Interfacial Characteristics and Cytocompatibility of Hydraulic Sealer Cements

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Abstract

Introduction: The stability and long-term success of root canal obturation depends on the choice of sealer because the sealer bonds to the dentin and stabilizes the solid cone. Furthermore, the sealer needs to be nontoxic because sealer toxicity will certainly lead to treatment failure. The aim of this study was to assess the sealer-dentin interface of 3 hydraulic root canal sealers and to evaluate their cytocompatibility compared with AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany). **Methods:** Four dental root canal sealers were assessed. AH Plus, MTA Fillapex (Angelus, Londrina, Brazil), BioRoot RCS (Septodont, Saint-Maur-des-Fossés, France), and Endoseal (Maruchi, Wonju-si, Gangwon-do, South Korea) were characterized using scanning electron microscopy and energy-dispersive spectroscopy. The sealer-tooth interface was assessed by confocal microscopy and scanning electron microscopy, and biocompatibility was measured by assessing the cell metabolic function using direct contact assays and alkaline phosphatase activity. **Results:** The tricalcium silicate–based sealers presented a different microstructure and elemental composition despite their similar chemistry and classification. BioRoot RCS was free of aluminum, and all sealers presented different radiopacifying elements. The sealer penetration in the dentinal tubules and interfacial characteristics were different. The migration of silicon was evident from sealer to tooth for all sealers containing tricalcium silicate. MTA Fillapex and BioRoot RCS exhibited the best cytocompatibility in both the direct contact test and alkaline phosphatase activity. **Conclusions:** The use of hydraulic calcium silicate–based sealers has introduced a different material type to endodontics. These materials are different than other sealers mostly because of their hydraulic nature and their interaction with the environment. Although the sealers tested had a similar chemistry, their cytocompatibility and bonding mechanisms were diverse. *(J Endod 2017;■:1–11)*

Key Words

Cell viability, characterization, hydraulic sealer cements, interfacial characteristics

Significance

The hydraulic calcium silicate–based sealer cement properties depend on the environment in which they are placed. The sealers are interactive rather than inert.

The success of root canal obturation depends on the sealer characteristics. The sealer stabilizes the solid cone and bonds to the dentin. At the root apex, the root canal sealer is in contact with the apical tissues; thus, its biocompatibility is also an important property.

Traditional root canal sealers were classified depending on their chemical composition. They are inert, and the interaction of the sealer to dentin occurs by sealer tags penetrating into the dentinal tubules. Thus, the bond is affected by the efficacy of the smear layer removal. During the last decade, the use of mineral trioxide aggregate (MTA) has been extended to fill the root canal either entirely or in conjunction with gutta-percha (1, 2). MTA was not indicated for use as a root canal sealer. Filling the root canal with MTA resulted in higher leakage apically than gutta-percha sealer obturation (2). Eventually, sealers based on MTA were developed, and a number are available clinically.

The bonding mechanism of MTA was not very well investigated, and was never reported. However, the bond strength was dependent on the environment's humidity (3), with higher values reported in contact with simulated tissue fluid (4). The biomineraliztion ability of MTA is responsible for the enhanced bond strength (5). Subcutaneous implantation of MTA resulted in the formation of mineralized tissues (6). The bonding mechanism was first described for Biodentine (Septodont, Saint-Maur-des-Fosses, France), and bonding occurred by alkaline etching and the development of the mineral infiltration zone in the material in contact with the tissues (7). In this research, using confocal laser microscopy and fluorescent markers, it was shown that mineral ions from the material cross over to the dentin and a layer is deposited at the interface. The mineral infiltration zone was also shown for BioRoot RCS (Septodont), a tricalcium silicate–based material developed to be used as a sealer (8). The bonding characteristics of BioRoot RCS were shown to be different than those of AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) because the latter only bonded by sealer tags, whereas the hydraulic calcium silicate–based materials also demonstrate the mineral ion–rich layer at the interface. No phosphate-based phases were shown in BioRoot RCS in contact with the tooth structure; however, beta calcium phosphate was

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deposited on the sealer surface when the material was immersed in simulated tissue fluids (9). This shows the mineral infiltration in dentin is unlikely to be hydroxyapatite.

A number of hydraulic calcium silicate sealers are premixed. Thus, their setting depends on the humidity of the root canal. EndoSequence BC Sealer (Brasseler, Savannah, GA) has been investigated and showed complete setting (9). Because this sealer contained a phosphate phase, its interaction with dentin and the development of mineral infiltration zone could not be assessed (9). There is a lack of knowledge on how hydraulic sealer cements interact with the dentin and whether changes in the presentation and sealer chemistry affect the interfacial characteristics of the materials. The aim of this study was to characterize 3 hydraulic calcium silicate sealers that have diverse chemistry and presentation and to assess the interfacial zone of these sealers. Furthermore, the biocompatibility of the sealers was investigated because sealer toxicity also affects the clinical success of endodontics. AH Plus, an epoxy resin–based sealer, was used as a control.

Materials and Methods

The following root canal sealers were used in this study:

1. AH Plus (Dentsply DeTrey GmbH)
2. MTA Fillapex (Angelus, Londrina, Brazil)
3. BioRoot RCS (Septodont)
4. Endoseal (Maruchi, Wonju-si, Gangwon-do, South Korea)

All sealers were mixed and manipulated in accordance with the manufacturers' instructions, except for Endoseal, which is a premixed root canal sealer that was syringed.

