A new quantitative method using glucose for analysis of endodontic leakage

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Objective. The purpose of this study was to introduce a new method for quantitative testing of endodontic leakage.

Study design. Eighty straight maxillary anterior teeth were divided randomly into 3 experimental groups of 20 samples each and 2 control groups. The experimental groups were prepared using the modified double-flared technique and obturated by lateral compaction of cold gutta-percha with Pulp Canal Sealer EWT, Sealapex, or AH Plus sealer. With the leakage test device, coronal 1 mol/L glucose solution was forced under a hydrostatic pressure of 1.5 kPa toward the apical part of the root. Leakage was measured by the concentration of leaked glucose in apical reservoir at 1, 2, 4, 7, 10, 15, 20, and 30 days with the enzymatic glucose oxidase method.

Results. No significant difference of sealing ability was found among 3 test groups at 1, 2, 4, and 7 days. From the tenth day, Pulp Canal Sealer EWT showed the highest leakage, and the leakage was not significantly different between Sealapex and AH Plus.

Conclusions. The quantitative method is sensitive, nondestructive, and clinically relevant. Pulp Canal Sealer EWT showed more leakage than Sealapex and AH Plus in most observation time.


Adequate obturation of the root canal system following intracanal preparation is a major objective of endodontic treatment. A great deal of attention has been given to the evaluation of sealing ability of root canal filling materials and associated obturation techniques. Various test methods have been described to evaluate the quality of seal by such methods as dye penetration,1 radioactive isotopes test,2 bacteria or bacterial metabolites leakage test,3,4 electrochemical technique,5 and fluid filtration.6 However, the published reports often reach different or even conflicting conclusions. As pointed out by Wu and Wesselink, there was a high level of variation in these results and it was difficult to draw firm conclusions as to which filling technique or material was the best in sealing the root canal system.7 It was suggested that more studies should be devoted to perfecting microleakage methodology.

When filling the root canal system, the sealer plays an important role in reducing microleakage.8 Many types and brands of sealers are available, which may be broadly classified into zinc oxide–eugenol based, calcium hydroxide based, epoxy resin, and glass ionomer cement. Numerous studies have been carried out to compare sealing property of various sealers, but there is hardly any common consensus. Some studies showed that the resin-based sealer provided a better seal than other sealers,9,10 and others indicated that there was no significant difference in leakage of different types of sealers.3,11 The variety of evaluation methodologies and test parameters might account for the varying and sometimes contradictory results.

The purpose of the present study was to introduce a new method for analysis of endodontic microleakage,
based on the filtration rate of glucose along the root canal filling. The amount of leakage was quantified with spectrophotometry. Three commonly used root canal sealers, Pulp Canal Sealer EWT, Sealapex, and AH Plus, were used to evaluate this method.

MATERIAL AND METHODS

Eighty recently extracted human maxillary anterior teeth with a single, straight root canal were selected for this study. Roots with cracks, open apices, resorptive defects, or large carious lesions approaching the pulp had been excluded. After removal of bony debris, calculus and soft tissues on the root surface, the teeth were stored in deionized water at 4°C until use. A trained operator performed the root canal instrumentation and obturation described below.

Instrumentation and obturation of root canals

The coronal portion of all teeth was removed with diamond disks so that each specimen was 15 mm long. A diamond bur was used to gain a straight-line entry to the root canal. After removal of the pulp tissue using a barbed broach, a size 15 K-Flexofile (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into the root canal until the tip was just visible at the major apical foramen. The working length was determined by subtracting 1 mm from this length. Apical patency was confirmed by inserting a size 15 file through the apical foramen before and after the root canal preparation. The coronal portion of each canal was preflared with Gates-Glidden drills (Dentsply Maillefer) of sizes 3 to 6 in a step-back manner. The apical portion of the canal was instrumented to a size 50 master file using the balanced force technique and Flexofile. A step-back flaring was then performed at 1 mm increments to size 80. The canal was irrigated with 2 mL freshly prepared 2.5% sodium hypochlorite solution with a 27-gauge needle after every instrument. A final rinse with 10 mL 17% EDTA (pH = 7.7) and 10 mL 2.5% sodium hypochlorite was given after completion of preparation to remove the smear layer. The canal was then dried with paper points.

