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Original Research

Evaluation of dentinal tubule penetration of two different sealer systems Endo seal MTA and Bioceramic sealer high flow using Confocal microscope

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ABSTRACT:
Aim: The aim of the study was evaluation of dentinal tubule penetration of two different sealer systems endo seal mta and bioceramic sealer high flow using confocal microscope. **Material and Methods:** A total of 100 recently extracted human single-rooted teeth without caries and with well-developed roots and closed apices were selected. Multi rooted teeth, teeth with curved roots, teeth with root resorption, fracture lines, endodontic filling, instrumented canals, and calcified canals were excluded from the study. All the selected teeth were cleaned, polished with pumice and stored in distilled water. **Results:** A significant difference was observed between MTA and bioceramic sealer at the 3 and 5 mm levels ($P < 0.05$), a significant difference was found between MTA and bioceramic sealer at the 3 and 5 mm levels ($P < 0.05$). **Conclusion:** At all root regions, bioceramic sealer exhibited more tubular penetration whereas MTA exhibited less penetration.
Keywords: Bioceramic sealers, confocal laser scanning microscopy, penetration depth

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INTRODUCTION

The complete sealing and filling of the cleaned and shaped root canal system are important steps that can affect the long term success of root canal treatment.[1,2] Because of the complexity of root canal system, sealers need to be used to fill the irregularities and to penetrate into dentinal tubules to obtain a hermetic seal of the root canal system. Meanwhile, root canal sealers should provide adherence between gutta-percha and dentinal walls to avoid gap occurrence at the sealer-dentine interface.[3]
Grossman [4] outlined the properties of an ideal sealer, including the following: provides good adhesion between it and the canal wall when set; establishes a hermetic seal; no shrinkage upon setting; insoluble in tissue fluids; tissue tolerant; and others. Current available commercial sealers can be broadly categorized into the following groups: zinc oxide eugenol-based, calcium hydroxide-based, glass ionomer-based, resin-based, silicone-based, and the recently introduced, calcium silicate based sealers. Zinc oxide eugenol-based,[5] calcium hydroxide-based[6] and glass ionomer-based sealers have the common problem of dissolving when in contact with periradicular tissues.
The access to areas such as isthmuses, ramifications, deltas, accessory, and lateral canals is difficult and residual bacteria are most often located there, due to the communication of accessory canals with the periodontal membrane, a potential periodontic-endodontic pathway for bacterial penetration to and from the periodontium can be created.[7] Therefore the penetration of sealer into these areas might have a role in the eradication of bacteria from the dentin tubules.[8] The analysis of the dentin/sealer interface can be done using confocal laser scanning microscope (CLSM). In comparison to conventional scanning electron microscope, CLSM provides the advantageof detailed information about the presence and distribution ofsealers inside dentinal tubules at a relatively low magnificationthrough the use of fluorescent Rhodamine-marked sealers.[9]

The aim of the study was evaluation of dentinal tubule penetration of two different sealer systems endo seal mta and bioceramic sealer high flow using confocal microscope.

MATERIALS AND METHODS

PREPARATION OF SAMPLES

A total of 100 recently extracted human single-rooted teeth without caries and with well-developed roots and closed apices were selected. Multi rooted teeth, teeth with curved roots, teeth with root resorption, fracture lines, endodontic filling, instrumented canals, and calcified canals were excluded from the study. All the selected teeth were cleaned, polished with pumice and stored in distilled water.

The crowns were decoronated close to the cementoenamel junction with a diamond disc mounted in straight hand piece under constant water cooling. The apical patency was maintained by no. 10 k hand file (Mani Inc., Tochigi, Japan).

