Sealing Ability of Three Different Materials Used as Retrograde Filling

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Purpose: To test the apical leakage prevention performance of three different materials through protein leakage procedures using bovine serum albumin (BSA) and Bradford protein reagent.

Materials and Methods: A total of 60 human single-rooted teeth were divided into 4 groups, and conventional root canal filling was done. The root was cut 3 mm from the apex, and a cavity was formed. Proroot MTA (MTA), Fuji II LC (GI), Fuji II LC with XP bond (GIA), and Caviton (CA) were used as experimental materials to fill the cavity in a retrograde filling manner. The extent of BSA leakage was then measured with a ultraviolet visible spectrophotometer 24, 48, and 72 hours after filling.

Result: After 24 hours, among the 15 teeth of each group, 2 in MTA, 4 in GI, 3 in GIA, and 7 in CA showed leakage. After 48 hours, 3 in MTA, 5 in GI, 5 in GIA, and 10 in CA had leakage and discoloration. After 72 hours, among the 15 teeth of each group, 3 in MTA, 6 in GI, 5 in GIA, and 10 in CA showed leakage. The leakage in the CA group was greater than that in the MTA group at 48 and 72 hours based on Fisher’s exact test (P=0.025), and the difference was statistically significant. Similarly, the leakage in the CA group was greater than that in the MTA group over time based on the Kaplan-Meier survival estimate (P=0.011), and the difference was statistically significant.

Conclusion: Glass ionomer, glass ionomer after adhesive application, and MTA all showed leakage. Caviton showed greater leakage compared to MTA 48 and 72 hours after filling, and the difference was statistically significant; thus suggesting that Caviton is not appropriate as retrograde filling material considering its sealing ability.

Key Words: Caviton; Glass ionomer; Leakage; MTA; Protein leakage procedure; Retrograde filling

Introduction

Apicoectomy and tooth transplantation procedures are effective methods of preserving a tooth in case root canal therapy fails - despite its high success rate - for reasons such as complexity of the
root canal system, microcrack, and periodontal disease. As a new solution that has been gaining popularity recently, teeth extracted for treatment, such as orthodontics and third molars, are excellent transplantation materials that can be used to fulfill the desire of both dentist and patient to preserve natural teeth.

Root end filling is important in procedures such as apicoectomy, tooth transplantation, and tooth replantation, and many materials including gutta-purca, amalgam, zinc oxide eugenol, IRM (Dentsply Maillefer, Tulsa, OK, USA), super eugenol and o-ethoxybenzoic acid, composite resin, gold foil, glass ionomer, and MTA have been used. The root end filling material should have characteristics such as bioaffinity, sealing ability, and convenience of manipulation. The unwashed antigen remaining in the root canal and leaking into apical tissue necessitates root canal treatment in most cases. Thus, one of the important characteristics of root end filling material is sealing ability that prevents the egress of antigens such as bacteria and bacterial endotoxin from the root canal to the periapical tissues.

MTA induces the appropriate healing of the apical tissue and biomineralization and has many excellent characteristics as a potential root end filling material that can replace other materials. Note, however, that MTA is difficult to manipulate, and washout may occur with too much water. Moreover, the disadvantage of long setting time of approximately 165 minutes causes problems such as leakage, surface separation, loss of continuity, and marginal adaptation.

Glass ionomer is one of the fillings with several merits such as good adhesive properties, fluoride release, antibacterial effect, etc. Hydraulic temporary sealing material such as Caviton (GC Corp., Tokyo, Japan), Cavit (3M ESPE, St. Paul, MN, USA), Lumicon (Heraeus Kulzer, Tokyo, Japan), and Hy-Seal (Shofu, Kyoto, Japan), is based on calcium sulfate, hardening when it comes into contact with water inside the mouth. Chemical reactions and dentin bonding occur when the material hardens and linear hygroscopic expansion takes place, providing good sealing ability. Hydraulic temporary sealing materials are commonly used in dentistry. Among these, Caviton shows good sealing ability when used as temporary filling material, and it is commonly used in dental clinics. Due to its good sealing ability and convenience of manipulation, we wanted to know if Caviton is appropriate as retrograde filling material.

Through this study, we evaluated the sealing ability of 3 different filling materials such as MTA, glass ionomer, and Caviton to investigate possible use as retrograde filling material.

Materials and Methods

1. Tooth Preparation
A total of 60 single-rooted human teeth (maxillary second premolar or mandibular premolars) that had been extracted for orthodontic and periodontal purposes and within 1 month of extraction were collected and kept in phosphate-buffered saline before carrying out the experimental procedures. Contaminants on the tooth surface were removed with an ultrasonic device.

