



## Comparative Evaluation of Root Surface Biomodification on Extracted Teeth Using Citric Acid, Laser And Photodynamic Therapy: A Field Emission Scanning Electron Microscopic (FESEM) study

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### Abstract

#### Introduction:

Regenerative periodontics is considered to be the panacea for periodontal disease alleviation. Root biomodification is considered a potential therapy for facilitating new attachment on the denuded and diseased root surface.

#### Objective:

The purpose of this ex-vivo study was to evaluate morphological changes at electron microscopic level that occur by altering the root surface using citric acid, Er-YAG laser & PDT

#### Methodology:

A sample size of thirty (30) was evaluated in this study. The null hypothesis was that there is no impact of root surface biomodification on increasing the diameter of dentinal tubules. Subsequently, randomization and blinding was performed and the root surfaces were treated by various root biomodifiers viz Citric acid, Er YAG laser, and PDT. Finally, surface morphological changes were analyzed under FSEM. Comparative statistical analysis was performed by using SYSTAT-13 software.

#### Results & Conclusion:

A statistically significant change in the diameter of dentinal tubules using PDT and Er-YAG was observed when compared with the control samples. However, the removal of smear layer was statistically not significant. PDT and Er-YAG have a greater effect on dentinal tubules & may act as a suitable conditioning agent.

**Keywords:** Root biomodification, Root conditioning, LASERS, Er-YAG, Citric acid, photodynamic therapy, Smear layer, Diameter of Dentinal tubules

### Introduction

Periodontal regeneration has been the most sought-after goal in periodontics. Periodontal pathogens and their toxins in periodontitis cause pathologic alteration on the root surface. This pathologically altered tissue cause fiber attachment loss and loses its

biocompatibility due to which chemotactic movement of regenerative cells are retarded [1]. The goal of periodontal therapy to provide a hospitable environment for epithelial and connective tissue cells to adhere and facilitate periodontal regeneration [2]. Studies have shown that scaling and root planing cannot completely remove diseased cementum

therefore approaches like root biomodification have been proposed [3,4]. Root biomodification after scaling and root planning shows promising results and effectively removes endotoxin and smear layer [5]. Many chemical agents, growth factors and laser have been used and effectivity have been tested in vitro [6,7] and in vivo [8,9].

The present study was carried out with the aim of evaluation of surface morphological changes and presence of smear layer after irradiation with Er:YAG laser, photodynamic therapy and citric acid on extracted human teeth using field emission scanning electron microscopy (FESEM). The null hypothesis was set that there is no impact of root surface biomodification on increasing the diameter of dentinal tubules.

The objectives of this study were:

1. To evaluate and compare the diameter of dentinal tubules at electron microscopic level that occur by using citric acid, Er-YAG laser & PDT
2. To assess and compare the presence and absence of smear layer at electron microscopic level that occur by altering the root surface using citric acid, Er-YAG laser & PDT

## Materials And Methods

A total five single-rooted teeth lost due to chronic periodontitis were selected for the study. The root surface was mechanically treated by hand scalar to obtain a smooth, clean surface then segmental grooving was performed on the radicular surface and divided into three equal parts (cervical third, middle third & apical third) subsequently roots were sectioned longitudinally using low-speed diamond bur under copious water irrigation. Randomization and blinding was done and divided into four groups.

Group 1: Treated by photodynamic therapy using methylene blue dye with low-level laser for 1 minute rinsed with distilled water

Group 2: Irradiation with Er -YAG for 10 seconds in noncontact mode with copious irrigation.

Group 3: Treated by citric acid of PH 1 for 3 minutes and then rinsed with distilled water.

Group C: Another part of a longitudinal section of the tooth has been taken as a control, divided into three segments.

All the specimens in each group were treated with the respective root biomodification procedure for respective time intervals followed by washing with distilled water.

## FESEM Sample Preparation

Samples were prepared for FESEM. Specimen were mounted on stubs by using conductive carbon tape. As a biological sample have a very low density and will lead to absorption of electron beam so platinum powder coating was done for making specimen surface conductive. Specimens were transferred into a scanning electron microscope and a high vacuum was created. A uniform electron microscope scanning has been performed at 5000, 10000, 25000 & 50000 resolution, and the diameter of dentinal tubules and smear layer were evaluated (Figure 1,2,3,4).

## Statistical Analysis

Statistical analysis was conducted by utilizing SYSTAT-13® software to analyse the results. Comparison of the diameter of dentinal tubules and presence or absence of smear layer at electron microscopic level that may occur by altering the root surface using above mentioned biomodifiers were analyzed and compared with the control group. Results were tabulated and Statistical analysis was performed. The mean values of the variables between the two groups were compared by Nonparametric Kolmogorov-Smirnov Test, post hoc analysis using Lilliefors's probability criteria was executed and 'p' values were also calculated. A 'p' value of less than 0.05 was set for statistical significance.

