Evaluation of pH and calcium ion release in capseal I and II and in two other root canal sealers

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Objective. The aim of this study was to compare the pHs and calcium ion releases of newly developed, calcium phosphate–based root canal sealers (Capseal I and II) with those of Sealapex (SA) and Sankin Apatite Root Sealer III (SARS III).

Study design. Each material (n = 10) was mixed, inserted into polyvinyl chloride (PVC) tubes, and immersed in a vial containing deionized water. The control group consisted of empty PVC tubes (n = 10). The pHs and calcium ion releases were measured by pH meter and ICP-AES (inductively coupled plasma atomic emission spectrometer) at 24 hours and 96 hours, and at 7, 15, and 28 days. The results were analyzed with the Kruskal-Wallis test.

Results. Capseal I and II produced pHs and calcium ion releases higher than or equal to those of SA and SARS III (P < .05).

Conclusions. Capseal I and II resulted in higher alkalinity and higher calcium ion releases than SA and SARS III. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:e23-e28)

Root canal sealer is essential to hermetically seal a root canal system.1 The ideal root canal sealer should be tissue tolerant, insoluble in tissue fluids, and bacteriostatic, or at least should not encourage bacterial growth.2 Furthermore, an ideal root canal sealer should induce mineralized tissue formation.3

Previous studies suggested that cements containing calcium hydroxide or calcium oxide can dissociate into calcium and hydroxyl ions, resulting in a higher pH in adjacent tissues and thus may induce mineralized tissue formation.4,8 Increased pH has also been reported to be bactericidal,9 to interfere with osteoclastic activity,10 and to promote experimental wound healing in human fibroblasts in vitro.11

Calcium ion release is another important characteristic of calcium hydroxide or calcium oxide–containing materials. Calcium ions in tissue have been reported to activate calcium-dependent adenosine triphosphatase12 and may react with tissue carbonic gas to form calcium carbonate crystals, which promote mineralization. Calcium ions also may be necessary for cell migration and differentiation.12

Although pH and calcium ion release from root canal sealers are 2 of their most important chemical characteristics for the promotion of mineralization, relatively few studies have evaluated these factors.3,4,7,13-18 Calcium hydroxide–based root canal sealers have been used successfully for many years,5 and many previous studies have reported their abilities to produce high pH and calcium ion release.3,7,14 Calcium phosphate cement is also well known for its biocompatibility19 and osteoconductivity,20 making calcium phosphate cement a successful bone substitute in medicine and dentistry.21-24 Several investigators have reported that mineral trioxide aggregate (MTA) produces alkalinity and calcium ion release.3,8,25 However, no study on the pHs and calcium ion releases of root canal sealers that contain both calcium phosphate and MTA (or Portland cement) has been published.

Capseal I and Capseal II are recently developed calcium phosphate–based root canal sealers. The main components of Capseal I and II are tetracalcium phosphate (TTCP), dicalcium phosphate dehydrate (DCPD), Portland cement, and zirconium oxide.
phate and Portland cement are both calcium-releasing materials. To the best of our knowledge, this is the first experimental root canal sealer to use both calcium phosphate and Portland cement. Previous studies performed with Capsales I and II have demonstrated that these sealers have favorable tissue responses, acceptable biocompatibilities, and good root canal sealing abilities. Furthermore, recently Bae et al. reported that Capsales I and II have superior mineralization potential than other commercial root canal sealers.

The aim of this study was to compare the pHs and calcium ion releases of newly developed calcium phosphate–based root canal sealers (Capsales I and II) with those of the other commercially used root canal sealers, Sealapex (calcium hydroxide–based) and Sankin Apatite Root Sealers III (calcium phosphate–based).

**MATERIAL AND METHODS**

**Materials used**

The materials used in this study were Capsale I, Capsale II (experimental root canal sealer), Sankin Apatite Root Sealer III (SARS III) (Dentsply-Sankin, Tokyo, Japan), and Sealapex (SA) (Sybronendo, Orange, CA) (Table I).

**Sample preparation**

Both commercial root canal sealers (SARS III and SA) were mixed according to the manufacturer’s instructions. Capsale I and Capsale II were mixed at a powder:liquid ratio of 1:1.5:1.0. To evaluate pH and calcium ion release, 10 samples of each material were prepared. Immediately after manipulating the materials, 10-mm long and 3.175-mm inner diameter polyvinyl chloride (PVC) tubes (Nalge Nune International Corporation, Rochester, NY) were filled and placed in lidded glass vials (Kimble, Vineland, NJ) containing 10 mL deionized water. Empty PVC tubes comprised the control group.

