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ORIGINAL ARTICLE

Quantitative microleakage analysis of endodontic temporary filling materials using a glucose penetration model

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Abstract

Objective. The purpose of this study was to analyze the sealing ability of different temporary endodontic materials over a 6-week period using a glucose penetration model. ***Materials and methods.*** Standardized holes were formed on 48 dentin discs from human premolars. The thicknesses of the specimens were distributed evenly to 2 mm, 3 mm and 4 mm. Prepared dentin specimens were randomly assigned into six groups ($n = 7$) and the holes in the dentin specimens were filled with two kinds of temporary filling materials as per the manufacturers' instructions as follows: Caviton (GC Corporation, Tokyo, Japan) 2 mm, 3 mm, 4 mm and IRM (Dentsply International Inc., Milford, DE) 2 mm, 3 mm, 4 mm. The remaining specimens were used as positive and negative controls and all specimens underwent thermocycling (1000; 5–55°C). The sealing ability of all samples was evaluated using the leakage model for glucose. The samples were analyzed by a spectrophotometer in quantitative glucose microleakage test over a period of 6 weeks. As a statistical inference, a mixed effect analysis was applied to analyze serial measurements over time. ***Results.*** The Caviton groups showed less glucose penetration in comparison with the IRM groups. The Caviton 4 mm group demonstrated relatively low glucose leakage over the test period. High glucose leakage was detected throughout the test period in all IRM groups. The glucose leakage level increased after 1 week in the Caviton 2 mm group and after 4 weeks in the Caviton 3 mm and 4 mm groups ($p < 0.05$). ***Conclusions.*** Caviton had better sealing ability than IRM in the glucose penetration model during 6 weeks. Temporary filling of Caviton to at least 3 mm in thickness is necessary and temporary filling periods should not exceed 4 weeks.

Key Words: *Caviton, glucose, IRM, microleakage, temporary filling materials*

Introduction

Coronal microleakage is a significant factor in the prognosis of root canal treatment [1,2]. The use of temporary restorative materials between therapeutic appointments is one of the factors that determine the success or failure of root canal treatment [3]. These materials act as a temporarily seal, preventing the entry of fluids, micro-organisms and other debris into the root canal space [4]. In addition, they prevent the escape of medicaments placed in the pulp chamber into the oral cavity [4].

A coronal temporary filling material is considered to be effective when it is able to fulfill certain properties, including good sealing of tooth margins, lack of porosity and dimensional changes to hot and cold temperatures, good abrasion and compression resistance, easy insertion and removal, compatibility with intra-canal medicaments and good esthetic appearance [5].

Microleakage of temporary restorative materials has been tested by several investigators using different methods, including dyes, radioisotopes and bacteria penetration methods [3,6–10], with most studies

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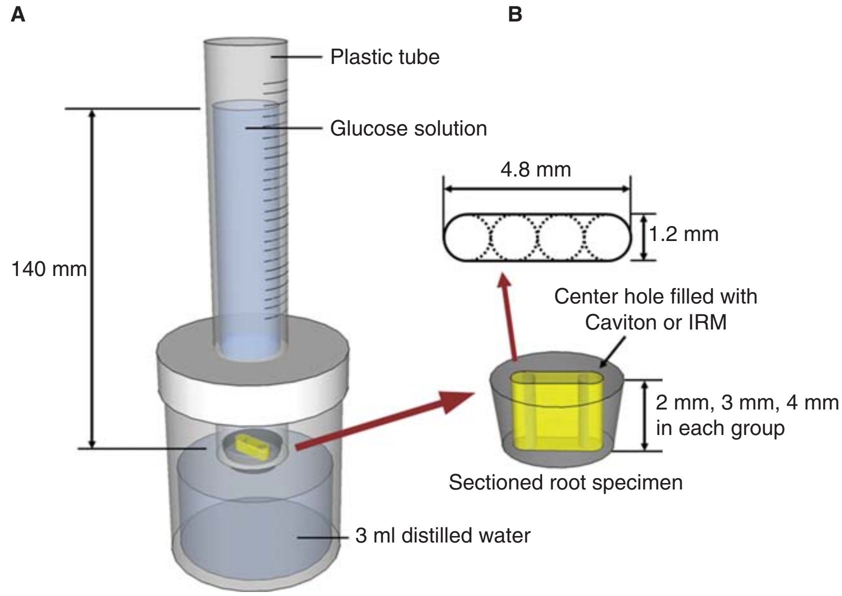

Figure 1. (A) Glucose penetration model used in this study. (B) Specimen design for microleakage test of temporary filling materials.

utilizing the dye penetration method. However, the published reports often reach different or even conflicting conclusions. Due to the variation in these results, it is difficult to draw firm conclusions as to which filling technique or material is the best for sealing the root canal system [11].

