Influence of Moisture on the Apical Seal of Root Canal Fillings With Five Different Types of Sealer

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Abstract
The aim of this study was to evaluate the influence of moisture on apical leakage using five different types of sealer. There were 120 single-rooted teeth instrumented to .02/#60 and randomly assigned to 10 experimental groups, one control group (AH Plus, lateral condensation) (n = 10) or positive/negative controls (n = 5). Before obturation teeth were dried thoroughly, followed by recontamination with moisture in a wet chamber (moist groups; 37°C for 7 days). The teeth of the experimental groups (a, dry; b, moist) were obturated with sealer (groups 1, AH Plus; 2, Apexit; 3, Ketac-Endo; 4, RoekoSeal; 5, Tubli-Seal) and a single gutta-percha cone .02/#55. Teeth were centrifuged (30 × g for 3 minutes) in 5% methylene blue. Linear dye penetration was measured under a stereomicroscope. Moisture led to less microleakage for Apexit, RoekoSeal, and Tubli-Seal and higher values for AH Plus and Ketac-Endo. Multifactorial ANOVA displayed a significant dependence of leakage on sealer (p < 0.001) and the combination sealer or moisture (p < 0.01). It depends on the sealer type in which way moisture affects the apical seal. (J Endod 2007;33:31–33)

Key Words
Apical microleakage, dye penetration test, moisture, root canal sealers

Root canal fillings aim to seal the root canal system to prevent reinfection of the periapex. Moisture and liquids can negatively affect sealing ability (1, 2). In some cases, the practitioner is unable to dry the root canal space completely before obturation, which is potentially caused by movement of fluids into the apical foramen after drying, or from the inability to sufficiently dry the apical extent of the root canal with paper points (2). Over time, fluid penetration can be detected through the dentinal tubules, leading to a surface contamination of the root canal dentin. Moisture may inhibit, prolong, or accelerate the setting process of root canal sealers, which may result in higher leakage.

The purpose of the present study was to determine the quality of the apical seal within dry or moisture-contaminated root canals using five different classes of sealers: an epoxy-resin (AH Plus; DeTrey/Dentsply, Konstanz, Germany), a calcium salicylate (Apexit; Ivoclar Vivadent, Schaan, Liechtenstein), a glass-ionomer cement (GIC) (Ketac-Endo; 3M-Espe, Seefeld, Germany), a polyvinylsiloxane (RoekoSeal Automix; Coltène Whaledent, Langenau, Germany), and a zinc oxide-eugenol sealer (Tubli-Seal; Kerr, Salerno, Italy). The null hypothesis tested was that neither the type of sealer nor contamination with moisture affects apical microleakage of root canal fillings.

Materials and Methods
There were 120 extracted human mandibular incisors containing one root canal selected for the study and stored for a maximum of 30 days in 0.5% chloramine T solution. After coronal access preparation, root canals were instrumented to a uniform .02/#60 shape with FlexMaster instruments (VDW/Dentsply, Munich, Germany) 1 mm short of the apex. Apical patency was verified by passing a #10 K-reamer (VDW/Dentsply) through the apical foramen. Canals were irrigated with 1 ml 5% NaOCl after each file using a 2 ml syringe with an endodontic needle (V.M.K. Endoneedle Buquet, Vedefar N.V., Dilbeek, Belgium). After finishing root canal instrumentation, irrigation with 2 ml 40% citric acid, activated by ultrasonics for 30 seconds (EMS Piezon-Master 400, Nyon, Switzerland) followed by 2 ml 5% NaOCl was carried out for smear layer removal. To accelerate and enhance the drying process, the root canals were finally flushed with 2 ml 70% ethanol as recommended by Weine (3) and Cohen and Burns (4). All root canals were dried until at least five paper points #55 (Coltène/Whaledent) no longer displayed any moisture. Fifty teeth were randomly assigned to five groups (groups 1a-5a) and obturated immediately after drying using the single-cone technique (SCT). Another 50 teeth were divided into five groups (1b–5b) and rehydrated by a 7-day storage at 37°C and 100% humidity in a wet chamber (Memmert B 80, Memmert GmbH, Schwabach, Germany) (Table 1). These teeth were obturated using the SCT immediately after they were successively taken out of the wet chamber. Ten teeth forming a control group (6a) were dried and filled with AH Plus using the lateral condensation technique (LCT). Five teeth (positive controls) were not obturated, another five (negative controls) were filled and coated completely with nail varnish to verify the experimental method.

Root Canal Obturation
Sealers were mixed and utilized according to the manufacturers’ specifications and placed into the root canals with a lentulo spiral #40 (Dentsply/Maillefer, Ballaigues, Switzerland). Gutta-percha cones #55 (Coltène/Whaledent) were adjusted with a gutta gauge (Dentsply/Maillefer) to fit at working length with tug back. Group 6a was laterally...
condensed with a #55 gutta-percha mastercone and fine-fine accessory cones (Dentsply/Maillefer) (5, 6).