The 60 teeth were randomly divided into 4 different groups of 15 teeth each. An access cavity was formed with a round bur under constant irrigation. Radiographic images were used for working length measurement, with each canal enlarged to Protaper F3 (Dentsply Maillefer); the master apical file was #35. Irrigation was done with 2.5% NaOCl after using each instrument. #35 gutta-purca cone was used as master cone, with the root canal filled without sealer using the cold lateral compaction method. The root was cut at a right angle under constant irrigation using a diamond bur 3 mm from the root apex, and a cavity with depth of 3 mm was formed using a #330 carbide bur for retrofilling.
The cavity was washed with sterile saline and dried with paper point. ProRoot MTA (MTA; Dentsply) was mixed following the manufacturer’s instructions and used as retrofilling material. Fuji II LC (GI; GC Corp.) was packed and light-cured for 20 s. For Fuji II LC with XP bond (GIA), a cavity was etched with Scotchbond Etchant (3M ESPE) for 15 s, washed with water for 15 s, and dried; XP bond (Dentsply De Trey, Konstanz, Germany) was then applied, followed by light curing for 20 s. After filling Fuji II LC, light curing was performed for 20 s. Caviton (CA; GC Corp.) was used as retrofilling material and packed into the cavity with wet cotton, with the excessive material removed. Two coats of nail polish were applied on each root surface except the cut root apex and crown portion to prevent leakage into the dentinal tubules. The teeth were placed in sterile saline for 24 h until the experimental procedure.

2. Leakage of Bovine Serum Albumin (BSA) Protein

A hole was formed at the bottom of a plastic bottle, and a tooth was buried with the root surface facing upward and subsequently fixed with acrylic resin and sealed. The root end was put inside the bottle for soaking in BSA solution, and a plastic vial filled with soaking Bradford protein reagent was placed at the bottom of the bottle so that the crown portion could be soaked (Fig. 1).

Bradford protein reagent was prepared by mixing 100 ml of Coomasive Brilliant Blue, 50 ml of 95% ethanol, and 100 ml of 85% phosphoric acid; distilled water was added to make the total volume 1 L. A commercially produced reagent (G-250; Bio-rad Corporation, San Francisco, CA, USA) diluted to 1/5 by mixing distilled water with 5 times’ concentrated solution was used in this experiment. The reagent shows maximum absorbance at 595 nm and turns blue when it comes into contact with protein.

The optical density of the experimental group was measured using an ultraviolet visible spectrophotometer (NanoDrop ND-1000; NanoDrop Tech Inc., Rockland, DE, USA) after 24, 48, and 72 hours. The device was calibrated with distilled water, and unreacted Bradford reagent was used as background solution. The amount of protein leakage can be calculated using the Bradford assay standard curve, but only the presence of leakage was investigated in this experiment.
PASW Statistics version 18.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. Fisher’s exact test was used to analyze statistically the difference in sealing ability between materials after 24, 48, and 72 hours. The Kaplan-Meier survival estimate was used to analyze statistically the difference in leakage between materials over time. The results were considered to be statistically significant when the P-value was smaller than 0.05.

Result

The leakage caused the blue discoloration of the Bradford protein reagent (Fig. 2).

The absorbance of the unreacted solution was around 0.1; protein microleak causing discoloration was deemed to have occurred if optical density was greater than 0.1 at 595 nm (Fig. 3). The number of teeth showing leakage after 24, 48, and 72 hours is shown in Table 1 and Fig. 4.

Fisher’s exact test was used to analyze statistically the difference in sealing ability between materials after 24, 48, and 72 hours, as a result of which no statistically significant difference was found among the MTA, GI, and GIA groups in terms of leakage.

The CA group showed significantly greater leakage compared to MTA at 48 and 72 hours (P=0.025), however.

The Kaplan-Meier survival estimate was used to analyze statistically the difference in leakage between materials over time. The leakage in the CA group was greater than that in the MTA group (P=0.011), and the difference was statistically significant.

Table 1. Number of teeth showing leak after 24, 48, and 72 hours

<table>
<thead>
<tr>
<th>Group</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTA</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>GI</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>GIA</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>CA</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

The values denote the number of samples that showed leak. *The leak in the CA group was greater than that in the MTA group over time based on the Kaplan-Meier survival estimate (P=0.011), and the difference was statistically significant, The leak in the CA group was greater than that in the MTA group at 48 hours based on Fisher’s exact test (P=0.025), and the difference was statistically significant, The leak in the CA group was greater than that in the MTA group at 72 hours based on Fisher’s exact test (P=0.025), and the difference was statistically significant.

