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ORIGINAL ARTICLE

Bacterial leakage assessment of bioceramic sealers

ABSTRACT

Aim: *To assess the bacterial leakage of two bioceramic sealers and compare them to an epoxy resin-based sealer.*
Methodology: *A sample of 94 one-rooted premolars was obtained and separated into three experimental groups of 30 teeth; two samples were used for negative controls and two for positive controls. The experimental groups were obturated using the hydraulic compaction technique: Group 1 AH Plus® sealer, Group 2 Bioroot® RCS and Group 3 TotalFill® BC Sealer. The teeth were mounted on bacterial leakage devices and incubated at 37 °C for 30 days in a bilin esculin agar culture with* Enterococcus Faecalis.
Results: *Leakage occurred in 27 roots (30%) of the entire sample. Group 1 (AH® Plus) showed the highest percentage of leaked samples (40%) and also took the least average days to leak (16,9) compared to Bioroot® (19,7) and TotalFill® (19,5), but the differences found were not significant (p>0,05).*
Conclusions: *None of the sealers analyzed in this study produced an effective apical seal in which no bacterial leakage occurred. A third of the total sample presented leakage, but the differences between groups were not significant.*

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Introduction

One of the main objectives of root canal treatment is the three-dimensional stable seal of the root canal system to avoid fluid, bacteria, and bacterial byproducts filtration (1, 2). This obturation is usually obtained through a gutta-percha core and an endodontic sealer that improves wall adaptation and fills irregularities and spaces inside the core (1). There is no endodontic sealer that allows a fluid-tight seal of the root canal system, and that also presents the ideal qualities of insolubility, biotolerability, fluidity, and physicochemical stability (3). Epoxy resin sealers are widely used in clinical endodontics (4), and they are considered the gold standard to compare new sealers (5), mainly because they present favorable qualities such as low solubility, adequate radiopacity (6) and dimensional stability (7). However, they have the disadvantage of producing a high initial inflammatory response (8).

Since biocompatibility and sealing ability of traditional sealers should be improved (3), bioceramic cements were developed few year ago to overcome these drawbacks. These sealers present several advantages over epoxy resin sealers, including bioactivity and biotolerability. Bioactivity refers to the stimulation of mineralization through the deposit of apatite crystals over the dentinal walls, generating a chemical bond between dentin and obturation material (9); and good biotolerability refers to the fact that, when compared to others sealers, they induce less citotoxicity (10, 22). They are hydrophilic, which allows setting in the presence of humidity, and they also present a significant rise of pH during the first few weeks after the initial setting (6, 9, 11).

TotalFill® BC Sealer (FKG Dentaire, SA, La-Chaux-de-Fonds, France) is amongst the most frequently used bioceramic sealers. It is a premixed calcium silicate-based sealer, composed of zirconium oxide, calcium silicate, monobasic calcium phosphate ($CaH_4P_2O_8$), calcium hydroxide, tantalum peroxide (9) and thickening agents. Its reported properties are adequate radiopacity, good fluidity because it is nanoparticulated, and a high pH of 11-12 for several days after its initial setting (9), giving the sealer an antibacterial effect. However, it has been reported that it presents higher solubility, porosity and water absorption than other sealers, possibly because of its longer setting time and hydrophilia (9); this factor may affect its capacity to produce a fluid-tight seal (12).

Bioroot® RCS (Septodont, Saint-Maur-des-Fosses, France) is a calcium silicate-based endodontic sealer presented commercially as a powder-liquid material. The powder comprises of tricalcium silicate, zirconium oxide as an opacifier, and excipients; the aqueous solution is composed of calcium chloride and excipients (6, 7). It is a hydrophilic sealer that presents a pH rise of up to 11-12 during the first 14 days after the initial setting (6). It has an antibacterial property (13) given by a sustained pH of 10 for up to six months (11). Moreover, it has good biotolerability (8, 14, 15) adequate radiopacity and excellent capacity to release calcium ions and apatite formation, but has higher solubility than other sealers such as AH Plus® and MTA Fillapex® (6). This high solubility has also been observed in TotalFill® BC Sealer (9); it is proposed for both cements that the sealing capacity could be unaffected by this solubility, because the apposition of apatite could mitigate it (11).

A treated root canal must be adequately sealed to prevent microleakage, because this could lead to the development of an apical pathology (16), mainly because bacteria, cell wall components and soluble byproducts of bacterial metabolism can easily penetrate inside the root canal (5). One of the methods used to evaluate the effectiveness of materials and obturation techniques is to verify the leakage of fluid, bacteria, or chemical substances through the interphase between the radicular wall and the obturation material or through the spaces inside the material itself (5).

