Sucrose as a stable tracer for quantifying endodontic leakage

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Abstract  Background/purpose: Coronal or apical leakage is a major cause of endodontic treatment failure. To evaluate sucrose as a tracer for leakage testing for various filling materials involved in endodontic treatment.

Materials and methods: The stability of sucrose and glucose was examined by immersing 11 common filling materials (1.5 × 3 mm blocks, cured for 1 week at 37°C in 100% relative humidity; n = 10 for each) in a 10 mM solution of either sucrose or glucose. The concentration of the solution was measured after 1 week, 2 weeks, 4 weeks, and 8 weeks of immersion and compared. Then, the two tracers were used to test the sealing ability of zinc oxide–eugenol cement (IRM) and amalgam root-end fillings. Each material (n = 40 for each) was equally divided into two subgroups, these were evaluated with either glucose or sucrose as the tracer substance, and the amount of leakage was determined after 24 h, and 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, and 8 weeks.

Results: Sucrose was stable with all materials at all time points. The concentration of glucose had significantly diminished after 1 week of immersion with mineral trioxide aggregate (MTA) or Sealapex (P < 0.001), but not with other filling materials (P > 0.05). Leakage results were similar (P > 0.05) when glucose and sucrose were used as the tracer substance, respectively. Amalgam leaked significantly less than IRM after 3 (sucrose test) and 4 weeks (glucose test) (P < 0.05).

Conclusion: Sucrose appears to be stable in the presence of various endodontic materials, and can be used as a stable tracer substance for detecting endodontic microleakage.

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Introduction

Leakage, either in the apical-to-coronal or coronal-to-apical direction, is implicated as a cause of endodontic treatment failure.\(^1,2\) Attaining a (hermetic) seal has been listed as a treatment goal that contributes to successful clinical outcomes.\(^3\) Numerous studies of the sealing ability of various obturation and (temporary) restorative materials have been reported, for which a variety of test methods and tracer substances were used to detect microleakage.\(^4-6\) There are controversies as to the choice of tracer particles to penetrate the interface between the restoration (or root canal filling) and root canal or cavity wall.\(^7,8\) The clinical relevance has also been debated.\(^9,10\) Alternatively, microleakage tests continue to be performed to compare the relative performance (sealing) achieved with different materials.

The glucose leakage test was first introduced in 2005 to evaluate endodontic leakage.\(^11\) This model allows long-term quantification of the cumulative amount of microleakage for an obturated root canal.\(^12,13\) However, a recent study revealed that glucose is slowly oxidized in an alkaline solution by oxygen into gluconic acid, which is then converted to gluconate.\(^14\) As gluconate cannot be detected by the glucose kit, the authors recommended that the sealing ability of alkaline materials like mineral trioxide aggregate (MTA) should not be evaluated with the glucose leakage model. The importance of having a tracer material that can remain stable in an alkaline condition cannot be overemphasized. Compared to glucose (180 Da), sucrose is relatively stable and is not readily oxidized in an aqueous environment; therefore, it is called a non-reducing sugar.\(^15\) It is nontoxic to humans and can be enzymatically detected at low levels.\(^16\) Sucrose is hydrophilic and has a low molecular weight (342 Da), compared to the rather large molecule (about 10^6 Da) for endotoxins. Therefore, sucrose has the potential to be a new and stable tracer.

The objectives of this study were to test sucrose as a replacement for glucose as a tracer particle for microleakage by evaluating the stability of sucrose with different endodontic materials, and compare leakage results of two root-end filling materials using both glucose and sucrose tests.

Materials and methods

Stability tests of sucrose and glucose

The stability of sucrose and glucose was examined in the presence of 11 endodontic materials (n = 20 for each): ProRoot MTA (Dentsply-Tulsa Dental, Tulsa, OK, USA), amalgam (Kerr, Romulus, MI, USA), composite resin (Clearfil, Tokyo, Japan), IRM (Caulk, Milford, DE, USA), Sealapex (Kerr), Tubli-Seal (Kerr), Pulp Canal Sealer (Kerr), AH Plus (Dentsply DeTrey, Konitz, Germany), RealSeal sealer (SybronEndo, Orange, CA, USA), gutta-percha (Dentsply DeTrey), and Resilon cones (Pentron, Wallingford, CT, USA).

