The effect of different acidic environments on the apical microleakage of different obturation techniques
(An in vitro study)

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ABSTRACT

Background: Pulpal and apical inflammation or infection decreases tissue pH in the region surrounding the involved tooth which might affect the sealing ability of different obturation systems. This study evaluate the apical microleakage of three obturation techniques (lateral condensation of Gutta-percha/AH 26, Soft-Core gutta-percha/AH 26 and lateral condensation of Resilon/Real Seal SE), when exposed to 7.3 6.5, 6.0 and 5.5 pH values.

Materials and method: One hundred and thirty two roots of freshly extracted teeth were selected. Teeth were decoronated, working length was established and the roots were instrumented using a crown down technique with ProTaper rotary files (SX-F3). The specimens were divided into three groups of 44 samples each. Group A: obturated using lateral condensation of gutta percha and AH 26. Group B: obturated using soft-core and AH 26. Group C: obturated using lateral condensation of Resilon and Real Seal SE. Each group was further subdivided into four subgroups, 10 samples each, which were exposed to pH values of 7.2, 6.5, 6.0 and 5.5 respectively. Microleakage was evaluated by longitudinal sectioning, and measurement of liner dye penetration.

Results: There was a non significant difference within each group regarding the different pH media. Both Soft-Core and Resilon showed less apical microleakage than lateral condensation of gutta percha with a highly significant difference in all the tested acidic media.

Conclusion: Resilon/Real Seal SE subgroups showed the least apical microleakage, however, it didn’t provide the complete sealing claimed by the manufacturer.

Key words: Soft-Core. Resilon, Real Seal SE, pH of the periapical area, apical dye penetration. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):18-24).

INTRODUCTION

Complete obturation of the root canal with an inert filling material and creation of a fluid-tight seal are among the major goals of successful endodontic treatment. Among numerous obturation techniques and filling materials available, gutta-percha continues to be the material of choice, owing to its unique chemical and physical properties (1). Lateral condensation technique has proven to be a very popular technique utilizing gutta-percha filling material; however its ability to conform to the internal surfaces of the root canal has been questioned (2).

Thermoplastized obturation techniques were introduced to improve the homogeneity and surface adaptation of gutta-percha. One of these techniques involves the use of a metal or plastic carrier coated with a layer of gutta-percha that is heated, to permit thermoplastized canal obturation. The Soft-Core System (CMS-Dental, Copenhagen, Denmark) uses a similar strategy to achieve root canal obturation. The Soft-Core obturator consists of a plastic core which is coated with thermoplastic alpha phase gutta-percha. It offers many advantages such as the reduction in chair-side time and rapid set of the gutta percha (3).

Many root canal sealers are currently being used in combination with gutta-percha to fill the root canal system. Epoxy resin sealers such as AH 26 and AH plus have been used because of their reduced solubility, apical seal and micro-retention to root dentin (4). Self-etch primers have been used for bonding to the root canal dentin, and as the epoxy resin sealers do not copolymerize with methacrylate resin-based adhesives, a dual-curable methacrylate resin sealer (Epiphany, Pentron, Wallingford, CT) or (Real Seal, SybronEndo, Orange, CA), was developed with a self-etch primer, and a new thermoplastic filled polymer (Resilon, Resilon Research LLC, USA), as an alternative to gutta-percha. Resilon contains dimethacrylates, which can bond to methacrylate-based resin sealers, such as Epiphany (5).

A new self-etch sealer (epiphany SE, Pentron, Wallingford, CT) or (RealSeal SE, SybronEndo, Orange, CA) has evolved which eliminates the priming step and has been claimed to have similar sealing abilities as the original system. It has been claimed that this system creates a mono-block effect with the canal wall. Such a mono-block eliminates the gaps associated with the core material and sealer, resists shrinkage and strengthens the root (6).