Fortunately, leakage tests are improving and Xu et al. [12] reported a new model that measured the leakage of glucose molecules. A smaller molecular size may be seen as more relevant to clinical outcomes and glucose was selected as the tracer because it has a small molecular size (MW = 180 Da) and is hydrophilic and chemically stable. Shemesh et al. [13] and Kececi et al. [14] reported that the glucose penetration model seemed to have a superior sensitivity to measure the sealing ability of root canal filling material than the fluid penetration model. The advantage of this method is the ability to perform continuous quantitative microleakage analysis, as such methods are rare in temporary restoration. Therefore, we selected the glucose penetration method as a possible coronal leakage evaluation during endodontic treatment.

The purpose of this study was to evaluate the quantitative microleakage of temporary filling materials using a glucose penetration model, testing the following hypotheses: (i) The null hypothesis of this study was that there would be no differences in microleakage, regardless of the thickness of filling materials; and (ii) The null hypothesis of this study was that there would be no differences in microleakage, regardless of the glucose penetration time.

Materials and methods

Preparation of specimen and temporary material filling

Forty-eight recently extracted human premolars with an intact coronal surface and that had been extracted for orthodontic treatment were used in this study. The crowns of the teeth were cut to leave only the root portion. The thicknesses of dentin discs were determined according to group and a hole was created in the centers of discs using a straight fissure bur 1.2 mm in diameter (FG558, MANI Inc., Tochigi, Japan). The shape of the hole was made by first placing four round holes 1.2 mm in diameter in a straight line, followed by smoothing the sides to form an oval shape (Figure 1B). Two temporary filling materials, Caviton (GC Corporation, Tokyo, Japan) or Intermediate Restorative Material (IRM) (Dentsply International Inc., Milford, DE) (Table I), were inserted into the holes and tested for microleakage. The specimens were randomly assigned to one of six different groups

Table I. Manufacturers, lot numbers and compositions of temporary filling materials tested in this study.

Cement	Manufacturer	Lot number	Composition
IRM	Dentsply International Inc., Milford, DE	Lot; 121207	Liquid: eugenol Powder: zinc oxide, polymethyl methacrylate
Caviton	GC corporation, Tokyo, Japan	Lot; 1202071	Zinc oxide, Plaster of Paris, vinyl acetate, others

and all specimens were coated with two layers of nail varnish applied 1 mm from the interface.

(1) Group 1 ($n = 7$): Caviton 2 mm;
(2) Group 2 ($n = 7$): Caviton 3 mm;
(3) Group 3 ($n = 7$): Caviton 4 mm;
(4) Group 4 ($n = 7$): IRM 2 mm;
(5) Group 5 ($n = 7$): IRM 3 mm; and
(6) Group 6 ($n = 7$): IRM 4 mm.

Caviton was placed incrementally with a plastic instrument, condensed with a plugger and excess material was removed with a sterile cotton pellet lightly dampened with sterile saline. IRM was homogeneously mixed with a hand spatula according to the manufacturer's instruction and incrementally placed with a plastic instrument and condensed with a plugger. Three tooth discs with temporary filling material were coated with nail varnish including the upper and lower surfaces and served as negative controls. Three positive controls consisted of tooth discs that were not applied with temporary filling material and whose holes were left empty. After 24 h in distilled water at 37°C, thermocycling was conducted in water that was between 5–55°C for 1000 cycles with 30 s of dwell time and 5 s of transfer time between baths.

Glucose penetration model

The model consisted of three components: the upper chamber, the prepared tooth discs and the lower chamber. The upper chamber consisted of a 10 mL disposable serological pipette (BD Falcon, Bedford, MA) that was cut to create an opening for the tooth disc. The gap between the pipette and disc was sealed with cyanoacrylate glue (Zapit, DVA Inc., Corona, CA) with a glue gun (GR-10 Mini Hot Melt Glue Gun, Stanley, CT). This upper chamber received 7 mL of a suspension of 1 mol/L glucose solution. The lower chamber consisted of a 20 mL glass bottle (Wheaton, Millville, NJ) which received 3 mL of sterile water (Daihan Pharm Co. Ltd, Ansan, Korea) (Figure 1A).

