Evaluation of cleanliness of dentinal tubules using scanning electron microscope in maxillary anterior teeth following gutta – percha removal with and without solvents: an ex vivo study

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ABSTRACT
Introduction:
Endodontic retreatment therapy has significantly improved in the last few decades due to the introduction of novel materials and techniques. The success of retreatment depends on the removal of the obturated material and gaining access to the full apical working length. The aim of this study was to evaluate the cleanliness of dentinal tubules following gutta-percha removal with and without solvent.

Material & Methods: 70 extracted teeth were stored in thymol, and were root canals were prepared and filled as per standard root canal procedure. Gates glidden drill was used to remove the gutta percha and the samples were divided in to 4 groups. Group I (Control-Unfilled) n=10, Group II (without solvent) n=20, Group III (Eucalyptol) n=20 and Group IV (chloroform) n=20. The samples were studied under SEM (Scanning Electron Microscopy) to assess the effect of different solvents on the cleanliness of dentinal tubules and effect of region of the root (viz. coronal, middle and apical third) on the cleanliness of dentinal tubules

Results: There was statistically significant difference in the cleanness if dentinal tubules in all groups, with Group II showing maximum open tubule compared to Group III (Eucalyptol) and Group IV (chloroform). There was no difference in cleanliness of the dentinal tubules among different regions (viz. coronal, middle and apical third). The use of solvent (Group III & Group IV) left behind more Gutta-percha compared to non-use of solvent. (Group II), thus it would be wise to assess before one use solvent for retretement.

Key words: Retreatment, solvent, chloroform, dentinal tubules.

INTRODUCTION
Non-surgical endodontic treatment is routinely practiced in modern dentistry. The Revolution of material science and techniques in root canal treatment has resulted in the retention of millions of teeth that would have otherwise been lost.1

With all the potential for endodontic success, the fact remains that clinicians are confronted with post-treatment disease.2,3 Retreatment of the previously obturated canal is becoming, more and more common in endodontic practice. The main causes of endodontic failure making retreatment necessary are insufficient cleaning and inadequate obturation.4 Additionally, teeth with inadequate obturation, unfilled root canals or under extended root fillings may require retreatment before coronal restorations, as failure may occur in the future.3

CJ Ruddle (CDA, 1997) Endodontic retreatment is a procedure performed on a tooth that diagnostically demonstrates incomplete treatment, yet the actual conditions require further endodontic treatment to achieve www.turkjphysiotherrehabil.org
successful results. The main goals of retreatment are regaining access to the apical foramen by complete removal of the root canal filling material thus facilitating sufficient cleaning and shaping of the complete root canal system and final obturation. Only if the filling material can be removed and root canal negotiated to the apical foramen, thus allowing thorough debridement, can the prerequisite for successful retreatment be filled. The success rate for retreatment ranges from about 65% (Molven 1974, Allen et al. 1989) to more than 80% (Strindberg 1956, Selden 1974, Sjogren et al. 1990).

A wide array of chemicals are available today as gutta-percha solvents, such as eucalyptus oil, turpentine, chloroform, xylol, methylene chloride, orange-wood oil, methyl chloroform, halothane, and tetrahydrofuran. As early as 1850, eucalyptol oil and chloroform were used as a gutta-percha solvent. Chloroform has been the most popular solvent and solubilizes gutta-percha more rapidly than eucalyptol, is less expensive and has a more pleasant odor.

In earlier studies, gutta-percha removal was assessed radiographically or roots were split longitudinally and residual gutta-percha and sealer were measured using evaluation scales. Recent studies have utilized scanning electron microscope (SEM) to evaluate the gutta-percha remnants at various microscopic levels. The study aims to determine and compare the cleanliness of dentinal tubules following gutta-percha removal with and without solvents and to assess the cleanliness effect of irrigants on the region of the root. (viz. coronal, middle and apical third).

**MATERIAL & METHODS**

**Specimen preparation**
A total of 70 freshly extracted human maxillary incisors and canine teeth were used for the study. The extracted teeth were kept under running water. The root surfaces were debrided and cleaned. Teeth were stored in 0.001% thymol for 6 months before the start of the study. Radiographs were taken to ensure patent canals and canal curvature angles of 0-10 degree.