Epidemiological studies of root-filled teeth in various countries and different populations have

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demonstrated the presence of apical periodontitis in a relatively high proportion of these teeth, which most of them are symptom-free \(^7\). The pH of the aspirated periapical periodontitis was found to be acidic (6 to 7.3) in a study done by Nekoofar et al in 2009\(^8\). Acidic pH may have an effect on the properties of the dental materials, which are routinely placed in environments that may be inflamed or infected \(^9\).

MATERIALS AND METHOD

Sample selection and preparation

One hundred and thirty two freshly extracted upper and lower first molar teeth with mature apices and semi straight roots were selected. The criteria for teeth selection included a straight root canal and a mature, centrally located patent apical foramen, and roots that devoid of any resorption, cracks and fractures by using an Illuminated magnifying lens (X10) \(^9\).

External soft tissue and debris were removed using periodontal curette, then the teeth were decoronated and the distal roots of the lower 1st molar and the palatal roots of the upper 1st molars were detached using a diamond disk bur. In order to get a flat reference point for measurement and to eliminate any variable in access preparation and to facilitate a straight line access in the coronal portion of the canal for both instrumentation and obturation, roots were sectioned through a line drown perpendicular to the long axis of the tooth at the cemento-enamel junction \(^10\).

All roots were stored in normal saline. In order to standardize the length of the root canals involved in each experimental group, the length of all roots were measured and root segments ranging from 13-16 mm were equally distributed to the groups \(^11\). The exact working length was established by passing a size 10 or 15 stainless steel file until its tip was just out of the apical foramen and then by subtracting 1 mm from the measured length.

The roots were placed in an assembly made of an acrylic block and a light body impression material as seen in Figure (1.1 A). This procedure would allow maximum simulation of the practiced clinical condition in a normal endodontic treatment especially regarding the bony socket with its periodontal ligament.

Instrumentation and irrigation

The canals were instrumented using a crown-down technique with NiTi rotary ProTaper files (Figure 1.1 B). Apical patency was maintained throughout instrumentation using a size 15 file \(^12\). The sequence of ProTaper instruments used in the present investigation is summarized in Table (1.1).

<table>
<thead>
<tr>
<th>Sequence</th>
<th>File name</th>
<th>Depth of insertion</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>S1</td>
<td>-6 mm of the working length</td>
</tr>
<tr>
<td>2.</td>
<td>Sx</td>
<td>-4 mm of the working length</td>
</tr>
<tr>
<td>3.</td>
<td>S1</td>
<td>Full working length</td>
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<tr>
<td>4.</td>
<td>S2</td>
<td>Full working length</td>
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<tr>
<td>5.</td>
<td>F1</td>
<td>Full working length</td>
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<tr>
<td>6.</td>
<td>F2</td>
<td>Full working length</td>
</tr>
<tr>
<td>7.</td>
<td>F3</td>
<td>Full working length</td>
</tr>
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All instruments were used in a low-torque motor with torque control and a constant speed of 250 rpm. The instruments were inserted into the root canal in a continuous in-and-out movement and were never forced apically. Maximum effort was made to take the files to length only one time for no more than 1 second \(^12\).

Irrigation was performed with 2 ml NaOCl (2.5%) after each change in instrument size. The smear layer was removed by rinsing with 2.0 ml of 17% ethylene diamine tetraacetic acid (EDTA) solution for 60 seconds \(^13\). The root canal was then flushed with 5 ml of distilled water and dried with ProTaper F3 paper points.

Canal obturation

The specimens were divided into three experimental groups of 44 samples each. Each group had two positive and two negative control samples.

**Group A:** 40 roots obturated using lateral condensation of gutta percha and AH 26 sealer. A master gutta-percha cone size 30 taper .06 was fitted to the working length with tug back.

The sealer was introduced to the full working length using a ProTaper F3 paper point with simultaneous rotary movement in a counter clock wise direction to coat the canal with a thin layer of sealer. The master cone was coated with the AH 26 sealer and gently seated to the working
length. Lateral condensation was carried out using accessory gutta-percha cones ISO size 20 taper 0.02 with an endodontic finger spreader size 20 by applying apical pressure (1-3 Kg) placed in the first instance to within 1 mm of the working length. The gutta-percha cones coated with sealer were laterally condensed until they could not be introduced more than 3 mm into the root canal. Following obturation, the excess gutta-percha was removed from the coronal cavity with a warm instrument (excavator) and vertically condensed with a straight endodontic plugger. The remaining cavity was filled with a temporary filling.

