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The effect of the final irrigant on the antimicrobial activity of root canal sealers

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ABSTRACT

Objective: Root canal irrigation is an essential step in root canal therapy as it enables the elimination of microorganisms. The final irrigant may affect the properties of the root canal sealer used during obturation particularly with tricalcium silicate-based sealers, which interact with dentine. The aim of this study was to assess the antimicrobial activity of tricalcium silicate-containing sealers in contact with different irrigants. Furthermore the sealer surface in contact with the irrigant was characterized.
Methodology: The antimicrobial activity of BioRoot RCS, MTA Fillapex and AH Plus in contact with water, ethylenediaminetetracetic acid (EDTA) and phosphate buffered saline (PBS) was assessed by agar diffusion test and by the intratubular infection test against *Enterococcus faecalis*. The sealer surface in contact with the three solutions was characterized after 1 min contact and also after simulation of *in vivo* sealer contact with irrigating solution inside a tooth model by grazing angle X-ray diffraction analysis.
Results: Irrigation with EDTA showed the highest antimicrobial properties of the three root canal sealers followed by water without significant differences. The antimicrobial activity of BioRoot RCS was significantly higher than the other sealers after exposition to the three root canal irrigants followed by MTA Fillapex. AH Plus lost its antimicrobial properties after irrigation with water and PBS.
Conclusions: BioRoot RCS showed the greatest antimicrobial activity. The root canal sealers exerted a higher antimicrobial activity when EDTA was used as final irrigant. PBS may be contraindicated as a final irrigant as it reduces the antimicrobial activity of sealers.

1. Introduction

A primary goal of root canal treatment is to reduce microorganisms from the infected root canal system to levels compatible with healing [1] and to induce a regeneration of the dental and surrounding periodontal tissues. Mechanical instrumentation and irrigation reduce significantly the bacteria, however viable bacteria often remain on the dentine walls and inside dentinal tubules. For this reason, the use of root canal filling material with antimicrobial properties is considered beneficial in order to reduce the residual infection or create an environment that renders bacterial colonization difficult [2]. Root canal sealers are used in conjunction with solid cones in root canal filling. The sealers fill the gaps and interact with dentine leading to a bond between the sealer and the dentine wall. The nature of the bond depends on the composition of the sealer used. For most sealer types the bond is usually mechanical and results from sealer tags penetrating the dentinal tubules [3,4]. The sealer penetration is enhanced by removal of smear layer.

Tricalcium silicate-based root canal sealers have been developed due to their promising biological and chemical-physical properties that have made them succeed in other endodontic procedures. In the presence of tissue fluids, bioactivity of tricalcium silicate occurs resulting in the deposition of hydroxyl apatite on the material surface [5–7]. This bioactivity induces hard tissue formation and healing of soft tissue. The interaction of Biodentine (Septodont, Saint Maur-des-Fosses, France), which is a tricalcium silicate-based dentine replacement material, with tooth tissue results in a mineral infiltration zone [8]. The mineral infiltration zone is the ion exchange layer that appears in the interface between dentine and tricalcium silicate-based cements attributed to a dual effect of the calcium-hydroxide-releasing cement: an alkaline caustic etching followed by mineral diffusion [8]. This zone has also been shown with BioRoot RCS (Septodont, Saint-Maur-des Fosses, France) a root canal sealer with similar formulation, by tagging the sealer with a fluorescent dye and assessing the interaction with dentine using confocal microscope [9]. This zone has not been demonstrated with AH Plus although

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resin tags were evident for this sealer [9] as have already been reported by previous investigators [3,4]. Apart from the bioactivity, tricalcium silicate-based cements have demonstrated antimicrobial properties due to the alkaline pH that occurs during the hydration reaction. This alkalinizing activity is correlated with the calcium hydroxide release. Thus, different tricalcium silicate-containing sealers such as MTA Fillapex and Endosequence BC sealer have demonstrated antimicrobial activity against endodontic pathogens using different *in vitro* and *ex vivo* studies [10–12]. The antimicrobial effect of the sealers has been mostly evaluated by the agar diffusion test (ADT). However limitations exist with this method as the results rely on the solubility of the materials and their interaction with the culture medium and they do not consider factors such as the chemistry of the tooth and the biofilm [12].

The irrigation regime is important when using tricalcium silicate-based sealers. EDTA has been shown to inhibit formation of calcium hydroxide [13] thus reducing the prospects of bioactivity. Both EDTA and BioPure MTAD caused surface loss of material when applied over MTA [14]. However the use of chelating agents enhances the push out bond strength of both tricalcium silicate-based and resin-based sealers [15,16]. The use of water as a final irrigant resulted in lack of chemical bonding of sealers to dentine [15]. Bond strength of sealers is differentially affected by the irrigation protocol. The epoxy resin sealer AH Plus chemically bonds to dentinal collagen. This interaction is influenced by the irrigation protocols [17]. The use of a phosphate-based solution in the root canal in the presence of tricalcium silicate-based cements results in the formation of hydroxyapatite [18]. This bioactivity enhanced the push out bond strength of the root canal filling [19]. Other researchers discredited the beneficial effects of irrigation with a phosphate containing solution [4].