The procedure used for each instrument and operator technique was standardized. Access cavity preparations were achieved using high-speed burs and water spray. A #10 K-file was used to negotiate the canal in watch-winding motion till it is visible through the apical foramen. The working length was established 1mm short of this length(18mm). The excess coronal structure was removed with the diamond disk. The root surfaces were grooved horizontally at a distance of 2, 6, and 10mm from the anatomic apex, in order to define the position for SEM images. The apical portion of the root tip was then sealed with sticky wax to generate a closed system.

**Root Canal Preparation**
All canals were prepared by the same operator using a standardized technique, using a combination of hand files and rotary files. All the canals were initially prepared with stainless steel K files till #20 followed by Hero shaper NiTi rotary files in a sequence 20(4%), 20(6%), 25(6%), 30(6%) were used followed by Apical enlargement was completed with #35 and #40 K hand file. Each instrument was replaced at the first sign of wear and tear. Irrigation was performed using 3% sodium hypochlorite (NaOCl) during instrumentation. Finally, root canals were rinsed for 1 minute using 17% EDTA, 5 ml, followed by 3% NaOCl,10 ml. A 27-gauge irrigation needle inserted 1-2 mm short of the working length was used for irrigation. All root canals were dried with #40 2% paper points.

All samples were then randomly divided into four groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Number</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>Control-Unfilled</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>without solvent</td>
<td>20</td>
</tr>
<tr>
<td>III</td>
<td>Eucalyptol</td>
<td>20</td>
</tr>
<tr>
<td>IV</td>
<td>chloroform</td>
<td>20</td>
</tr>
</tbody>
</table>

**Root Canal Filling**
The root canal of each tooth in groups II to group IV was filled using lateral compaction of gutta-percha. AH Plus sealer (Dentsply) was coated on the master cone size 40(tug back checked) and positioned into the canal. Thereafter, accessory gutta-percha cones size 20 were laterally compacted using nickel-titanium finger spreaders size 20. The roots were radiographed in buccolingual and mesiodistal directions in order to confirm the adequacy of root filling. Temporary restoration (Coltosol, Coltene Whaledent) was then placed in the access cavity. All teeth were stored in a humidor at 37-degree centigrade and 100% humidity for 2 weeks to allow the complete setting of the sealer.
Retreatment Techniques

In group II to group IV, 6 mm of root fillings was removed from the cervical part of the canal using gates-glidden burs sizes 2, 3 and 4, to create space for the solvent. In the middle and apical part of the canal, H-files size 25-50 were used in a circumferential quarter-turn push-pull filing motion in order to remove gutta-percha and sealer from the canal. No solvent was used for GP (gutta-percha) removal in group II. Eucalyptol served as a solvent in group III, and chloroform in group IV.

15µL of solvent was applied four times in each canal (overall: 60-µL solvent per canal). During reinstrumentation, 3% NaOCl was used as an irrigant. After gutta-percha removal, the canals were irrigated for 1 minute with 17% EDTA (5 ml), followed by 3% NaOCl (10 ml) using the a forementioned irrigation needle 1-2 mm short of the working length. Finally, all canals were dried with #40 2% taper paper points.

Figure 1: The process of retreatment
A: Radiographic assessment after root canal obturation  
B: Use of gates glidden drill to remove the GP Points  
C: Removal of remaining material with H file  
D: Longitudinal splitting of samples with chisel and mallet

EVALUATION

The teeth were grooved with a diamond disk and split longitudinally with the help of a chisel and mallet. For, SEM analysis, the specimen was dehydrated at 37-degree centigrade for 7 days and sputtered with palladium. The coronal, middle and apical third of all root halves were examined using an SEM at 10Kv and at a magnification of 2000X. One image was made at the position of each groove prepared in the root surface. For statistical analysis, the total no. of dentinal tubules and the clean/open dentinal tubules were recorded. Tubules completely or partially filled with the material were considered as unclean. (Fig 2, 3 ,4)
Figure 2: Scanning Electron Microscopy Images of Coronal third of tooth
A: Group I - Control Group                    B: Group II - Without Solvent
C: Group III - Eucalyptol Group            D: Group IV - Chloroform Group