**Group B**: 40 roots obturated using Soft-Core and AH 26 (carrier system). The size 30 Soft-Core obturator was verified using the ‘Size Verifier’. At first the oven was adjusted on the button H (for regular heat obturators) and then heated (Soft-Core regular needed 110°C to melt). The stopper was adjusted to the premeasured working length and the obturator was placed in its specific hole in the oven. While the obturator was heated, AH 26 sealer was mixed according to manufacturer’s instruction and the canal wall was lightly coated with the sealer using ProTaper F3 paper point. When the oven indicated that the obturator was ready with a beep sound and a green diode light, the obturator was carefully removed from one of the slots in the top of the oven. The plasticized Soft-Core was then inserted to the apical stop with apical pressure. After the gutta-percha had been cooled, the handle and insertion pin were removed by a twisting motion. Any excess plastic core was cut away with a small inverted cone bur and the extra gutta-percha was trimmed away. The remaining cavity was filled with a temporary filling.

**Group C**: 40 roots obturated using lateral condensation of Resilon and Real Seal SE. A size 30 taper .06 Resilon cone was fitted to the working length with tug back. The dual syringe (with mixing tip) was used to express the self-etch root canal sealant onto the cement slab which was then introduced to the canal on ProTaper F3 paper point to coat the canal with a thin layer of sealer according to manufacturer’s instruction. The lateral condensation was made in the same manner mentioned in the gutta percha group (group A). Following obturation, the excess Resilon was removed from the coronal cavity with a warm instrument and vertically condensed with finger plugger. The orifice was light cured for 40 seconds according to manufacturer’s instruction. The remaining cavity was filled with a temporary filling.

After obturation samples of all groups were stored in an incubator for 48 hours in a 37°C and 100 % relative humidity to ensure complete setting of the sealer, then the roots were removed from their assembly and each group was further subdivided into four subgroups, 10 samples each (1, 2, 3 and 4) which were exposed to (7.2, 6.5, 6.0 and 5.5 pH solutions) respectively.

Each group had two roots acting as positive controls and two roots as negative controls. The negative control roots were obturated and completely coated with one layer of nails varnish and two layers of sticky wax, while positive controls where left uncoated and unobturated. All experimental roots were coated with one layer of nails varnish and two layers of sticky wax except for the apical 3mm.

An ELIZA test micro-plate was used to immerse about 7.5 mm of the root apically in the freshly prepared acidic solutions according to their subgroups, and the assembly was incubated for two days. Indian ink was used as the leakage indicator, and all samples including the positive and negative controls were immersed in the ink and then the assemblies were re-incubated for another one week. At the end of this period, the roots were removed from the ink and washed and by using lacron carver the coating layers were removed from the roots. Longitudinal sectioning was made using chisel and mallet taking care to include the apical foramen in the fracture line. The linear extent of dye penetration from the apical end coronally was measured by means of stereomicroscope at (X40) magnification with calibrated grid. Apical micro-leakage was measured independently by two evaluators one of them not aware of the obturation technique used and the average of the two measurement of each sample was considered for statistical analysis.

**Figure 1.3**: Dye penetration in samples of the experimental groups. (Group A) lateral condensation GP/AH 26, (Group B) Soft-Core/AH 26, (Group C) lateral condensation Resilon/Real Seal SE.
Figure 1.2: Mean values of apical microleakage in mm with the highest and lowest mean values in all tested subgroups.

Statistical analysis of the data was done using the analysis of variance (ANOVA). The results showed that there was no statistical significant difference within each group regarding different pH media ($P > 0.05$). ANOVA test was also done between groups A, B and C to establish the difference in microleakage among the different obturation techniques. It showed a highly statistical significant difference ($p \leq 0.01$).