This study aimed to evaluate the sealing ability through the bacterial leakage of two bioceramic sealers: TotalFill® BC Sealer

and Bioroot® RCS and compare them to an epoxy resin-based endodontic sealer: AH Plus® (Dentsply-Sirona, Ballaigues, Switzerland).

Materials and Methods

An experimental ex vivo study was conducted on extracted human one-rooted lower premolars obtained on the public dental services of Curicó province, Chile, with the previous approval of the ethics committee of the San Sebastian University (resolution Nº 2019-53). The teeth were cleaned of organic residue with a Scaler (NSK Varios 570, Japan), stored in saline solution, and a periapical x-ray was taken. Inclusion criteria consisted of roots with small curvatures (according to the Schneider Method), the presence of only one canal, no intracanal calcifications, complete radicular formation, and absence of resorptions.
The obtained sample consisted of 94 teeth, which were then decrowned 1 mm coronal to the cemento-enamel junction. Working length was obtained through introducing a K10 File until it was visualized through the apical foramen, and 1 mm was subtracted of this length. Canal shaping was performed with WaveOne® Primary files (Dentsply-Sirona, Ballaigues, Switzerland) and frequent irrigation and aspiration, of 5% Sodium Hypochlorite.
The final irrigation protocol was performed with 17% EDTA for 60 seconds, followed by 5% Sodium Hypochlorite and Saline Solution. Canals were dried with paper points and then distributed randomly in three different experimental groups of 30 samples each, to be obturated with the Synchronized Hydraulic Condensation Technique with WaveOne® Primary gutta-percha cones (Dentsply-Sirona, Ballaigues, Switzerland). Group 1: AH Plus® sealer (Dentsply-Sirona, Ballaigues, Switzerland); Group 2: Bioroot® RCS® (Septodont, Saint-Maur-des-Fosses, France); Group 3: Total-Fill® BC Sealer® (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland). After root canal obturation, each root's coronal end was sealed with a glass ionomer self-curing cement (Chemfill® Superior, Dentsply, Germany) and exteriorly sealed with methacrylate varnish leaving the most apical two millimeters uncovered. Two roots were sealed entirely with glass ionomer cement after obturation, and were used as negative controls. One root was not obturated and was covered only with methacrylate varnish, and used as positive control.
According to the bacterial leakage model, the roots were mounted on a device built specifically for this study (Figure 1). The device consisted of two chambers connected by the endodontically treated tooth (17) (De Deus, 2007): the superior chamber was an Eppendorf tube (Biologix Research Company, USA) cut to a capacity of 1.5 ml of volume with a hermetic seal lid, and the inferior chamber was a 5ml glass bottle with a plastic lid. The union between the tube and the tooth was sealed with industrial silicone (Silicon Seal, Lanco, Orlando, Florida, USA). After that, the Eppendorf tubes were fixed to the glass chambers with cyanoacrylate incorporating a 30G Luer type needle, which allowed both the entry of fresh broth and the liberation of gases (Figure 1).
The devices were left to settle for 24 hours and then were sterilized with Ethylene

Figure 1
Bacterial leakage device composed of two chambers connected by the endodontically treated tooth.

Oxide; they were posteriorly opened in a sterile environment obtained through the use of three burners, and Bile Esculin Agar broth (Becton Dickinson and Co. Sparks, USA) was loaded into the superior chamber.
The inferior chamber was loaded with an *Enterococcus Faecalis* suspension in bile esculin agar broth, adjusted to 0,5 McFarland (1,5x108 CFU/ml) (Probac do Brasil, Sao Paulo, Brasil) leaving the root apex submerged in the contaminated broth. Then all the samples were incubated for four weeks at 37 °C in a Binder culture incubator (Tuttlingen, Germany), with frequent introduction of fresh nutritional broth. Bacterial growth was determined through the appearance of turbidity in the superior chamber. The number of days in which turbidity occurred was registered according to the group, and the data was analyzed with IBM SPSS 24.0 statistics program. A sample was taken of each broth that presented turbidity and again cultured in bile esculin in 6.5% NaCLO agar to confirm the bacterial strain.
A Shapiro-Wilk test was applied determining that data distribution was non-normal ($p<0,05$), proceeding then to the application of Kruskal-Wallis non-parametric test to analyze independent samples.

