

Comparison of the root-end seal provided by bioceramic repair cements and White MTA

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Abstract

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Aim To compare the ability of Ceramicrete, BioAggregate and white ProRoot MTA (mineral trioxide aggregate) to prevent glucose leakage through root-end fillings.

Methodology After root canal instrumentation, the apical 3 mm of maxillary incisors were resected and retropreparations, 3 mm in depth, were created with ultrasound. Root-end cavities were filled with the tested materials (15 roots per group). All roots were mounted in a double-chamber system to assess glucose penetration using 15 psi pressure application. After 1 h, glucose concentrations in the lower chamber were

measured following an enzymatic reaction. Four roots were used as controls. One-way ANOVA verified differences in glucose leakage between groups and Tukey test performed multiple comparisons. Significance was set at $\alpha = 5\%$.

Results There was a significant difference between the three materials (ANOVA, $P < 0.05$). Ceramicrete had significantly lower glucose penetration than BioAggregate (Tukey, $P < 0.05$). There was no difference between the two bioceramic cements and white MTA ($P > 0.05$).

Conclusions Both endodontic bioceramic repair cements displayed similar leakage results to white MTA when used as root-end fillings materials. Ceramicrete had significantly lower glucose penetration.

Keywords: bioceramic cement, MTA, root-end filling.

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Introduction

Both grey MTA and white MTA contain potentially hazardous amounts of toxic substances (Duarte *et al.* 2005). A new water-based cement has been developed recently (DiaRoot BioAggregate; Innovative BioCeramic Inc., Vancouver, BC, Canada). That is said to be produced under an environment free of contamination to ensure that the final product results in a pure and fine white hydraulic cement-like powder composed of biocompatible ceramic nano-particles ([\[diadent.com/products/diaroot.html\]\(http://www.diadent.com/products/diaroot.html\)\). BioAggregate has similar biocompatibility to white ProRoot MTA when in contact with mesenchymal human cells \(De-Deus *et al.* 2009\). In addition, its hydrophilic powder is claimed to promote cementogenesis and to form a hermetic seal inside the root canal \(<http://www.diadent.com/products/diaroot.html>\). Although the manufacturer states that trace amounts of naturally occurring potentially harmful constituents may be detected during chemical analysis, Bioaggregate has the same indications for use and is composed of similar materials and exhibits comparable technological characteristics to white MTA \(<http://www.diadent.com/products/diaroot.html>\). However, its composition includes tantalum oxide as a radiopacifier agent \(Park *et al.* 2010\), the removal of aluminium owing to its toxic effect](http://www.</p></div><div data-bbox=)

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(<http://www.diadent.com/products/diaroot.html>; Duarte et al. 2005) and the incorporation of hydroxyapatite as the main bioactive component (<http://www.diadent.com/products/diaroot.html>).

Another bioceramic phosphate-based cement developed at the Argonne National Laboratory (Chicago, IL, USA) [Ceramicrete, Wagh et al. (2003)] has been modified recently for dental applications by the incorporation of hydroxyapatite and radiopacifiers to the original composition (Wagh & Primus 2006). This material displays high resistance to compression (compared with Portland cement), low water permeability, low porosity and rapid setting reaction (5–15 min) (Wagh et al. 2003, Wagh & Primus 2006), which are desired characteristics for reparative purposes. Because of the presence and the formation of hydroxyapatite during the setting reaction (72 h) (Wagh & Primus 2006), Ceramicrete is claimed to display elevated bioactivity. Tay et al. (2007) demonstrated superior root-end sealing quality for Ceramicrete when compared with MTA. Its bioactivity has also been demonstrated by the deposition of crystals in the dentine material interface. It is currently under development (Dentsply Tulsa Dental Specialities, Tulsa, OK, USA).

This study was designed to assess whether bioceramic repair cements display similar sealability when used as root-end filling materials. White ProRoot MTA (Dentsply Tulsa Dental Specialities) was used as reference material. The recently developed glucose leakage model (Souza et al. 2008) was used to test two null hypotheses that there is no significant difference in the sealability between the two bioceramic cements and that both bioceramic cements have similar leakage results to White MTA.

Materials and methods

Pilot tests assessing the glucose reactivity

A well-known problem related to the use of a tracer for evaluating leakage is that the tracer could chemically react with MTA-based materials (Shemesh et al. 2008). Shemesh et al. (2008) reported that MTA significantly reduced glucose concentration after 24 h contact with the glucose solution. To verify whether shorter periods of contact would also influence glucose concentrations, a pilot test with the three cements (BioAggregate, Ceramicrete and white ProRoot MTA) was performed in this study to determine the most appropriate experimental time.

