Sealing ability of a bioceramic sealer used in combination with cold and warm obturation techniques

D. I. K. PONTORIERO[1], G. MADARO[2], V. VANAGOLLI[2], S. BENEDICENTI[2], G. VERNIANI[3], E. FERRARI CAGIDIACO[3], S. GRANDINI[4], M. FERRARI[3]

[1] Department of Prosthodontics, University of Siena - Department of Endodontics, University of Genoa, Italy
[2] Department of Endodontics, University of Genoa, Italy
[3] Department of Prosthodontics, University of Siena, Italy
[4] Department of Endodontics, University of Siena, Italy

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ABSTRACT

Aim The aim of this study was to evaluate the sealing ability of a bioceramic endodontic sealer and a ZOE sealer at the apical third when used in combination with three different obturation techniques.

Materials and methods A sample of 80 recently extracted intact human single-rooted maxillary premolars were included in the study. After instrumentation, teeth were randomly divided into five groups and accordingly filled with different techniques and materials. Group 1 was filled using Thermafil® Obturators (Dentsply/Maillefer) with Pulp Canal Sealer™ (Kerr), Group 2 was filled using Thermafil® Obturators (Dentsply/Maillefer) with BioRoot™ RCS (Septodont) bioceramic sealer, Group 3 was filled using warm vertical condensation with traditional Pulp Canal Sealer™ (Kerr), Group 4 was filled using a Single GP cone with BioRoot™ RCS (Septodont) bioceramic sealer and Group 5 was filled using warm vertical condensation with BioRoot™ RCS bioceramic sealer (Septodont). Apical leakage, using silver nitrate for 24 hours, was evaluated and statistical analysis was applied (Bonferroni and Anova tests with the significance level set at $P < 0.05$).

Result The groups using bioceramic sealer showed a lower amount of apical leakage, and Group 5 the lowest amount of dye penetration apically.

Conclusion Under *in vitro* conditions, bioceramic endodontic sealers can seal the root apex similarly to traditional ZOE endodontic sealers and can be used in combination with any obturation technique, both warm and cold gutta-percha.

INTRODUCTION

Root canal obturation aims to fill and seal, in all the three dimensions, the endodontic space, in order to prevent penetration and growth of microorganisms within it, as well as percolation of tissue fluids susceptible to degradation (1,2). To achieve a proper obturation of the root canal, two main factors are crucial: the root canal obturation technique and the endodontic cement.

Several root canal obturation techniques are available, and those most commonly used can be divided into two groups.

1. Warm gutta-percha techniques, such as carrier-based obturation (3,4), continuous-wave compaction technique, plasticized technique, warm vertical compaction (5-9).
2. Cold techniques, namely lateral compaction, and single cone technique (10-13).

The purpose of the cement (14,15) is to facilitate the flow of the gutta-percha, to fill the spaces not filled by the gutta-percha itself, and to create an adhesive interface on the canal walls. However, the cement appears to be a locus minoris resistentiae for bacterial infiltration, since all types of cement currently available on the market are slightly resorbable, with the formation of empty spaces; consequently, they should be used in the smallest possible quantity.

Bioceramics are non-metallic, biocompatible, and inorganic materials that include zirconia and alumina, bioactive glass, coatings and composites, hydroxyapatite, and resorbable calcium phosphates (16). Today, new bioceramic cements can be used as substitutes of MTA or as root canal sealers (16).

BioRoot RCS (Septodont) has been reported to induce, *in vitro*, the production of osteogenic and angiogenic growth factors by human periodontal ligament cells (17). Moreover, it has lower cytotoxicity than other conventional root canal sealers, and it may induce hard tissue deposition (18,19). This material also has antimicrobial activity (20). The powder contains tricalcium silicate, zirconium oxide, and povidone; the liquid is an aqueous solution of polycarboxylate and calcium chloride. Bioceramic cements are inductive materials, meaning that during hardening, when they come in contact with tissue fluids, calcium hydroxide reacts with phosphatase enzymes, thus inducing the formation of hydroxyapatite (21).

It was also demonstrated that these types of cement could be used as either root filling materials or root canal sealers in combination with gutta-percha for filling root canals. *In vivo* studies show excellent results, although Torabinejad emphasizes that the new bio-ceramic cements are indicated by recent clinical research as the future of root canal obturation, but they still need further investigation, as there is no long-term *in vivo* and *in vitro* studies in the literature (21).