Results

Out of the 90 experimental samples observed, 27 presented bacterial leakage, which corresponds to 30%. Table 1 shows leakage results per group, including the average day in which turbidity occurred. Group 1 (AH Plus®) showed the highest bacterial leakage with 12 samples (40%), Group 3 (TotalFill® BC Sealer) presented eight samples with leakage (27%), and Group 2 (BioRoot® RCS) presented seven leaked samples (23%). The group that took the least amount of days to leak was Group 1 (AH Plus®) with 16,9 average days, followed by Group 3 (TotalFill® BC Sealer) with 19,5 days average and Group 2 (Bioroot® RCS) with 19,7 days. No turbidity was observed on the negative controls, and on the positive controls, turbidity was observed on the third day for both samples. The differences within the samples were not statistically significant ($p>0.05$).

Discussion

Endodontic sealers should ideally produce a long-term fluid and bacteria-tight seal of the root canal system, but this has not been achieved in the past with the available sealers. One of the objectives of obturation is the entombment of remaining bacteria, which cannot occur if there is apical filtration. To overcome these limitations, new materials and obturation systems are continuously developed.
In the present study, leakage was observed in all groups with no statistical differences between them; 30% of all samples presented bacterial leakage, which in general terms means that none of the sealers produced an effective seal. These results agreed with Yanpiset et al. were leakage was observed in 20 to 45% of all studied groups (18).
The group that presented the highest amount of leaked samples was the AH Plus® group (Group 1). It also showed the shortest median time to leak, without significant differences compared to the other groups. These results matched with what was observed by Viapiana et al. who evaluated the sealing ability of BioRoot® RCS and AH Plus® through fluid transportation and microsphere leakage, and obtained similar results in both groups (4). Zhang and Yanpiset et al. compared Bioceramic Sealers to AH Plus® sealer, and they also did not found any significant differences between groups (1, 18).
Contrary to the results obtained in this study, Pawar et al. observed that the bioceramic cement' sealing ability was better than AH Plus® sealer (19). But the method they used to measure leakage was Blue Methylene dye, which has a low molecular weight, and has a different penetration pattern (5). El Sayed et al. found that leakage was significantly less in a Bioceramic sealer (Endosequence BC Sealer, Brasseler, USA, Savannah, GA) when combined with a hydrophilic gutta-percha C-point (Brasseler, USA, Savannah, GA). However, when they used bioceramic sealers with conven-

Table 1
Bacterial leakage results per group and average day in which turbidity occurred with standard deviation

	Bacterial Leakage		Average days to leak
	YES	NO	
Group 1 (AH Plus)	12 (40%)	18	16,9 d (9,5 SD)
Group 2 (Bioroot)	7 (23%)	23	19,7 d (9,5 SD)
Group 3 (Totalfill)	8 (27%)	22	19,5 d (7,3 SD)

tional gutta-percha, they registered no significant difference between them and epoxy resin sealers (20). Muedra et al. (21) found higher sealer penetration for Endosequence BC sealer when compared to Bioroot and AHPlus sealer, although the difference was not significant. They attributed the difference between the syringe sealer and the powder/liquid sealer, possibly to particle size, presentation and fluidity.
Leakage occurred on average at 16 to 19 days for all groups, which is earlier than what was observed in other studies in which leakage occurred mainly after the fifth week of observation (5) and after nine weeks for 30% of the resin sealer samples (17). This early leakage may have occurred because the synchronized hydraulic technique uses just one tapered gutta-percha cone and a considerable amount of sealer, which could compromise the obturation if and when some parts of the sealer contracts or dissolves.
The mentioned studies used lateral compaction technique for their samples (5, 17), which might require further evaluation, because lateral compaction would be a more favorable technique for bioceramic sealers. A study conducted by Yanpiset et al. used a similar obturation protocol, but filtration occurred on days 42 to 52 on average (18). The difference with the outcomes reported in the present study could be due to the use of distobuccal roots instead of one-rooted premolars, which have a more irregular transversal shape.
There are different methods to measure leakage, such as dye penetration and diffusion, bacterial and endotoxins leakage, fluid, glucose, caffeine, and radioisotope filtration (2). The methods that use bacterial or bacterial byproduct penetration are considered more clinically and biologically relevant than the dye penetration method (16). *Enterococcus Faecalis* could be even more appropriate because it is one of the microorganisms related to apical periodontitis in previously treated teeth (13).

Conclusions

Under the conditions of this study, it is possible to conclude that none of the materials used in this study offer a 100% effective seal. All groups presented leakage, without significant differences between them, but with slightly better behavior for bioceramic sealers.

Clinical Relevance

The use of Bioceramic Sealers is widespread because of their biocompatibility. This study shows that their sealing ability is similar to resin sealer AH Plus, which is the gold standard to measure endodontic sealers. This result gives more confidence to use these relatively new sealers.

Conflict of Interest

The authors declare no conflicts of interest.

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None.

Ethics approval

The ethics committee of the San Sebastian University (resolution Nº 2019-53).