A total of 50 μL of solution was drawn from the lower glass bottle using a micropipette at 24 h, 1 week, 2 weeks, 4 weeks and 6 weeks. After drawing the sample, 50 μL of sterile water was added to the glass bottle to maintain a constant volume of 3 mL. Each sample was transferred to a cuvet and the optical density (OD) was measured at 410 nm with a spectrophotometer (Bio-tek Instruments Inc., Winooski, VT; power wave x340) using a Glucosa Kit (Glucose assay kit GAGO-20, Sigma-Aldrich, St. Louis, MO).

SEM analysis

The same specimens used for microleakage analysis were processed for scanning electron microscope (SEM) observations of the temporary filling material–tooth interfaces. For SEM evaluation, the specimens were mounted on aluminum stubs with carbon adhesive. The specimens were twice sputter-coated with gold palladium at 20 mA for 30 s by means of an ion sputtering coater and observed under an S-4700 FESEM (Hitachi, Tokyo, Japan) at an accelerating voltage of 15.0 kV and working distance of 12 mm. Representative 500× and 1000× images of the specimens from each group were used for evaluation.

Statistical analysis

We measured the leakage five times, at 24 h, 1, 2, 4 and 6 weeks after sealing. Since the serial measurements were correlated, a mixed effect analysis using the R statistical language (R Foundation for Statistical Computing, Vienna, Austria) was performed. The level of significance was set at $p < 0.05$.

Results

Figure 2 shows the glucose penetration in interfaces between dentin discs and temporary filling materials. The amounts of penetrated glucose were represented with the OD. All the measurements after 24 h from all the lower chambers of the Caviton groups and negative control group showed a low glucose leakage. However, the IRM groups showed high glucose leakage at 24 h and a leakage level as high as that of the positive control throughout the experimental period.

The Caviton 2 mm, 3 mm and 4 mm groups showed the least leakage at 24 h and the glucose leakage level significantly increased after 1 week in the Caviton 2 mm group ($p < 0.05$). The Caviton 3 mm and 4 mm groups showed significantly low leakage levels for the first 2 weeks and the leakage level was significantly increased after 4 weeks ($p < 0.05$). After 4 weeks, the Caviton 3 mm group showed a high leakage level that was not significantly different from that of the IRM groups and only the Caviton 4 mm group demonstrated relatively low glucose leakage over the test period (Table II).

In the SEM results, the IRM groups exhibited distinct gaps at the interface of filling material and dentin surface (Figure 3). The Caviton groups showed a relatively tight seal at the interface and a homogeneous state of the Caviton material itself (Figure 4).

Discussion

The purpose of this study was to evaluate the sealing ability of temporary dental filling materials. The Caviton 4 mm group showed the least microleakage, while all the IRM groups showed the highest microleakage in the glucose penetration model. This model enabled the continuous quantification of microleakage of the temporary filling materials over time.

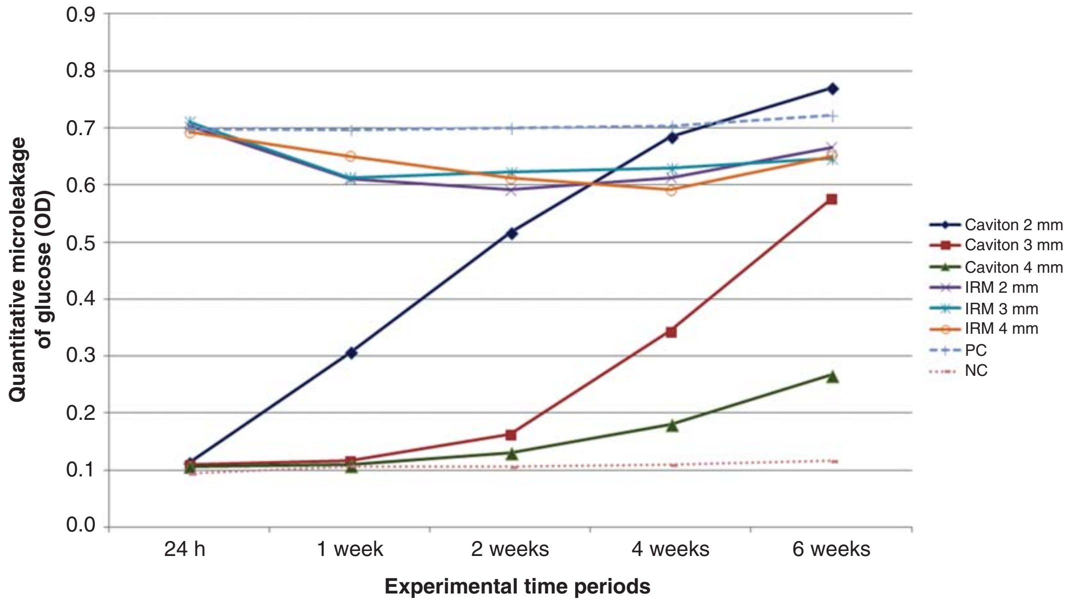

Figure 2. Time periods at which glucose was detected and quantified in the lower chamber of model systems, indicating penetration of the temporary filling materials. PC, positive control; NC, negative control; OD, optical density.

