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EFFICACY OF CO_2 LASER ON REMOVAL OF SMEAR LAYER – AN IN-VITRO STUDY

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ABSTRACT

Aim: The purpose of this in vitro study was to evaluate the effect of CO_2 laser on the periodontally involved root surface, and to compare its efficacy with hydrogen peroxide, EDTA and citric acid on removal of smear layer. **Methodology:** 50 specimens from the proximal surface of periodontally involved extracted human teeth were used. Group A specimens received CO_2 laser. Groups B, C and D were treated with 6% hydrogen peroxide, EDTA (pH 7.4), and citric acid (pH 1) respectively. The specimens were then scanned using Scanning Electron Microscopy**. Results:** The CO_2 laser was not able to remove the smear layer on the sites that were irradiated for 0.2, 0.4 or 0.6 seconds at 3W power. Irradiation time of 0.8 seconds at 3W power was able to remove the smear layer, but the dentinal tubules were partially exposed. The surface irradiated for 1 second showed a flat appearance with many clear orifices of dentinal tubules. Irradiation time of 1.2 and 1.4 seconds produced surface charring and was totally ineffective in exposing the dentinal tubules. Hydrogen peroxide did not remove the smear layer completely. EDTA and citric acid were found to be effective in removing the smear layer and exposing the dentinal tubules, however the exposed dentinal tubules showed funnel shaped widening.
Conclusion: Results suggest that CO_2 laser produced increased exposure of dentinal tubules more effectively as compared to hydrogen peroxide, EDTA and Citric acid.

Keywords: CO_2 laser, periodontal regeneration, Root biomodification

INTRODUCTION

A concerted effort has been made in the field of root conditioning to improve the outcome of regenerative periodontal therapies by favoring the attachment of the regenerated periodontal structures. Mechanical instrumentation like scaling and root planing leaves a smear layer, which inhibits cell re-attachment and can serve as a reservoir for microbial growth[1].Therefore, chemical conditioning of the roots is performed in order to remove the smear layer and to improve their biocompatibility. After the removal of the smear layer, the dentinal tubules are exposed and these serve as chemo-attractants for periodontal fibroblasts[2]. Apart from surgical options, various adjunctive agents have been applied to promote healing and further enhance clinical outcomes. These include root conditioners (e.g., citric acid, tetracycline HCI, EDTA, phosphoric acid, and hydrogen peroxide) [3], enamel matrix proteins, recombinant human growth factors, platelet-rich plasma, and dentin

bonding conditioner. In addition to chemical conditioning, the applicability of different laser systems such as CO_2, Nd:YAG, diode and Er:YAG laser in the removal of the smear layer have been demonstrated[4-9] . Only few reports exist on the use of CO_2 laser for root conditioning. The aim of this in vitro study was to evaluate the efficacy of CO_2 irradiation towards removal of smear layer on the periodontally involved root surface and to compare its efficacy with H_2O_2, EDTA and citric acid, using scanning electron microscope.

MATERIAL AND METHODS:

The study sample consisted of 50 single-rooted extracted teeth with hopeless periodontal prognosis showing an absence of caries and/or filling material with no hypoplastic defects.

Sample Preparation

Following extraction, teeth were washed with normal saline to remove blood and debris. The root surfaces were then scaled and root planed using hand curets to obtain a smooth hard surface. The test area on each tooth was the proximal surface. The specimens approximately 1 mm thick to the size of 5 mm × 5 mm were sliced from the proximal region 3 mm apical to the cervical line using a water cooled high speed bur. The specimens were then washed and cleaned with normal saline.

Experimental Design

Specimens thus collected were divided into 4 groups randomly as below. Group A (35 specimens) was divided into 7 sub groups (A1, A2, A3, A4, A5, A6, A7) of 5 specimens each and irradiated with CO_2 laser at different energy densities (0.2sec, 0.4sec, 0.6sec, 0.8sec, 1.0sec, 1.2sec, 1.4sec). Group B consists of 5 specimens treated with 6% hydrogen peroxide. Group C consists of 5 specimens treated with EDTA (pH 7.4). Group D consists of 5 specimens treated with citric acid (pH 1).