The specimens were moved to new vials containing 10 mL of deionized water at 24 hours, 96 hours, 7 days, and 15 days for subsequent measurements. The vials were maintained at 37°C throughout the experiment.

The pHs and calcium ion releases of each root canal sealer were measured at 24 hours and 96 hours, and 7, 15, and 28 days after initial immersion. A schematic diagram of the experimental design is shown in Fig. 1.

**Contamination control during sample preparation and analytical operation**

To eliminate trace elements leaching from the walls of the sample bottles and from the pipette tips, equipment was soaked in an 8:1:1 mixture of water, nitric acid, and hydrochloric acid for at least 48 hours. The equipment was then rinsed several times with ultrapure water (Millipore, Milli-Q Plus), dried at room temperature on a clean bench, and stored in a clean air environment until use. The analyst wore polyvinyl chloride gloves during sample preparation, including cleaning and analysis.

**pH analysis**

The pHs of the solutions were measured with a pH meter (Orion 520 A, Orion Research Inc., Boston, MA). The apparatus was calibrated with known standard pH solutions. As previously described, the pHs of the solutions were measured at 24 hours and 96 hours, and 7 days, 15 days, and 28 days after sample immersion. After removing the specimen from the vial, the solution was homogenized by a magnetic stirrer (IKA Werke GmbH & Co. KG, Staufen, Germany). Between each measurement, the pH meter electrode was washed with ultrapure water (Millipore, Milli-Q Plus) and blotted-dried. Each measurement was carried out twice, and the mean value was recorded.

**Calcium ion release analysis**

*Reagents for calcium ion release analysis.* A calcium ion stock solution (1000 mg/L) was purchased from SCP Science (Baie D’Urfé, Québec, Canada) as an atomic absorption standard solution in 4% HNO₃. Working standard solutions were prepared by serially diluting the stock solution with high-purity water. High-purity deionized water (18 MΩ) was obtained.

<table>
<thead>
<tr>
<th>Name</th>
<th>Powder</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsale I</td>
<td>Tetracalcium phosphate &amp; dicalcium phosphate dehydrate, Portland cement, zirconium oxide, others</td>
<td>Hydroxypropyl methyl cellulose in sodium phosphate solution</td>
</tr>
<tr>
<td>Capsale II</td>
<td>Tetracalcium phosphate &amp; dicalcium phosphate dehydrate, white Portland cement, zirconium oxide, others</td>
<td>Hydroxypropyl methyl cellulose in sodium phosphate solution</td>
</tr>
<tr>
<td>SARS III</td>
<td>α-tricalcium phosphate, hydroxyapatite, iodoform (5%)</td>
<td>Polyacrylic acid, water</td>
</tr>
<tr>
<td>Sealapex</td>
<td>Mixed sealer: carbon oxide 20%, bismuth trioxide 29%, zinc oxide 2.5%, submicron silica 3%, titanium dioxide 2%, zinc stearate 1%, tricalcium phosphate 3%, blend 39%</td>
<td></td>
</tr>
</tbody>
</table>
from a Milli-Q water purification system (Millipore, Milli-Q plus).

**Determination of calcium ion release.** Each sample solution was analyzed by ICP-AES (inductively coupled plasma atomic emission spectrometry) (ICPS-1000IV, Shimadzu Corporation, Kyoto, Japan). The conditions for analysis were 1.2 kW of power from a radio frequency generator, a plasma argon flow rate of 1.2 L/min, a cooling gas flow of 14.0 L/min, a carrier gas flow of 0.7 L/min, an entrance slit of 20 μm, an exit slit of 30 μm, an observation height of 15 mm, and an integration time-lapse of 5 seconds.

**Calibration for calcium release analysis.** The equipment was calibrated using a “5-point” calibration method. The calibration was verified using the standard solutions described previously. One representative calibration curve is shown in Fig. 2.

**Statistical analysis**

The data were statistically analyzed using a Kruskal-Wallis test with a Bonferroni correction to compare pH and calcium ion release at each time point. If the difference was significant, individual comparisons were performed using the least significant difference test (LSD test) by rank with a Bonferroni correction.

**RESULTS**

**pH measurement**

The mean pH values measured for materials at different time points are shown in Fig. 3 and Table II. The controls showed no noticeable change over the experimental period. Capseal I and Capseal II showed significantly higher pH than those of SARS III and SA at all time points (P < .05). At 96 hours, Capseal II had the highest pH of all the materials (P < .05). At 15 and 28 days, Capseal I had the highest pH of all the materials (P < .05).