The results of previously published sealability studies in endodontics are controversial and have unclear clinical implications [15,16]. Moreover, these studies are often non-reproducible with relatively large standard deviations [11]. Therefore, more objective and quantifiable microleakage analysis methods are necessary [17].

The glucose penetration model is based on measurements of glucose concentration in a lower chamber using a sensitive enzymatic reaction. A colored substance is produced and OD is determined by a spectrophotometer and later translated to concentration units. The advantages of this model are the relative ease of assembly and operation, the general availability of the materials and equipment, the high sensitivity of the test and the ability to continuously quantify leakage amount using spectrophotometry, independent from the observer [12,13,18–20].

The choice of tracer material should be carefully chosen because its size and physicochemical properties may influence the result. A smaller molecular size and stricter test ability may be seen as more relevant to clinical outcomes. In the present study, glucose was selected as the tracer because it has a small molecular size (MW = 180 Da) [12] and is a nutrient for micro-organisms and, even at very low concentration, a biofilm is able to survive [21]. If glucose can enter the canal from the oral cavity, bacteria that might survive after root canal preparation and obturation can multiply, potentially leading to periapical infection and inflammation [12]. Therefore, glucose is thought to be more clinically relevant than other tracers used in microleakage tests of temporary restoration.

In this study, the Caviton 4 mm group showed the least microleakage and all IRM groups showed the highest microleakage level. Caviton in general resulted in low leakage levels, confirming previous findings [3]. Caviton is composed of pre-mixed temporary filling materials that set on contact with

Table II. Mean optical density of each experimental group during the 6 weeks period.

	24 hours	1 week	2 weeks	4 weeks	6 weeks
Negative control	0.094^{a}	0.107^{a}	0.106^{a}	0.109^{a}	0.116^{a}
Caviton 2 mm	0.112^{a}	0.304^{b}	0.516^{b}	0.684^{e}	0.771^{e}
Caviton 3 mm	0.110^{a}	0.118^{a}	0.162^{a}	0.344^{c}	0.577^{c}
Caviton 4 mm	0.106^{a}	0.109^{a}	0.131^{a}	0.180^{b}	0.266^{b}
IRM 2 mm	0.703^{b}	0.610^{c}	0.591^{c}	0.613^{d}	0.666^{cd}
IRM 3 mm	0.711^{b}	0.613^{c}	0.623^{c}	0.629^{d}	0.647^{cd}
IRM 4 mm	0.693^{b}	0.651^{cd}	0.613^{c}	0.591^{d}	0.651^{cd}
Positive control	0.699^{b}	0.697^{d}	0.700^{d}	0.704^{e}	0.723^{de}

Different superscripts indicate statistically significant sub-groups after performing the Student-Newman-Keuls multiple comparisons test ($p < 0.05$).

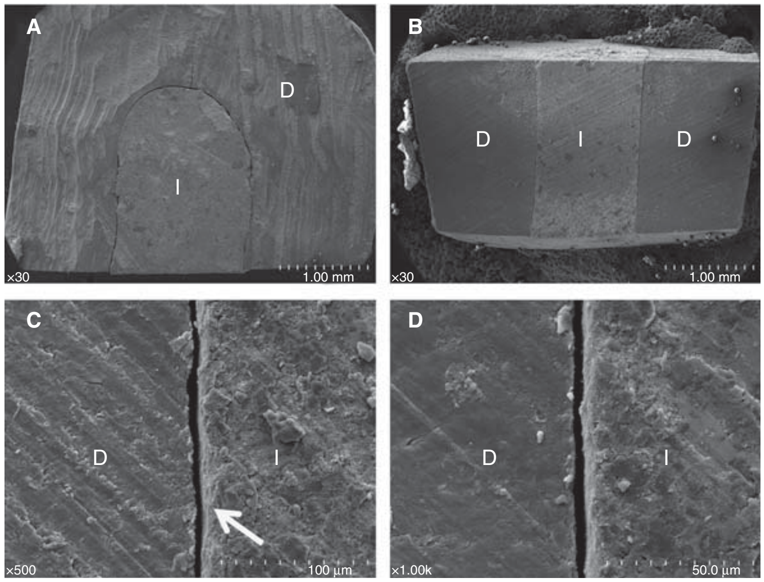

Figure 3. A scanning electron micrograph of the interface between dentin and Intermediate Restorative Material (IRM). (A) horizontal section view. (B) longitudinal section view. (C) magnification, 1000×. (D) magnification, 500×. D, dentin; I, IRM. The arrow indicates the gap between dentin and IRM.