The glucose reactivity analysis carried out in this work was based on the previous report by Shemesh et al. (2008).

Six human maxillary incisor root ends were filled with the materials (two per material). Two additional roots remained unfilled. The apical portion of each sample was inserted into small containers filled with 4 mL of 0.2 mg mL⁻¹ glucose solution, one container per root. Two more containers were used as controls containing only 4 mL of glucose standard (0.2 mg mL⁻¹) solution. All containers were kept at 37°C, and a sample of 0.1 mL was taken after 30, 60 and 120 min of contact from each container. Each sample was analysed using a glucose kit (Megazyme, Wicklow, Ireland) in a spectrophotometer (Camspec M 330, Leeds, UK) at a wavelength of 340 nm and the optical density (OD) statistically compared with the aid of SPSS 17.0 software (IBM Corporation, New York, USA). Univariate analysis of variance was used to verify the effect of time of contact and cement on the OD of the glucose solution. Tukey post hoc test was used to compare the time-points and Dunnett post hoc used to compare the OD's between cements and glucose standard.

Univariate analysis of variance demonstrated that neither time of contact nor cement influenced the OD of the glucose solutions ($P > 0.05$). There were no difference in OD between the time frames ($P > 0.05$) and no difference between the cements and glucose standard ($P > 0.05$). Based on the results of the pilot study, even though the 2-h contact had demonstrated no significant glucose reduction in any of the tested cements, an experimental time of 60 min was chosen to ensure that no glucose reaction would occur.

Specimen selection and preparation

Forty-nine human left and right maxillary incisors were selected, autoclaved and kept in 0.2% sodium azide for no longer than 30 days. Standard access cavities were prepared and the canal orifices located and apical patency confirmed with a size 15 K-file (Dentsply Maillefer). The working length was established 1 mm from the apex.

Instrumentation of the cervical and middle thirds of the canal was carried out using a crown-down flaring technique with numbers 5-3 Gates Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland). The apical third was instrumented using K-Flexofiles sizes 60, 55, 50, 45 and 40 (Dentsply Maillefer). After each instrument, 1 mL of freshly prepared 5.25%

sodium hypochlorite (pH 10.8) was used at a rate of 1 mL per 1 min. A total amount of 8 mL per 8 min of NaOCl was used during instrumentation. Three microlitres of distilled water was then used and the smear layer removed using 17% EDTA per 3 min. Three microlitres of distilled water was used as a final rinse. The canals were dried with paper points. Crowns were then removed to standardize root length in 15 mm.

Root-end resection and preparation

The apical 3 mm was resected perpendicularly to the root long axis by means of a diamond disc no. 7020 (KG Sorensen, São Paulo, Brazil). A ML accessory gutta-percha cone was inserted (without cementation) into the canal and through the apical foramen until tug-back was achieved. The apically extruded gutta-percha was sectioned. The root-end cavities were created with the aid of an ultrasonic device (NSK – Nakanishi Inc., Tokyo, Japan) and a 3-mm-length retro tip E32D (NSK, Nakanishi Inc.).

The roots were randomly distributed with the aid of a computer algorithm (<http://www.random.org>) into three equal experimental groups ($n = 15$) and two control groups ($n = 2$).

Root-end filling procedures

The prefitted gutta-percha cone served as a barrier for the condensation of the root-end filling materials. BioAggregate and white ProRoot MTA were manipulated according to manufacturers' recommendations, whilst Ceramicrote powder was added to the liquid (20% of phosphoric acid and 60% of magnesium phosphate) until a consistency resembling white MTA was retrieved. Three groups were formed as follows: group 1 (G1), Ceramicrote (Dentsply Tulsa Dental, Tulsa, OK, USA); group 2 (G2), BioAggregate (DiaDent, Burnaby, BC, Canada); and group 3 (G3), white ProRoot MTA (Dentsply Tulsa Dental).

All materials were placed into the root-end cavities using a number 5 spatula (Odous de Deus, Minas Gerais, Brazil) and further compacted with a specific plugger (Paiva compactor; Odous de Deus). Immediately after filling, all samples were stored in 100% humidity for 72 h to allow the final set of the sealers. However, after the initial set (24 h) of the tested cements, the gutta-percha was removed from each root canal. Root-end resection, preparation and filling were performed by a single operator.