There is not a standard method used to measure the bonding of a sealer to the root dentin; for this reason, the quality of the adhesion of the root filling material is commonly tested using bond strength tests and microleakage (22). Microleakage is described as the "spread of bacteria, oral fluids, ions, and molecules into the tooth and filling material interface" (23).

Apical leakage is regarded as the most common cause of endodontic failure and is influenced by several variables, such as different obturation techniques, chemical and physical properties of the root canal filling materials, and the presence or absence of the smear layer. This is referred to as primary failure or primary infiltration.

In a recent study (24) micro-CT scans were used to compare the 3D filling performance and the presence of radiographic translucencies of three different root canal filling techniques: warm vertical condensation, carrier-based technique, and single cone with bioceramic sealant; obturation volume, pore-volume, and pore rate were investigated. No obturation technique revealed completely filled root canal systems. The filling techniques employed in this study have similar features: they were biocompatible, radiopaque, and inert, providing a stable apical and intracanal seal.

Both continuous-wave and carrier-based techniques are built on the concept of minimum sealant interface, whereas the single-cone technique of bioceramic sealant originates from Grossman's concept of the maximum interface of the sealant with the gutta-percha cone intended as support (15).

Still, few studies are available concerning this category of cements, and most of them focus on the analysis of their excellent biological properties. For this reason, in this study, it was decided to focus on their behavior within the root canal when used in association with different obturation techniques.

The aim of this study was to evaluate the sealing ability of a bioceramic endodontic sealer (BioRoot RCS) and a ZOE sealer (Pulp Canal Sealer) at the apical thirds when used in combination with three different obturation techniques.

The null hypothesis tested was as follows.

1. The bioceramic endodontic cement can seal the apex similarly to traditional endodontic ZOE cement.
2. The bioceramic endodontic cement can similarly seal the apex when used in combination either with cold or warm obturation technique.

MATERIALS AND METHODS

Eigthy intact human single-rooted maxillary premolars, recently extracted for orthodontic reasons, were included in the study. After the cleaning of remaining tissues, a ×4.5 stereomicroscope (Nikon SMZ645; Nikon, Tokyo, Japan) was employed to rule out any external radicular cracks.

Samples were stored in 0.9% saline solution (SALF SPA, Cenate Sotto, Italy) at a temperature of 37°C to prevent dehydration. After preparing the access cavity, pre-curved stainless steel manual K-files # 8, and # 10 (Sweden & Martina, Due Carrare, Italy) were used to define the working length.

All chemo-mechanical procedures for preparation of the samples were performed by two calibrated operators (GM and VV).

NiTi Mtwo File (Sweden & Martina, Due Carrare, Italy) instruments were used for root canal shaping following the manufacturer's instructions with an endodontic motor (X-SMART TM Plus; Dentsply Maillefer, Ballaigues, Switzerland) set at 250rpm; 2.5 ml of 5% sodium hypochlorite (Niclor 5 dental; Ogna Lab, Muggiò, Italy) was used after each rotary instrument by syringe-needle irrigation with a 31-gauge needle tip. Sodium hypochlorite was preheated at 50°C. Apical patency was maintained by using a # 10 K-type file after each larger file. The final irrigation phase was carried out with 2 ml of 17% EDTA (Ogna Lab, Muggiò, Italy) for 2 minutes, followed by the final rinse of 5 ml of 5% NaOCl for 5 minutes, to optimize the removal of inorganic and organic components. After instrumentation, canals were dried with paper points.

Teeth were randomly divided into five groups and filled with different techniques and materials.

- Group 1 was filled using Thermafil® Obturators (Dentsply/Maillefer) with Pulp Canal Sealer™ (Kerr), n=20.
- Group 2 was filled using Thermafil® Obturators (Dentsply/Maillefer) with BioRoot™ RCS (Septodont) bioceramic sealer, n=20.
- Group 3 was filled using warm vertical condensation with traditional Pulp Canal Sealer™ (Kerr), n=20.
- Group 4 was filled using a Single GP cone with BioRoot™ RCS (Septodont) bioceramic sealer, n=20.
- Group 5 was filled with warm vertical condensation with BioRoot™ RCS bioceramic sealer (Septodont), n=20.