Hydrogen peroxide, EDTA and citric acid of Groups B, C and D were rubbed vigorously on the prepared specimen using cotton pellets for 3 minutes. The pellets were changed every minute. Immediately after chemical treatment the specimens were rinsed thoroughly with normal saline. A CO_2 laser with helium – neon laser guide(Aarvam medical systems, pondicherry, india) was used for irradiation of the specimens at a measured power of 3 watts.

Each subgroup of group A was exposed to laser irradiation for 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 seconds time intervals respectively.The specimens thus treated were fixed in 2% glutaraldehyde in 0.1M cacodylate buffer at room temperature for 1 hour and then washed with the same buffer and post fixed in cacodylate buffered 1% osmium tetra oxide for 1 hour.

The specimens were then etched with cacodylate buffer and dehydrated in a graded series of aqueous ethanol and finally were immersed in iso amyl acetate and dried. The specimens were then mounted on brass stubs and sputter coated with platinum for 2 minutes in sputter coater. All the specimens were examined under a scanning electron microscope and were photographed at X 3,500. magnification. The photographs were analyzed for the number and diameter of dentinal tubules exposed per 100 μm^2. The diameter of the dentinal tubules exposed was measured using a vernier calipers.

RESULTS

Mean and standard deviation of mean number and diameter of dentinal tubules exposed per 100 μm^2 were estimated from the sample for each study Groups (Table 1 and 2). Mean values were compared by kruskel-Wallis and one way Anova. Mann Whitney u-test was employed to identify the significant Groups (Table 3 and 4).

Mean number of exposed dentinal tubules in Groups A4, A5, B, C and D were significantly higher than in Groups A1, A2, A3, A6 and A7 at $p < 0.05$. Further, the mean number of exposed dentinal tubules in Group A4 was significantly lower than Group A5 ($p < 0.05$). Finally, the mean number of dentinal tubules exposed in Group B was significantly lower than the mean number of dentinal tubules in Groups C & D ($p < 0.05$). However, no other contrasts were significantly different. (Table 3 and 4)

Mean diameter of exposed dentinal tubules in Groups A1, A2, A3 and A7 were significantly lower than that in A4, A5, B, C and D at $p < 0.05$. Also, the mean diameter of dentinal tubules in A6 was significantly lower than A5, C and D at $p < 0.05$. However; no other contrasts were significantly different (Table 3 and 4)

DISCUSSION

The aim of the study was to evaluate the efficacy of CO_2 laser and other chemical agents in removing smear layer on periodontally involved root surfaces. The efficacy of laser and chemical agents was determined by measuring the number and diameter of dentinal tubules exposed per 100 μm^2 at the experimental site using scanning electron microscopy.

The surface of specimens which received laser irradiation for 0.2 seconds of subgroup A1 failed to expose any dentinal tubules .The surface also showed rough topography with irregular and uneven surface texture. In certain areas, the surface appeared granular in appearance, which corresponded with the presence of smear layer (Fig. 1). Similar findings were observed in specimens irradiated with 0.4 and 0.6 seconds of subgroups A2 and A3 (Fig-2 and 3).

The specimens of subgroups A6 and A7 which were irradiated for 1.2 and 1.4 seconds, respectively, also showed absence of exposed tubules, but, unlike subgroups A1 to A3, surface charring was visible (Fig-6 and 7) .The charring was more pronounced in sub- group A7. The surface of these specimens exhibited cracking and pitting and crater formation.

