphate.[11,17] The calcium phosphate improves the biosealers setting properties, resulting in a chemical composition and crystal structure equivalent to tooth and bone hydroxyapatite.[17,18] Such properties contributed to the widespread use of these materials in endodontics. However, the most significant disadvantage of their use is the difficulty of removing the material from the root canal if retreatment is required or during canal preparation for a dental post.

Using different activation techniques, the current *in vitro* study will evaluate a bioceramic sealer's penetration ability inside dentinal tubules. The null hypothesis was that no difference exists between the two activation techniques and the traditional obturation method.

MATERIALS AND METHODS

The present research was conducted as per the guidelines of the Declaration of Helsinki and permitted by the Institutional Review Board: Protocol code 00008975-March 28, 2023, Naples University, Federico II, Italy.

Sample selection

In the current study, the sample size ($n = 60$).

The teeth used in this research were extracted during an orthodontic treatment plan and were irrelevant to the present experiment.

Inclusion criteria

Mandibular premolars, single root, and patient's age (20–25 years).

Exclusion criteria

Presence of root resorption, immature apices, caries, fractures, or root fillings.

Before the experiment started, informed consent was obtained from the patients.

Immediately after extraction, the soft tissue attached to the outer surface of the teeth was removed using a curette. Then, the specimens were stored in individual vials containing 5 mL of 10% formalin until use.

Teeth preparation

The selected premolars were decoronated at the cementoenamel junction level to acquire roots of standardized length (16 mm). A size 10 K-type file (Coltène/Whaledent AG Feldwiesenstrasse 20 9450 Altstätten, Switzerland) was inserted into each canal until it was seen through the apical foramen. The final working length was reached by subtracting 0.5 mm from this measurement. Next, all the root canals were mechanically instrumented with nickel-titanium rotating files (Hyflex EDM, Coltène/Whaledent AG Feldwiesenstrasse 20 9450 Altstätten, Switzerland). For the previously mentioned step, only the 10/0.05, 20/0.05, and 25/0.08 Hyflex EDM files were used for the full working length. After completion of the apical preparation, the size and taper of the apical area was 25/0.08. Throughout the entire canal instrumentation phase, chemical irrigation was conducted using 3% NaOCl (Canal pro, Coltène/Whaledent AG Feldwiesenstrasse 20 9450 Altstätten, Switzerland) via a 30 G needle in a syringe (Canal pro irrigating tips, Coltène/Whaledent AG Feldwiesenstrasse 20 9450 Altstätten, Switzerland). A total of 5 mL NaOCl solution was used per tooth and refreshed every 60 s. The root canals were then flushed with sterile saline, pursued by irrigation with 3 mL of 17% EDTA (Canal pro-EDTA, Coltène/Whaledent AG Feldwiesenstrasse 20 9450 Altstätten, Switzerland) for 1 min to clear the smear layers. All root canals received a final rinse of 3 mL of sterile saline.

The samples were randomly divided into three groups ($n = 20$) using www.randomizer.org.

All samples were painted with a layer of nail varnish (Revlon, New York, USA).

To test the sealer penetration, the biosealer selected for this study was bioseal (Coltène/Whaledent AG Feldwiesenstrasse 20 9450 Altstätten, Swiss) marked with 0.1% Rhodamine B (Rhodamine B, VWR International Srl, Via San Giusto 85-20153 Milano, Italy) and the same quantity was used. The bioseal syringe tip was inserted 6 mm from the working length, and 2 mm of biosealer was injected for each premolar.

In Group A, the ultrasonic activation technique was applied. An ultrasonic tip (25/02, 38,000 hz, Ultra Smart AI, Coxo, China) for 6 s was used. Specifically, the tip was placed 2 mm away from the working length, making up and down motions with an amplitude of 4 mm.

In Group B, the sonic activation technique was employed. A sonic tip (Eddy tip, 6000 Hz, VDW, Munich, Germany) for 6 s was used. The tip was positioned 2 mm from the working length, creating up and down moves with an amplitude of a wave of 4 mm.

Group C used a single-cone obturation technique without prior activation. The procedure included inserting the gutta-percha cone (25.08, Coltène/Whaledent AG Feldwiesenstrasse 20 9450 Altstätten, Swiss) to the working length.

The coronal access opening was sealed with a temporary filling material (Cavit, 3M; ESPE, St. Paul, MN, USA). Then, the samples were stored at 100% humidity and 37°C for 2 weeks to set entirely and ensure the sealer's complete setting.