After root canal obturation, all dental surfaces were covered with nail varnish, except 2 mm around the area of the tooth apex which were left exposed. A solution

of ammoniacal silver nitrate (ammoniacal silver nitrate and distilled water 1:4) was prepared, and the diluted solution was filtered with a millipore filter (0.22-nm filter, Carrigtwohill, County Cork, Ireland) mounted on a syringe. Under laboratory light, each tooth was placed in a test tube with diluted ammoniacal silver nitrate solution. After 24 h, specimens were thrice rinsed in water for 10 min. Nail varnish around the tooth was removed with acetone, and each tooth was placed in a test tube with a diluted solution of photo-developer (Kodak, Rochester, NY, USA) (ratio of 1:10 photo-developer:distilled water). After 8 hours, teeth were rinsed thrice in water for 10 min. Each tooth was embedded in transparent self-curing acrylic resin. The teeth were then sliced with a low-speed diamond saw under water cooling to prevent frictional heat (Isomet; Buehler, Lake Bluff, NY, USA) into two slices along their long axis and perpendicularly to the root.
Samples were examined with a digital microscope. Two observers (DP, MF) independently scored the amount of tracer along the interface. The apical leakage was evaluated following the procedure described by Limkangwalmongkol et al. (25). In case of a score discrepancy, the sample was evaluated again by the two operators, and a common decision was taken.
The Bonferroni and Anova tests were used to assess differences among groups, and to separately determine whether leakage significantly differed among groups. The significance level was set at $P < 0.05$, and the analyses were performed with the software package SPSS IBM Statistics version 21 for Mac (SPSS Inc., Chicago, IL, USA).

RESULTS

Apical leakage scores are reported in Tables 1 and 2.
The groups using bioceramic cement showed a lower amount of apical leakage (Table 1), independently from the obturation technique used.
The average of apical leakage score was found in Group 4 (single cone technique and Bioroot bioceramic endodontic sealer), which is 0.59 mm (Fig. 1). Group 2 (same sealer applied with Thermafil technique) reached an average of 0.665 mm, 12.71% higher than that of Group 4 (Fig. 2).
The apical leakage of Group 5 (vertical condensation with Bioroot cement) was 0.450 mm, the lowest recorded in this study (Fig. 3). The highest results belong to Group 1 (endodontic Pulp canal sealer with Thermafil obturation technique) (Fig. 4) and Group 3 (warm vertical condensation) (Fig. 5), with an average of 0.780 mm (32.20% more than the best average) and 0.706 mm (19,66% more) respectively. However, no statistically significant differences were found among the 5 groups (Table 2). Also, no statistically significant differences were found in the sealing ability of the two types of endodontic cement.

DISCUSSION

In this study, the sealing ability of a bioceramic endodontic sealer (BioRoot RCS) and that of a traditional ZOE cement (Pulp Canal Sealer) in the apical third of root canals, when used in combination with three different obturation techniques, was tested. Recent literature provides information about the new family of bioceramic endodontic sealers.
Sealing at the interface with the root canal dentin is facilitated by the fact that, due to their calcium phosphate content, bioceramic cements have the

	Mean apical leakage	Standard Deviation
Group 1 (n=20)	0.780 mm	0.235
Group 2 (n=20)	0.665 mm	0.256
Group 3 (n=20)	0.706 mm	0.362
Group 4 (n=20)	0.590 mm	0.197
Group 5 (n=20)	0.450 mm	0.184

TABLE 1. Apical leakage of the 5 groups.

FIG. 1 The average of apical leakage was found in Group 4.

