Original Article:
A comparative study of apical microleakage of different root canal sealers by apical dye penetration
Ayer A1, Manandhar T. R.2, Agrawal N3, Vikram M4, Suwal P5

Abstract:
Objective: This study was designed to compare the level of apical microleakage of root canal sealers; Acroseal, AH Plus, Endoflas FS and Endomethasone N, with laterally condensed gutta percha by level of apical dye penetration. Materials and Methods: Freshly extracted sixty permanent maxillary anterior teeth were divided randomly into four groups. Shaping and cleaning of teeth was done followed by obturation with gutta percha and four different root canal sealers. Samples were immersed in 2% methylene blue dye solution in individual dappen dish and stored in the solution for 30 days. The roots were split longitudinally with a chisel in two halves and observed under stereomicroscope. Apical microleakage measured from the apex to the most coronal extent of dye penetration. Results: Minimum microleakage was observed with AH Plus with mean value 2.140 mm, standard deviation 0.817. The maximum microleakage was observed with Endomethasone N with mean value 3.858 mm, standard deviation 1.840. There was no statistically significant difference in microleakage between Acroseal, AH Plus, and Endoflas FS. Endomethasone N showed highest level of microleakage than other three groups under the test condition, which was statistically significant. Conclusion: The microleakage was the lowest for the AH Plus and increased in the following order, Endoflas FS, Acroseal, Endomethasone N.
Keywords: (MeSH Heading): Calcium Hydroxide, Epoxy Resins, Gutta-Percha, Root Canal Filling Materials, Zinc Oxide-Eugenol Cement.

Introduction
The success of endodontic therapy depends on shaping and cleaning of root canal system followed by total obturation with perfect coronal and apical seal including accessory canals.1 Treatment failures in endodontics could be attributed to inadequate shaping, cleaning and obturation.2 Even after thorough chemomechanical preparation of the root canal system, presence of microorganisms in the dentinal tubules, lateral canals, and apical ramifications has been seen.3,4 These residual organisms, together with those entering from oral cavity if the root canal system is not sealed adequately, rapidly repopulate the empty canals and can induce or sustain apical periodontitis.5 Obturation of the root canal system may be conducted in a number of ways but the most commonly advocated method is by the application of gutta-percha as the core obturation material combined with a root canal sealer. According to Weine, failure in endodontic therapy occurs when

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the apical foramen is not completely obturated and sealed.\textsuperscript{3} Sealer is essential with all core obturation materials although behavior of sealer with different obturation material and technique may differ.\textsuperscript{6} The sealer can fill the irregularities of the root canal wall and the dentinal tubules which cannot be filled by gutta-percha.\textsuperscript{7} Regardless of the technique used, studies have shown that gutta-percha without sealer will not seal the root canal.\textsuperscript{8}

Sealers are resorbable when exposed to tissues and tissue fluids,\textsuperscript{9} but breakdown products from the sealers may have an adverse effect on the proliferative capability of periradicular cell populations.\textsuperscript{10} Although an osteogenic response has been observed,\textsuperscript{11} the ability of these sealers to sustain a high pH over time has been questioned.\textsuperscript{12} All sealers exhibit some degree of toxicity until they have set, thus extrusion of sealers into periradicular tissue should be avoided.\textsuperscript{13} Sealer penetration into the tubules increases the contact surface between filling material and dentin thus enhancing the sealability.\textsuperscript{14}

The apical leakage of the endodontic sealers has been measured by several ways; by degree of penetration of a dye, microbial leakage test, scanning electron microscopy, electrochemical means, radioisotope penetration, fluid filtration method. Among them linear measurement of tracer dye penetration technique is most frequently used.\textsuperscript{15}

In this study we compare, in vitro, sealing ability of four different root canal sealers; Acroseal (Septodont, Saint-Maur, France), AH Plus (Dentsply De Trey GmbH, Konstanz, Germany), Endoflas-FS (Sanlor and CIA S. en C.S. Cali Colombia) and Endomethasone N (Septodont, Saint Maur, France) by the measurement of linear dye penetration using stereomicroscope (Olympus, USA). Acroseal is a calcium hydroxide based sealer with epoxy resin. It has good radiopacity,\textsuperscript{16} excellent film thickness,\textsuperscript{17} however, it presents a lower calcium ion release and pH compared with Sealapex.\textsuperscript{18} AH Plus is an epoxy-bis-phenol resin that comes in two tubes of epoxide paste and amine paste. Endoflas FS is iodoform incorporated zinc oxide eugenol sealer that also contains calcium hydroxide. Endomethasone N is a modification of Endomethasone sealer that does not contain paraformaldehyde in its composition. It is supplied as powder and liquid.

