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# Sonic Activation Improves Bioceramic Sealer's Penetration into the Tubular Dentin of Curved Root Canals: A Confocal Laser Scanning Microscopy Investigation

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**Abstract:** Background—The aim was to determine the influence of sonic activation in the tubular dentine penetration of bioceramic sealers. Methods—Forty mesiobuccal curved root canals of mandibular molars with an apical diameter smaller than #30 were prepared, divided into two groups, and filled with EndoSequence BC sealer, with or without sonic activation during its placement. Roots were sectioned at 3 mm, 6 mm, and 9 mm from the apex, producing a sample size of 120. The samples were evaluated using a confocal laser scanning microscope and comparing these images to the images obtained from an operatory microscope. The percentage of sealer penetration and maximum sealer penetration were evaluated. Statistical analysis was performed using the two-tailed Mann–Whitney *U* test, where statistical significance was set to $p < 0.05$. Results—Sonic activation showed higher values for the percentage of sealer penetration when compared at the 9 mm level ($p = 0.03$). A higher value of maximum sealer penetration was observed at all levels when the sealer was activated. Conclusions—The sonic activation of bioceramic cement resulted in higher sealer penetration into dentinal tubules.

**Keywords:** CLSM; Endoactivator; EndoSequence BC sealer; sonic activation; tubular penetration

## 1. Introduction

Mineral trioxide aggregate (MTA) has been described as a bioactive and versatile material [1,2] and is used for procedures such as root-end fillings, apexification, perforation, vital pulp therapy, and regenerative procedures. Lately, it has been developed to be used as an endodontic sealer [3].

Bioceramic-based materials, which generally contain calcium silicate or calcium phosphate, have attracted substantial attention because of their biological and physical properties. Endosequence® BC sealer (Brasseler USA, Savannah, GA, USA) is a tricalcium-silicate-based root canal sealer; since it is premixed, it requires dentinal moisture to cure and can act as a stand-alone obturation system. This material is hydrophilic, highly radiopaque because it contains zirconium oxide and tantalum oxide, has no shrinkage, and forms hydroxyapatite upon setting [4]. Endosequence BC sealer has been shown to increase the fracture resistance of roots [5].

The capacity to penetrate the tubules is a measure of a sealer's efficiency and alludes to the amount of sealer that is able to enter them. Moreover, with the tubular penetration, a sealer is able to form a physical barrier [6], improve root filling material retention [7], and entomb any residual bacteria [8]. The dentist's knowledge of the sealer's behavior improves the clinical outcome [9]. A sealer's antibacterial activity is proportional to its tubular penetration [10]. In fact, BC Sealer eliminates bacteria beyond the tubular penetration of the sealer [11]. This could be carried out through ultrasonic or sonic activation of endodontic sealers, which have been proposed to improve the quality of root filling [11,12]. Sonic activation uses low-frequency vibration (1–6 kHz) through flexible tips, which when combined with short pecking movements inside the root canal, act in a synergistic way by creating a hydrodynamic phenomenon that is responsible for the increased penetration of the sealer into lateral canals [12]. The aim of the present study was to determine whether sonic activation increased the dentinal tubule penetration of EndoSequence BC sealer in curved root canals.

## 2. Materials and Methods

### *2.1. Specimens Collection and Experimental Groups Assignment*

An in vitro randomized trial was schematized in order to obtain the approval of the Ethical and Research Committee of the institution in which the investigation was carried out. The sample size calculation was also determined before performing the study (CIPI/006/14). The following exclusion criteria were applied: open apex canals and/or with radicular caries, calcified canals, internal or external root resorption, cracks and/or fractures, previously endodontically treated root, and canals with double curvature; after applying these exclusion criteria, 40 human mandibular molars were selected. The methodology used was described in previous studies [13]. Calculus was eliminated (ultrasonic tip mounted on a Suprasson P5 Booster® (Satelec) (Acteon, Merignac, France)) and specimens were stored in a 2% thymol solution. Crowns and distal roots of the mandibular molars were discarded and the working length of the mesiobuccal canals was standardized (13 to 15 mm).