![Fig. 3. Spectrophotometric graph. In the Bradford assay, protein microleak causing discoloration was deemed to have occurred if the absorbance was greater than 0.1 at 595 nm. All samples belong to the MTA group at 24 hours. Only 2 samples discolored.](image)

![Fig. 4. The Kaplan-Meier survival estimate was used to analyze statistically the difference in leak between materials over time. The leak in the CA group was greater than that in the MTA group, and the difference was statistically significant (P=0.011).](image)
Discussion

Among the many methods used to test the sealing ability of a material, radioisotope and dye penetration method have been commonly used. The autoradiograph (radioisotope) method traces the pathway of a radioactive material that releases radiation by conventional printing methods, and the results may be affected by factors such as the type of isotope used, distance between the radiation source and emulsion, and film exposure time. Another disadvantage is that the size of the radioisotope tracer is much smaller than bacteria, thereby resulting in a different leakage pattern. The dye penetration method is the most popular method due to its simplicity, and methylene blue and India ink are generally used. In particular, methylene blue is cheap and easy to handle. The dye results are also good; hence its popularity. Note, however, that the molecular size is much smaller than bacterial toxin, and the method can draw incorrect results due to osmotic and capillary action. India ink is also commonly used but has problems, i.e., occurrence of dissolution during the decalcification and washing process and occasional difficulty in measuring maximum penetration depth. Furthermore, factors such as pH and chemical reactivity are known to be likely to affect the level of penetration.

A leakage measurement method that is gaining popularity nowadays is the albumin protein leakage procedure. This method is based on the discoloration that takes place when protein comes into contact with Coomasive Brilliant Blue as well as on the increase in optical density at 595 nm. The extent of leakage can be measured on every surface; another advantage is that the size of bovine albumin protein is similar to bacterial lipopolysaccharide molecule, thereby prompting us to decide on this method for this study.

As a pre-mixed temporary filling material whose components are zinc oxide, calcium sulfate, glycol acetate, polyvinylacetate resins, polyvinyl chloride acetate, and triethanolamine, Caviton has the advantage of minimizing the error that may occur during the mixing process. Moreover, the gypsum component in the material absorbs water and expands when the material comes into contact with water, and hardening occurs. This characteristic helps stop the leakage of bacteria and oral fluid by tightly bonding to the cavity. Note, however, that the hygroscopic characteristic of Caviton causes the material itself to absorb water, triggering dye penetration into the material itself in addition to the border between the material and cavity and causing viscous liquid component to be pushed out of the opposite side that is in contact with water by the absorbed water. Previous studies showed that Caviton has better sealing ability compared to Cavit and IRM. Other studies evaluating the change in leakage according to various external factors found that the sealing ability of Caviton is not affected by heat and loading. As a result of our study, however, more teeth filled with Caviton showed leakage compared to MTA.

MTA is divided into the gray type and the white type. The white MTA used in this study mainly consists of tricalcium silicate and bismuth oxide; when mixed with water, calcium hydroxide and calcium silicate hydrate are formed, changing into porous gel that is not totally crystallized. It changes from gel state into a hard structure over time. MTA is hydrophilic, slightly expanding when it hardens. This characteristic is known to provide its superior sealing ability.

Another study reported that pH affects the property of MTA. Low pH decreases the hardness and strength of MTA, and its compressive strength decreases when mixed with 2% lidocaine HCl (with 1 : 100,000 epinephrine). pH changes caused by in vivo inflammation will likely affect the property of MTA, wielding harmful influences on the treatment results. When MTA is exposed to low pH as in the case of direct contact with an apical lesion, a
mixing calcium phosphate cement matrix should be considered to prevent direct contact with an acidic environment\textsuperscript{30}.

The glass ionomer used in this study was Fuji II LC, and the difference in leakage in its use was compared with that in the use of other materials in case of sole use and use with dentin bonding agent. A previous study wherein Fuji II LC was used as filling of the root apex area showed that the difference between the use of bonding agent and non-use of bonding agent was not statistically significant; the same result was found in this experiment. Moreover, compared with composite resin, the Fuji II LC group was reported to show less leakage than the Admira (VOCO, Cuxhaven, Germany) resin filling group\textsuperscript{31}.

There are many factors that affect sealing ability besides the type of material. Gilheany et al.\textsuperscript{32} performed root resection at various degrees of angulations (0\textdegree, 30\textdegree, 45\textdegree), formed cavities of various depths, and tested the sealing ability of various root end filling materials by doing retrofilling. The results showed that the amount of leakage decreased significantly with greater depth of apical filling and smaller amount of bevel. Torabinejad and Chivian\textsuperscript{33} studied the effect of thickness of the root end filling material using MTA and concluded that MTA thickness of 3\textendash4 mm is suitable. They showed in another study that MTA 4 mm thick has superior sealing effect compared to the 3 mm-thick filling\textsuperscript{16}.

The results of this study show that glass ionomer, glass ionomer after adhesive application, and MTA all showed leakage. In terms of sealing ability, Caviton - which is commonly used as temporary dental filling material for its ease of use and minimal leakage - cannot be used as retrograde filling material. As a limitation of this study, the statistical model used cannot do in vivo replication; dynamic and further in depth studies considering the interaction between the root canal system and tissue surrounding the root apex are necessary.

Conclusion

Glass ionomer, glass ionomer after adhesive application, and MTA all showed leakage. Caviton showed greater leakage compared to MTA at 48 and 72 hours after filling, suggesting that Caviton is not appropriate as retrograde filling material considering its sealing ability.

References

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