**Preparation of the Specimens**

The access cavities were filled with GIC (Ionofil Molar, Voco, Cuxhaven, Germany). The sealers were allowed to set for seven days in 100% humidity at 37°C. All teeth were completely coated with two layers of nail varnish. The apices of the teeth were removed with a water-cooled diamond bur until the masterpoints were visible. The negative controls were completely coated being sure to include the apical foraminar area. The teeth were put into test-tubes containing 5% methylene blue dye and centrifuged at 30 G for 3 min (Varifuge K, Heraeus-Christ, Osterode, Germany). After drying they were embedded in epoxy resin (Biresin, Sika B.V., Utrecht, Netherlands). Serial cross-sections in distances of 1 mm were manufactured using a water-cooled diamond saw (Roditi International Corp., Hamburg, Germany).

**Measurement of Leakage**

The cross-sections were investigated for dye penetration under a stereomicroscope at 40-fold magnification (Wild stereomicroscope, Leica Geosystems AG, Heerbrugg, Switzerland) by three independent, calibrated observers. For evaluation of linear dye penetration, at each cross-section a dye penetration line was traced (10, 11), it allows a detection of dye penetration within the root canal filling material and enables a good visualization of sealer distribution, penetration depth, and penetration area (12). Entrapped air inside the root canal system may inhibit the penetration of dyes (13, 14). Thus, dye penetration tests under negative or high pressure were recommended like in the present study working with centrifugation (13, 15).

The use of a higher number of paper points used and additional drying methods such as bursts of warm air or insertion of a heat probe resulted in a higher degree of dryness within the root canals and a better apical seal (16). Obturation of moist root canals resulted in tendentially more leakage (17). In the present study, moisture contamination was obtained by storage of the teeth in a wet chamber, which does not result in seepage of fluid into the root canal but to a moist surface of the root canal wall. Although the amount of moisture was certainly less than the volume used in the study of Horning and Kessler, who used a volume of 0.05 ml of saline solution per canal for moisture recontamination (2),

**Statistical Analysis**

Statistical analysis was computed with SPSS 11.0 (SPSS, Chicago, IL), using Kolmogorov-Smirnov tests, Kruskal-Wallis tests, Mann-Whitney tests, and multifactorial ANOVA. The level of significance was set at \( \alpha = 0.05 \).

**Results**

Mean leakage values are displayed in Table 2. The positive controls yielded dye penetration along the entire length of the root canal, whereas none of the negative controls showed any dye penetration. When moisture was present, Tubli-Seal, Apexit, and RSA revealed less leakage compared to the noncontamination group. In contrast, the sealability of AH Plus and Ketac-Endo was negatively affected by moisture. Ketac-Endo provided the poorest apical seal when used to obturate moisture-contaminated root canals. Multifactorial ANOVA did not exhibit any influence of moisture on apical microleakage (\( p = 0.857 \)) but displayed a significant influence for sealer type (\( p < 0.001 \)) and the combination sealer/moisture (\( p < 0.01 \)). Kruskal-Wallis test revealed significant differences between groups for linear dye penetration (\( p < 0.001 \)). Comparing the SCT and LCT group, no significant difference in leakage was present but the LCT group displayed tendentially more leakage (Mann-Whitney test; \( p > 0.05 \)).

**Discussion**

Both sealer type and the combination of sealer/moisture significantly altered microleakage of root canal fillings. Thus, the null hypothesis tested in this study was rejected.

Dye penetration tests as an easy and fast method are common to gauge microleakage of root canal fillings (7–9). Methylene blue is a very small molecule that shows a deeper penetration than other dyes (8), and therefore was used in this study. Cross-sectioning the specimens reveals significantly higher dye penetration values compared to the clearing technique (10, 11), it allows a detection of dye penetration within the root canal filling material and enables a good visualization of sealer distribution, penetration depth, and penetration area (12). En trapped air inside the root canal system may inhibit the penetration of dyes (13, 14). Thus, dye penetration tests under negative or high pressure were recommended like in the present study working with centrifugation (13, 15).