The presence of irrigants inside the root canal can affect the sealer chemistry particularly with reactive materials like tricalcium silicate-based sealers. Furthermore the changes in surface chemistry may affect the antimicrobial properties of the sealers within the root canal. The aim of this study was to assess the antimicrobial activity of tricalcium silicate-containing sealers in the presence of different irrigants. The sealer surface in contact with the irrigant was also characterized.

2. Materials and methods

The following sealers were assessed: BioRoot RCS (Septodont, Saint Maur-des-Fosses, France); MTA Fillapex (Angelus, Londrina, Brazil); AH Plus (Dentsply International, Addlestone, UK). The sealers were used in conjunction with different irrigation protocols as final rinse within the root canal that included one of the following irrigants: water; ethylene diamine tetracetic acid (EDTA; Calasept, Wykle Research, Carson City, NV, USA); phosphate buffered saline (PBS; Sigma-Aldrich, Gillingham, UK).

2.1. Agar diffusion test

Specimens 1 mm height and 6 mm diameter were molded for each sealer. Six specimens per group, for a total of 9 groups according to the irrigating solution and the sealer, were prepared. The materials were allowed to set for 24 h at 100% humidity and 37 °C. For the ADT, a previously described methodology was used [20]. Briefly, 100 μL of a 0.5 McFarland *Enterococcus faecalis* ATCC 29212 suspension were spread evenly on brain-heart infusion (BHI, Scharlau Chemie S.A., Barcelona, Spain) agar plates. After 30 min at room temperature three samples of each root canal sealer were immersed in any of the three solutions for 1 min, dried on sterile filter paper and then placed on the BHI agar plates. Ten microliter drops of each irrigating solution were also included as controls. After incubation for 24 h at 37 °C, microbial inhibition zones were measured in millimeters (±1 mm) with a precision rule.

2.2. Intratubular infection test

2.2.1. Tooth preparation

Thirty freshly extracted non-carious human maxillary premolars were selected and stored at 4 °C until use. The Ethics Committee of the institution where the experiment was performed approved the protocol (UGR-438). Specimen preparation followed a previously described protocol [21]. Cylindrical root segments of 4 mm length were obtained by sectioning the root horizontally 1 mm below the cemento-enamel junction using an Accuton-50 machine (Struers, Copenhagen, Denmark). Each root canal was enlarged to the size of a Gates-Glidden bur #4. The root segments were then sectioned longitudinally into halves by means of a low-speed hand piece with a diamond disk (355514220 HP, Edenta AG, AU/SG, Switzerland). The outer cementum of each half was then removed, and the size was adjusted using a calibrator and polishing with 220–800-grit silicon carbide papers to obtain $4 \times 4 \times 2$ mm specimens. The smear layer formed during preparation of the dentine specimens was removed by treating the surface with 17% EDTA for 5 min. Then, samples were washed with distilled water for 10 min and sterilized by autoclave for 20 min at 121 °C. The sterility of the dentine was checked by incubating each specimen in 5 mL of BHI broth at 37 °C for 24 h. Each prepared dentine specimen was placed in the upper chamber of a filter tube (VWR International Eurolab SL, Barcelona, Spain) with the canal side up, and gaps with the inner wall of the tube were sealed with flowable composite resin (Tetric EvoFlow, Ivoclar Vivadent, Schaan, Liechtenstein) and light-cured for 20 s. A sterile tweezers was used for the specimens manipulation.

2.2.2. Dentine infection with E. faecalis

A previously described protocol [22] was used for dentinal tubule infection. Briefly, 500 μL of an *E. faecalis* suspension of approximately 1×10^7 CFU/mL was added to the upper chamber of each filter tube with the dentine specimen inside. The tubes were centrifuged at 1400*g*, 2000*g*, 3600*g*, and 5000*g* in sequence, twice each for 5 min. Between the centrifugations, the suspension was replaced with 500 μL of a fresh one. The same procedure was repeated after two days. The samples were incubated for a total of 5 days at 37 °C.

2.2.3. Antimicrobial test

Dentine specimens were taken out of each tube and were randomly divided into twelve groups (n = 5) according to the final irrigating solution and the sealer: Group 1: water and BioRoot RCS; Group 2: EDTA and BioRoot RCS; Group 3: PBS and BioRoot RCS; Group 4: water and MTA Fillapex; Group 5: EDTA and MTA Fillapex; Group 6: PBS and MTA Fillapex; Group 7: water and AH Plus; Group 8: EDTA and AH Plus; Group 9: PBS and AH Plus. Control groups were also included (n = 5) which consisted of dentine specimens treated with water, EDTA and PBS without sealer.

Fifteen μL of the irrigating solutions were placed on the root canal lumen of each sample for 1 min. The specimens were then dried by placing them on sterile absorbent papers and each freshly prepared sealer was placed on the surface of the root canal wall using a cavity liner applicator to achieve an approximate thickness of 0.5 mm. The dentine samples were placed at 37 °C in 100% relative humidity for 7 days. The sealer was then removed from the root canal surface with the aid of a spatula, and the specimens were vertically cut into two halves through the root canal using an Accuton-50 machine with saline solution as irrigant.