The working length was established by subtracting 1 mm from the total root length (root length mean 12.00 ± 0.2 mm). The root canals were prepared with crown down technique, using ProTaper Universal system (Dentsply-Maillefer, Ballaigues, Switzerland) incrementally up to size F3 (30, 0/6) to working length. The root canals were irrigated with 2 ml 3% sodium hypochlorite at the change of each instrument. Final irrigation was performed by 2 ml 17% Ethylenediaminetetraacetic acid for 3 min and 2 ml 3% sodium hypochlorite for 1 min followed by a final rinse of 2 ml normal saline. After completion of the biomechanical preparation of root canals, the samples were dried and randomly divided into three experimental groups of n = 50 according to the sealer placed.

Group 1- endo seal mta

Group 2- bioceramic sealer high flow

The sealers were manipulated according to the manufacturer'sinstructions. To allow analysis under the confocal microscope, each sealer was labeled with Rhodamine B dye to an approximate concentration of 0.1%. The sealer was placed with size 30 lentulo spiral in the canals keeping it 1 mm short of the working length and size F3 master gutta-percha cone was coated with sealer and placed in the canal up to the working length. The root canals were obturated with the lateral compaction technique using an endodontic finger spreader and accessory gutta-percha cones with a. 02 taper until the entire length of the root canal was filled. Excess gutta-percha was removed using a heated plugger and vertical compaction was performed 1 mm below the orifice level and then the teeth were sealed with temporary cement barrier and stored in incubator in 37°C at 100% humidity for 1 week. Each specimen was sectioned perpendicular to its long axis with diamond disc at slow speed under constant water cooling to avoid friction. Roots were sectioned at 3 mm and 5 mm from apex and two slices were obtained of two mm thickness. The coronal facing surface of each section was polished for 10 s on each side with abrasive sandpaper. The polished sections were imaged using a confocal laser scanning microscopy.

PENETRATION DEPTH MEASUREMENT

The samples were then mounted then mounted onto glass slides and examined with Nikon A1R inverted confocal microscope (Nikon Corp, Japan). The respective wavelength for Rhodamine B dye was 561 nm. Photos of each section were evaluated with NIS-Elements Br 3.0 imaging software (Nikon, Tokyo, Japan). The digital images were imported into ImageJ program (ImageJ software NIH) to measure the total dentinal tubule penetration, using a calibrated measuring tool. The maximum depth of sealer penetration was measured directly in micrometers (μm), from the canal wall to the deepest point at which the sealer was visualized. The depth of sealer penetration was measured and recorded at four standardized points (mesial, distal, buccal, and lingual) on each section. The measured readings were averaged to obtain a single mean value for each section and tabulated.

STATISTICAL ANALYSIS

The depth of sealer penetration in the apical area for three different sealers was analyzed at 3 mm and 5 mm level. The two groups were compared by ANOVA followed by post hoc multiple comparisons test (Tukey honestly significant difference). All the statistical tests were two-sided and wereperformed at a significance level of $\alpha = 0.05$. Statistical analysis was conducted using IBM SPSS Software (version 22.0). (IBM SPSS Inc., Chicago, IL, USA). With the significance level established at 5% ($P < 0.05$).

RESULTS

Table 1: The mean±standard deviation of the deepest tubular penetration of MTA, and bioceramic sealer at 3 mm and 5 mm from the apex

Group	Mean±SD		Significant
	At 3 mm	At 5 mm	
Group 1	258.65±95.76	461.82±101.77	0.339
Group 2	280.81±99.99	551.16±138.35	0.075

A significant difference was observed between MTA and bioceramic sealer at the 3 and 5 mm levels ($P < 0.05$), a significant difference was found between MTA andbioceramic sealerat the 3 and 5 mm levels ($P < 0.05$). No statistical significance in the depth of sealer penetration was found between MTA and bioceramic sealer at 3 mm level ($P > 0.05$) but a significant difference was observed at 5 mm level ($P < 0.05$), with bioceramic sealer showing greater dentinal tubule penetration. (Table 1)