FIG. 2 Group 2 reached an average of 0.665 mm leakage.

ability to take on a crystalline structure similar to that of hard biological tissues, when fully cured (26).
The sealing ability of a sealer is linked to its solubility and to its bonding to the gutta-percha cone to the dentin. Several studies have evaluated the sealing

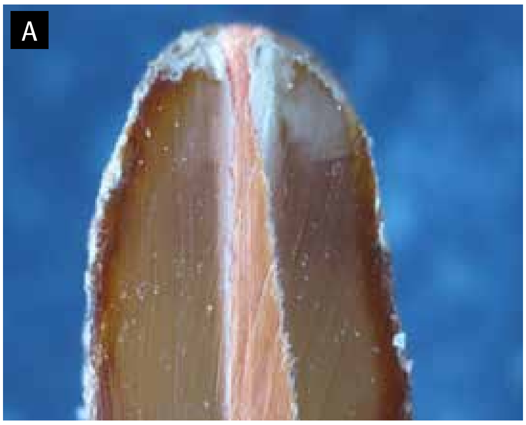
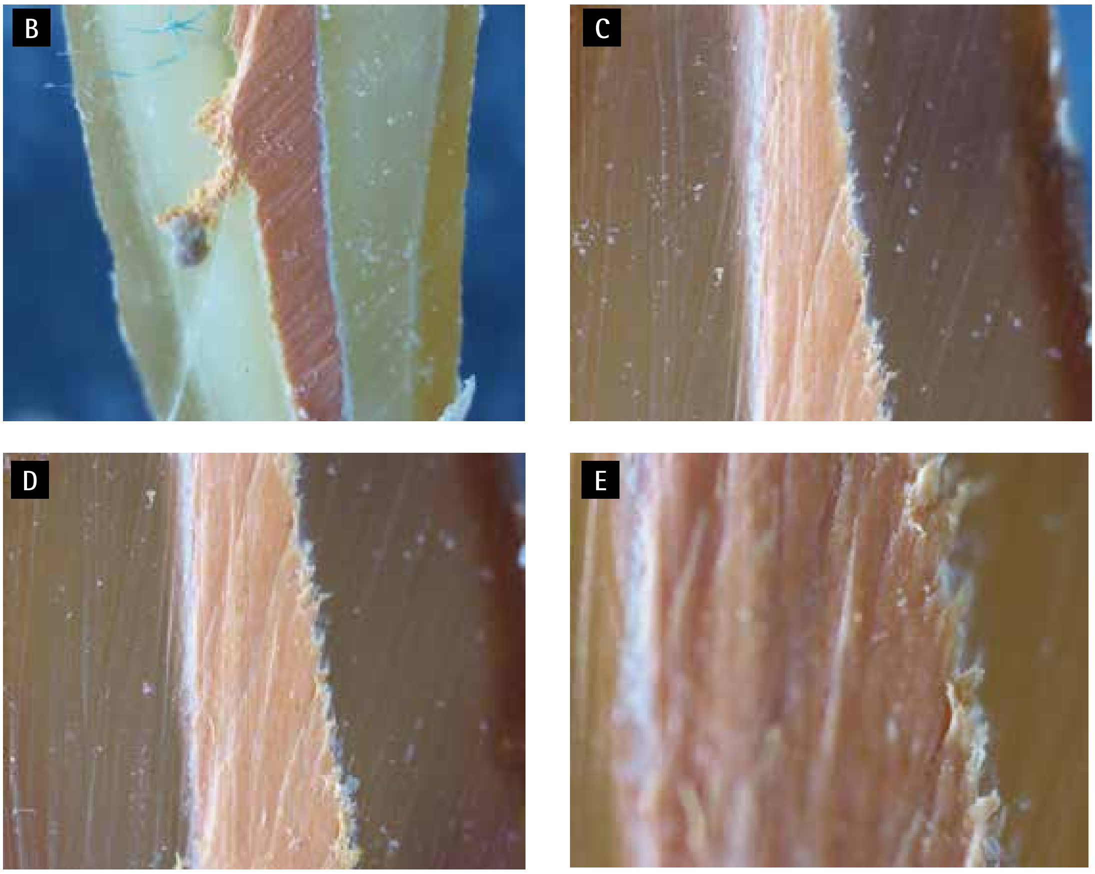


FIG. 3 The apical leakage of Group 5 was 0.450, the lowest recorded in this study (A). The bioceramic sealer extruded at the end of a lateral canal in the medium third of the root and warm gutta-percha filled it (B).
The bioceramic sealer was able to penetrate into the tubules' opening of root dentin (C-E).