Student’s t-test was performed between subgroups that were subjected to the same acidic environments. The results of this test revealed a highly significant difference between all subgroups of both Soft-Core and Resilon with all subgroups of gutta percha (lateral condensation); this result was constant in all the tested acidic media, while a non significant difference was observed between all sub groups of Soft-Core and Resilon in all the tested acidic media.

DISCUSSION

In the current study, sodium hypochlorite was not used as the final irrigation solution since it might result in reduced resin bond strengths (23). EDTA (17%) was used as the final irrigation to remove the smear layer which may improve the apical seal (24). The Soft-Core System (a carrier system that consists of a Thermoplasticized alpha phase gutta-percha coating a plastic carrier) was evaluated in this study since it was introduced to improve the homogeneity and surface adaptation of gutta-percha (2). AH26, an epoxy resin sealer, was combined with gutta-percha obturated groups in this study, because it performed better than quite a lot of sealer types (20, 25) and AH 26 (an improved epoxy resin sealer in the AH series) (26). The second generation of the Resilon/Epiphany obturation system (an alternative system that offers the promise of adhesion to dentine) replaced the original sealer and primer with the self-etch Real Seal SE, that’s why Resilon combined with Real Seal SE was used in this study to evaluate the benefit of this self-etch dual-cure, hydrophilic resin sealer that omitted the separate priming step (27).

However the canals were instrumented with the ProTaper system, obturation was not made with ProTaper gutta-percha points because the Resilon core material is available only in tapers 0.02, 0.04 and 0.06 (28). And since the last instrumentation file used was F3 which have a D0 diameter and apical taper of 30/0.09, and a decreasing percentage taper from D4 to D14 (29). In order to obtain maximum geometrical apical fitness, the greatest taper of Resilon cones available (size 30/0.06 taper) were used in this study, and for standardization reasons gutta-percha cones with the same geometry were utilized. Since the pH of the periapical periodontitis was found to be acidic ranging from 6 to 7.3 (8), the obturated roots in the current study were subjected to solutions ranging from a neutral pH value of (7.2) to an increased acidity of (5.5).

**Comparison between the different acidic media in each group**

Statistical analysis of the data showed a non significant difference within each group regarding different pH media.

Since, to our knowledge, this is the first time to assess the effect of an increased acidity on the apical microleakage encountered in obturation, it is of benefit to compare our results with other studies that evaluated the effect of decreased pH on dental materials. The effect of an acidic environment (pH 5) was found to be of a non significant difference on the microleakage of various root-end filling materials in two in vitro studies (30, 31) which is the same conclusion drawn by the current study regarding the obturation materials.

However the statistics revealed a non significant difference, the data showed an increase in dye penetration with the decreased pH but to a mild extent. This mild effect can be hypothesized in two ways:

1. Short exposure time (48 hours) which might reflect the mild effect.
2. The use of resin sealers (AH26, Real Seal SE) which have a relatively short setting time and low solubility in aqueous solutions (11, 32) which might explain the mild effected of the acidic solutions on these resin sealers.

**Lateral condensation of (Gutta-Percha + AH26) versus (Resilon + Real Seal SE)**

Student’s t-test revealed a highly significant difference between all subgroups of Resilon +
Real Seal SE with all subgroups of gutta-percha + AH 26. These results were in agreement with many studies that compared the same materials using the same dye penetration methodology (33, 34), and the microbial leakage and fluid filtration methods (13, 35, 36, 37), and when comparing Resilon with gutta-percha and the other AH sealer (AH plus) (38).

Excellent sealing capability of Resilon may be attributed to the “mono-block” which is created by the Resilon filling material being closely adapted to the Epiphany sealer and in turn the Epiphany sealer adhering to the dentin walls. In contrast, the high-power SEM micrograph showed how the gutta-percha filling pulled away from the AH 26 sealer, whereas the resin tags held the sealer against the dentin wall which created a gap between gutta-percha and sealer forming an avenue for microleakage (39).

