A scanning electron microscopic study of the presence of pores and vacuoles in set endodontic sealers – in vitro

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Abstract

Introduction: The present study was carried out to evaluate the presence of defects on the external surface termed ‘Pores’ and defects on the fracture surface termed ‘Vacuoles’ in the structure of various endodontic sealers when set.

Materials and Method: Four Endodontic Sealers namely EndoFlux, Endomethasone, AH Plus and Apexit Plus as Experimental Group and Zinc Oxide Eugenol cement as control group were included in this study. The study involved the assessment of surface defects by Scanning Electron Microscope.

Results: The frequency of Pores was maximum in Endomethasone sealer, and minimum in AH Plus sealer followed by Zinc oxide eugenol cement (control), EndoFlux sealer, then by Apexit Plus sealer. The frequency of vacuoles in different sealers was also found maximum in Endomethasone sealer while it was minimum in AH Plus sealer followed by Apexit Plus and Zinc oxide eugenol cement (control) then by EndoFlux sealer. The diameter of pores was found largest in Endomethasone sealer whereas the smallest was found in Apexit Plus sealer.

Conclusion: Based on this study, resin based sealer-AH Plus showed best structural features while zinc oxide eugenol based-Endomethasone sealer had the poorest structural features in respect to the presence of pores and vacuoles.

Keywords: Obturation, Hermetic seal, Vacuoles.

Introduction

It is of vital importance to know the properties of sealing materials for the success of root canal treatment because a hermetic seal of root canal is essential in endodontics. Sealers play an important role in obturation of root canal. As per available literature, numerous materials have been used for the purpose of filling and sealing the root canal. A perfect sealer would form a chemical bond between the core materials and the dentin walls, but currently there are no sealers available that can achieve this ideal.

Sealing of root canal completely may increase the clinical success to a rate as high as 96.5%. Several studies have addressed different aspects of the properties and characteristics of sealers such as their granularity, the dimensional changes they undergo after setting, their solubility, and their sealing capacity. However, few reports have addressed the structural features of sealers. Apical permeability has been extensively studied, but remarkably little attention has been given to the cause of such permeability.

The present study was therefore conducted to evaluate and estimate the frequency of pores and vacuoles and to measure the dimension of pores and vacuoles in set endodontic sealer under scanning electron microscope.

Materials and Method

Four Endodontic Sealers (EndoFlux, Endomethasone, AH Plus and Apexit Plus) as Experimental Group and Zinc Oxide Eugenol cement as control group were included in this study. Forty (40) samples were prepared and were divided into two main groups and again subdivided into subgroups ‘A’ and ‘B’. Subgroup ‘A’ were employed to study the external surface (Pore). Subgroup ‘B’ were used for analysis of fracture surface (Vacuoles).

1. Control group: (Zinc oxide eugenol cement):- 8 samples
   - Sub group ‘A’ (4 samples)
   - Subgroup ‘B’ (4 samples)
2. Experimental group: (32 samples)
   - Group I (EndoFlux)
     - Subgroup 1A (4 samples)
     - Subgroup 1B (4 samples)
   - Group II (Endomethasone)
     - Subgroup 2A (4 samples)
     - Subgroup 2B (4 samples)
   - Group III (AH Plus)
     - Subgroup 3A (4 samples)
     - Subgroup 3B (4 samples)
   - Group IV (Apexit Plus)
     - Subgroup 4A (4 samples)
     - Subgroup 4B (4 samples)

Each group made up of eight plastic rings were placed on glass slab separately by group. The name of the sealer was written on the side of the glass slabs for identification purpose with the help of permanent marker. The rings were filled with the corresponding sealers. Control group was made up of Zinc oxide eugenol cement, Group I was made up of...
Endoflux, Group II was made up of Endomethasone; Group III was made up of AH plus: Group IV was made up of Apexit Plus. A single operator prepared the samples in keeping with the instruction of the manufacturers. The samples of prepared sealers were stored in a moist chamber (Incubator) at 37°C and at least 95% relative humidity until completely set. Sealer hardening was confirmed by visual inspection. A 27 gauge needle was inserted to evaluate surface set at varying times. The sealer was considered to have set when the needle did not adhere or leave an imprint.