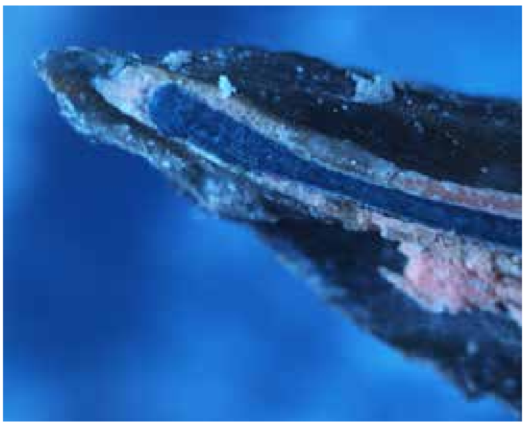


FIG. 4 The highest results belong to Group 1.

FIG. 5 The second highest results belong to Group 2.

Group 1 = Therm/PCS; Group 2 = Therm/Bio; Group 3 = WVC/PCS; Group 4 = Sc/Bio; Group 5 = WVC/Bio. Oneway leakage3_n Group, bonferroni tabulate					
	Summary of Leakage 3				
Group	Mean	Std. Dev.	Freq.		
1	78	.23475756	10		
2	665	.25603248	20		
3	70555556	.36213781	18		
4	59	.19708401	20		
5	.45	.18408935	10		
Total	**.64230769**	**.27232488**	**78**		
Analysis of Variance					
Source	SS	df	MS	F	Prob > F
Between groups	.696440171	4	.174110043	2.53	0.0473
Within groups	5.01394444	73	.06868417		
Total	5.71038462	77	.074160839		
Bartlett's test for equal variances: $chi^2(4)$ = 8.7882 Prob>chi^2 = 0.067					
	Comparison of leakage 3 by Group (Bonferroni)				
Row Mean-|					
Col Mean |	0	1	2	3	
1 | -.115					
	1.000				
2	-.074444	.040556			
	1.000 1.000				
3	-.19	-.075	-.115556		
	0.652	1.000		1.000	
4	-.33	-.215	-.255556	-.14	
	0.063	0.376	0.158	1.000	

TABLE 2 No statistically significant differences were found among the 5 sealer/obturation technique combinations.

abilities of various bioceramic-based sealers *in vitro* (16-18,20,26). Regardless of the several methodologies used, the sealing ability of bioceramic sealers has been found to be satisfactory, and similar to that of other commercially available cements. In fact, most bioceramic cements have great flowability and very small particle size, averaging 0.2 µ. Third-generation bioceramic cements have hydrophilic properties and a low contact angle that allows the sealants to easily spread over the dentinal wall and to penetrate into the tubules and irregularities of the root dentin. The penetration of the sealant into the dentinal tubules has the advantage of improving the mechanical retention of the sealant on the dentinal walls. This retention could function as a physical barrier to prevent microinfiltration of the root canal system (26).

This mechanism of action is well described by Pashley and Tay (27): when hydrated, the main components of the cement, tricalcium silicate and dicalcium silicate react with water to produce needles of calcium silicate, hydrate phase and calcium hydroxide. The latter provides a slowly releasing source of calcium and hydroxyl ions, in the presence of a phosphate-containing fluid, for the precipitation of calcium-deficient apatite via the formation of initial amorphous calcium phosphate (26). Also, Yamamoto at al. (28) suggest that all calcium silicate hydrates when in contact with human fluids containing phosphates, and then release OH- and Ca2+ ions producing hydroxyapatite. To date, bioceramic sealers are considered an advantageous technology in endodontics and they are revolutionizing the former endodontic principles that favored more gutta-percha

