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ORIGINAL ARTICLE

Interfacial characterization of hydraulic calcium silicate-based sealers by Scanning Electron Microscopy

ABSTRACT

Aim: *To characterize the sealer-dentin interface of hydraulic calcium silicate-based sealers TotalFill and Bioroot and compare it to an epoxy-resin sealer AH Plus.*
Methodology: *An experimental ex vivo study was conducted were 15 single-root extracted premolars were divided into three experimental groups. The teeth were prepared and filled using a single tapered gutta-percha cone. Samples were cut and analyzed using scanning electron microscopy. The images were analyzed using ImageJ software.*
Results: *The median interface value for the three sealers was between 0.6 and 2.5 µm. The largest interface was observed in the TotalFill group in the middle third, and this difference was significant compared to AH Plus ($p<0.05$). The largest sealer area was for Bioroot in the apical third, but the difference was not significant.*
Conclusions: *Within the limitations of this study, AH Plus had better marginal adaptation in the middle third. The interfacial gaps were similar in the apical third for all sealers.*

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Introduction

Root canal filling materials are necessary to avoid root canal reinfection and to entomb remaining bacteria (1), and to fulfil these purposes, root canal sealers must provide excellent sealing ability, adequate dimensional stability, a reasonable setting time to ensure proper handling, insolubility in tissue fluids and ideally, high cytocompatibility (1, 2). However, most materials fail to provide an effective seal (3) and moisture inside the canal before obturation appears to be critical (4). Conventional resin-based sealers are hydrophobic, and their properties are altered by moisture (4), but this is not the case for Bioceramic (BC) or hydraulic sealers because they are hydrophilic, and root canal wall and tubule moisture is necessary for setting (2).

BC sealers were created to overcome several disadvantages of traditional resin-based sealers. They present properties such as a non-existent setting contraction (5) or slight expansion and an alkaline pH, which gives them antibacterial capacity before setting and chemical stability (6). Other properties include biocompatibility and the ability to create a link between dentine and the obturation material (7). All of these qualities make them, in theory, excellent obturation materials.

Among the most frequently used BC sealers is TotalFill BC SealerTM (FKG Dentaire, La Chaux-de-Fonds, Switzerland), a premixed tricalcium silicate-based sealer available in a syringe presentation. Its composition includes monoclinic zirconia, calcium silicate, monobasic calcium phosphate, calcium hydroxide, and tantalum pentoxide (8). Its working time is over four hours at room temperature, and the setting time is more than four hours; however, in an environment with low humidity, the final setting time may be up to 23 hours (8).

Other properties reported are the ability to produce an appropriate seal, adequate radiopacity, flow and bioactivity produced by ion release (8).

BioRoot RCS™ (Septodont, Saint-Maur-des-Fosses, France) is a sealer based on tricalcium silicate and zirconium oxide (9). Its presentation is powder-liquid. According to the manufacturer, the powder is based on tricalcium silicate, zirconium oxide and iodine, and the liquid is composed of calcium chloride and polycarboxylate. It has ten minutes of working time and a final setting time of five hours (10). Other reported properties include adequate radiopacity, bioactivity and an alkaline pH (10).

Considering the lack of contraction upon setting and the possibility of obtaining a chemical bond to the root canal wall, obturation with hydraulic calcium silicate-based sealers can work differently than obturation with resin sealers. Its qualities make it unnecessary to maintain the sealers' thickness to its minimum (6). It is used in a hydraulic technique based on a tapered 4 or 6% gutta-percha point of the calibre of the last used instrument that acts as a piston allowing the sealer to flow into the canals' irregularities leaving a higher percentage of sealer (6). However, to allow a higher percentage of sealer, it should be first determined whether the interface between dentine and sealer is minimum or ideally non-existent.

This study aims to characterise the sealer-dentine interface in teeth obturated with hydraulic calcium silicate-based sealers: TotalFill BC Sealer™ and BioRoot RCS™ analysed under a scanning electron microscope (SEM).

Materials and Methods

An experimental ex-vivo study was conducted prior to the approval of the ethics committee of San Sebastian University (Resolution Number 2018-14).

Sample Selection

The sample consisted of fifteen one-rooted mandibular premolars extracted for orthodontic reasons, with a type I Vertucci configuration (11), complete apical formation and a radicular curvature of no more than 10°, of patients between the age of 15 and 30.

Teeth that presented canal obliteration, internal or external radicular resorption, or endodontic treatment, were discarded.