References

1. Zhang W, Li Z, Peng B. Assessment of a new root canal sealer's apical sealing ability. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 107: e79-82.
2. Jafari F, Jafari S. Importance and methodologies of endodontic microleakage studies: A systematic review. J Clin Exp Dent. 2017; 9: e812-e9.
3. Wang Z. Bioceramic materials in endodontics. Endodontic Topics. 2015; 32: 3-30.
4. Viapiana R, Moinzadeh AT, Camilleri L et al. Porosity and sealing ability of root fillings with gutta-percha and BioRoot RCS or AH Plus sealers. Evaluation by three ex vivo methods. Int Endod J. 2016; 49: 774-82.
5. Monardes Cortés H, Abarca Reveco J, Castro Hurtado P. Microfiltración apical de dos cementos selladores: un estudio in vitro. Int J Odontostomat. 2014;8: 393-8.
6. Siboni F, Taddei P, Zamparini F et al. Properties of BioRoot RCS, a tricalcium silicate endodontic sealer modified with povidone and polycarboxylate. Int Endod J. 2017; 50: e120-e36.
7. Khalil I, Naaman A, Camilleri J. Properties of tricalcium silicate sealers. J Endod. 2016; 42: 1529-35.
8. Eldeniz AU, Shehata M, Hogg C, Reichl FX. DNA double-strand breaks caused by new and contemporary endodontic sealers. Int Endod J. 2016; 49: 1141-51.
9. Zamparini F, Siboni F, Prati C et al. Properties of calcium silicate-monobasic calcium phosphate materials for endodontics containing tantalum pentoxide and zirconium oxide. Clin Oral Investig. 2019; 23: 445-57.
10. Chang SW, Lee SY, Kang SK et al. In vitro biocompatibility, inflammatory response, and osteogenic potential of 4 root canal sealers: Sealapex, Sankin apatite root sealer, MTA Fillapex, and iRoot SP root canal sealer. J Endod. 2014; 40: 1642-8.
11. Urban K, Neuhaus J, Donnermeyer D et al. Solubility and pH Value of 3 different root canal sealers: a long-term investigation. J Endod. 2018; 44: 1736-40.
12. Poggio C, Dagna A, Ceci M et al. Solubility and pH of bioceramic root canal sealers: a comparative study. J Clin Exp Dent. 2017; 9: e1189-e94.
13. Arias-Moliz MT, Camilleri J. The effect of the final irrigant on the antimicrobial activity of root canal sealers. J Dent. 2016; 52: 30-6.
14. Camps J, Jeanneau C, El Ayachi I et al. Bioactivity of a calcium silicate-based endodontic cement (BioRoot RCS): interactions with human periodontal ligament cells in vitro. J Endod. 2015; 41: 1469-73.
15. Dimitrova-Nakov S, Uzunoglu E, Ardila-Osorio H et al. In vitro bioactivity of Bioroot RCS, via A4 mouse pulpal stem cells. Dent Mater. 2015; 31: 1290-7.
16. Savadkouhi ST, Bakhtiar H, Ardestani SE. In vitro and ex vivo microbial leakage assessment in endodontics: a literature review. J Int Soc Prev Community Dent. 2016; 6: 509-16.
17. De-Deus G, Brandao MC, Fidel RA, Fidel SR. The sealing ability of GuttaFlow in oval-shaped canals: an ex vivo study using a polymicrobial leakage model. I Int Endod J. 2007; 40: 794-9.
18. Yanpiset K, Banomyong D, Chotvorrarak K, Srisatjaluk RL. Bacterial leakage and micro-computed tomography evaluation in round-shaped canals obturated with bioceramic cone and sealer using matched single cone technique. Restor Dent Endod. 2018; 43: e30.
19. Pawar SS, Pujar MA, Makandar SD. Evaluation of the apical sealing ability of bioceramic sealer, AH plus & epiphany: an in vitro study. J Conserv Dent 2014; 17: 579-82.
20. Mohamed El Sayed MAA, Al Husseini H. Apical dye leakage of two single-cone root canal core materials (hydrophilic core material and gutta-percha) sealed by different types of endodontic sealers: An in vitro study. J Conserv Dent. 2018; 21: 147-52.
21. Muedra, P et al. Could the Calcium Silicate-Based Sealer Presentation Form Influence Dentinal Sealing? An In Vitro Confocal Laser Study on Tubular Penetration. Materials. 2021;14(3), 659.
22. Sanz, JL et al. Cytocompatibility and Bioactive Properties of Hydraulic Calcium Silicate-Based Cements (HCSCs) on Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs): A Systematic Review of In Vitro Studies. Journal of Clinical Medicine. 2020; 9(12), 3872.