Preparation of the roots

After 14 days, the roots were fixed centrally and vertically in orthodontic resin (Dentsply Caulk, Milford, DE, USA).

Each specimen was sectioned horizontally utilizing a diamond disk (Buehler, Lake Bluff, IL, USA) attached to a low-speed handpiece (25,000 rpm) at 2 and 4 mm from the apex. The cut specimens were then mounted onto glass slides, and the coronal surface underwent a polishing phase using sandpapers of 500, 700, and 1200 grit under running water to eradicate the dentin debris that could be created during root preparation to produce a clear reflective surface. The samples examined with confocal laser microscopy were 2 mm thick, and analysis of the roots using confocal laser scanning microscopic imaging.

Root canal segments were studied with a Zeiss confocal laser scanning microscope (CLSM) (Carl Zeiss, LSM 780, Jena, Germany) at 10 magnifications and set in fluorescent mode (at a wavelength of 514 nm) in Figure 1. Digital images were uploaded into ImageJ software (National Institutes of Health, Bethesda, MD, USA). The sealer penetration depths into the dentinal tubules were measured at the maximum depth for each specimen.

The measurements were made by operators blinded to which samples corresponded to which Group (A, B, or C). The measurements were repeated twice to ensure reproducibility.

Data presentation and statistical analysis

The data for biosealer penetration were assessed for normality with the Shapiro–Wilk test. Since they were not normally distributed, nonparametric tests were applied for multiple comparisons between the groups (Kruskal–Wallis), and Mann–Whitney tests were used to compare pairs of groups.

RESULTS

The results of the current study showed the deepest penetration of biosealer in Group A, followed by Group B ($P < 0.05$).

In Group A, ultrasonic distribution, at the 5 mm level, the highest penetration depth was 0.35 mm and, at the 3 mm level, was 0.21 mm.

In Group B, sonic distribution, at the 5 mm level, the highest penetration depth was 0.21 mm and, at the 3 mm level, was 0.11 mm.

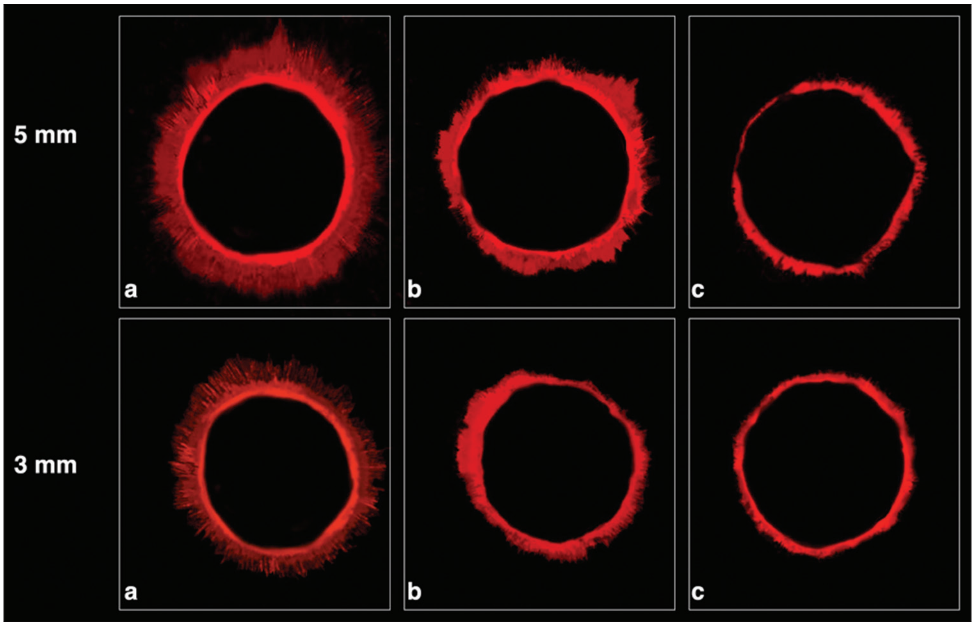

Figure 1: Representative images of dentinal tubular penetration of biosealer in groups. (a) Ultrasonic activation, (b) sonic activation, and (c) single-cone technique at the 3 and 5 mm levels

The penetration depth of the Group C, single cone, was at 5 mm level, 0.06 mm and, at 3 mm level, 0.04 mm [Figures 1-3 and Table 1].