A size 8 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into the canals and the working length was established as follows: once the file was visible in the apical foramen, 0.5 mm was subtracted from this length. A number was assigned to each specimen and they were radiographed to establish their angle of maximum curvature, as described in previous studies [14]. According to Pruett et al. [15], the angle of curvature was measured using AutoCad 2014® (Autodesk Inc, San Rafael, CA, USA) for this purpose. Specimens with angles of curvature between 20° and 90° were included in the study, arranged based on this parameter, and divided into 2 groups of 20 samples each via stratified random sampling. A group name was randomly assigned: BC with sonic activation (BC+) and BC without sonic activation (BC−).

### *2.2. Root Canal Preparation and Root Canal Filling*

A precurved size #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into the canal and MTwo rotary system basic sequence files (VDW, Munich, Germany) were used to working length. In order to increase the apical diameter, a Profile size #30/0.04 taper file (Dentsply Maillefer, Ballaigues, Switzerland) was used. All the rotary instruments were mounted on a 16:1 handpiece with an X-smart device (Dentsply Maillefer) following the manufacturer´s indications. Between each instrument, 1 mL of 5.25% NaOCl (masterful formulation) was used to irrigate the canals with a Monoject syringe. Patency of the canal was maintained with a size #10 K-file during all the procedures. Each instrument was used three times (three canals). Reus wax (Cera Reus SA, Reus, Spain) was used to seal the apexes, as described in previous studies [16], in order to create a closed system [16,17]. For the final irrigation protocol, 3 mL of 5.25% NaOCl followed by 1 mL of ultrasonically activated 17% EDTA (Irri-S; VDW, Munich, Germany) (three cycles of 20 s), and a final irrigation with 1 mL of 5.25% NaOCl was performed. The root canals were then dried with 30/0.02 paper points (Dentsply Maillefer). Subsequently, a #30 verifier (Dentsply Maillefer) was used to ensure the apical caliber. Canals with an apical diameter wider than size #30 were discarded and replaced with another root; the replacements were then prepared to Profile #30/0.04 taper file. Fluorescent Rhodamine-B was weighted (laboratory microweighing scale) and added to Endosequence BC sealer at 0.1% in order to obtain fluorescence using confocal laser scanning microscopy [18]. An insulin syringe was used to deliver 0.05 mL of sealer into the canal [19]. In the group with sealer sonic activation (BC+), the cement was extended with a pecking motion with a tip 25.04 of Endoactivator (Dentsply Maillefer) for 10 s and placed 2 mm shorter than the working length. An EndoSequence BC Gutta-percha point number 30/0.04 was placed to the working length.

Cavit (3M ESPE AG.Dental Products, Seefeld, Germany) was used for provisional coronal sealing of the canals. A radiograph of each specimen was taken to confirm that the filling was homogeneous.

### 2.3. Sectioning and Confocal Laser Scanning Microscopy (CLSM) Analysis

Specimens were enclosed in Eppendorf test tubes and filled with a photo-curable methacrylate resin (Technovit 7200 Heraeus Kulzer GMBH, Wasserburg, Germany) and positioned in an Exakt 402 (Exakt Aparatebau GMBH, Norderstedt, Germany) polymerization unit to prevent the specimens' temperature rise. Afterward, they were sectioned horizontally at 3 mm, 6 mm, and 9 mm from the clinical apex using a 0.2 mm wafering blade circular saw (Isomet 100 precision saw, Buehler, Lake Bluff, IL, USA) at 100 rpm and under water cooling [20]. A total of 120 specimens' slices were fixed on slides to be observed. The surfaces of the samples were then polished using 1200 g and 4000 g sandpapers under running water mounted on an Exakt 400 Micro Grinding System (Exakt Aparatebau GMBH). The samples were evaluated by CLSM and epifluorescence method (Leica TCS SP5; Leica Microsystems GmbH, Mannheim, Germany) with 540/590 nm Rhodamine B absorption and emission wavelength [21]. All samples were scanned at 10 micrometers below the surface with 10× magnification. Digital images with 262,144 pixels were obtained from the 120 samples using the fluorescent mode (resolution of 512 × 512 pixels). In addition, another 120 optic microscopy 30× images (Zeiss, Zeiss OPMI® pico, Oberkochen, Germany) were obtained in order to create a reference for the CLSM images [22] (Figure 1). Adobe Photoshop CS6 (Adobe Systems, San Jose, CA, USA) software was used to analyze all the images. To evaluate the percentage of sealer penetration, each root canal wall's circumference was traced and measured with the Adobe Photoshop CS6 measuring tool. Then, all the areas along the canal walls where the sealer penetrated the tubules were outlined and measured. The measured distance was divided by the circumference in order to calculate the percentage of sealer penetration. To describe the maximum depth penetration of the BC sealer, the point of maximum length penetration was traced. Each measurement was performed twice to confirm agreement.