**Released calcium ion concentration**

Figure 4 and Table III show the mean calcium ion releases at each time point. At all time points, Capseal I and Capseal II showed significantly higher calcium ion releases than did SARS III (P < .05). Likewise, at all time points, Capseal I and Capseal II showed calcium ion releases significantly higher than or equivalent to that of SA (P < .05).

**DISCUSSION**

The specimen preparation method used in this investigation was adopted and modified from methods used in 2 previous studies. Although this method does not exactly simulate the clinical situation, where the diameter of the apical foramen is smaller than 1 mm in most cases, it does offer the advantage of simplicity, reproducibility, and time savings. These advantages allow for easy in vitro comparisons between different materials. Distilled water at a pH 7 was used as a solvent, as in previous studies, as a buffered solution or saline could affect different materials in dissimilar ways.
In this experiment, Capseals I and II produced higher pHs from the beginning and continued to do so throughout the experimental period. Calcium phosphate and Portland cement, components of Capseals I and II, might play an important role in this pH increase.

The hydroxyl ion–releasing ability is not necessarily a positive characteristic of a root canal sealer. Increased pH has been reported to have beneficial effect.4-11 However, releasing of hydroxyl ion requires the solubilization of the root canal sealers, which can jeopardize the dimensional stability. On this concern, Eldeniz et al.7 stated that the problematic dissolution of root canal sealer is limited because the surface area of the root canal sealer that is exposed to the tissue fluid is limited. In a previous study, Capseal has been reported to have as good sealing ability as other commercial root canal sealers.23

Table II. Mean pH values with standard deviations for 4 sealers at 5 different times

<table>
<thead>
<tr>
<th>Material</th>
<th>pH 24h</th>
<th>SD</th>
<th>pH 96h</th>
<th>SD</th>
<th>pH 7d</th>
<th>SD</th>
<th>pH 15d</th>
<th>SD</th>
<th>pH 28d</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaic I</td>
<td>11.205</td>
<td>0.61769</td>
<td>11.345</td>
<td>0.83018</td>
<td>10.932</td>
<td>0.12515</td>
<td>11.412</td>
<td>0.0939</td>
<td>11.424</td>
<td>0.07849</td>
</tr>
<tr>
<td>Capsaic II</td>
<td>11.052</td>
<td>0.22225</td>
<td>11.772</td>
<td>0.14382</td>
<td>11.009</td>
<td>0.22258</td>
<td>11.314</td>
<td>0.08566</td>
<td>11.298</td>
<td>0.03162</td>
</tr>
<tr>
<td>ARS III</td>
<td>7.047</td>
<td>0.2071</td>
<td>9.782</td>
<td>0.16158</td>
<td>9.21</td>
<td>0.25914</td>
<td>9.558</td>
<td>0.09199</td>
<td>9.306</td>
<td>0.23186</td>
</tr>
<tr>
<td>Sealapex</td>
<td>10.532</td>
<td>0.2304</td>
<td>10.102</td>
<td>0.68621</td>
<td>9.78</td>
<td>1.00904</td>
<td>10.44</td>
<td>0.1241</td>
<td>10.181</td>
<td>0.6862</td>
</tr>
<tr>
<td>Control</td>
<td>7.266</td>
<td>0.22387</td>
<td>7.467</td>
<td>0.20271</td>
<td>7.431</td>
<td>0.21153</td>
<td>7.621</td>
<td>0.10571</td>
<td>7.461</td>
<td>0.26006</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant differences between materials.
*Indicate statistically significant differences between Capsaic II and Sealapex but not between Capsaic I and Sealapex.

Fig. 3. Change of pH at each time point in experimental and control groups.

Fig. 4. Calcium ion release at each time point in experimental and control groups.
Capseal I and Capseal II might stem from the component. Camilleri et al.32 evaluated the pH changes attributed to the Portland cement, another major component. Duarte et al.8 investigated the pH of MTA and reported that MTA immersed in deionized water produced a pH of 9.07, 24 hours after immersion. This result is lower than that in the present study. The calcium phosphate and Portland cement might have acted synergistically to produce a higher pH than that seen by Duarte et al.8 MTA and Portland cement also contain calcium oxide, which is converted to calcium hydroxide ion upon contact with tissue fluid or water, thus raising the pH.

The other materials used in this study were calcium hydroxide– or calcium phosphate–based root canal sealers, which have a known potential for alkalization. SARS III showed a lower pH than those of Capseal I and Capseal II at all time points. The lower pH might be explained in part by the different liquid constituents. SARS III uses polyacrylic acid as a constituent of the setting solution, which might contribute to the lower pH. On the contrary, Capseal I and Capseal II use hydroxypropyl methylcellulose (HPMC) in the setting solution, the hydroxyl group of which might contribute to the alkalization potentials of Capseals I and II.