**Materials and Methods:**

Permanent maxillary anterior teeth extracted due to caries or periodontal problem were collected. The teeth were evaluated and were discarded if any of the following was noted: Incompletely formed apex, carious involvement apical to cementoenamel junction, fracture of root, root curvature greater than 5\textdegree, pathologically affected root, bifurcating canals, fins, and ribbon- shaped canals calcification of root canals. Pre-operative radiographs (Intra Oral Periapical) were taken to look into the patency and negotiability of the root canals. Teeth with bifurcating canals, fins, and ribbon- shaped canals or extreme calcifications were discarded from the study. **Sampling:** The study sample consisted of sixty permanent maxillary anterior teeth. The teeth were randomly divided into four groups of fifteen teeth each. To eliminate any variability in access preparation, the crowns of the teeth were removed at the cementoenamel junction by a diamond disc (Axis dental, USA) fitted with a mandrel to an Airmotor handpiece (NSK, Japan) in slow speed with constant water supply. Canal shaping has been done according to ‘Step-back’ instrumentation technique. In this instrumentation process, the apical preparation of the canal was enlarged to a minimum of no. 30-40 k file (Mani, Inc, Japan) and maximum no. 50 k file depending upon the original size of the canal. Any tooth requiring a size larger than # 50 k file for adequate cleaning was discarded and replaced with another tooth. During instrumentation, the canals were irrigated copiously with 2.5% sodium hypochlorite solution and root canal conditioner (Glyde file prep, Dentsply, Maillesfer, USA). Drying of canal was done with absorbent paper points (Dentsply, Maillefer) and standardized master gutta percha cones were selected as master cone. The teeth were obturated with gutta percha points (Dentsply, Maillesfer) using root canal sealers as follows. **Group 1:** Root canal obturation with Acroseal sealer and gutta percha. **Group 2:** Root canal obturation with AH Plus sealer and gutta percha. **Group 3:** Root canal obturation with Endoflas-FS sealer and gutta percha. **Group 4:** Root canal obturation with Endomethasone N sealer and gutta percha.

The sealers were mixed according to the manufacturer’s direction and introduced into the canal by lentulospiral root filler (Mani, Inc. Japan). Gutta percha points were introduced using lateral condensation technique. After obturation final intra oral periapical radiograph was taken to evaluate the root canal fillings. Obturation was considered adequate when no voids discernible and all visible canal spaces were filled completely. The access cavity was sealed with intermediate restorative material (IRM, Dentsply caulk, Milford, USA) and
the teeth were stored at room temperature for two days, followed by storage in normal saline (0.9%, W/V) for 1 week at room temperature. Roots were coated with two layers of nail varnish, except for the area surrounding the apical foramen and allowed to dry for 24 hours. Samples were immersed in 2% methylene blue dye (HIMEDIA, Mumbai, India) solution in individual dappen dish and stored in the solution for 30 days. The roots were grooved in a buccolingual direction without penetrating root canal to split longitudinally with a chisel in two halves. Each section was viewed under stereomicroscope (Olympus, USA) at ×20 magnification; linear apical leakage measured from the apex to the most coronal extent of dye penetration. Ethical approval: Prior the study, the ethical approval has taken locally.

Results
Collected data were entered in Microsoft Excel 2007 and coded accordingly. Groups were formed based on microleakage. The statistical analysis was performed by statistical package for social science (SPSS) version 11.5; IBM, Incorporation, USA. For descriptive statistics; mean, standard deviation and percentage were calculated For Inferential statistics; one way ANOVA with Post Hoc test was applied to find out differences among and between the groups at 95% confidence interval where p = 0.05.

Table: 1: Distribution of extent of microleakage by number and percentage of sealer.