### 2.4. Statistical Analysis

Quantitative variables were expressed as median (interquartile range (IQR)) following the confirmation of the nonparametric behavior using the Shapiro–Wilk normality test. To test the statistically significant differences between the groups, the two-tailed Mann–Whitney *U* test was used. Differences were considered significant with a probability higher than 95% ($p < 0.05$). The data analysis was carried out with IBM SPSS statistics version XXIII (IBM Corp, Armonk, NY, USA).

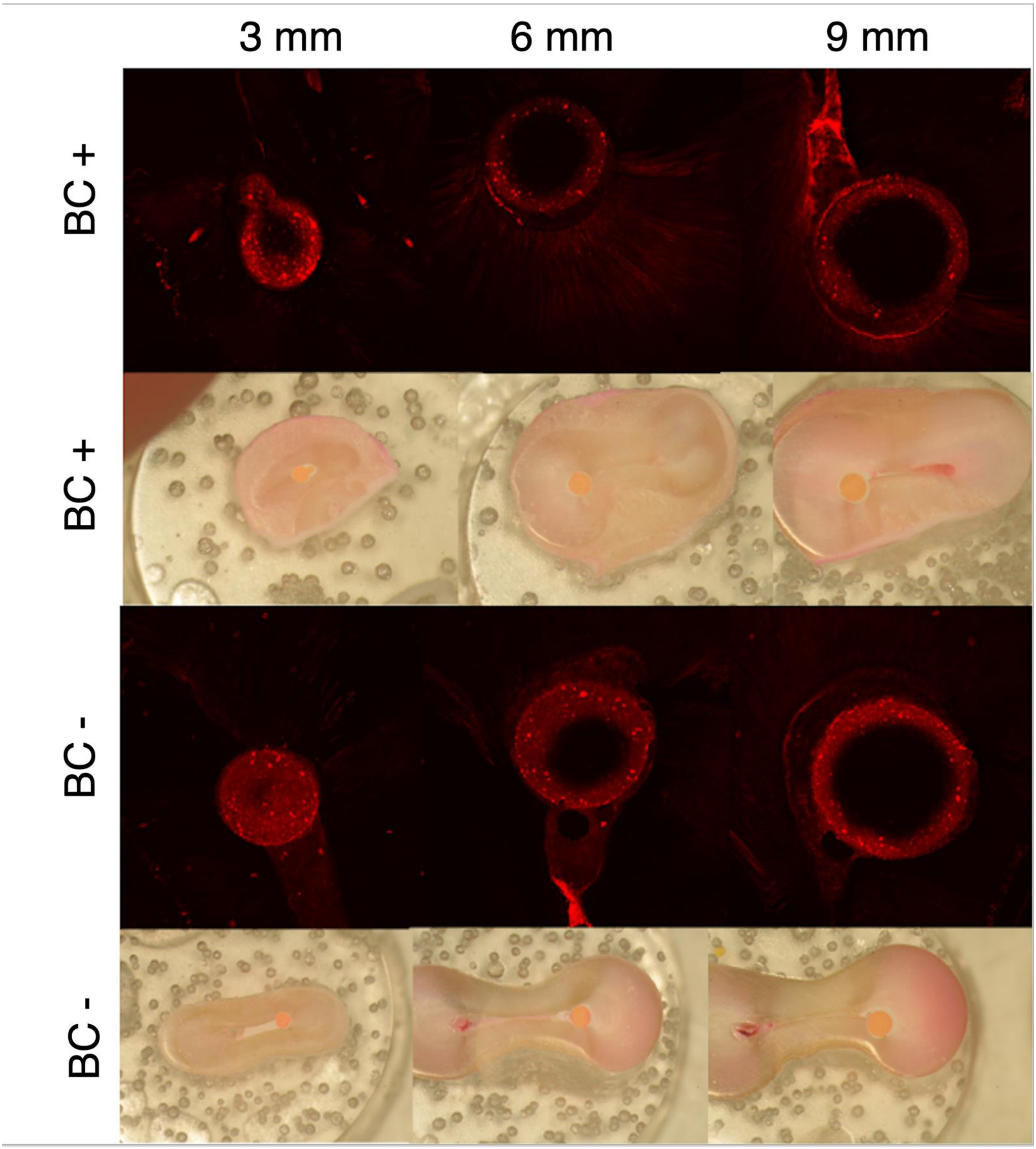


**Figure 1.** Representative confocal laser scanning microscopic images and their optical images from each experimental group.

## 3. Results

The values obtained for each group of the evaluated parameters are represented in Table 1. There was no statistical difference ($p > 0.05$) between the angles of both groups (BC+ = 31.5° ± 15.35, BC− = 30.6° ± 15.42). Regarding the percentage of sealer penetration (infiltration), significant differences were found between the groups only at the 9 mm level ($p = 0.03$), where a better performance of the cement was observed when using the