Figure 3: Scanning Electron Microscopy Images of Middle third of tooth
A: Group I - Control Group                    B: Group II - Without Solvent
C: Group III - Eucalyptol Group            D: Group IV - Chloroform Group
Figure 4: Scanning Electron Microscopy Images of Apical third of tooth
A: Group I - Control Group  
B: Group II - Without Solvent  
C: Group III - Eucalyptol Group  
D: Group IV - Chloroform Group

STATISTICAL ANALYSIS

The data obtained was graded according to the following criteria:

Grade 1: 100% of dentinal tubules are clean  
Grade 2: 75 to <100% of dentinal tubules are clean  
Grade 3: 50 to <75% of dentinal tubules are clean  
Grade 4: 25 to <50% of dentinal tubules are clean  
Grade 5: <25% of dentinal tubules are clean

The quantitative variable was presented in the form of mean, standard error (SE) of ratio evaluated in SEM images and the number of evaluated images (N).

The analysis was carried out broadly under two categories i.e. effect of different solvents on the gutta-percha removal on the cleanliness of dentinal tubules at between coronal, middle and apical third and effect of region of the root (viz. coronal, middle and apical third) on the cleanliness of dentinal tubules.

Comparison among various study groups for SEM analysis at various levels (coronal, middle, apical) was done with the help of a One-way analysis of variance (ANOVA) or Kruskal Wallis test depending on the result of normality and Tukey’s multiple comparison test or Dunn’s multiple comparison test. P<0.05 was taken as significant. Pair wise comparison between coronal, middle and apical third was further analyzed by Mann Whitney Test and results were confirmed.

RESULTS

Prepared samples were split longitudinally with the help of a chisel and mallet and were examined under scanning electron microscope. Out Of 420 SEM images only 333 images could be assessed.

Out of which 5 samples (Group I: 1 of 10, Group II: 2 of 20, Group III: 2 of 20) were damaged while splitting the tooth samples and another 57 SEM images couldn't be assessed because of tubular sclerosis and/or artifacts (Group I: 3 of 60, Group II: 8 of 120, Group III: 16 of 120, Group IV: 30 of 120). SEM images were analyzed to calculate the ratio of total number of open dentinal tubules/ total number of dentinal tubules and mean was calculated.

Table 1 shows the comparison among different groups across different tooth regions i.e Coronal, Middle and Apical
third and overall. It was seen that at each level i.e. Coronal, Middle and Apical third there was a statistically significant difference between each groups. Most no. of open dentinal tubules was observed in the following order: Group I > Group II > Group III > Group IV. Difference of cleanliness between any two groups is also statistically significant as per the Tukey’s test.

Table 1: Group wise comparison of effect of different solvent used for gutta-percha removal on the cleanliness of Coronal, Middle, and Apical third and overall dentinal tubules.

<table>
<thead>
<tr>
<th></th>
<th>Coronal</th>
<th>Middle</th>
<th>Apical</th>
<th>Overall</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Group I (Control Group)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Group II (Without Solvent)</td>
<td>0.7878</td>
<td>0.01631</td>
<td>0.7489</td>
<td>0.01519</td>
</tr>
<tr>
<td>Group III (Eucalyptol)</td>
<td>0.2262</td>
<td>0.01037</td>
<td>0.238</td>
<td>0.009973</td>
</tr>
<tr>
<td>Group IV (Chloroform)</td>
<td>0.1521</td>
<td>0.008399</td>
<td>0.1463</td>
<td>0.009286</td>
</tr>
<tr>
<td>F value</td>
<td>910.6</td>
<td>1130</td>
<td>411.3</td>
<td>1862</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Post Hoc test</td>
<td>Group I &gt; Group II &gt; Group III &gt; Group IV</td>
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Table 2 shows the pair wise comparison for each section of the tooth i.e coronal, middle and apical thirds which were graded for cleanliness by different methods. The collected data was analyzed statistically to know the effect of site of observation on the cleanliness. Ranks given to the three sites were compared in each group by Mann Whitney test. As shown in the above tables, sites of observation do not have significant effect on the cleanliness of the dentinal tubules.