The evaluation of setting was carried out at the periphery of the core material that contacted the ring to avoid potential alteration in the external or internal structures of the samples induced by the contact, pressure or force of indenters. Each group (control group and experimental group consisting of four groups of sealers) made up of eight samples and identified by the name of the sealer was subdivided into subgroups 'A' and 'B' having four sample each. Sub group 'A' was used to study the structure of the external surface of the specimens and subgroup 'B' was employed to study the structure of the fractured surfaces. As the specimens were fragile, the fracture was performed manually.

The study of both surfaces was performed by Scanning Electron Microscope. A team of three observers analysed the external and fracture surface of the samples. Only one of the three observers was associated with the study, two other observers were unfamiliar to the study but they were instructed in detail the technique of evaluation of the sample. They performed an initial overall assessment at a magnification of X160 to establish a gross estimate of the frequency of pores and vacuoles.

The quantitative evaluation was performed by using four point scale:
1. When structural defect was minimum or absent it was considered 'Exceptional'
2. When very careful observation was required to detect the defects it was considered 'Scarce'
3. When the defects could be detected easily it was considered 'Frequent'.

4. When the defects were found in all the observed fields it was considered 'Abundant'.

At a magnification of X 320 each surface was analyzed in more detail. Photographs were taken and employed to measure the diameter of pores and vacuoles. The most representative areas in the central, more reliable area of each block were selected for detailed analysis of pores and vacuoles. The diameter of pores and vacuoles were measured by millimeter scale which was converted into micrometer.

**Statistical Analysis:** The data thus obtained were subjected to statistical analysis using mean, standard deviation, student-t test and analysis of variance keeping p value significant at 99% confidence limit. P defines the level of significance, with P > 0.05 being not significant and P < 0.001 being highly significant.

**Statistical formulae Used:** Following formulae were used in statistical analysis-

\[
\overline{X} = \frac{\sum X}{N}
\]

(Mean)

\[
SD = \sqrt{\frac{\sum (x - \overline{x})^2}{N-1}}
\]

(Standard Deviation)

\[
t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}
\]

(Student t Test)

**Result**

The results thus obtained are presented in the following tables. The values of the findings of the study should be read in terms of micrometer.

In Table 1 the mean pore score in different materials was maximum for Group II (Endomethasone) and minimum for Group III (AH Plus).

**Table 1: Mean pore scores in different sealants**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>Mean (µm)</th>
<th>SD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control Group</td>
<td>1.67</td>
<td>0.49</td>
</tr>
<tr>
<td>2.</td>
<td>Group I (Endoflux)</td>
<td>2.58</td>
<td>0.51</td>
</tr>
<tr>
<td>3.</td>
<td>Group II (Endomethasone)</td>
<td>4.00</td>
<td>0.00</td>
</tr>
<tr>
<td>4.</td>
<td>Group III (AH Plus)</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>5.</td>
<td>Group IV (Apexit Plus)</td>
<td>3.00</td>
<td>0.43</td>
</tr>
</tbody>
</table>

The mean square variance between groups was found to be 16.317 while the mean square variance within groups was calculated as 0.138. The ratio of these two was calculated as 118.341, depicted as “F” statistic in the Table 2. Analysis of variance of pore scores in different materials showed a statistically significant difference (P < 0.001) thus implying that the pore scores were different for different materials.

**Table 2: Analysis of variance for pore scores in study materials**

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>&quot;p&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>65.267</td>
<td>4</td>
<td>16.317</td>
<td>118.341</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within Groups</td>
<td>7.583</td>
<td>55</td>
<td>0.138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>72.850</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of different groups revealed a statistically difference from the control group. The control group had a significantly higher mean score when compared to Group III while all other groups had significantly higher pore score as compared to control group as shown in Table 3.

**Table 3: Comparison of Pore Scores as Compared to Control Group**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Control Group vs.</th>
<th>&quot;t&quot;</th>
<th>&quot;p&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group I (Endoflux)</td>
<td>-4.457</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2.</td>
<td>Group II (Endomethasone)</td>
<td>-16.417</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3.</td>
<td>Group III (AH Plus)</td>
<td>4.690</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4.</td>
<td>Group IV (Apexit Plus)</td>
<td>-7.091</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

On the basis of above evaluation the mean pore scores in different groups can be graded as: **Group II > Group IV > Group I > Control > Group III.**

As shown in Table 4 the mean vacuole score was maximum for Group II and minimum for Group III.