Sample Preparation
Teeth were cleaned of organic residues with an ultrasonic scaler and then stored in distilled water for no longer than 30 days. The roots were sectioned to a length of 12mm from the apex with a diamond bur under constant refrigeration. Working length was determined with a K10 file observed under a 3.5x loupe when it came out of the apical foramen; 1mm of this length was subtracted. Root canals were then prepared with the Reciproc system to R40 file (VDW, München, Germany), using 10 mL of 5.25% sodium hypochlorite (NaClO), activated by the Endoactivator (Dentsply Maillefer, Ballaigues, Switzerland) for one minute. Final irrigation was performed with 3mL ethylendiaminetetracetic acid (EDTA) 17%, 3 mL of NaClO for 60 seconds, and 5 mL of saline solution for 60 seconds. The canals were dried with aspiration and paper points.

Experimental Groups Preparation
Teeth were divided into three groups of five premolars each.
Group 1: TotalFill BC Sealer™
Group 2: BioRoot RCS™
Group 3: AH Plus™ (Dentsply DeTrey, Konstanz, Germany)
All groups were obturated using the hydraulic technique with an R40 gutta-percha point. The sealers were manipulated according to the manufacturers' indications. The samples were coronally sealed using Vitremer ionomer (3M Espe, St Paul, USA) and stored at 37 °C and 100% relative humidity for seven days. After this period, the teeth were mounted in translucid epoxy resin (Fibratec, Santiago, Chile), to be sectioned at 3 and 6mm from the apex using a precision sectioning saw (ISOMET 1000, Buehler Ltd., Lake Bluff, IL, USA).

Sample Observation and Analysis
The samples were sent to the Advanced Microscopy Unit of the Catholic University of Chile for processing (golden shadowing on the coronal surface) and observation under SEM (Hitachi, TM3000, Tokyo, Japan). Images were obtained at 60 and 100x to completely visualise the obturation material and dentine, and two quadrants were chosen because they presented the lesser distortion of gutta-percha produced by the saw's cut. Close-ups at 500x 1,000x and 2,000x were taken. The interfacial distance was measured from the edge of the sealer to the edge of the dentine.
Before the observation, criteria for analysis was defined, and measuring methods were considered between the examiner and expert observer. To measure the interface horizontally and vertically, the examiner observed a total of twelve images of 2,000x randomly chosen, in the same conditions of daytime, light and screen resolution, and ten days later repeated the entire process. With the acquired data, an intra-class correlation coefficient was obtained using the statistical program EPIDAT 4.2 (Xunta de Galicia, Universidad CES, 2016), which showed an intra-examiner agreement of 0,97 on the horizontal plane and 0.91 on the vertical plane, which indicates a high concordance. For the sealer area percentage, the examiner observed a total of eight images at 60x that were randomly chosen, obtaining an intra-examiner correlation coefficient of 0.99 in the canal area and 0,95 for the gutta-percha point, which indicates a high concordance.
The digitalised images were observed by one operator on the same computer, with a screen resolution of 1,366x768 in a 14-inch screen with the ImageJ program (ImageJ, Wayne Rasband, National Institutes of Health, USA). Twenty different images of each group at 2000x were selected to measure the sealer-dentine interface. A printed scale of an image at 2,000x representing 30 µm was used to measure the interface. A grid was traced with 20 vertical and horizontal lines that guided the location during measurement. The images where the interface did not coincide with the vertical line were rotated (Figure 1A).
The horizontal measurement was obtained by tracing 20 segments perpendicular to the interface selected to reference the horizontal lines on the grid. After that, for the vertical measurement, segments were traced parallel to the interface taken as