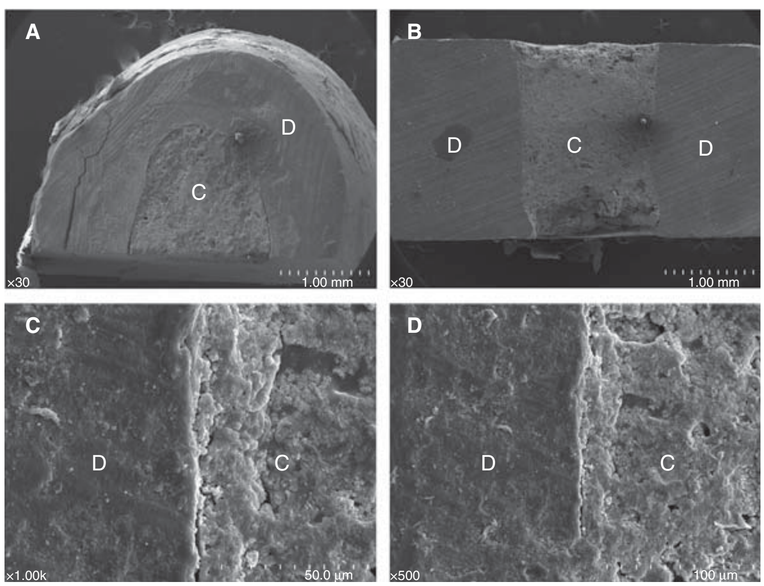

Figure 4. A scanning electron micrograph of the interface between dentin and Caviton. (A) horizontal section view. (B) longitudinal section view. (C) magnification, 1000×. (D) magnification, 500×. D, dentin; C, Caviton.

moisture and possess hygroscopic properties. This enables the material to provide a tight seal in endodontic access cavities, thereby preventing seepage of bacterial, oral fluids and other debris into the pulp chamber, which is essential for successful root canal treatment [3]. Comparative studies have indicated that Cavit-like materials provide an adequate seal [22–24]. The Caviton 2 mm group showed high leakage after 1 week and the Caviton 3 mm and 4 mm groups showed high leakage after 4 weeks. Therefore, a minimum 3 mm thickness of Caviton is recommended for temporary filling. However, after 4 weeks, all the Caviton groups showed high leakage levels, suggesting that temporary filling periods between endodontic procedures should not exceed 4 weeks.

Several studies [25–27] have analyzed thickness and duration of time between procedures as factors in temporary restoration leakage. The study of Weston et al. [27] used an acrylic tooth model to measure penetration of *Streptococcus mutans*. Their results showed no bacterial contamination at 2 weeks in any of the class I samples with a 4 mm thickness. Three of 14 class II samples with 2–3 mm thickness showed contamination at day 1 and all were contaminated at day 7. The results suggest that a 4 mm thickness of Cavit should prevent bacterial ingress for at least 2 weeks, but microbial leakage may occur if the thickness is less than 3 mm or in a complex access preparation. These results are corroborated by the results of the present study; however, study conclusions of bacterial leakage may depend on the bacterial species used and the maintenance of aseptic conditions throughout the experiment [12].

The sealing ability of all the IRM groups was poor, confirming previous reports [5,9]. Extensive material degradation was observed, with the presence of dye observed within the body of the material [28]. Studies have pointed out that stress, such as that caused by thermocycling, promotes significant degradation of IRM [29,30], whilst others indicate that variations in volume resulting from contraction of the material and inhomogeneous mixing could partially explain the poor sealing results with this filling [5]. In the IRM groups in which thermocycling was applied, IRM was severely affected, with resultant gap formation noted between the filling materials and the tooth structure in the SEM images. This finding may be attributed to the instability of zinc oxide when subjected to extremes of temperatures, as well as inconsistencies in the mixing process and the resulting lack of homogeneity.

In conclusion, Caviton showed less microleakage than IRM when used as temporary dental filling materials. A Caviton thickness of at least 3 mm is necessary and the temporary filling period should not exceed 4 weeks. If the temporary filling period is extended to 4 weeks, then over a 4 mm thickness of Caviton is required. In this study, the glucose penetration model enabled the quantitative microleakage analysis of temporary endodontic filling materials.

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