The materials were mixed, if necessary, according to the manufacturer’s instructions and packed into tubular plastic molds (n = 20 for each) 3 mm long and 1.5 mm in diameter. For the composite resin, two 1.5-mm-thick layers were packed into the mold, with each layer being cured with a curing light (3M, St. Paul, MN, USA) for 20 seconds. For gutta-percha and Resilon, the cones were fitted into the molds with a warm instrument. All molds with individual materials were stored at 100% relative humidity and 37°C. After 1 week, the set materials were carefully removed from the molds. Ten specimens of each material were put into separate glass tubes containing 4.0 mL of 10mM sucrose, and the other 10 specimens were placed into 4.0 mL of a 10mM glucose solution. Tubes containing only the sucrose or glucose solution served as controls. The concentration of the solution in each tube was determined after 1 week, 2 weeks, 4 weeks, and 8 weeks using a sucrose kit (BioSenTec, Toulouse, France) in an ultraviolet/visible recording spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of 340 nm, or with a glucose kit (DiaSys, Shanghai, China). Data for the reactivity of glucose or sucrose with the different endodontic materials was tested with the Mauchly test of sphericity. If sphericity was assumed, a repeated-measures analysis of variance (ANOVA) was used to analyze the data. If not, a multivariate ANOVA was used. If the interaction of time and effect was significant in the repeated-measures ANOVA, simple effects between groups were analyzed by a one-way ANOVA, and multiple comparison tests were performed with Dunnett’s tests at a significance level of P < 0.05.

Microleakage of two root-end filling materials

Coronal-to-apical leakage using glucose or sucrose as the tracer particle was examined for two retrograde filling materials: amalgam and IRM. One hundred freshly extracted human teeth with a single, straight root canal were used. These teeth were free from root fracture, open apices, resorptive defects, or large carious lesions extending close to the pulp. The teeth were kept in a 2.5% sodium hypochlorite solution overnight and then in phosphate-buffered saline at 4°C until used.

A trained operator performed both the root canal therapy and retrograde cavity preparation and filling. Briefly, each tooth was sectioned near the cementoenamel junction using a diamond disk under water cooling, leaving a 15 mm-long tooth. Pulp tissues were removed with a barbed broach. A size-15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted to measure the working length and verify the canal patency. Root canals were prepared using the ProFile rotary system (Dentsply Maillefer) with the crown-down technique recommended by the manufacturer. A size-15 K-file was used in between the rotary files to verify canal patency, followed by irrigation with 2 mL of freshly prepared 2.5% sodium hypochlorite with a 27-gauge needle. The master apical dimension was fixed at size 40 and a 0.04 taper. Then, the apical 3 mm of each root was sectioned perpendicular to the long axis of the tooth using a high-speed diamond bur with water spray coolant. The root end was prepared to a depth of 3 mm using ultrasonic tips powered by an ultrasonic unit (Excellence in Endodontics/Analytic Technology, Orange, CA, USA) with water spray.
After root-end preparation, a random selection of 80 teeth was divided into two groups. A gutta-percha cone (size 70 or 80) that fit snugly at 3 mm from the resected root-end was held in place to serve as a matrix against which the retrograde material could condense. The root-end cavities were filled with either amalgam or IRM (n = 40 for each), both mixed according to the manufacturer’s instructions. After that, the gutta-percha cone was removed leaving only the root-end filling material in place; radiographs were taken to ensure that it was indeed 3 mm deep with no voids within the material. For the remaining 20 teeth in which root-end cavities were prepared, 10 were filled with sticky wax (Kerr) to serve as negative controls: five for the glucose and five for the sucrose leakage test. The last 10 specimens were left unfilled and served as positive controls for the two leakage tests.