Similar results were reported by Sharite et al [10]where in the effect of CO_2 laser on root surface with continuous mode of irradiation was studied and reported that an increase in power setting and exposure time resulted in corresponding increase in the width and depth of damage to hard tissues. They concluded that the use of pulsed laser may induce less thermal damage to root surface. These changes are probably related to temperature rise associated with longer exposure time. These observations provide cause for concern regarding potential thermal, pulpal and periodontal damage in clinical situations.

Barone [5] studied the influence of continuous, pulsed, focused and defocused modes of CO_2 laser on periodontally involved root surfaces and concluded that continuous, focused mode caused damages to dentinal surfaces such as craters and fissuring whereas in our present study CO_2 laser on continuous, focused mode did not produce the above stated effects. Those effects might be related to optimal wavelength used.

Specimens irradiated with CO2 laser at 3 watts for 0.8 and 1 second of subgroups A4 and A5, showed presence of exposed dentinal tubules (Figs. 4 and 5). The openings of dentinal tubules exposed were not clear in the specimens irradiated with laser for 0.8 second (Fig. 4), whereas with laser irradiation for 1.0 second at 3 watts power the surface appeared smooth and flat with many clear orifices of the dentinal tubules (Fig. 5). The granular smear layer was totally absent.

Misra[11] in their study using CO_2 laser for 1.0sec on periodontally involved root surfaces stated that root conditioning favors attachment for periodontal regeneration due to the removal of the smear layer. The result of the above study is

similar to our findings where CO_2 irradiation resulted in complete detoxification of the root surface.

These findings are in accordance with the previous study by V. Pant[6]Tani [12]and et al. who reported that CO_2 laser irradiation removed the smear layer completely and enhanced periodontal regeneration on bovine teeth.

When mean diameter of dentinal tubules exposed to CO_2 laser subgroups were compared, subgroup A5 showed greatest diameter as compared to the rest with the mean value of 4.8 ± 0.9. The dentinal tubules appeared less clear in A4 suggesting that they are only partially exposed. This difference between A4 and A5 subgroup might be attributed to the existence of the smear layer deep in the dentinal tubule. Complete elimination of smear layer was achieved when the exposure time was increased subsequently to 1.0 sec as in A5 subgroup.

Crespi[4] studied the effect of CO_2 laser on periodontally involved root surface in terms of the ability of fibroblast to migrate and attach to laser treated root surface. The result is concurrent to our study in that the CO_2 laser not merely favored better attachment of fibroblast through root conditioning but also produced bactericidal effect when used at low energy powered level. The mean number of dentinal tubules exposed per 100 μm^2 in specimens treated with H2O2 was 0.753, which was the lowest of all the groups. The surface of these specimens presented an amorphous surface along with exposed dentinal tubules (Fig. 8). This amorphous surface could be due to incomplete removal of smear layer by H2O2. The orifices of exposed dentinal tubules were not as clear as that observed groups A5, C, and D.

The specimens of Group C (EDTA) showed a speckled surface with clear funnel shaped openings of dentinal tubules. The presence of smear layer was not detected in these specimens (Fig. 9). Similar findings were reported by Blomlöf [1].

The specimens treated with Group D (citric acid) showed a smooth surface with no traces of smear layer. Funnel-shaped opening of dentinal tubules was observed in these specimens (Fig. 10). These findings are in conformity with the studies conducted by Polson [2].

Even though Hydrogen peroxide, EDTA and Citric acid conditioning agents are effective in removing the smear layer there was funnel shaped widening of exposed dentinal tubules and wide dentinal tubules reduces the attachment area and hence are not favorable for enhancing periodontal regeneration as compared to the CO_2 laser Group.

Wilder-Smith [8] et al reported that EDTA causes removal of smear layer with surface cracking and were not effective in opening the dentinal tubules. In the present study, EDTA was found to be effective in removing the smear layer but with the corresponding increase in the diameter of the dentinal tubule.