**Table 1. Experimental groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sealer</th>
<th>Number of teeth</th>
<th>Condition</th>
<th>Obturation technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>AH Plus</td>
<td>( n = 10 )</td>
<td>Dry</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>1b</td>
<td>AH Plus</td>
<td>( n = 10 )</td>
<td>Moist</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>2a</td>
<td>Apexit</td>
<td>( n = 10 )</td>
<td>Dry</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>2b</td>
<td>Apexit</td>
<td>( n = 10 )</td>
<td>Moist</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>3a</td>
<td>Ketac-Endo</td>
<td>( n = 10 )</td>
<td>Dry</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>3b</td>
<td>Ketac-Endo</td>
<td>( n = 10 )</td>
<td>Moist</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>4a</td>
<td>RoekoSeal</td>
<td>( n = 10 )</td>
<td>Dry</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>4b</td>
<td>RoekoSeal</td>
<td>( n = 10 )</td>
<td>Moist</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>5a</td>
<td>Tubli-Seal</td>
<td>( n = 10 )</td>
<td>Dry</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>5b</td>
<td>Tubli-Seal</td>
<td>( n = 10 )</td>
<td>Moist</td>
<td>Single-cone technique</td>
</tr>
<tr>
<td>6a</td>
<td>AH Plus</td>
<td>( n = 10 )</td>
<td>Dry</td>
<td>Lateral condensation</td>
</tr>
<tr>
<td>Negative control</td>
<td>AH Plus</td>
<td>( n = 5 )</td>
<td>Dry</td>
<td>—</td>
</tr>
<tr>
<td>Positive control</td>
<td>AH Plus</td>
<td>( n = 5 )</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Table 2. Dye penetration scores**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dye penetration [mm] (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>1.4 (0.7)</td>
</tr>
<tr>
<td>1b</td>
<td>2.3 (1.8)</td>
</tr>
<tr>
<td>2a</td>
<td>2.5 (0.7)</td>
</tr>
<tr>
<td>2b</td>
<td>5.7 (1.1)</td>
</tr>
<tr>
<td>3a</td>
<td>3.6 (1.1)</td>
</tr>
<tr>
<td>3b</td>
<td>6.1 (2.9)</td>
</tr>
<tr>
<td>4a</td>
<td>2.2 (2.4)</td>
</tr>
<tr>
<td>4b</td>
<td>1.3 (0.7)</td>
</tr>
<tr>
<td>5a</td>
<td>4.2 (2.1)</td>
</tr>
<tr>
<td>5b</td>
<td>2.8 (1.1)</td>
</tr>
<tr>
<td>6a</td>
<td>3.0 (2.3)</td>
</tr>
</tbody>
</table>
the present study also exhibited significant differences regarding dye penetration. The drying procedure seems to represent a critical step in endodontic treatment, because of the fact, that already small amounts of moisture can alter the apical seal.

Higher standard deviations were evident when AH Plus and Ketac-Endo group (1h/5h) were used in moist canals. Moisture may be the relevant factor for these higher standard deviation values. In contrast, the absence of moisture in the RoekoSeal and Tubli-Seal groups (4a/5a) resulted in higher standard deviation values. Moisture may work as a lubricant for these sealers that allows a better attachment to the root canal wall. Thus, a complete drying of the root canal dentin may have adversely affected linear dye penetration.

Calcium salicylate sealers show an acceleration of the setting reaction and a decrease of sealing abilities under moist conditions (2). A premature setting impedes the lubricating effect for the placement of gutta-percha cones, thus preventing a complete coverage of either gutta-percha cone or canal wall by the sealer. These findings disagree with those of the present study but can be explained by the higher amount of contaminant used (2).

In contrast to our study, Pitt Ford did not find any leakage in root canals obturated with GIC sealer (18), may be because of different test conditions or different products. In another study, the GIC sealer Ketac-Endo revealed significantly more apical leakage than Apekit and Diakiet (19). GIC exhibit a poorer sealing quality than other materials under moist conditions (18). GIC showed a significant decrease in their overall mechanical properties if contaminated by moisture after mixing and before final set (2), because of their high water solubility in the early stage of the setting reaction (20). A decrease of sealing ability was reported for GIC sealers but not for zinc oxide-eugenol type sealers (16). Kuhre and Kessler determined no significant difference between the apical seal produced by zinc oxide-eugenol sealers and LCT in moist and dry root canals (17).

Like in the present study, silicone-based and epoxy resin-based sealers were shown to effectively seal the root canal (21–25). This may be related to the slight expansion during their setting reactions (24). Silicones seem to profit, epoxy resins to suffer from traces of moisture, because of the hydrophobic structure of the latter (25). In general, regarding our results, the effect of moisture is dependent on the type of sealer.

In previous studies including Ketac-Endo, the other sealers were used in LCT technique, but only Ketac-Endo was used with SCT because of its very quick setting reaction leaving no time for lateral condensation (2, 18). In the present study in all experimental groups the SCT was used to exclude the obturation technique as a possible factor of influence. Facer and Walton had shown that the use of the LCT led to a sealer distribution very differently than the traditional belief (26). The inserted spreader was shown to squeeze out the sealer, resulting in gutta-percha being in direct contact with the canal wall. Missing areas of sealer were also reported in other studies (12, 26–29). The percentage of sealer coated canal perimeter was shown to be significantly higher when SCT was used (12). SCT using a nonshrinking sealer produced an apical seal similar to that of the LCT (30–35) or warm vertical compaction (25). Regarding the data of the present study that reveals less leakage when the SCT was used, the necessity to use the LCT for a reference group should be reconsidered. These findings are in correlation to Lussi and colleagues (1999) who concluded, that the sealer but not the obturation technique is the relevant factor for apical microleakage (11).

References