DISCUSSION

Currently, root canal sealer penetration into dentinal tubules was mainly tested by scanning electron microscopy (SEM) and CLSM. Methods to evaluate root canal filling quality include root sections followed by stereomicroscope, SEM, CLSM, and micro computerized tomography (micro CT).The CLSM was used to evaluate the penetrationability of iRoot SP because it could provide a detailed view of the presence and distributionof sealers inside dentinal tubules when fluorescent Rhodamine B was added into the sealers. Another advantage of using CLSM is that the samples can be visualized in various depths, and it can differentiate the genuine interfacial failures from artificial gaps that could be produced after high vacuum desiccation under a scanning electron microscope.[10,11]

The complete sealing and filling of the cleaned and shaped root canal system are important steps that can affect the long-term success of root canal treatment.Inadequate obturation could result in re-entry and re-growth of microorganisms in the root canal system causing irritation to the periapical tissues and compromising the treatment success.[12] A hermetic seal reduces coronal leakage and bacterial contamination, prevents apical periodontitis, and entombs the remaining irritants in the root canal. Gutta-percha is considered as gold standard filling material, despite of its many desirable properties, it solely fails to provide an effective three-dimensional seal, to overcome this insufficiency endodontic sealers are used along with a core filling material to attain an impervious seal. Sealer fills the residual spaces and creates a union between the core material and the canal wall.[13] Various root canal sealers used in endodontic practice are categorized according to their main chemical constituents: Zinc oxide eugenol, calcium hydroxide, glass ionomer, silicone, resin, and bioceramic-based sealers.[14]

Bioceramic sealers have excellent biocompatibility due to their similarity with biological materials, like hydroxyapatite. BioRoot RCS (Septodont, Saint-Maur-des Fosses, France) is a powder/liquid hydraulic tricalcium silicate based cement, it is composed of tricalcium silicate, zirconium oxide, and calcium chloride. When comes in contact with the physiologic solution, these sealers release calcium and forms an interfacial calcium phosphate (apatite) layer, thus developing a chemical bond with the dentinal walls. It has a lower cytotoxicity than other conventional root canal sealers, may induce hard tissue deposition and has antimicrobial activity.[15]

The ideal outcome in root canal obturation is to have high volume of gutta-percha and minimal volume of sealer within the root canal space and enhanced penetration into the canal irregularities and dentinal tubules.[16] The removalof the smear layer of the root canal walls is considered tobe fundamental to allow sealer penetration into dentinaltubules irrespective of the root canal sealer used.[17,18] Bacteria may penetrate into dentinal tubules up to 100–1,000 μm.

Sealer penetration into the dentinal tubule would serve as a reasonable blocking agent that may prevent bacterial repopulation and can maintain their bactericidal action, which is favorable for healing periapical lesions and preventing reinfection.[16,19]

The deepest tubular penetration was observed at 5 mm from the apex than at 3 mm level of the root canal for all the tested sealers. These findings are similar to those reported by Camilleri.[20] and McMichael et al.[21] this may be because the number and diameter of dentinal tubules decrease apically in the root canal. To achieve tubule penetration, the particle size of the material must be smaller than the tubule diameter; it can be assumed that because the particles for bioceramic sealer are <1 mm in diameter, they will be well suited for tubule penetration. In the current study, the lesser penetration at 3 mm can be attributed to smaller tubules nearer the apex. The small particle size, hydrophilicity, and low contact angle of bioceramic sealer explains why it penetrated the deepest at this level.

The physical properties of root canal sealers could have an impact on the quality of the final root filling and the healing of periapical lesions. The flowing ability of root canal sealers results in better penetration leading to mechanical interlocking between the sealer and dentin.In clinical situations, bioceramic sealer absorbs water from dentinal tubules in the canal walls to expand laterally and adopt the canal shape. As water absorption induces expansion of the material, it improves the seal between the material and dentin.

According to the present study bioceramic sealer showed maximum sealer penetration at 3 mm and 5 mm level than MTA. This study did not examine the interface between the gutta-percha and dentin wall. The incidence of voids in root canal filling material can result in the proliferation of residual microorganisms and it may jeopardize the treatment outcomes. Further studies are necessary to analyze the interfacial adaptation of these sealers to root canal walls and the long-term results of these materials.

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