In this study, we also compared calcium ion releases from Capseals I and II and commercial root canal sealers. Twenty-four hours after immersing the specimen in distilled water, Capseal I had released significantly more calcium ions than any other material used in this study (P < .05). Capseal II released significantly more calcium ions than SA or SARS III, but significantly less than Capseal I. The difference in calcium ion release between Capseal I and Capseal II might stem from the compositions of these 2 materials. Capseal I contains gray Portland cement, whereas Capseal II contains white Portland cement. Our laboratory intends to investigate the abilities of gray and white Portland cements to release calcium ions. The ability of Capseal to release calcium ions might be attributed to the Portland cement and the TTCP in its composition. Previous studies3,33 have reported that both MTA and calcium phosphate produce calcium hydroxide during the hydrolyzing and setting reactions, which could provide calcium ions to the surrounding environment. The unique and interesting finding of the present experiment is that calcium phosphate (TTCP/DCPD) and Portland cement had a synergistic effect on the increase of calcium ion release, as supported by comparing the results of the present study with those of previous studies. Tanomaru-Filho et al.3 studied the calcium ion release from MTA using a similar experimental design. The calcium ion release from MTA at 28 days was 10.6 mg/kg, which was much lower than that of Capseal I (44.1 mg/kg) or Capseal II (37.3 mg/kg) in this study. SARS III, a calcium phosphate–based root canal sealer, was used in this study to compare its calcium ion release with that of the Capseals. The calcium ion release from SARS III at 28 days was 0.8 mg/kg, far less than that of Capseal I (44.1 mg/kg) or Capseal II (37.3 mg/kg). Considering these findings, the high calcium ion release from the Capseals may be a result of a combined, synergistic effect of calcium phosphate and Portland cement.

In this experiment, in the case of Capseal, the calcium release decreased at 7 days, whereas the pH values remained stable. This phenomenon was not easy to interpret. It might be the result of Portland cement–calcium phosphate–water interaction, although the exact mechanism is still unknown. Further study to investigate Portland cement–calcium phosphate–water chemical reaction is necessary. This study has other limitations. Positive and negative controls with basic and acidic samples with known pH would have better confirmed the validity of the method used in this experiment. Furthermore, using zero calcium-releasing samples, such as zinc oxide– and eugenol-based root canal sealers would have strengthened the validity of this study.

### Table III. Mean calcium ion release and standard deviations for 4 sealers at 5 different times

<table>
<thead>
<tr>
<th>Material</th>
<th>Ca&lt;sup&gt;P&lt;/sup&gt;/H&lt;sub&gt;11021&lt;/sub&gt; (ppm)</th>
<th>24 h Mean</th>
<th>SD</th>
<th>96 h Mean</th>
<th>SD</th>
<th>7 d Mean</th>
<th>SD</th>
<th>15 d Mean</th>
<th>SD</th>
<th>28 d Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capseal I</td>
<td>54.8086&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.4177</td>
<td></td>
<td>39.2786&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.0551</td>
<td></td>
<td>25.2735&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.2681</td>
<td></td>
<td>25.4145&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7143</td>
</tr>
<tr>
<td>Capseal II</td>
<td>58.0006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.1944</td>
<td></td>
<td>55.4594&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.3163</td>
<td></td>
<td>25.3945&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0027</td>
<td></td>
<td>29.9857&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4417</td>
</tr>
<tr>
<td>ARS III</td>
<td>10.9337&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.9899</td>
<td></td>
<td>0.4582&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2335</td>
<td></td>
<td>0.3417&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1136</td>
<td></td>
<td>0.6306&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1638</td>
</tr>
<tr>
<td>Sealapex</td>
<td>18.3179&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.211</td>
<td></td>
<td>22.9023&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8062</td>
<td></td>
<td>17.1692&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0544</td>
<td></td>
<td>25.6334&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.6843</td>
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<tr>
<td>Control</td>
<td>0.4023&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.5625</td>
<td></td>
<td>0.1525&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1074</td>
<td></td>
<td>0.243&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.121</td>
<td></td>
<td>0.3687&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.305</td>
</tr>
</tbody>
</table>

Different letters indicate significant differences between materials.
CONCLUSIONS

Based on the results of this study, the newly developed Capsels I and II produced pHs and calcium ion releases higher than or equivalent to those of other tested sealers, which might be promising chemical characteristics of these newly developed root canal sealers.

REFERENCES


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