All specimens were examined under an operating microscope (Carl Zeiss, Oberkochen, Germany) to ensure the absence of any cracking of the root after retrofilling, before being stored in 100% relative humidity at 37°C for 1 week. Then, sticky wax (Kerr) was applied to all external tooth surfaces except for the coronal access cavity and the apical end of the root. For the negative control group, the entire specimen was sealed with sticky wax.

Quantification of microleakage

The 40 teeth that were filled with either of the two test materials were divided randomly into two subgroups (n = 20) and tested for leakage using either glucose or sucrose as the tracer. Teeth in the first subgroup were mounted in an assembly similar to that described by Xu et al.\textsuperscript{11,12} and tested with a 1M glucose solution. For teeth in the second subgroup, a 1M sucrose solution containing 0.2% NaN\textsubscript{3} (as a preservative), instead of glucose, was mounted in a similar setup. The level of the solution in the vertical column was 14 cm above the top of the filling material in the glucose test and 13.6 cm higher in sucrose test group; this created hydrostatic pressure of 1.5 kPa. A container with 2 mL of 0.2% NaN\textsubscript{3} was attached to the root end to collect any solution that leaked through. All 100 teeth were assembled this way before being transferred to an incubator with 100% relative humidity at room temperature. The amount of sucrose and glucose that leaked through the root-end filling to the solution beneath the root was evaluated after 24 h, and 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, and 8 weeks. At each designated interval, 0.5 mL of the solution was withdrawn from the container. After sampling, a new container with 2 mL of 0.2% NaN\textsubscript{3} was installed. The cumulative amounts of leakage at the various intervals were compared between the two root-end filling materials, and between the two leakage tests, using the non-parametric Kruskal–Wallis test. When a significant difference was found, further multiple comparisons were performed with the Mann–Whitney test.

Results

Table 1 shows results of the stability test for sucrose and glucose with 11 endodontic materials. A multivariate ANOVA was used for the sucrose groups, and a repeated-measures ANOVA was used for the glucose groups. There were no significant differences in sucrose concentrations among all materials at all time points (P > 0.05). In the glucose groups, however, the interaction of time and effect was found to be significant. The simple effects between groups were then analyzed; it was found that MTA and Sealapex significantly altered the concentration of glucose at all time points (P < 0.001). No significant difference in the glucose concentration was noted between the other filling materials and the control (P > 0.05).

For the leakage tests of the two root-end filling materials, the level of sucrose or glucose solution in the plastic tubes dropped sharply, indicating high leakage rates in the positive control groups. Alternatively, no leakage was detected in the negative controls throughout the time course, indicating that the seal with sticky wax was effective and reliable. Both the glucose and sucrose tests showed similar leakage values for the two retrograde filling materials at various intervals (P > 0.05) (Table 2). Amalgam

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration of the tracer solution (glucose or sucrose) immersed with different materials (mM/L).</th>
<th>1 wk</th>
<th>2 wk</th>
<th>4 wk</th>
<th>8 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sugar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.98 ± 0.19</td>
<td>10.02 ± 0.10</td>
<td>9.98 ± 0.12</td>
<td>10.03 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>MTA</td>
<td>9.99 ± 0.24</td>
<td>9.96 ± 0.08</td>
<td>9.95 ± 0.10</td>
<td>10.02 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Amalgam</td>
<td>9.88 ± 0.18</td>
<td>9.95 ± 0.17</td>
<td>9.99 ± 0.12</td>
<td>9.94 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>10.03 ± 0.18</td>
<td>10.05 ± 0.11</td>
<td>10.05 ± 0.08</td>
<td>10.02 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>IRM</td>
<td>10.07 ± 0.11</td>
<td>10.06 ± 0.09</td>
<td>9.94 ± 0.12</td>
<td>9.94 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Sealapex</td>
<td>10.03 ± 0.16</td>
<td>10.06 ± 0.12</td>
<td>10.07 ± 0.25</td>
<td>10.06 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Tubli-Seal</td>
<td>9.87 ± 0.21</td>
<td>10.06 ± 0.13</td>
<td>10.05 ± 0.12</td>
<td>10.09 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>PCS</td>
<td>10.04 ± 0.11</td>
<td>10.09 ± 0.17</td>
<td>9.98 ± 0.13</td>
<td>9.99 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>RealSeal</td>
<td>9.96 ± 0.17</td>
<td>9.98 ± 0.14</td>
<td>9.99 ± 0.13</td>
<td>10.03 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>AH Plus</td>
<td>10.08 ± 0.05</td>
<td>10.08 ± 0.15</td>
<td>9.99 ± 0.12</td>
<td>10.03 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>10.00 ± 0.08</td>
<td>10.07 ± 0.17</td>
<td>9.96 ± 0.18</td>
<td>9.94 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Resilon</td>
<td>10.01 ± 0.07</td>
<td>10.07 ± 0.14</td>
<td>10.04 ± 0.08</td>
<td>10.01 ± 0.23</td>
<td></td>
</tr>
</tbody>
</table>