The obtained results could be related to the type of sealer used, since Epiphany performed better than both of the epoxy resin sealers (AH26, AH plus) which could be explained by the inferior adaptation and penetration ability of gutta-percha with AH26 and AH plus across the root canal (39). In addition AH26 is hand-mixed and the formation of voids is a common finding which might explain the increased leakage (40).

The results of this study disagree with studies that either showed a non significant difference or proved better results in gutta-percha (19, 41, 42, 43).

Shemesh et al concluded that Resilon/Epiphany had more glucose penetration than gutta-percha and AH 26 (41); while equivalent apical leakage of Resilon/Epiphany and gutta-percha/AH Plus sealed roots was also shown by Biggs et al using a silver nitrate microleakage study (42), however the smear layer was not removed in their study compared to our study since Cobankara et al showed a significant decrease in leakage when the smear layer was removed (44).

Paque´ & Sirtes in 2007 showed that roots filled with Resilon/Epiphany allowed significantly more fluid movement when compared to gutta-percha/AH plus after 16 months (long-term) compared with the immediate measurement in which they allowed relatively less fluid movement (19). However these results disagree with our study in long term but still it agrees with our results in the short term, since (in the long term) the susceptibility of a polycaprolactone-based root canal filling material to degradation can be an important factor (45). Oliveira et al, in 2011, used bacterial leakage and found that Epiphany SE had intermediate results in contrast to AH Plus which had the least leakage (43). The cause of disagreement in all of the previously mentioned studies could be attributed to the differing methodologies applied in instrumentation and obturation and to the microleakage testing methods that were not used in the present study.

Lateral condensation of (Gutta-percha + AH26) versus carrier coated gutta-percha (Soft-Core + AH26)

Soft-Core showed less apical microleakage than lateral condensation in all subgroups with high significant difference. This was in agreement with two in vitro studies (2, 17) that used different techniques to measure microleakage (computerized fluid filtration meter and dye infiltration ratio in horizontal cross sectioning respectively, while the same results was reached by Nema, who utilized the same dye penetration method (18).

These results can be explained either by the use of heat softened GP that created a better homogenous mass with less voids and better adaptation of the GP to the canal wall (46), or by the sealer content since Genço˘lu, in 2003, established that Soft Core technique had more gutta-percha content than lateral condensation technique and that higher sealer content might lead to higher leakage amounts, since endodontic sealers are soluble materials and the shrinkage may result in potential leakage pathways in root canal fillings (47).

The results of this study disagree with two in vitro studies (15, 20) that used dye penetration and fluid filtration respectively. This might be explained by the differing instrumentation and obturation techniques, since the root canals were prepared using Gates Glidden/step-back technique without removing the smear layer in contrast to NiTi ProTaper files and smear layer removal in the current study, also AH 26 was sparingly introduced into the coronal third of each canal using the rotary paste filler while in our study a thin layer of sealer was introduced to the full working length using a paper point according to manufacturer’s instruction.

Lateral condensation of (Resilon + Real Seal SE) versus carrier coated gutta-percha (Soft-Core + AH26)

A non significant difference was observed between these two obturation techniques, still they showed a highly significant difference when compared to lateral condensation of gutta-percha.

It should be noticed that both of the obturation techniques didn’t prevent apical microleakage completely, which can be attributed
in case of Resilon to the polymerization shrinkage and failure to generate a complete mono-block without gaps when examined under SEM (23), while in case of Soft-Core it could be attributed to the thermal shrinkage of the softened gutta-percha (18).

Within the circumstances of this in vitro study the following conclusions could be withdrawn:

1. The increased pH did not affect the apical microleakage of the examined obturation techniques.
2. Both lateral condensation of Resilon/Real Seal SE and Soft-Core/AH 26 provided less microleakage when compared to the lateral condensation of gutta-percha/AH 26.
3. Lateral condensation of Resilon/Real Seal SE showed the least apical microleakage, however, it didn’t provide the complete sealing claimed by the manufacturer.

REFERENCES