Table 2: Pair wise comparison between cleanliness of Coronal & Middle; Middle third & apical third and apical third and coronal third among different solvent used for gutta-percha removal.

<table>
<thead>
<tr>
<th>Pairwise Comparison</th>
<th>Group</th>
<th>Site</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Uvalue</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Coronal &amp; Middle Third</td>
<td>Group I (Control Group)</td>
<td>Coronal</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II (Without Solvent)</td>
<td>Coronal</td>
<td>2.375</td>
<td>2</td>
<td>0.4903</td>
<td>575</td>
<td>0.2419</td>
<td>Not significant</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Middle</td>
<td></td>
<td>2.542</td>
<td>3</td>
<td>0.5054</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III (Eucalyptol)</td>
<td>Coronal</td>
<td>4.568</td>
<td>5</td>
<td>0.5022</td>
<td>645</td>
<td>&gt; 0.9999</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td></td>
<td>4.571</td>
<td>5</td>
<td>0.5021</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV (Chloroform)</td>
<td>Coronal</td>
<td>4.971</td>
<td>5</td>
<td>0.1715</td>
<td>594.5</td>
<td>&gt; 0.9999</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td></td>
<td>4.971</td>
<td>5</td>
<td>0.169</td>
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<td></td>
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</tr>
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</table>

**DISCUSSION**

Nonsurgical root canal therapy has become a routine procedure in modern dentistry. Unfortunately, not all treatments result in optimum long term healing. Instrumentation through the apex and overfills from retreatment were factors associated with a decreased frequency of complete healing. Sjogren et al.\(^{14}\) found 62% success rate for retreatment of teeth which originally had apical pathology present. Van Nieuwenhuyzen J P et al.\(^{15}\) found 71.8% success rates from conventional endodontic retreatment of symptomatic roots, with a further 18.9% showing some healing. Many endodontists believe that endodontic success depends more on what is taken out of the canal (i.e. bacteria and necrotic tissue) than on what is put in it. The same is true for endodontic retreatment. Many studies have documented the penetration of plastic filling materials into the dentinal tubules in the absence of the smear layer.\(^{16-18}\)