CR = composite resin; GP = gutta-percha; IRM = intermediate restorative material; MTA = mineral trioxide aggregate; PCS = pulp canal sealer.
leaked significantly less than IRM from week 3 onwards when tested with sucrose, and from week 4 onwards with the glucose test (P < 0.05).

Discussion

Various test methods have been described in the endodontic literature to assess the quality of seals; all were based on the penetration of a tracer substance along the filled root canal of an extracted tooth. The use of small tracer molecules has been favored by some authors. The smaller the molecular size, the stricter the test; this was considered to be more relevant to clinical outcomes.

Glucose has been proposed as a tracer substance because both materials are non-alkaline, and were once popular for root-end filling. When a glucose or sucrose solution was used to assess the extent of leakage of the two neutral (in pH) root-end filling materials (amalgam and IRM), no significant differences were found. The sucrose molecule is 1.06 nm in diameter, which is slightly larger than that of glucose (0.86 nm), but is still very small compared to methylene blue, bacterial cells, and endotoxin. The results of our study indicated that the modest difference in the size of glucose versus sucrose did not affect the conclusions of the leakage test.

Amalgam and IRM were used to verify the new tracer molecule for a leakage test because both materials are non-alkaline, and were once popular for root-end filling. When comparing the sealing ability of these two materials, amalgam appeared to perform better than IRM after the initial few weeks. Amalgam leaked significantly less than IRM from week 3 onwards when tested with sucrose, and from week 4 onwards with the glucose test.

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Amalgam and IRM were used to verify the new tracer molecule for a leakage test because both materials are non-alkaline, and were once popular for root-end filling. When comparing the sealing ability of these two materials, amalgam provided a more-effective barrier than IRM after the initial few weeks. Amalgam leaked significantly less than IRM from week 3 onwards when tested with sucrose, and from week 4 onwards with the glucose test. Thereafter, amalgam appeared to perform better than IRM, which differed from the results of Fogel and Peikoff who reported no difference between amalgam and IRM using a fluid filtration system.

A meta-analysis of root-end filling materials (amalgam and IRM), no significant differences were found. The sucrose molecule is 1.06 nm in diameter, which is slightly larger than that of glucose (0.86 nm), but is still very small compared to methylene blue, bacterial cells, and endotoxin. The results of our study indicated that the modest difference in the size of glucose versus sucrose did not affect the conclusions of the leakage test.
mixing IRM to the same consistency can be difficult at all times, thus potentially producing inconsistent results. Amalgam was tested in this study as a control, because, since the report by Dorn and Gartner, it is a material that is used to substitute for IRM and super EBA cement. Amalgam is rarely used clinically as retrograde filling material because of its disadvantages, such as corrosion, release of ions, dimensional instability, and poor biocompatibility.

Within the limitations of the present study, it is concluded that sucrose appears to be stable in the presence of various endodontic materials, and can be used as a stable tracer substance for detecting endodontic microleakage.

References