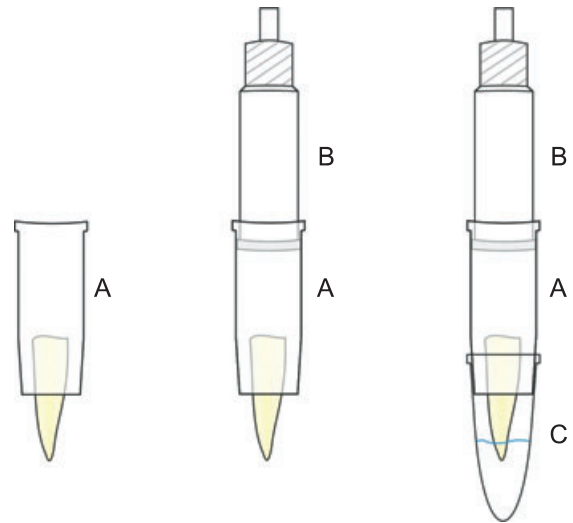


Figure 1 Sequence of assembled double-chamber dispositive. (A) upper chamber, (B) syringe connector, (C) lower chamber.

Assembled double-chamber and glucose leakage measuring

Teeth were placed into a device designed to measure glucose leakage (Xu *et al.* 2005, De-Deus *et al.* 2008) (Fig. 1). Teeth were individually inserted into an Eppendorf tube (1.5 mL) with the apical 7 mm protruding through the end. The upper portion of the Eppendorf tube was connected to a plunger-less syringe containing 0.75 mL of 1 mol L⁻¹ glucose solution (pH = 7.0/density = 1.09 × 103 g L⁻¹/viscosity = 1.18 × 10⁻³ Pa s⁻¹ at 37°C). The lower portion of the Eppendorf was inserted into another Eppendorf tube containing 0.75 mL of deionized water in such a way that 3 mm of the root apex was immersed in the water. Low-viscosity cyanoacrylate adhesive was used to seal all the interfaces and connections.

In the negative control group, two layers of nail varnish were applied over the root surface of teeth with intact crowns. In the positive control group, root ends were not filled nor covered. Before the beginning of the experiment, all samples were sterilized in ethylene oxide (BIOXXI Sterilization Services Ltd, Rio de Janeiro, Brazil).

The upper part of the syringe containing glucose solution was connected to a pressure source to create a headspace pressure of 103 kPa per 60 min. The present experimental set-up was constructed to run 30 experimental samples plus 2 controls simultaneously (Fig. 2). After that, a 10-μL aliquot of solution was drawn from the inferior Eppendorf using a

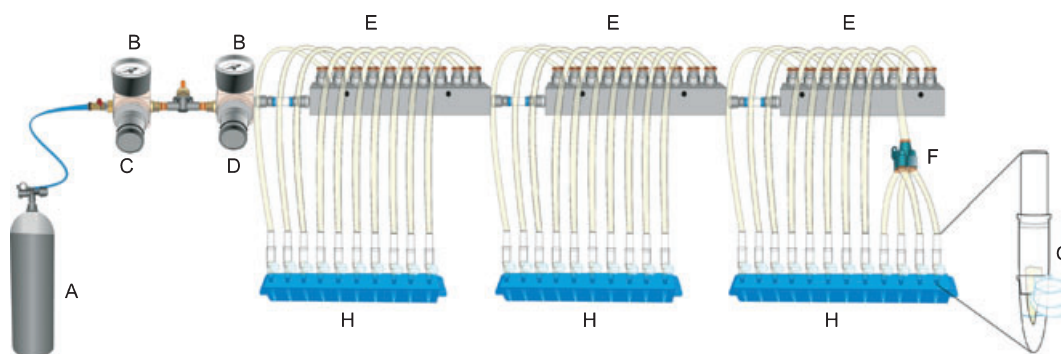


Figure 2 Experimental set-up for pressure distribution. (A) Compressed air source, (B) manometer, (C) pressure controller of low sensitivity, (D) pressure controller of high sensitivity, (E) distribution device with 9 or 10 exits, (F) connector with 4 exits, (G) double-chamber apparatus, (H) laboratorial support for eppendorfs.

micropipette and then analysed using a glucose kit (Megazyme) in a UV/VIS spectrophotometer (Camspec M 330) following a kinetic assay at 340-nm wavelength to obtain the specific OD value for each sample. OD values were converted in glucose concentrations (g L^{-1}). All the readings were taken in duplicate, and the mean value was considered for statistical analysis.