Citric acid was also equally efficient in removing the smear layer as A5 subgroup with a mean number of dentinal tubules exposed ranging from 2.8 ± 0.4. However, the exposed dentinal tubules showed funnel shaped widening. Wilder-Smith[8] et al. reported that funnel shaped widening of the dentinal tubules reduces the surface area available for reattachment and hence was not favorable as compared to CO_2 laser A5 subgroup.

Differences between our results and those of other studies may be related to diseased status of the dentine specimen utilized, the extent of instrumentation and the concentration of demineralizing solution, the time of exposure to the demineralizing agents or a combination of these variables. Additional studies on these variables might add to the knowledge necessary for clinical use.

These findings are in accordance with the previous study by V. Pant[6] Tani [12] and et al. who reported that CO_2 laser irradiation removed the smear layer completely and enhanced periodontal regeneration of bovine teeth.

The above findings suggest that laser irradiation of 1.0 second is able to remove the smear layer with minimal change in the diameter of the dentinal tubules. On the other hand, the diameter of the dentinal tubules in the specimens treated with H_2O_2, EDTA, and citric acid was found to be increased. Maximum increase was noticed in citric acid treated specimens.

CONCLUSION

The surface of specimens that received laser irradiation for 0.2, 0.4, 0.6 sec failed to expose any dentinal tubules and no surface alteration were visible. Laser irradiation of 1.2 and 1.4sec produced surface charring. The surface of specimens that received laser irradiation for 0.8 and 1.0sec completely removed the smear layer. 0.8 sec irradiation exposed maximum number of dentinal tubules per 100 μm^2 followed by citric acid, EDTA, 1.0sec irradiation and H_2O_2. The diameter of dentinal tubules exposed by citric acid and EDTA was drastically higher than 0.8, 1.0sec and H_2O_2.

In view of these findings, it is concluded that CO_2 laser can produce better regeneration of periodontal tissues if it is used as a root conditioner during periodontal regeneration procedures. Further research must be undertaken with tissue culture studies to substantiate the reported findings of the present study, to observe whether root surfaces irradiated with a CO_2 laser enhances or inhibits the fibroblast migration and attachment to the root surface.

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Table 1 – Mean, Standard Deviation And Test Of Significance Of Mean Number Of Dentinal Tubules Per 100 μm^2 Of Group A (A1, A2,A3,A,4,A,5,A6,A7)

Group	No. of Tubules	p - Value	Significant groups at 5% Level
	Mean ± S.D		
A1	0.0 ± 0.0	<0.0001 Significant	A1 vs A4,A5
A2	0.0 ± 0.0		A2 vs A4,A5
A3	0.0 ± 0.0		A3 vs A4,A5
A4	1.2 ± 1.3		A4 vs A5,A7
A5	3.0 ± 0.7		A5 vs A6,A7
A6	0.2 ± 0.4		
A7	0.0 ± 0.0		

Kruskel – Wallis one way Anova was used to calculate the P- value.
Mann-Whitney u-test was employed to identify the significant groups at 5% level

TABLE- 2 – – Mean, Standard Deviation And Test Of Significance Of diameter Of Dentinal Tubules Per 100 μm^2 of Group A (A1, A2,A3,A,4,A,5,A6,A7)

Group	*Diameter* *Mean ±S.D*	*p - Value*	Significant groups at 5% level
A1	0.0 ± 0.0	<0.0001 Significant	*A1 vs. A4, A5*
A2	0.0 ± 0.0		*A2 vs. A4, A5*
A3	0.0 ± 0.0		*A3 vs. A4, A5*
A4	3.4 ± 3.4		*A4 vs. A7*
A5	4.8 ± 0.9		A5 vs. A6, A7
A6	0.6 ± 1.3		
A7	0.0 ± 0.0		

Kruskel – Wallis one way ANOVA was used to calculate the P- value.
Mann-Whitney u-test was employed to identify the significant groups at 5% level