<table>
<thead>
<tr>
<th>Sealers</th>
<th>Number of samples and Percentage</th>
<th>% of Sealer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acroseal</td>
<td>&lt;1 mm* 1 – 2 mm 2 – 3 mm 3-4 mm  ≥ 4 mm</td>
<td>[33.33%] [33.33%] [20%] [13.33%]</td>
</tr>
<tr>
<td>AH Plus</td>
<td>1 [6.66%] 6 [40%] 7 [46.66%] 1 [6.66%] 0</td>
<td>[6.66%]</td>
</tr>
<tr>
<td>Endoflas FS</td>
<td>1 [6.66%] 3 [20%] 7 [46.66%] 3 [20%] 1 [6.66%]</td>
<td>[6.66%]</td>
</tr>
<tr>
<td>Endomethasone N</td>
<td>0 2 [13.33%] 4 [26.66%] 4 [26.66%] 5 [33.33%]</td>
<td>[33.33%]</td>
</tr>
</tbody>
</table>

*Microleakage in millimeter.

Table: 2: Mean comparison among sealer and their level of significance.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Categories</th>
<th>Mean</th>
<th>Standard Deviation (SD)</th>
<th>P- value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microleakage of each group</td>
<td>Acroseal 2.792 mm 1.153</td>
<td>0.004*</td>
<td>Significant</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AH Plus 2.140 mm 0.817</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endoflas FS 2.596 mm 0.938</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endomethasone N 3.858 mm 1.840</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.847 mm 1.375</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

While comparing the root canal sealers it was observed that all the sealers show microleakage under the test condition. The result obtained was statistically significant.

Table: 3: Correlation matrix between sealers.

<table>
<thead>
<tr>
<th>Categories</th>
<th>P Values</th>
<th>Acroseal</th>
<th>AH Plus</th>
<th>Endoflas FS</th>
<th>Endomethasone N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acroseal</td>
<td>-</td>
<td>0.159</td>
<td>0.670</td>
<td>0.023*</td>
<td></td>
</tr>
<tr>
<td>AH Plus</td>
<td>0.159</td>
<td>-</td>
<td>0.323</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Endoflas FS</td>
<td>0.670</td>
<td>0.323</td>
<td>-</td>
<td>0.008*</td>
<td></td>
</tr>
<tr>
<td>Endomethasone N</td>
<td>0.023*</td>
<td>&lt;0.001*</td>
<td>0.008*</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*P value significant.
Correlation between Acroseal, AH Plus and Endoflas FS doesn’t show significant difference. Endomethasone shows statistically significant microleakage when compared with other sealers.

Discussion:
All root filling materials allow marginal infiltration. They are not impenetrable. Although the potential for an extremely high success rate for endodontic treatment is widely accepted, epidemiological studies demonstrated that success rate varies between 40-50%.\(^1\) Thus improvements in the technique and materials must be everlasting. Any material should be tested by different methods and have its performance compared to that of other materials before its clinical use. When obturating root canals with a solid core material, some form of cement is required that fills the minor gaps between the core material and the dentinal wall of the canal to prevent leakage.\(^2\) Shrinking of gutta-percha and lack of adhesion of the root filling materials to dentinal root canal walls are factors creating enough predispositions for microleakage. Considering the main purpose of using sealers is to fill gaps within the irregular root canal system, their solubility and disintegration should be as low as possible.\(^3,4\) Disintegration of the sealer or undetected voids in the filling mass may create leakage channels that allow periapical tissue fluids to reach residual bacteria within tubules and provide nutrient for their growth.\(^5\) There are chances of seepage of fluids into the apical foramen following drying, or due to the inability of the paper points to reach the full apical extent of extremely small or tortuous canals.\(^6\) Therefore, the effect of residual moisture on the apical seal produced by the obturation of the root canal space with gutta-percha and the various classes of endodontic sealers should be determined.