The prepared roots were randomly divided into 3 experimental groups of 20 roots each and 2 control groups of 10 each. The sealers used in 3 test groups were Pulp Canal Sealer EWT (Kerr, Romulus, Mich), Sealapex (Kerr), and AH Plus (Dentsply DeTrey, Konztanz, Germany). The root canals were filled using lateral compaction technique. The sealers were mixed according to the manufacturers’ instructions and introduced into the canal using a size 45 file with counterclockwise rotation. A size 50 master gutta-percha (GP) cone (Dentsply Maillefer) lightly coated with sealer was then placed to the full working length. Lateral compaction was achieved using size 25 accessory gutta-percha cones and a size B finger spreader (Dentsply Maillefer) that initially reached to within 1 mm of the working length. The tip of each accessory GP cone was lightly coated with sealer, placed, and compacted laterally. The process was repeated until the cone could not be inserted more than 3 mm into the canal. The 10 positive control teeth were filled in a manner identical to that of the experimental teeth but without sealer. The 10 negative control teeth were obturated with GP cones and AH Plus.

The gutta-percha at the root canal orifice was removed with a hot plugger leaving 10 mm of the filling material in the canal. All teeth were stored in aqueous solution containing 0.1% sodium azide (NaN₃) for 1 week.

Preparation of specimens

The entire specimen of the negative control group, including the root canal orifice and apical foramen, was completely coated with sticky wax (Kerr). The roots of the experimental groups and the positive controls were covered with sticky wax, except for the coronal access cavities, root canal orifices, and apical apices.

The coronal part of each root was glued to the end of an Eppendorf vial using cyanoacrylate. Leakage at this connection was eliminated by the generous use of sticky wax.
wax. A hole was created in the cap of the Eppendorf vial, through which a plastic tube of at least 15 mm long was connected. A seal was obtained using cyanoacrylate glue and sticky wax. The assembly was then placed in a sterile 5 mL glass bottle with a screw cap and sealed with sticky wax (Fig 1).

The tracer used in the present study was a 1 mol/L glucose solution (pH = 7.0), which density was $1.09 \times 10^3$ g/L and viscosity $1.18 \times 10^{-3}$ Pa·s at 37°C. Glucose has a low molecular weight of 180 Da, and is hydrophilic and chemically stable. About 5 mL of the glucose solution, containing 0.2% NaN₃, was injected into the Eppendorf vial from the plastic tube until the top of the solution was 14 cm higher than the top of gutta-percha in the canal, which created a hydrostatic pressure of 1.5 kPa (15 cm H₂O). The glass bottle contained 1 mL 0.2% solution of NaN₃, in which glucose that passed through the obturated canal would be collected. The NaN₃ was used here to inhibit the proliferation of microorganisms that might decompose glucose. The seal at all junctions of the system was checked by connecting the open end of the plastic tube to compressed air. Any bubbles would indicate leakage of the assembly.

The model was then transferred to an incubator that provided 100% humidity and 37°C temperature for the duration of observation periods. To examine if there might be any evaporation of solution in the tube/vial, an additional assembly containing 1 mL 0.2% NaN₃ was placed in the incubator under the same condition and weighted every day during test time.

**Measurement of microleakage**

A 10 μL aliquot of solution was drawn from the glass bottle using a micropipette at 1, 2, 4, 7, 10, 15, 20, and 30 days. After drawing the sample, 10 μL of fresh 0.2% NaN₃ was added to the glass bottle reservoir to maintain a constant volume of 1 mL. If there was any decrease in volume in the control bottle due to evaporation, corresponding amount of sterile deionized water was added to the glass bottle. The sample was then analyzed with a Glucose kit (Diasys, Shanghai, China) in a UV-Vis recording spectrophotometer (Shimadzu; Kyoto, Japan) at 500 nm wavelength. Two blinded independent evaluators conducted the spectrophotometric determination of glucose concentration.

The results of leakage in all groups were calculated as mmol/L at that particular time after obturation. Since the data were not normally distributed, analysis by means of the Kruskal-Wallis test was adopted. When the difference of leakage in terms of glucose concentration among groups was significant, further multiple comparisons by means of the Nemenyi test were performed.

**RESULTS**

The positive control group showed high values of glucose leakage from the first day and it increased rapidly over time. In the negative control group, no glucose was detected in all apical reservoirs throughout the experiment (Fig 2). This indicated that the seal of the system was effective and reliable.