Table 3 – Comparison Of Mean Number Of Dentinal Tubules Exposed Per 100 μm^2 between Group A (A1,A2,A3,A4,A5,A6,A7) And Groups B, C And D

Group	No. of tubules Mean ± S.D	p - value	Significant groups at 5% level
A1	0.0 ± 0.0	< 0.0001 Significant	A1 vs. A4,A5,B,C,D
A2	0.0 ± 0.0		A2 vs. A4,A5,B,C,D
A3	0.0 ± 0.0		A3 vs. A4,A5,B,C,D
A4	1.2 ± 1.3		A4 vs. A5,A7,D
A5	3.0 ± 0.7		A5 vs. A6,A7,B
A6	0.2 ± 0.4		A6 vs. C,D
A7	0.0 ± 0.0		A7 vs. B,C,D
B	0.6 ± 0.5		B vs. C, D
C	1.8 ± 1.1		
D	2.8 ± 0.4		

* One way ANOVA was used to calculate the P value.

Tukey – HSD procedure was employed to identify the significant groups at 5% level.

Table 4 – Comparison Of Mean Diameter Of Dentinal Tubules Exposed Per 100μm^2 between Group A (A1,A2,A3,A4,A5,A6,A7) And B, C And D.

Group	Diameter	p – value	Significant groups at 5% level
	Mean ± S.D		
A1	0.0 ± 0.0		A1 vs. A4,A5, B,C,D
A2	0.0 ± 0.0		A2 vs. A4,A5 B,C,D
A3	0.0 ± 0.0		A3 vs. A4,A5,B,C,D
A4	3.4 ± 3.4		A4 vs. A7
A5	4.8 ± 0.9		A5 vs. A6,A7
A6	0.6 ± 1.3		A6 vs. C,D
A7	0.0 ± 0.0		A7 vs. B,C,D
B	4.6 ± 4.3		B vs. C, D
C	5.9 ± 1.6		
D	5.9 ± 1.1	<0.0001 Significant	

* One way ANOVA was used to calculate the P value.

\# Tukey – HSD procedure was employed to identify the significant groups at 5% level

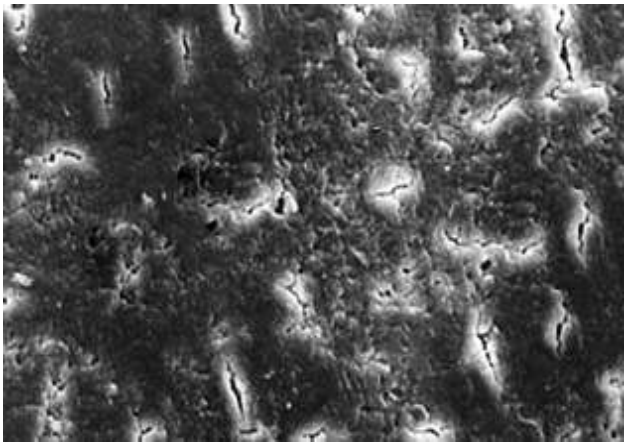

Fig 1: Scanning electron micrograph showing no exposure of dentinal tubules for CO_2 laser at 0.2sec

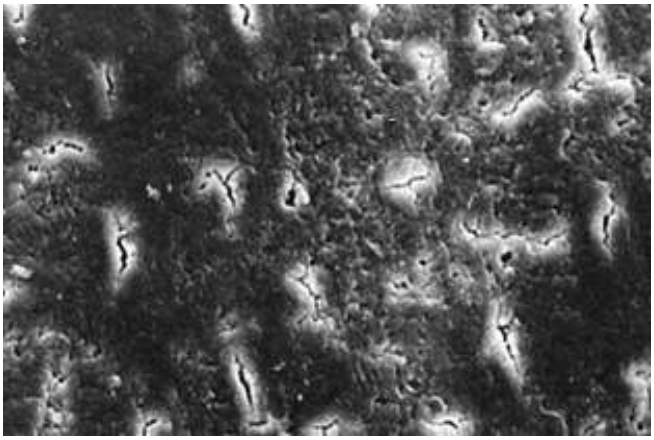

Fig 2: Scanning electron micrograph showing no exposure of dentinal tubules for CO_2 laser at 0.4sec