Endoactivator to place the sealer. At the 3 and 6 mm levels, a trend for increased infill was observed for the sonic activation group (BC+); however, this did not reach a statistically significant difference. On the other hand, activation of the sealer with Endoactivator showed significant difference at all levels regarding the maximum sealer penetration (maximum infiltration).

**Table 1.** Median (25% and 75% quartiles) of the infiltration (%) and maximum infiltration (µm) of the sealer in the 9, 6 and 3 mm levels of the tested groups. BC−, no sonic activation; BC+, sonic.

| | Infiltration (%) | | | Maximum Infiltration (µm) | | |
|---|---|---|---|---|---|---|
| | Experimental Group | | | Experimental Group | | |
| **Level** | **BC−** | **BC+** | ***p*-Value** | **BC−** | **BC+** | ***p*-Value** |
| 9 mm | 50.3 (37.49/67.85) | 70.5 (53.54/75.11) | 0.030 | 239.9 (176.8/304.3) | 291.1 (242.7/368) | 0.037 |
| 6 mm | 57.1 (41.14/65.85) | 64.6 (44.44/70.8) | 0.474 | 193.8 (175.9/248.4) | 260.2 (192.4/321.7) | 0.044 |
| 3 mm | 47.8 (33.98/56.61) | 49.7 (39.23/56.46) | 0.653 | 105.6 (68.16/189.5) | 193.1 (112.2/221.7) | 0.005 |