Statistical analysis

The preliminary analysis of the raw pooled data from the experimental groups showed a bell-shaped distribution (D'Agostino & Person omnibus normality test). As all groups displayed Gaussian distribution, further statistical analysis was performed using parametric methods. One-way ANOVA test and post hoc analysis were performed using Tukey test for multiple comparisons. The alpha-type error was set at 0.05, and Prisma 5.0 (GraphPad Software Inc, La Jolla, CA, USA) was used as analytical tool.

Results

After 1 h of pressure application, no sign of glucose was detected in the inferior Eppendorfs in the negative control groups, whereas the samples in the positive control group displayed substantial concentrations of glucose leakage after the same time period.

Mean and standard deviation of glucose concentrations from the cements studied were 0.744 g L^{-1} (± 0.5) for the Ceramicrete group, 1.2 g L^{-1} (± 1.1) for the ProRoot MTA group and 1.858 g L^{-1} (± 1) for the BioAggregate group. A significant difference was observed comparing the three groups ($P = 0.02$, $F = 6.915$, one-way ANOVA). Tukey test detected

difference between Ceramicrete and BioAggregate groups ($P < 0.05$). White ProRoot MTA displayed intermediate glucose concentrations and no difference was observed comparing this group with both Ceramicrete and BioAggregate groups ($P > 0.05$, Tukey).

Because no significant difference was observed between ProRoot MTA and both Ceramicrete and BioAggregate, a *post hoc* power statistic calculation was made [G*Power for Macintosh (Heinrich-Heine Universität, Düsseldorf, Germany)] to verify whether the sample size used and F value observed had sufficient power (minimum 80% power) to reveal any significant difference. The test resulted in 100% power statistics.

The graph in Fig. 3 displays the mean, median, standard deviations and the data distribution for each experimental group.

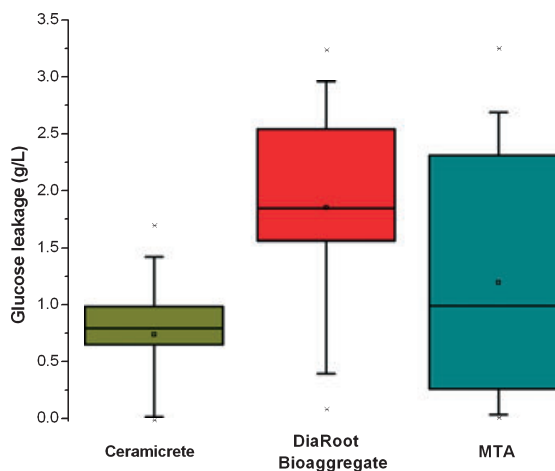


Figure 3. Box plots of the leakage results, illustrating the mean traces, minimal and maximal fluid flow traces as well as the variance in each experimental group.

Discussion

Various models including bacterial penetration, fluid transport and glucose leakage model (GLM) are currently used for sealability tests. None of them is universally accepted owing to different limitations presented by each model. In the bacterial leakage model, the difficulty in maintaining aseptic conditions, the variability of bacteria species used and the relative large size of the bacteria are some of the drawbacks (Wu *et al.* 1993). On the other hand, the glucose molecule is a known nutrient source for bacteria that might remain in the root system. It has a low molecular weight (180 Da) (Xu *et al.* 2005) and is considered more clinically relevant (Xu *et al.* 2005). Furthermore, the enzymatic reading of glucose concentration (minimum 0.08 μL) is more sensitive than the bubble movement (minimum 1 μL) in the fluid transport model (Shemesh *et al.* 2006). Therefore, the GLM is currently considered to overcome many of the limitations displayed by other models (Xu *et al.* 2005).

However, GLM itself also presents some limitations such as the extended experimental period, the difficulty in keeping a bacteria-free system to avoid glucose consumption and the risk of water evaporation, which may alter glucose concentrations. Souza *et al.* (2008) proposed pressure amplification in the upper chamber of the GLM system. This way, glucose leakage was accelerated (from weeks to hours) reducing the risk of bacteria growing and long-term water evaporation with no reduction in the capability of detecting leaking samples (Souza *et al.* 2008).