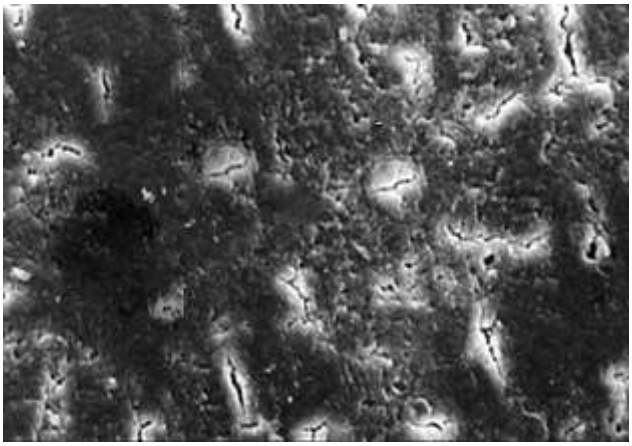

Fig 3: Scanning electron micrograph showing no exposure of dentinal tubules for CO_2 laser at 0.6sec

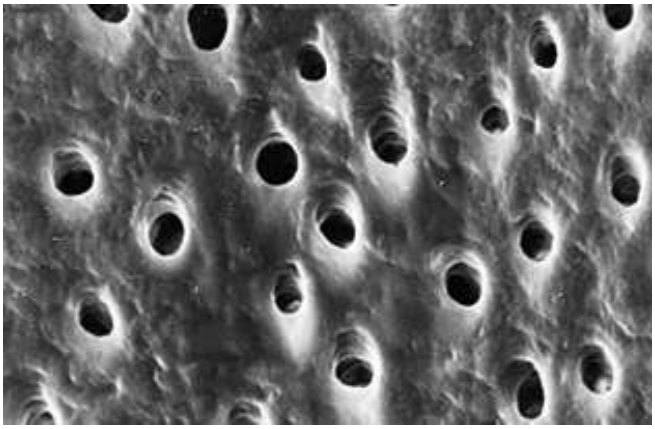

Fig 5: Scanning electron micrograph showing exposure of dentinal tubules for CO_2 laser at 1.0sec

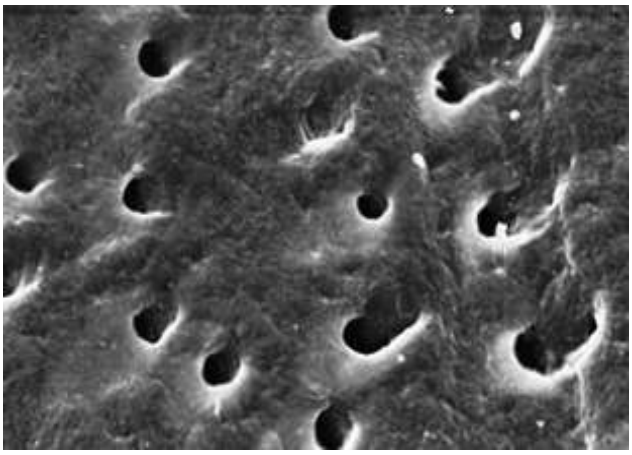

Fig 4: Scanning electron micrograph showing exposure of dentinal tubules for CO_2 laser at 0.8sec