## 4. Discussion

Since Duarte et al. [23] discovered that the pH level of CaOH was higher when it was activated with ultrasound and suggested that this was due to the increased penetration of calcium hydroxide inside dentinal tubules, other investigations have been carried out in order to assess the effect produced by the ultrasonic or sonic activation of different obturation materials. There does not appear to be any controversy regarding the ultrasonic activation of cement producing a significantly higher tubular penetration of sealers [11,12,24–26]. Nevertheless, sonic activation has been questioned. In research conducted by Arslan et al. [12], the authors observed that, although ultrasonic activation of the cement had the best results, regarding the penetration of sealer into lateral canals, sealer sonic activation exhibited better performance than the non-activated group. On the other hand, sonic activation has been shown to have the same effect as the control group of non-activation of the cement [26]. The results of the present investigation show that sonic activation had an influence on the tubular penetration of the EndoSequence BC sealer and this could be due to some differences in the methodology; Wiesse et al. [26] explained that differences in their results compared with those obtained by Arslan et al. [12] could be due to worse performance of the cement when activated for a longer period with a sonic device. As in Arslan et al. [12], the time taken for activating the sealer was 10 s in our study. In contrast, Nikhil and Singh [25], found worse results when activating with Endoactivator for 5 s. This could suggest that the time spent using the Endoactivator is a factor of crucial importance and possibly directly related to the heat generated. It is assumed that heat could accelerate the hydration of a bioceramic sealer and influence some of its properties, such as setting time, flow, or porosity [27]. DeLong et al. [28] observed that MTA plus had a lower bond strength when used with a continuous-wave obturation technique compared with a single-cone technique. Nevertheless, in another study [29], where both techniques were assessed, they found similar results for tubular penetration of tricalcium silicate sealers; however, as in our study, these authors did not examine the interface between the gutta-percha and the dentin wall.

In addition to these methodological differences, Wiesse et al. [26] utilized straight canals with an apical diameter #50/0.02 taper in which the activator tip was considerably smaller than the width of the canals. In the present study, activator tips used were size #25/0.04, which were manually and loosely fitted within 2 mm of the working length, as per the manufacturer's recommendation, into #30/0.04 canals. Besides this, it has been demonstrated that the ultrasonic activation of irrigants can improve the efficacy of those in removing both organic and inorganic debris from root canal walls [30–32], releasing debris from the tubules and allowing the sealer to penetrate. For this reason, we used passive ultrasonic activation; maybe this is one of the reasons why we obtained better results. Conversely, neither Wiesse et al. [26] nor Nikhil and Singh [24,25], reported the use of an ultrasonic disinfection protocol in their investigation.

The results showed an increased sealer penetration at 9 mm from the apical foramen of the root canal, which may also imply an influence of the microstructure on the dentine, as the tubular diameter was wider coronally than apically. These data could support the evidence that the activation of the bioceramic sealers was more effective when the tubule diameter is greater. Clinically, this finding is relevant, as coronal leakage occurs in a corono-apical fashion. Previous studies have shown an increased number of voids at the coronal aspect for single-cone techniques [33,34]. Hence, an increased sealer penetration at this level, as promoted by activation, may prevent tubular reinfection and limit the reservoir potential of coronal dentine for secondary endodontic infections.

From a clinical point of view, the efficacy of ultrasonic activation in curved canals has been discussed and seems to be effective when the instrument is pre-bent and can reach the established length [35]. Moreover, ultrasonic irrigation may not be as passive as claimed and may ledge the canal walls in the presence of a curved canal [36]. Nevertheless, the flexibility of the polymer tips of Endoactivator permits them to follow the root curvature and thus reach the full working length of curved canals without altering the root canal morphology [37].

In this study, CLSM was used. As it has been defined in previous studies, it represents a versatile method in the study of dentin and/or cement interface and the vitality of bacteria [38,39]. FISH techniques represent a valid alternative for assessing biofilms and other biological structures; however, for hard tissue, CLSM appears to be the most appropriate [40,41]. Furthermore, it provides relevant details about the adaptation/distribution of the cement within dentinal tubules since this microscopy technology (CLSM) is carried out by using fluorescent dyes and it permits the visualization of materials with various compositions [42]. Samples evaluated with CLMS do not require any special treatment and the observations can be accomplished in regular environmental conditions. This suggests that the possibility to produce artifacts with this technology is smaller compared with other methods [38]. Moreover, the CLMS has been defined as a non-destructive technique since it allows the use of the same sample for additional analysis.

## 5. Conclusions

Within the limitations of this study, the use of sonic activation of a bioceramic cement promoted a greater sealer penetration into dentinal tubules. More studies are needed in order to better understand the effect of different activation methods on bioceramic sealers.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Conflicts of Interest:** The authors declare no conflict of interest.

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