Quantitative Image textural analysis of Time dependent Surface structural changes of Gutta Percha in two different solvents -- SEM study

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Abstract— Gutta Percha (GP) has been the choicest endodontic restoration, when coated with a sealant for better binding and adhesion with the canal walls. The filling hardens on setting eliminating any chance of micro leakage. In the event of a failure of the treatment, removal of the filling to reattempt endodontics is a necessary mandate. Eugenol an organic solvent and xylene, a chemical solvent have been in use, to soften the GP to aid removal. Removal, however is not easy, and quite laborious even with the contemporary rotary instruments and the search for an efficient solvent to aid rotary is an ongoing research. To understand the effect of eugenol or xylene on the surface of Gutta Percha each when used as a solvent. To understand the time dependent structural changes of Gutta Percha on exposure with eugenol and xylene. The time dependent structural changes of GP when exposed to solvents, needs to be understood, to enable innovations in this area of dissolving GP for rotary endodontic retreatment.

Index Terms— Root canal filling, Gutta Percha, endodontic failure, endodontic retreatment, Xylene, Gutta Percha solvents.

1 INTRODUCTION

Gutta Percha, [5] a naturally occurring polymer, is a very popular endodontic filling material when used with appropriate sealants. Endodontics, however fails occasionally, due to reasons like, an unnoticed anatomical ramifications in root canal, or errors in obturation, over extended fillings, imperfect lateral condensation, resulting with apical infection [16]. Non-surgical retreatment, aims at re-establishing the healthy apical tissues after retreatment of an infected endodontically treated tooth. The situation warrants retreatment with extensive reinstrumentation and disinfection of the root canal [11]. In such instances, mechanical removal of the intracanal Gutta Percha filling is the most opted conservative mode [12] of management.

Removal of the set Gutta Percha with its sealant has always been a challenge to an endodontist, particularly remnants of the GP adhered to the canal walls. The commonly used methods are being manual, thermal, chemical, ultrasound [10][7] and more recently rotary [15]. The most common method adopted in day to day clinical practice has been the use of chemical solvents to soften GP followed by the mechanical removal using manual or rotary instruments. Chemical and organic solvents most commonly used are chloroform, eucalyptol oil, eugenol, xylene, turpentine oil and pine needle oil [4]. Other organic solvents have also been tried [14]. The solvents when used in conjunction with hand /rotary instruments have shown to be most efficient and time saving than using just rotary[2]. Xylene has been a very popular chemical solvent [6][18], to remove Gutta Percha, but being chemical in nature, the search for an organic solvent to optimally soften the GP is on, as Bergenholtz [19] has opined that excessive softening of GP could result with GP being pushed apically. A perusal of the literature reveals that SEM [3] has been extensively used to study the various aspects of root canal sealants and efficiency of various endodontic techniques.

This paper is an invitro analysis using SEM, to qualitatively analyse, the time dependant surface structural changes, in Gutta Percha, when exposed to two different solvents eugenol and xylene. The images were then subjected to a quantitative analysis for further confirmation. Image analysis by extracting texture features of Gutta Percha exposed to eugenol and xylene using Spyder, Anaconda version 3(Python 3.7) software was used to quantitatively evaluate the time dependant surface structural changes, in Gutta Percha.

2 METHODOLOGY

Gutta Percha pieces of size 21of Dentsply Co. was used in the study. Only the first 5mm of the nontaper end of the GP was used to maintain homogeneity in dimension when exposed to the solvent. One Gutta Percha piece was taken as a control (Group A) and the rest were used for experimentation in separate petri dishes. Two experimental groups B and C were included. Each group was further divided into 3 subgroups based on the time of dissolution allowed in eugenol and xylene as B1, B2, B3 and C1,C2,C3 respectively.

Group B contained Gutta Percha pieces that were exposed to eugenol for 1 minute (B1), 3 minutes (B2) and 5 minutes (B3) in different petri dishes.

Group (C) was exposed to xylene for a period of 1 minute (C1), 3 minutes (C2) and 5 minutes (C3) in different petri dishes. Experiment was repeated in triplicate in a sample of 21

pieces of GP.

One Gutta Percha piece from each petri dish was subjected to sputter coating for scanning electron microscope (SEM) study. Areas in the approximate centre of the GP were identified wherever possible and studied under SEM microscopy.

2.1 Microscopic SEM procedure analysis:

Fresh samples were observed under dissection microscope and identical samples without any surface aberration and deformity were picked out with the help of a fine forcep. A small portion of the sample was placed on the stub with double side adhesive carbon tape. The samples were sputter coated with palladium gold at a thickness of 200*A in ion sputtering device (Quorium technologies) and scanned under scanning electron microscope (Tescan) at an accelerating voltage of 5-10 kV.

The GP surface was studied under 100x for gross morphological change (Fig 1: 1A, 1B, 1C), and other areas subjected to the solvent were studied under a standardised 10kx for finer details.