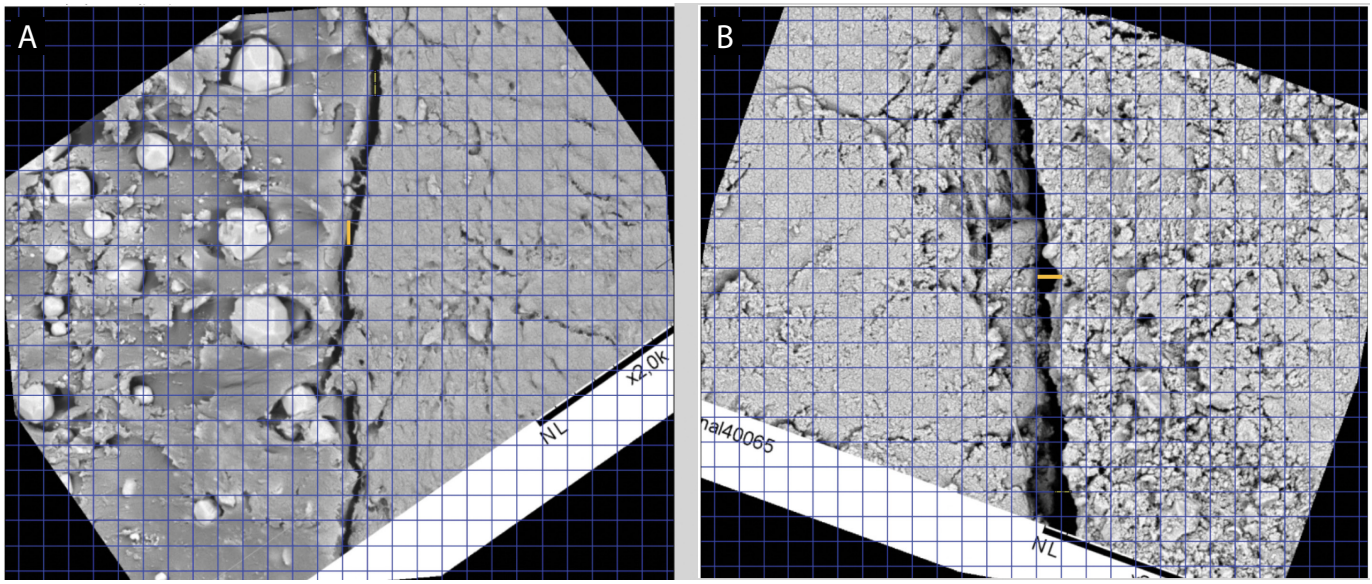

Figure 1
A) Horizontal measuring and **B)** vertical measuring of the sealer-dentin interfase through the tool provided by ImageJ Software (2,000x).

reference for the horizontal lines (Figure 1B). Ten images of each group were selected to calculate the sealer percentage inside the canal. The surface occupied by the canal was first measured, and then the surface occupied by the gutta-percha point; the difference between these determined the sealer area (Figure 2). All measurements were registered in μm and adjusted to three digits after the decimal using a measuring tool provided by the software.

Results

A total of 240 images were obtained: 60 at 2,000x, 60 at 1,000x, 60 at 500x, 30 at 100x and 30 at 60x. For the interfacial measurement, 60 images at 2,000x were analysed, founding the largest horizontal interface in the TotalFill BC Sealer™ group in the apical zone (1,091 μm on average), and the smallest vertical interface was in the BioRoot RCS™ group in the middle third (57,1%) (Table 1).

Sealer area was measured in relation to the canal area; the images taken were analysed at 60x, and the most substantial proportion of sealer was found on the BioRoot RCS™ group in the middle third (Table 1).

To verify that the differences observed were statistically significant, the Shapiro Wilk test was applied, which showed that data had a non-normal distribution ($p<0.05$). Secondly, Kruskal Wallis non-parametric test was applied and showed significant differences in the horizontal interface of AH Plus™ middle third

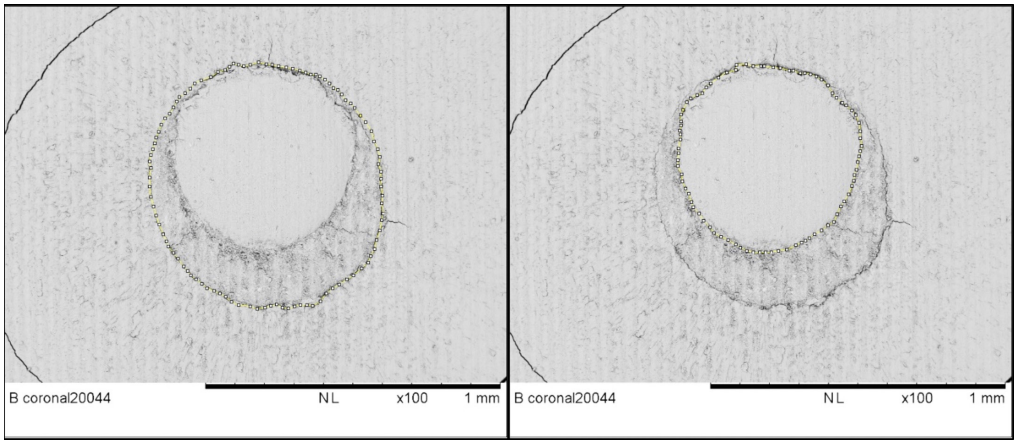

Figure 2
Sealer area measurement through the tool provided by the ImageJ software (60x).