**Table 4: Mean Vacuole Scores in different materials**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>Mean (µm)</th>
<th>SD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control Group</td>
<td>2.75</td>
<td>0.45</td>
</tr>
<tr>
<td>2.</td>
<td>Group I (Endoflux)</td>
<td>3.00</td>
<td>0.60</td>
</tr>
<tr>
<td>3.</td>
<td>Group II (Endomethasone)</td>
<td>3.33</td>
<td>0.49</td>
</tr>
<tr>
<td>4.</td>
<td>Group III (AH Plus)</td>
<td>1.83</td>
<td>0.39</td>
</tr>
<tr>
<td>5.</td>
<td>Group IV (Apexit Plus)</td>
<td>2.75</td>
<td>0.45</td>
</tr>
</tbody>
</table>

The mean square variance between groups was 3.725 and that within groups was 0.233. The ratio of these two was calculated as 15.964, depicted as “F” statistic in Table 5. Analysis of variances revealed a statistically significant difference among the various groups.
Table 5: Analysis of variance for vacuole scores in study materials

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>&quot;p&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>14,900</td>
<td>4</td>
<td>3.725</td>
<td>15.964</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within Groups</td>
<td>12,833</td>
<td>55</td>
<td>0.233</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27,733</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of control group with Group I and Group IV revealed no significant difference in the mean vacuole values as shown in the Table 6. However, the mean vacuole value of the control group was significantly lower as compared to Group II and significantly higher as compared to Group III.

Table 6: Comparison of Vacuole Scores as Compared to Control Group

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Control Group vs.</th>
<th>&quot;t&quot;</th>
<th>&quot;p&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group I (Endoflux)</td>
<td>−1.149</td>
<td>0.263</td>
</tr>
<tr>
<td>2.</td>
<td>Group II (Endomethasone)</td>
<td>−3.023</td>
<td>0.006</td>
</tr>
<tr>
<td>3.</td>
<td>Group III (AH Plus)</td>
<td>5.322</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4.</td>
<td>Group IV (Apexit Plus)</td>
<td>0.000</td>
<td>1</td>
</tr>
</tbody>
</table>

On the basis of above evaluation the mean vacuole scores in different groups can be graded as: **Group II = Group I > Group IV = Control > Group III**

**Discussion**

Pores and Vacuoles in set endodontic sealers seem to be consistent structural features. As Hovland EJ & Dumsha TC(19) mentioned, all root canal sealers tend to leak to some extent either at the interface of dentin and sealer or at the interface of the solid core and sealer or through the sealer itself in the form of voids, the present study detected vacuoles and pores in varying degrees in all the sealers examined. The studies conducted by Mutal L & Gani O(20) also detected pores and vacuoles of varying degrees in all the sealers they examined.

The presence of structural deficiencies depends on the physical properties of the sealers. If the mixture is dense and barely flows, only a few bubbles will open up at the interface, yielding depression with elevated border that resembles craters. Conversely, if the mixture is fluid, the bubbles will burst open on the surface more easily. All the sealers under the present study exhibited pores and vacuoles. This present finding was almost similar to the previous study done by Mutal L & Gani O,(20) where they also detected more incidence of pores in set endodontic sealers in the present study than that of the pores found. This is true to the previous study done by Mutal L & Gani O(20) where they also detected more incidence of vacuoles in each sealer examined than the pores.

Within this context, the questions arise as to the fate of the vacuoles within the sealers when the filling components are compressed within the canal. The pressure of spreaders and pluggers will probably expel the air. The air might also be trapped within the thickness of the sealer layer because most of the time, the distribution of sealer were not uniform; however thin, not as bubbles but as voids may be present between the cones and the dentin walls.

It is thus reasonable to assume that these structural defects – pores and vacuoles, detected in set endodontic sealers in the present study or previous study Mutal L & Gani O,(19) Their solubility as observed by Peters et al.(21) Wu et al(10) and Kaplan et al.(11) might affect the integrity, stability, durability and impermeability of the sealer which is an important component of root canal fillings. Considering that there are several kinds of sealer materials, there is need of more studies to comparatively evaluate them.

**Conclusion**

Based on this study, resin based sealer-AH Plus showed best structural features while zinc oxide eugenol based-Endomethasone sealer had the poorest structural features in respect to the presence of pores and vacuoles. It may be assumed that these structural defects – pores and vacuoles detected in the set endodontic sealers might affect the solubility, integrity, stability, durability and impermeability of the sealer which are important for the successful root canal filling. However, further research and clinical study is necessary to find out whether these structural defects found in set endodontic sealers clinically affect the performance of root canal obturation.

**References**