There was a tendency of increase in leakage in all experimental groups from the first day to the end of experimental period. The mean values of leakage are given in Table 1. No significant difference was found among the 3 experimental groups at 1, 2, 4, and 7 days ($P > .05$). From the tenth day onward, there was a significant difference among the groups ($P < .001$). The results of Nemenyi test indicated that Pulp Canal Sealer EWT showed the greatest amount of leakage, whereas the difference between Sealapex and AH Plus groups was not significant at 10, 15, 20, and 30 days ($P > .05$) (Fig 3).

**DISCUSSION**

Various methods have been developed to assess sealing ability of root canal filling materials, usually based on the same principle, ie, to evaluate the penetration of a tracer along the obturated canal of an extracted tooth.4,14 Several tracers, such as dye,
radioisotope, and bacteria and their products, had been used for evaluation of microleakage. The dye penetration test is the most popular, probably because it is simple and inexpensive. However, this method often yielded a large variation of the result, and could hardly be reproducible and comparable. Assessment of bacterial leakage might be more biologically relevant than that of dye or radioisotope penetration, but the conclusions might vary with the bacterial species used. Maintaining aseptic conditions throughout all steps of the experiment can be difficult. Radioisotope labeling and electrochemical technique were less frequently employed because they pose a radiation hazard and require sophisticated materials and apparatus. The fluid filtration method, which was developed by Derkson et al for measuring dentin permeability, and later modified by Wu et al to evaluate endodontic leakage, is gaining popularity because it is sensitive and nondestructive and permits repeated observation of the same specimen over times. However, there was no standardization of the methods, such as the measurement time, the applied pressure, the diameter of the tube containing the bubble, and the length of the bubble, which might influence the results. The choice of tracer should be carefully chosen because its size and physicochemical properties may influence the result. The use of tracer of a small molecular size was favored by some authors. The smaller molecular size, and the stricter test, may be seen as more relevant to clinical outcome. In the present study, glucose was selected as the tracer because it is of small molecular size (MW = 180 Da) and is a nutrient for bacteria. If glucose could enter the canal from the oral cavity, bacteria that might survive root canal preparation and obturation could multiply and potentially lead to periapical inflammation. Glucose, therefore, was thought to be more clinically relevant than other tracers used in microleakage tests. Quantitative analysis of leakage was possible by determining the concentration of glucose in the apical reservoir that leaked through the filled root canal.

To determine the concentration of glucose, the enzymatic glucose oxidase method was chosen because it provided the ultimate degree of specificity and high sensitivity when compared with other methods, such as copper or ferricyanide methods. With this method, glucose is oxidized by the enzyme glucose oxidase in the presence of oxygen to gluconic acid with formation of hydrogen peroxide as shown in reaction (1) below. Then in the presence of a peroxidase enzyme, a chromogenic oxygen acceptor (4-aminoantipyrine and phenol) is oxidized by the hydrogen peroxide, resulting in the formation of a red product (oxidized chromogen). The quantity of this oxidized chromogen is proportional to the glucose present initially in the first reaction, which quantity is determined by spectrophotometry.

\[
\text{Glucose} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{Gluconic acid} + \text{H}_2\text{O}_2 \quad (1)
\]

\[
\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{phenol} \rightarrow \text{Oxidized chromogen} + \text{H}_2\text{O} \quad (2)
\]

With this model, it was possible to quantify the endodontic microleakage continuously over time. The amount of microleakage was the cumulative value of leaked glucose. In addition, the coronal low pressure used in the study could help rule out entrapped air or fluid and seemed to be sufficient for a device with high sensitivity.

The results of the present study indicated that the 3 types of sealer all showed similar level of leakage in the first 10 days during the observation time. Thereafter, the greatest amount of leakage was observed with Pulp Canal Sealer EWT. That might be owing to the dissolution of the sealer in the solution. Sealapex and AH Plus showed similar behavior of long-term seal; although AH Plus seemed to perform better, the difference was not significant. With our leakage testing device, most canals showed low degree of leakage at day 2 and all samples showed leakage to a small extent at day 7. This suggested the need to reconsider those studies that reported on short-term leakage patterns of different obturation materials or techniques. The authors note the need, however, to test the validity and reproducibility of the current method.

In summary, under the conditions of this study, this new in vitro method allowed a nondestructive, long-term, quantitative evaluation of endodontic microleakage. It appeared that Pulp Canal Sealer EWT showed a greater amount of leakage than Sealapex and AH Plus after an extended period of observation.

REFERENCES


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