Material Characterization

The sealers were mixed following manufacturers' instructions and were allowed to set at 100% humidity for 48 hours at 37°C. Endoseal was covered with moist gauze. Three discs 10 mm in diameter were prepared for each sealer type. The set sealers were characterized by scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS). Disc-shaped specimens (10 mm in diameter and 2-mm high) were prepared from each sealer type. They were vacuum impregnated in resin (Epoxyfix; Struers GmbH, Ballerup, Denmark). The resin blocks were then ground with progressively finer diamond discs and pastes using an automatic polishing machine (Tegramin 20, Struers GmbH). Specimens were mounted on aluminum stubs, carbon coated (Agar Scientific, Stansted, UK), and viewed under a scanning electron microscope (Zeiss MERLIN Field Emission SEM; Carl Zeiss NTS GmbH, Oberkochen, Germany). Scanning electron micrographs at high magnification of the different material microstructural components in the backscatter electron mode were captured, and EDS was performed.

Assessment of Interfacial Characteristics

The root dentin–sealer interface was assessed using confocal microscopy with 0.1% fluorescein dye (Sigma-Aldrich, St Louis, MO) and scanning electron microscopy and energy-dispersive mapping.

Tooth Preparation. No ethical approval or patient consent was sought because the country legislation did not restrict the collection of extracted teeth. Sixteen single-rooted human teeth with fully formed apices (including bicuspids, canines, and incisors) were collected anonymously from dental offices (general dentists, oral surgeons, and periodontists) and stored in distilled water until use. All teeth were decoronated, standardizing the root length to a 15-mm length. Roots were prepared using ProTaper Universal instruments (Dentsply Maillefer, Ballaigues, Switzerland) in a modified crown-down manner up to F4 as the master apical file, 1 mm shorter than the root length (14 mm).

The canals were irrigated with 2 mL 5% sodium hypochlorite between the changes of the rotary files using a 30-G Miraject Endotec Luer (Hager & Werken GmbH & Co KG, Duisburg, Germany) tip attached to the plastic syringe and introduced 3 mm shorter than the working length. The final rinse was performed with 5 mL 5% sodium hypochlorite for 5 minutes followed by 5 mL distilled water and 5 mL 17% EDTA followed by 5 mL saline. The root canals were dried with paper points and then randomly divided for SEM and confocal examination.

Confocal Microscopy Examination. The sealers were mixed according to the manufacturers' instructions. Fluorescein (Sigma-Aldrich) was added to the sealers in a 0.1% proportion. The sealers were placed inside the root canals using a Lentulo spiral. The coronal and apical access was restored with glass ionomer cement (Fuji IX; GC Europe, Leuven, Belgium). Two teeth were prepared for each material and were immersed in Hank's Balanced Salt Solution (Sigma-Aldrich, St. Louis, MO) for 28 days at 37°C. At the end of the immersion period, the teeth were removed from solution, dried and embedded in resin, sectioned longitudinally using a hard tissue microtome (Accutom 50, Struers GmbH), and polished using an automatic polishing machine as indicated previously. The root dentin–cement interface was assessed using a confocal microscope (Nikon Eclipse, Tokyo, Japan) with an oil immersion ×60 magnification objective lens. The fluorescein was visible at an excitation/emission wavelength of 494/521 nm.

Scanning Electron Microscopic Examination. The teeth used for scanning electron microscopic investigation were filled with sealers as mentioned earlier but without fluorescein and immersed in Hank's Balanced Salt Solution for 28 days at 37°C. They were processed in a similar way to the previous experiment. The sections were then mounted on aluminum stubs and carbon coated. The root dentin–cement interface at different levels along the root canal was then viewed with a scanning electron microscope (Zeiss MERLIN Field Emission SEM) in the backscatter electron mode at ×2000 magnification. EDS analysis was performed over the materials and tooth structure in order to determine the elemental constitution. Furthermore, elemental maps across the interface were performed with each element being mapped in a different color.

Investigation of Sealer Biological Properties

The biocompatibility was assessed by evaluating the cell activity and proliferation of gingival fibroblasts in contact with the different sealers. Human gingival fibroblasts were obtained from gingival tissue from healthy patients who underwent oral surgery. They were isolated and grown in cell culture medium (Dulbecco modified Eagle medium) supplemented with 10% fetal bovine serum, 100 mg/mL penicillin G, and 50 mg/mL streptomycin at 37°C in air with 5% CO_2 in a humidified incubator under ambient atmospheric pressure. At 70% to 80% confluence, cells were detached using 0.25% trypsin and 0.05% EDTA for 5 minutes at 37°C and replated or counted.

The cytocompatibility of the test materials was evaluated *in vitro* according to ISO 10993-5; 2009 (10) using a direct testing method. The 3-(4,5 dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide assay was used to assess cell metabolic function (11). The 4 sealers were mixed in strict compliance with manufacturers' instructions and shaped with 1-mm-thick nonreactive plastic molds with a diameter of 10 mm under aseptic conditions.

For direct testing, 1.5×10^5 gingival fibroblast cells were seeded in 1 mL in a 24-well plate after including the discs in each well. This was done in triplicate. After 1 day of incubation, the disc was removed