It has been reported that when MTA remained in contact with glucose solution for 24 h, glucose concentrations decreased (Shemesh *et al.* 2008). Thus, in the present study, a pilot test was conducted aiming to establish a suitable time period of contact between glucose and MTA-based filled roots that result in no reduction in glucose concentrations. Afterwards, the pressure application was adjusted to provide an appropriate pressure/time ratio enough to provide the passage of glucose by a through-and-through void (Pommel & Camps 2001). After the pilot test, it was decided to apply 103 Kpa of pressure per 1 h, as no reduction in glucose concentration was observed. Shemesh *et al.* (2008) stated that glucose in an alkaline solution is slowly oxidized by oxygen, forming gluconic acid. It might be that after the 2-h contact in the pilot test no significant reduction had taken place. Additionally, Shemesh *et al.* (2008) used large discs of materials in contact with glucose solutions tested. Rather, in the

present pilot study, root-end filled teeth were used, which means that a considerably smaller quantity of material was in contact with glucose solution. This might explain the non-significant reduction even after 2 h of contact.

The pressure used in this study was three times higher than the pressure applied in the 24-h experiment originally performed by Souza *et al.* (2008). However, pressure application may be able to alter the seal of a root filling. De Gee *et al.* (1994) subjected root canal sealers to 120-kPa fluid pressure and shear tests. The results were compared with those of controls that were not subjected to fluid pressure before the shear force was applied. Similar shear strengths observed between groups indicated that the 120 kPa had not created avenues of leakage or damage to the structure of sealers tested. Thus, it may be assumed that the 103 kPa of fluid pressure used in the present study was probably not detrimental to the integrity of root-end fillings.

Sealability studies are still important in endodontics, especially as an initial screening for newly developed filling materials. Because MTA is ranked with good sealability results in several studies (Bates *et al.* 1996, Wu *et al.* 1998, Yatsushiro *et al.* 1998, Fogel & Peikoff 2001, Lamb *et al.* 2003, Valois & Costa 2004, Al-Hezaimi *et al.* 2005, Bortoluzzi *et al.* 2006, De Bruyne *et al.* 2006, Hamad *et al.* 2006), it is important that new endodontic materials display at least similar ability to prevent leakage as MTA.

The mechanism that provides MTA with superior sealability results is not completely understood. Analysing the contact of MTA with a synthetic tissue fluid and root dentine, Sarkar *et al.* (2005) suggested that MTA initially produced a mechanical seal and further dissolved leading to the formation of hydroxyapatite crystals, which reacted with dentine to create a chemical adhesion (Sarkar *et al.* 2005) The fact that both bioceramic cements (Ceramicrete and Bioaggregate) contain hydroxyapatite may explain the comparable leakage results to white MTA observed in the present study ($P > 0.05$).

However, Tay *et al.* (2007) were unable to verify similar root-end filling leakage results between Ceramicrete and white MTA in a fluid transport model. In theory, the divergent results cannot be explained by the variation in the leakage model used, as GLM is supposed to be more sensitive than fluid transport (Shemesh *et al.* 2006). Tay *et al.* (2007) mixed Ceramicrete powder with deionized water rather than the liquid provided by the manufacturer. This liquid is composed by 20% of

phosphoric acid and 60% of magnesium phosphate. It may be that the use of water instead of the manufacturer's liquid resulted in better sealing ability. Furthermore, observing Fig. 3, it is possible to verify lower mean glucose concentration for Ceramicrete compared with White MTA. However, it might be that significant difference could not be detected by the statistical model owing to the elevated standard deviation displayed by the white MTA group, even though normally distributed (D'Agostino & Person omnibus normality test), contrasting with the results of Tay *et al.* (2007). On the other hand, the power statistic of 100% for the sample size used in this study indicates that any difference between groups, if existed, could be detected in the present set-up (Schuurs *et al.* 1993).

Although the second null hypothesis of this study was accepted, the first null hypothesis was not because there was a significant difference between the leakage patterns of the two bioceramic cements tested ($P < 0.05$). Root-end cavities filled with Ceramicrete displayed significantly less glucose leakage compared with BioAggregate. This is the first study to compare the sealing ability of those bioceramic cements, and a clear background to explain this difference has to be established.

Importantly, both endodontic repair cements displayed similar leakage results to the current gold-standard repair cement (White MTA). Additional screenings of these new materials on their biocompatibility, dimensional stability and bone formation induction are still to be performed.

Conclusion

On the basis of the present laboratory study, it was possible to observe that bioceramic-based endodontic repair cements, Ceramicrete and BioAggregate, had a similar ability to white ProRoot MTA in preventing glucose leakage as root-end fillings and that Ceramicrete provided significant better results than BioAggregate.

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