at the expense of a very thin film of cement (29).
If it were possible to fill the canal homogeneously, the need for a base material becomes questionable. At present, gutta-percha is primarily used as a plugger to allow hydraulic movement of cement into the irregularities of the root canal and accessory canals, and also serves as a pathway for eventual subsequent retreatments, if needed. However, these new materials may become the main component of root filling in the future.
Although Gade et al. (30) did not show a significant difference in the quality of obturation when they compared single-cone, lateral condensation, and Thermafil techniques using the BC sealer, the literature is still in favor of the use of bioceramic cements with cold techniques and little inclined to exploit them with hot techniques.
The single cone technique has always been unsuccessful among endodontists because it involves the use of a lot of cement and it is particularly susceptible to the formation of voids in the canal; actually, with the introduction of bioceramic cements, the technique has aroused new interest (31). Also, in favor of cold techniques (32) it was argued that bioceramic cements should be used without heat, as this may accelerate the reaction causing an increase in film thickness.
In addition, a study (33) that evaluated push-out bond strengths of bioceramic cements when used with warm techniques showed that the bond strength was more favorable in cases where the single cone technique was chosen compared to thermoplastic techniques. In contrast, another study (34) points out that considering the bond strength alone, bioceramic cements seem to perform better with hot techniques. A systematic review (35) shows that the prevalence of postoperative pain, long-term results, and seal quality of the filling are similar whether a cold or a warm technique is used.
The findings of this study do not show statistically significant differences in the sealing ability of the two types of cement at the apex and consequently the first null hypothesis was accepted. The second null hypothesis tested was that bioceramic endodontic cement can seal better the apex when used in combination with a cold obturation technique. The findings of this study showed no statistically significant differences among the five tested groups in which the two sealers were used in combination with hot and cold obturation techniques and consequently the second null hypothesis was accepted.
The results of this study are in contrast with another laboratory study (36) regarding the chemical changes affecting different types of gutta-percha and endodontic sealers during heating, and correlate changes with the heating capacity of different heat carriers. It was reported that an increase in the temperature to 100 °C can provoke a degradation of epoxy resin-based sealers and evaporation of water from calcium silicate-based sealers (7,9). Mainly, it was shown that the temperature affects physical properties and in particular sealer's flowability, thickness and setting time (37-42) The duration of heat treatment was also shown to affect the stability of sealers (37).
The outcomes of this study strongly support the importance of identifying the real temperature levels of endodontic heat carriers by dental professionals, and the suitability of sealers to be used at the temperature achieved. Usually, all carriers reached temperature levels below 60 °C when used in cut-out mode, which is a safe level for most tested gutta-percha and all sealers. However, the use of some devices, in continuous mode, can produce a warmer temperature that can exceed the recommended safe levels. The hot gutta-percha techniques tested in this study were the 'carrier-based' Thermafill and the vertical condensation technique. It can be speculated that the temperature of the gutta-percha in both techniques can go down quickly during the time of insertion of the carrier and the hot spreader as well into the canal. That might let the bioceramic sealer to flow into the root canal and lateral canals as well and to seal the dental walls, without losing its biochemical properties. This speculation is supported by the fact that in this study the bioceramic sealer was used inside the root canal simulating the real clinical situation.
The findings of the present study are in agreement with other recent studies (43-45); one investigated the apical sealing ability of a newly introduced bioceramic sealer in combination with continuous-wave condensation technique, single cone technique, and with AH plus by means of the continuous wave condensation technique and did not find significant differences in terms of apical seal (43). De Angelis et al. showed that in terms of microleakage, the warm continuous wave of condensation technique seems promising even when combined to a bioceramic sealer (44).
Also, Jeong et al. pointed out that the choice of the obturation technique used with a calcium silicate-based sealer may not necessarily influence sealer penetration in the apical portion of the root canal (45).
This study was performed *ex vivo*; therefore, in order to confirm or not the findings of this study, and because, to date, there had been very little information regarding the long-term sealing ability or clinical outcomes associated with bioceramic sealers, randomized clinical trials are desirable to clarify the performance of bioceramic materials when used in daily practice.

CONCLUSIONS

From the findings of this in vitro study, the following conclusions can be drawn.
1. Under in vitro conditions, bioceramic endodontic sealers can seal the root apex similarly to traditional ZOE

endodontic sealers.

2. Bioceramic sealers can be used in combination with any obturation technique that involves both warm and cold gutta-percha.

Authors contributions

Conceptualization, MF and DP; methodology, DP, MG, VV; software, EFC; validation, MF, DP, and SB; formal analysis, SB; investigation, MG, VV, DP, GV; resources, EFC; data curation, SB, SG; writing—original draft preparation, MG, VV; writing—review and editing, DP, MF, SG; visualization, SB; supervision, MF, SG, EFC; project administration, GV. All authors have read and agreed to the published version of the manuscript.

Disclaimers

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Data availability

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and protection of intellectual property.

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