Table 1 Depth of penetration (mean ± standard deviation; mm) of the biosealer into the dentinal tubules in all the experimental groups

Groups		3 mm level	5 mm level
A	(ultrasonic distribution)	0.21 ± 0.02^a	0.35 ± 0.02^a
B	(sonic distribution)	0.11 ± 0.03^b	0.21 ± 0.02^b
C	(single cone)	0.04 ± 0.001^c	0.06 ± 0.001^c

Mean values with different superscript letters, along each column, indicate statistically significant difference ($P < 0.05$)

DISCUSSION

One of the crucial steps to achieving success in endodontics is the diagnosis, which, in turn, leads to the correct treatment plan. Equally important are the subsequent phases: the access cavity, shaping, cleaning, and, finally, the 3D filling.

During the chemomechanical preparation, the bacterial load present in the complex endodontic space is eliminated or reduced. Moreover, activating the irrigants is of fundamental importance to allow better cleansing in the more complex root canal system. The goals of this therapeutic phase are dual; one objective is to create an apical seal and to fill the canal three-dimensionally, and the other is to leave no gaps.[11]

Furthermore, this complete seal is challenging because canals have complex and unique anatomies, making them all different. Many materials and filling techniques have been developed to seal better and fill the complex anatomical areas of the endodontic. Most techniques involve using a root canal sealant in conjunction with a solid core filler.[19,20]

The sealer plays a key role in achieving the goal of 3D sealing and obturation, as it fills the void between the gutta-percha and the dentinal walls and can even penetrate inside the tubules.[20] In addition, it acts on the bacterial component by providing antimicrobial action.[11] However, it should be noted that sealants exhibit toxicity before the setting phase, so cautiousness must be considered to avoid extrusion beyond the apex into the periapical tissues. They are partially resorbed after contact with the periodontium's tissue fluids.

Bioceramic types of cement have calcium silicate or phosphate as the main material. Mineral trioxide aggregate (MTA) is used, in addition to many established techniques in dentistry, as a root canal sealant. Its chemical formulation involves tricalcium silicate, to which a radiopaque substance, bismuth oxide, is added to improve radiographic monitoring of the material. One characteristic of MTA is the environment in which it must act; in fact, the presence of water is required. The contact between the material and the wet environment leads to a basic pH (pH = 12) mixture, which leads to calcium silicate hydrates. The chemical reaction leads to the formation of more hydrates on the surface of existing calcium silicate molecules, with centripetal growth. This process creates an efficient barrier, which is useful for endodontic seal formation.[21] Nevertheless, a disadvantage to being considered regarding

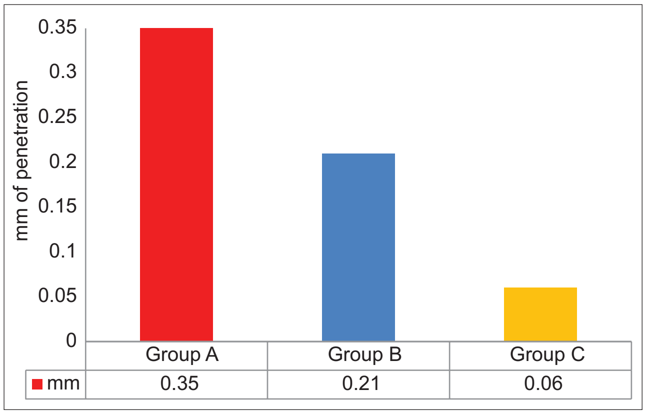

Figure 2: Depth of penetration of the biosealer into dentinal tubules (5 mm)

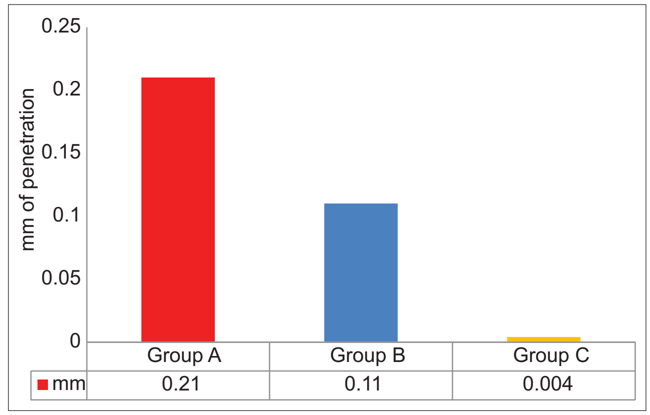

Figure 3: Depth of penetration of the biosealer into dentinal tubules (3 mm)

bioceramic sealer is the degree of adhesion with the dentin walls; the very tight bond between the dentin walls and the cement, while it is an advantage in forming the seal, is also a major obstacle in that it makes the material removal and shaping phase very difficult.