In this study, after obturation the samples were stored in physiologic saline for one week to evaluate the solubility after setting. Low solubility of root canal sealers has been introduced as a requirement in the International standard ISO 6876 for root canal sealing materials. Since the root canal filling materials may be in direct contact with periapical tissue for prolonged period of time, the components leaching from the root canal may have undesirable biological effects on the surrounding tissue.\(^7\) In this study samples were kept in contact with dye for 30 days. This was done to demonstrate the microleakage measurement after long period, because the relative differences between the materials change over time, and this change has a clinical relevance.\(^8\) The results demonstrated the least leakage of dye with AH Plus sealer followed by Endoflas FS, Acroseal and Endomethasone N. There was statistically significant difference between the leakages of Endomethasone N with other three groups. Sung- Eung Yang et al.\(^9\) performed an in vitro evaluation of the sealing ability of different root canal sealers using an anaerobic bacteria leakage model with Prevotella nigrescens. They observed that AH plus showed the most prominent sealing ability followed by calcium hydroxide based sealer, whereas the zinc oxide based sealer showed a greater tendency for leakage from early during observation period. The results are in accordance with our finding. Eric Balguerie et al.\(^7\) also observed AH Plus with best tubular adaptation and penetration followed by Acroseal, when compared with glass ionomer and zinc oxide eugenol sealer. The sealer penetration depth in the dentinal tubules depend on factors like smear layer removal, dentinal permeability, root canal dimension, and the physical and chemical properties of the sealer.\(^7,25\) An acceptable flow within the working time is important for any root canal sealer in order to reach and seal the apical foramen and lateral dentinal wall irregularities.\(^20\) Studies have shown that the flow of AH Plus is comparable or significantly higher than other sealers tested.\(^7,20\) In contrast some studies reported AH Plus exhibited greater, but not statistically significant leakage. This was explained by the faster setting time of AH Plus, which caused shrinkage stress and earlier debonding from dentinal walls. Also, some ingredients of AH Plus, such as silicone oils, can affect the sealing ability of this material.\(^8\) AH Plus being oil based material could prevent complete wetting of the root canal wall and adhere poorly to humid dentine,\(^6\) so canal must be dry before placement of sealer. The most advantageous and more predictable results are obtained if the root canal system is as dry as possible before obturation.\(^27\) Any material which slowly releases therapeutic substance will lose some of its original mass. In addition its physical properties such as; dimensional stability, porosity, compressive and shearing strength or wear resistance are expected to be compromised.\(^4\) Djurica Grga et al.\(^1\) observed that ability to absorb fluid of Acroseal and AH Plus is greater than Apexit. Water sorption after polymerization of Acroseal and AH Plus was reported which is in contrary with other studies.\(^26\) While the difference in mass after immersion in solution for long period (greater than 96 hours), Acroseal loose more mass compared with AH Plus, may be due to calcium ion release as a result of solubility.\(^1\) Hence results are in agreement with our study that show
more dye penetration in the samples with Acroseal as compared to AH Plus and Endoflas FS, but they were not statistically significant. Voids might occur in Acroseal as a result of formaldehyde release during setting and through ionization of calcium hydroxide. The composition of the sealers seems to be the major factor related to their flow characteristic but their final consistency also have significant influence. Norberto Batista de Faria- Junior et al. assessed the flow rate of root canal sealers, reported that the flow rate of Acroseal, AH Plus conformed to ISO specification 6876/2001 for endodontic filling material, whereas the flow value of Endomethasone N was lower than those considered acceptable for the ISO specification. Anca Torcatoru et al. examined the group filled with Endomethasone and found a greater leakage in the apical third up to 90% and in the coronal third up to 80%. When solubility in water and artificial saliva was tested, AH Plus showed the least weight loss than other sealers (AH 26, Apexit, Sealapex, Zinc oxide eugenol sealer, Ketac- Endo, Diaket) independent of the solubility medium used. Zinc oxide eugenol sealer had a marked weight loss in all liquids. These results might explain the highest linear tracer dye penetration in Endomethasone N group in this study. The thickness of the endodontic sealer film ranges from 4 to 180 µm. Since all sealer show microleakage to certain extent, this implies the necessity to limit its presence to a thin film and increasing the mass of gutta- percha, because the sealer might shrink during setting and dissolve over time producing leakage.

**Conclusion:**
All the endodontic sealers tested showed measurable microleakage under the study condition. Samples were kept in solution for 30 days to demonstrate the change in material property over time. AH Plus sealer performed best apical sealing ability with minimum microleakage. Microleakage increased in the following order AH Plus, Endoflas FS, Acroseal, Endomethasone N. Although there was no statistically significant difference between the microleakage among AH Plus, Endoflas FS and Acroseal, compared to them Endomethasone N showed significantly higher microleakage score.
A comparative study of apical microleakage of different root canal sealers by apical dye penetration

References