Retreating previously filled canal systems demand that antimicrobial irrigants and medicaments gain access to all
ramifications of the canal system which may be harboring organic matter and microorganisms. It is desirable that all materials employed are amenable to complete removal during retreatment. Various removal methods are available, including the use of solvents, heat, and mechanical instrumentation, alone or in combination. For this study, 70 extracted human maxillary anterior teeth were collected and treated according to OSHA and CDC regulations. Thymol a known bactericidal and fungicidal agent was used at a concentration of 0.001% and samples were cleaned and kept no longer than 6 months. Decoronation was done to assure the standardization of specimens eliminating some variables, such as anatomy of the coronal area and access to the root canals allowing a more reliable comparison between retreatment groups. Grooves were made at 2, 6, and 10 mm from the apex to define the position for SEM images, to avoid the operator bias. Preparation was undertaken by a single operator following a predetermined protocol. By using the hybrid technique, a certain amount of dimensional uniformity was produced in the final canal shape within the confines of the natural tooth used. This meant that obturation could also be standardized. The apical portion of the root tip was sealed with sticky wax to simulate the in vivo counter pressure and to prevent seepage of irrigant through the apical foramen during canal preparation. Teeth were grooved with a diamond saw, split longitudinally with the help of a chisel and mallet. Takahashi et al. mentioned that longitudinal cleavage of root is a practical method to evaluate the effectiveness of the retreatment method and it is advisable that the chisel should not touch the root canal walls. Wilcox et al. and Wilcox (1989), Hulsmann & Stotz (1997), Sae-Lim et al. (2000), Baratto et al. (2002) used a method of splitting teeth longitudinally with the help of chisel and mallet; splitted samples were photographed, magnified and traced. Most of the studies done on gutta-percha removal, assessed samples radiographically However, radiographs provide a two-dimensional image and only allow a semi-quantitative evaluation of the amount of debris remaining. Also, evaluation is subjective and observer performance is known to be variable. Therefore in this study, we choose scanning electron microscopy was used with a magnification of 2000X which allows for highly detailed observation of dentinal tubule and filling material therein. Group I was left unfilled. Group II-group IV was obturated with gutta-percha with a cold lateral compaction technique using the AH-Plus resin sealer. In group II to group IV retreatment was performed. The cervical part of gutta-percha was removed with gates-glidden bur sizes 2, 3 and 4. The use of Gates- glidden drills is a well-known technique to remove gutta-percha from the coronal and middle part of the canal. Gutta-percha from the middle third and apical third was removed with H-files sizes 25-50. During reinstrumentation, 3% NaOCl was used as an irrigant. The final flush was done with 17% EDTA (5 ml) for 1 min, followed by 3% NaOCl (10 ml). All canals were then dried with paper points. In group II solvent was not used. In group III eucalyptol and group IV chloroform were used as solvents, respectively. When assessing the significance of region of the root (viz. coronal third, middle third and apical third) on gutta-percha removal, the results of the present study showed that the difference between coronal, middle and apical regions is not statistically significant (p>0.05). However, a slightly better cleaning was observed in the apical third. The Middle third showed the least cleaning of dentinal tubules of all the thirds. The results were confirmed with graded data analysis Mann Whitney test (Table 2). However, contradictory to our study; some studies found more debris in apical third, while no significant differences were found in the coronal and middle portions. The present study showed that not using a solvent showed more open dentinal tubules compared to using a solvent. (Table 1). None of the solvents tested was able to completely remove the root filling materials. This is in accordance with studies done by Fereira et al. 2001, Ezzie et al. 2006, Schirmerste et al. 2006b,d,e, and Takahashi CM et al.2009. Amongst the solvents, eucalyptol showed significantly more open dentinal tubules than chloroform. (Table 1) The results of our study were in contrast to Tamse et al. 1986, Wennberg & Orstavik 1989, Kaplowitz 1990, Wilcox 1995, in which Chloroform is the most effective gutta-percha solvents when compared to most of these solvents. The use of chloroform as a solvent for gutta-percha is controversal to Zakariasen et al. 1990, McDonald & Vire 1992. It has been reported to be locally toxic when contacting periradicular tissues. Additionally, it is hepatotoxic and nephrotoxic and is classified as a carcinogen. However, McDonald & Vire 1992 and Allard & Andersson 1992, there seem to be no or only low risks of occupational hazards for the patients and the dental team when the solvent is stored in a closed dappen dish. It seems that more remnants were found in irregularities of the root canal wall and in dentinal tubule with the increasing dissolution of the root filling material. This might be explained by the fact that softened root filing material
may easily be compacted into these irregularities and into the dentinal tubules from where they no longer can be removed. This effect was observed more often in the solvent group than in the non-solvent group and more often in the chloroform group than in the eucalyptol group (with regard to SEM images). The difference between the solvent groups might be explained by the higher solubility of gutta-percha in chloroform compared with eucalyptol. However, Hunter et al. 1991 demonstrated equal softening abilities between chloroform, halothane, and eucalyptol. Several different solvents, among them halothane, eucalyptol, xylol, methyl chloroform, tetrahydrofuran, methylene chloride and others have been recommended as an alternative to chloroform for gutta-percha retreatment cases.

CONCLUSION

Within the limitations of this study, it could be concluded that the use of solvent leaves more gutta-percha and sealer residues in the dentinal tubules as compared to the specimens where the solvent was not used. Therefore, solvents should not be standard practice during endodontic retreatment. They should only be applied if the working length cannot be reached without a solvent. Eucalyptol performed better than chloroform, also it should be chosen to chloroform as it is much safer than chloroform. Also, the region of the root has no influence on gutta-percha removal. Further studies should evaluate the effect of rotary instrumentation on the cleanliness of dentinal tubules during endodontic retreatment.

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