	1minute	*3 minutes*	*5 minutes*
A - Control group GP	Homogenous surface	Homogeneous surface	Homogenous surface
B-GP treated with Eugenol	B1 not much change in surface structure of GP	B2 not much change in surface structure of GP	B3 considerable change in surface structure compared to B1 and B2
C-GP treated with xylene	C1 not much difference in surface structure of GP	C2 considerable change in surface structure of GP	C3 considerable change in surface structure of GP

Exposure of GP to eugenol and xylene began to show time dependent surface changes on softening with solvents, as shown in the (Fig 2: 2, Fig 3: 3A,3B,3C,Fig 4: 4A,4B,and 4C) under 10kx resolutions.

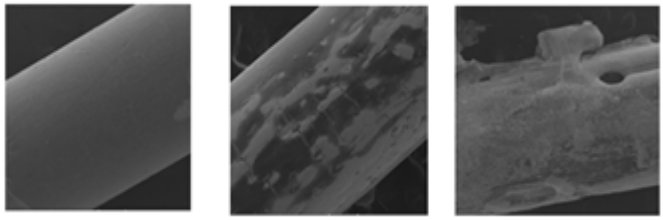
TABLE 1
QUALITATIVE SURFACE ANALYSIS OF GUTTA PERCHA OF GROUP A, B AND C

Qualitative surface analysis of Gutta Percha of Group A, B, and C were done using SEM.

The microphotographs of Group A was seen to be homogenous at 100x magnification (Figure 1: Fig 1A) and 10 kx (Figure 2: Fig 2) and began to appear fibrilous with exposure to solvents (Figure 1: Fig 1B, Fig 1C).

The microphotographs of the samples in group B were evaluated, Group B1 (Figure 3: Fig 3) appeared less homogenous than Group A (Figure 2: Fig 2). Group B1 (Figure 3: Fig 3A) and B2 (Figure 3: Fig 3B) did not show much difference in the surface structure of Gutta Percha but whereas group B3 (Figure 3: Fig 3C) showed some amount of surface alterations when compared to B1 and B2.

The microphotographs of all the samples in group C were evaluated. The surface appeared less homogenous even at 1 min (Fig 4: 4A) and the fragmentation progressed with time. There was not much difference in the surface structure of group C at 1 min (Fig 4: 4A) when compared with Fig 2: 2, but there was a considerable change in surface structure of Gutta Percha when exposed to 3 (Fig 4: 4B)and 5 minutes (Fig 4: 4C).



(a) 1A (b) 1B (c) 1C

Fig 1 : 1A, 1B, 1C

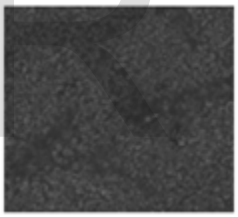
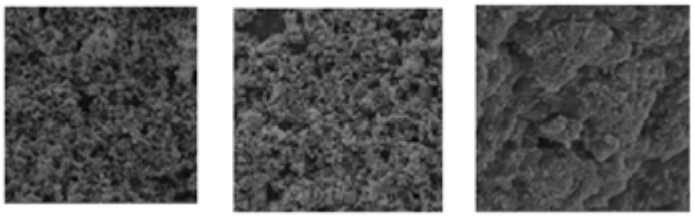
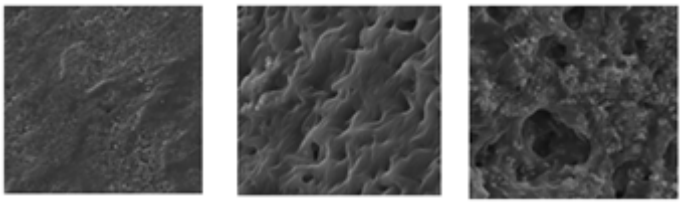


Fig 2: 2



(a) 3A (b) 3B (c) 3C

Fig 3: 3A, 3B, 3C



(a) 4A (b) 4B (c)4C

Fig 4: 4A, 4B, 4C

3 IMAGE ANALYSIS USING TEXTURE FEATURES

The SEM pictures were further quantitatively assessed for better definition of the findings. Image analyzed by extracting texture features of Gutta Percha exposed to eugenol and xylene was evaluated using Spyder, Anaconda ver 3 (Python 3.7) software and the statistical measures were interpreted using IBM SPSS Statistics version 20 with the system configuration of Window 7 Operating System, Intel Pentium CPU 2127U@ 1.90 GHz and 4 GB RAM.