The current *in vitro* study compared the degree of penetration of bioceramic cement inside dentinal tubules with different filling techniques, such as single-cone, and those with sonic and ultrasonic frequency activation. To our knowledge, few studies in the literature evaluate the degree of intratubular penetration of activated bioceramic cement. However, none compares the single-cone filling technique with those in which sonic and ultrasonic instruments are used.

Confocal laser scanning has been used to assess the degree of penetration of bioceramics within dentinal tubules.[12] As described in a recent study conducted by Tedesco *et al*.,[20] confocal laser scanning allows a better assessment of the degree of penetration of the sealant in terms of quantity and depth. Bioceramic cement was labeled with Rhodamine B to visualize the sealant within the dentinal tubules. Rhodamine B was used because it does not affect the physical and chemical properties of the material.[20]

The protocol initially described for biosealers involved using the single-cone technique, and contact between a heat source and the sealant was not recommended, as it could lead to an instant hardening of the material. Recently, a new filling technique for bioceramic sealers termed the modified heat technique has been described that aims to use bioceramics as 3D sealants, promoting penetration into lateral anatomies with greater depth than the single-cone technique.[11] The technique described by Abdellatif *et al*.[11] did not cause chemical and physical alteration of the material, and no heating of the sealant in the apical third was observed.

It is also true, however, that this technique, if not well performed, could lead to changes, such as rapid hardening due to heat, in bioceramic cement. Therefore, we evaluated the degree of bioceramic cement penetration using sonic and ultrasonic sources, comparing it with the traditional single-cone technique.

Ultrasonic is used in many branches of dentistry, from periodontology to endodontics. Ultrasonic is defined as sound energy with a wave frequency above the values audible to the human ear, precisely above 20 kHz.

In modern endodontics, ultrasonics has its main applications during the pulp chamber opening stage for calcification removal, canal identification, the cleansing phase with the ultrasonic irrigant activation technique, and the obturation stage.[22]

Based on these considerations, our study evaluated the penetration ability of bioceramic cement inside dentinal tubules, taking advantage of the possibility of managing and controlling the heating of the material by ultrasonic tips. It can be seen that the degree of penetration of biosealers into the dentinal tubules is greater for Group A, that is, the one subjected to ultrasonic action, both by comparing it with Group B (tested by sonic action) and Group C, performed by the single-cone technique. Specifically, at 5 mm from the working length, the maximum material penetration is 0.35 mm for Group A, 0.21 mm for Group B, and 0.06 mm for Group C. At 3 mm from the working length, the maximum material penetration results in 0.21 mm for Group A, 0.11 mm for Group B, and 0.04 mm for Group C.

Normalized the values with the Shapiro–Wilk test and performed the nonparametric tests for multiple comparisons between groups (Kruskal–Wallis) and Mann–Whitney test to compare pairs of groups; these differences in the degree of penetration of bioceramics within the dentinal tubules between the different groups results statistically significant for $P < 0.05$.

Therefore, the data showed that using ultrasonic during the filling stages with bioceramic cement can be a technical element that can improve the 3D closure of the anatomical root canal complex.

It is described in the literature that the main cause associated with endodontic failure is the persistence of intracanal bacteria due to inadequate shaping, cleansing, and obturation.[11] In particular, the lack of an adequate apical seal leads tissue fluids, rich in glycoproteins, to enter the canal and act as a substrate for the surviving bacteria, specifically in the dentinal tubules. Consequently, a better obturation phase with an adequate apical seal (both lateral anatomies and dentinal tubules) increases the success rates of endodontic treatment.[23,24] The protocol adopted in this article aims to accomplish this result, which, from the data that emerged, is achievable with the activation of bioceramic cement utilizing ultrasonic tips.

It should be emphasized, however, that this study's main limitation is the exclusively *in vitro* development of the method, which must be applied and analyzed *in vivo* in a manner that complies with the guidelines and ethics committee approvals.

The current research found that the new ultrasonic and sonic activation techniques had the highest dentinal tubular penetration compared to the single-cone technique. Thus, the null hypothesis was rejected.

CONCLUSIONS

This study found that activation with ultrasonic tips is the most effective technique to improve the degree of penetration of bioceramic cement within the dentinal tubules. Further studies, not only *in vitro* but also *in vivo*, will be needed to verify and confirm the effectiveness of the described technique to achieve better root canal obturation and, thus, a higher success rate of endodontic treatment.

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Conflicts of interest
There are no conflicts of interest.

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