Table 1

Measurements of central tendency of the sealer-dentin interfase in the horizontal plane, in the vertical plane and sealer area inside the root canal

Sealer-Dentin Interfase and Sealer Area									
Horizontal Interfase Measurements in µm						Vertical Interfase Measurements in µm			Sealer Area
Sealer		Median	DS	Minimum	Maximum	Interfase Length	Total Interfase	% Interfase	% Sealer
TotalFill	Apical	1,091	0,720	0,020	2,609	52,711	66,298	79.7%	23.2%
	Middle	0,393	0,330	0,000	1,161	48,100	67,593	71.1%	32.7%
Bioroot	Apical	0,681	0,544	0,000	1,898	39,317	67,260	58.6%	29.4%
	Middle	0,801	0,615	0,000	2,003	40,703	70,977	57.1%	35.6%
Topseal	Apical	0,640	0,519	0,042	1,756	41,580	66,654	62.8%	28.1%
	Middle	0,357	0,281	0,044	0,983	32,472	66,344	48.8%	35.4%

vs BioRoot RCS™ middle third (p=0.001). No significant differences were observed for the vertical interface and sealer area (p<0.05).

Discussion

This study aimed to determine the sealer-dentine interface in teeth filled with BC sealers TotalFill BC Sealer™ and BioRoot RCS™ under SEM. There were no differences in the sealers' vertical and horizontal interfacial adaptation in the apical third. These results agree with what was observed by Mohammadian (2016), who also found no differences in the apical third when comparing hydraulic calcium silicate-based sealers to AH Plus™ (12).

AH Plus™ presented significantly better adaptation in the middle third when compared to BioRoot RCS™. The results obtained in this study agree with Al-Haddad and Aziz (2015) when they compared hydraulic calcium silicate-based sealers to AH Plus and found that the interface was slightly smaller for AH Plus™, but the differences were not significant. (13) In a study conducted by Arikatla (2018), they also observed better adaptation for AH Plus™ when compared to hydraulic calcium silicate-based sealers (BioRoot RCS™ and MTA Fillapex™ sealer) (14). One of the reasons why AH Plus™ might have presented better adaptation in the middle third could be because of its chemical bonding to dentine (13) and higher flow (15); premolars with one canal tend to have irregular shapes in the middle third, and this ability would prove helpful to fill this area.

BioRoot RCS™ presented the highest sealer percentage in the apical third, but the difference was not significant. Theoretically, the sealer percentage should be minimal, considering that most sealers contract upon setting (13). However, this is not the case for Hydraulic calcium silicate-based sealers, in which a slight expansion is observed (6); a large percentage of sealer in the apical third would not be detrimental to the sealing ability (16).

The three sealers studied presented gaps in the apical and middle third; this agrees with Eltair (2018) in that both TotalFill BC Sealer™ and AH Plus™ showed visible gaps in all root thirds (3). Marginal adaptation is critical in root canal treatment. Root canal obturation aims to produce a bacteria-tight seal and entomb possible remaining bacteria (1). Root canal biofilm has a variable thickness between a few and hundreds of cells, and the microorganisms

that regularly take part in biofilm foundation, such as *Enterococcus faecalis*, have a diameter between 0,6-2,5 μm (17). The interface in this study had a median value of 0,357 to 1,091 μm, which is minimal. Even though all sealers present an interface, it is unlikely that a biofilm could form in such a confined space, indicating that all the sealers studied would be effective obturation materials.

Conclusions

This study compared the sealer-dentine interface of three root canal sealers: TotalFill BC Sealer™, BioRoot RCS™, and AH Plus™. All sealers presented a minimal interface in the horizontal and vertical planes. Within the limitations of this study, AH Plus™ had better marginal adaptation in the middle third. The interfacial gaps were similar in the apical third for all sealers. The sealer percentage was slightly higher for BioRoot RCS™ in the apical third, but the difference was not significant.

Clinical Relevance

Endodontic sealers are an essential part of canal obturation since they effectively seal against bacteria. Under the conditions of this study, it can be inferred that BC sealers TotalFill BC Sealer™ and BioRoot RCS™ provide a proper marginal adaptation that could lead to successful obturations.

Conflict of Interest

None.

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Nothing to declare.

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