TABLE 2
QUANTITATIVE TEXTURE ANALYSIS OF GUTTA PERCHA OF GROUP A, B AND C

Groups	*Control*	*1minute (B1/C1)*	*3 minutes (B2/C2)*	*5 minutes (B3/C3)*
GP (Group A)	55.28±8.95			
GP (Group B)		63.153±9.42	71.127±10.26	74.140±9.14
t value		0.857	1.64	2.083
p value		p = 0.219	p = 0.088	p = 0.052*
GP (Group C)		70.826±9.44	78.903±9.22	84.03±10.14
t value		1.691	2.602	3.008
p value		p = 0.083	p = 0.029*	p= 0.014**

p value<0.01**

p value< 0.05*

INFERENCE:

SEM analysis of Gutta Percha images for three groups (A, B, C) taken with 1 minute, 3 minutes and 5 minutes exposure in solvent . Images were examined using GLCM (Gray Level Co-occurrence Matrix) feature extraction. To perform this feature extraction, images had to be converted to gray scale image. Then, GLCM feature measures such as contrast, homogeneity, energy, dissimilarity and Angular Second Moment (ASM) were computed from the pixel values (pixel intensity value) of gray images, and these measures were taken as quantitative data for statistical analysis. t-test for specified mean or single mean were used to analyse the image data with respect to mean and standard deviation and P-value was tabulated in the above table.

Data are represented as mean value± standard deviation for example, the mean value 55.282±.95 of group A smooth surface was compared to mean value of group B and group C surface for 1 minute (63.153±9.42 and 0.826±9.44) (Figure 3: Fig 3A, Figure 4: Fig 4A) which shows not much variation in images.(Table 2)

The same mean value of group A smooth surface 55.282±.95 (Figure 2: Fig 2) was compared to mean value of group B and group C surface for 3 minutes (Figure 3: Fig 3B, Figure 4: Fig 4B) was 71.127± 10.26 and 78.903 ±9.22 which showed a statistically significant variation in group C (p = 0.029*) more than group B.

The mean value 55.282±.95 of group A , smooth surface was compared to mean value of group B and group C surface for 5 minutes(Figure 3: Fig 3C and Figure 4: Fig 4C) which was 74.140±9.14 and 84.033±10.14 respectively, which confirms that there was a considerable change in group B and group C at 5 mins . Since P value is less than 0.01, the null hypothesis (H_0) was rejected at 1 % level . Hence there is significant difference between surface structures which means there is much changes in the surface structure of C3. Since P value is less than 0.05, the null hypothesis (H_0) is rejected at 5% level with respect to B3 and C2. Hence there is significant difference between surface structures which means there is much changes in the surface structure of GP sample, B3 and C2 and C3. However, on comparing values of B3 with C2, no significance was noticed.

4 DISCUSSION

The effect of solvents on GP have been evaluated and proved beyond doubt to be efficient for removal of GP in an event of a failed endodontics in clinical practice [7]. There are not many studies that have reported the time dependent surface structural changes of GP when exposed with eugenol and xylene.

The intervals chosen in this study was 1, 3 and 5mins according to the findings reported by Mubir [8], where no significant dissolution was evinced between 2 and 5 mins .

From the above results, it has been shown that the surface structure changes studied qualitatively and quantitatively reflected the same. The surface structure of the control group A was homogenous. The findings were quite the same as reported by Afaf Al-Haddad [1]. The changes in Group B and Group C are a reflection of time dependent changes due to the effect of eugenol and xylene respectively. When Group C evinced changes in 3mins itself Group B showed changes at 5mins only. This reveals that longer exposure of the solvent showed more surface structural changes in both Group B and in Group C, more in C than B. In other words, a 5mins exposure with eugenol, softened only as much as xylene could achieve in 3 mins itself. (Table 1 and 2).However no statistical significance seen between B3 and C2 shows that given an even longer time of exposure, eugenol an organic solvent can be as efficient as xylene.

Studies have revealed that chemical solvents and the speed of the protaper files, contribute to the plasticizing effect

that contributes to softening and thereby easy removal of the set root canal filling materials [1]. Small protaper files sometimes have the disadvantage that the root canal debris can be pushed apically [18]. The time dependant structural changes seen on the surface of treated Gutta Percha in the present study , probably explains the softening or plasticisizing effect on GP that aids in allowing mechanical lock with H files ,when rootcanal retreatment is attempted.

Studies have shown that Xylene dissolves Gutta Percha more slowly in manual extripation ensuring a better control and removal of softened Gutta Percha [7]. Softening followed by mechanical removal of Gutta Percha rather than dissolving it completely, may prove to be efficient and a biologically safer procedure [17]. The same can be accomplished by a cotton pellet moistened with eugenol and left in the chamber and removing the root canal filling at the following appointment. Because the aged root canal filling tends to be harder and more difficult to remove, softening the GP slowly, over a longer period of exposure may be more safe and efficient with eugenol rather than xylene.

Studies have reported that as the interval increases xylene being a chemical solvent can cause periapical tissue irritation, hence further studies are required in this direction to prove the effect of eugenol on tissue reaction even when placed in the root canal for a long duration of time.

5 Conclusion

Xylene has shown good surface alterations on Gutta Percha, more than eugenol as a solvent. Within the limitations of our in vitro analysis the present study suggests that surface alterations of Gutta Percha, when exposed to chemical solvent xylene is mainly time dependent and more efficient than eugenol. But, eugenol being an organic solvent is certainly a better choice as results can be paralleled given more time than xylene.

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