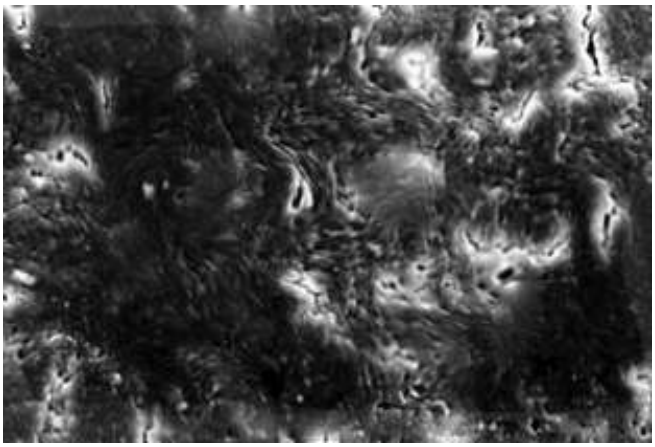

Fig 6: Scanning electron micrograph showing surface charring for CO_2 laser at 1.2 sec

Fig 7: Scanning electron micrograph showing surface charring for CO_2 laser at 1.4sec

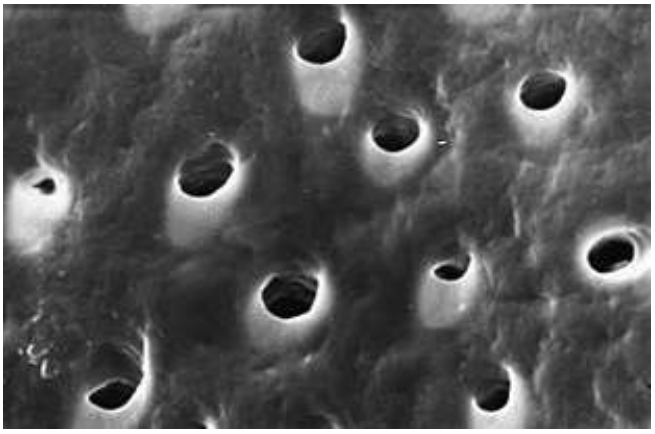

Fig 9: Scanning electron micrograph showing exposure of dentinal tubules with EDTA

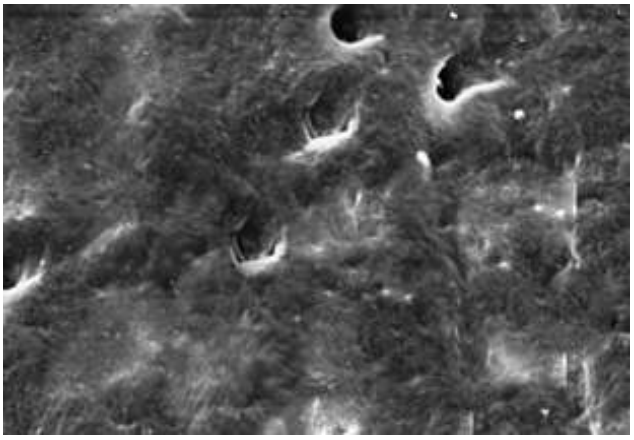

Fig 8: Scanning electron micrograph showing exposure of dentinal tubules with H_2O_2

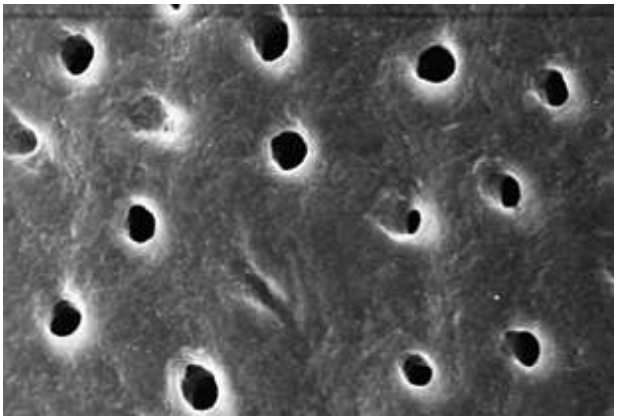

Fig 10: Scanning electron micrograph showing exposure of dentinal tubules with citric acid