## Results

### Diameter of Dentinal Tubules: -

Comparison of the mean diameter of dentinal tubules by altering the root surface using above mentioned biomodifiers were analyzed and compared with the test group. To access the normality of sample distribution the normalized graphical representation (Graph A) of mean diameter was adopted it showed a positive skewed because of the small sample size. To reduce the outlier, effect a post hoc analysis using Lilliefors probability criteria was executed. P values for PDT & Er YAG are less than 0.05 i.e., the test is

statistically significant (Table 1). A statistically significant change in the diameter of dentinal tubules using PDT and Er-YAG was observed. Er-YAG has a greater effect on dentinal tubules followed by PDT & thereafter citric acid.

### Smear Layer :-

Comparison of the presence or absence of smear layer at electron microscopic level that may occur by altering the root surface using above mentioned biomodifiers were analyzed and compared with the test group. The variables between the two groups were compared by Nonparametric Kolmogorov-Smirnov Test, post hoc analysis using Lilliefors's probability criteria was executed and comparative graphs (Graph B) were plotted between all three biomodifiers and the control group. P values (Table 2) for the presence of smear layer for all three biomodifiers were more than 0.05. Removal of smear layer was statistically not significant in any group but the maximum amount of smear layer removal seen in Er-YAG group.

### Discussion

Periodontitis causes substantial pathological alteration on the root surface which restricts healing of a wound that does not fully restore the architecture and function of the lost periodontium [10]. The pathologically exposed root contains bacteria and their endotoxins restrict collagen fibers insertion on the affected root surface and may no longer serve as an acceptable root surface for cell attachment which can produce periodontal regeneration [11]. Successful periodontal regeneration can be achieved by biomodifying the roots so that firm initial clot stabilization occurs on the exposed collagenous root surface and firm clots facilitate regenerative cells to migrate and adhere to the root surface [12].

The concept of acid demineralization on root surface was demonstrated by Urist way back in the nineteenth century and opened a new era in relation to the inductive properties of demineralized dentin. It was evident from his study that biomodification had the ability to induce the differentiation of regenerative cells [13]. The mechanisms of biomodification that can facilitate regeneration include effective removal of smear layer, the removal of diseased cementum, and the exposure of the root-associated collagen [14]. These mechanisms lead to

early fibrin linkage, increased fibronectin-binding sites as well as accelerated mesenchymal cell adhesion, chemotaxis, and growth. Simultaneously the apical growth of epithelium is inhibited by initial clot stabilization and early cementogenesis [15].

The present study was undertaken after considering the current fact that pathologically altered root surfaces do not favour regeneration of the periodontal tissues due to the altered surface characteristics under FESEM to assess the smear layer and diameter of dentinal tubules. The Study was conducted in the Department of Periodontology of a Tertiary Care Dental Centre of Armed Forces in collaboration with Indian institute of technology, New Delhi as basic research to know the morphological changes by PDT as compare with Er-YAG and citric acid. This FESEM study demonstrated that a significant change in the diameter of dentinal tubules using PDT and Er-YAG was observed, However, removal of smear layer was statistically not significant in any of group but the maximum amount of smear layer removal seen in the Er-YAG group.

Studies have been conducted which shows that Er: YAG laser facilitate removal of smear layer after root planing, removes calculus and diseased cementum, and leave a surface similar to an acid-etched appearance [16].

In this present study, the average diameter of the exposed tubules of a specimen was calculated by dividing the total diameter of tubules in the specimen by the total number in the specimens. In the present study, it was observed that the mean average of the Er-YAG group was higher than the PDT group and then after citric acid group. The difference in the tubule diameter for Er-YAG and PDT was found statistically significant ( $p < 0.05$ ). Studies have been done and concluded that PDT has a bactericidal effect on the root surface. it facilitates regenerative cells to migrate and adhere to the root surface and promote regeneration [17,18]. however, the present study concluded that PDT has a bactericidal effect as well as a morphologically altered surface and provides a conducive surface for periodontal regeneration.

The available relevant literature highlighted the fact that exposed collagen optimizes the biological

mechanisms essential for successful new attachment and regeneration. The limitations of the present study are; being an in vitro study with limited sample size and lack of histological evidence the findings cannot be extrapolated directly to an in vivo situation. The definitive way to ascertain the clinical efficacy of root biomodification, further studies with larger sample sizes and histologic studies should be carried out to substantiate the current findings.

### Conclusion

The successful outcome of periodontal regeneration therapy can be achieved when initial wound healing over a viable root surface devoid of any smear layer or biofilm. Even though controversies exist but literature reveals the potential of root biomodification. However, lasers in periodontal therapy showing a revolutionary change in regenerative therapy.

The results of the present study conclude that Er-YAG & PDT alter the diseased root surface, creating a more acceptable surface that can influence events in wound healing. Er-YAG has a greater effect on dentinal tubules followed by PDT & thereafter citric acid. The present study was conducted on a smaller sample size, further studies with a larger sample size and histologic studies should be carried out to substantiate the current findings.

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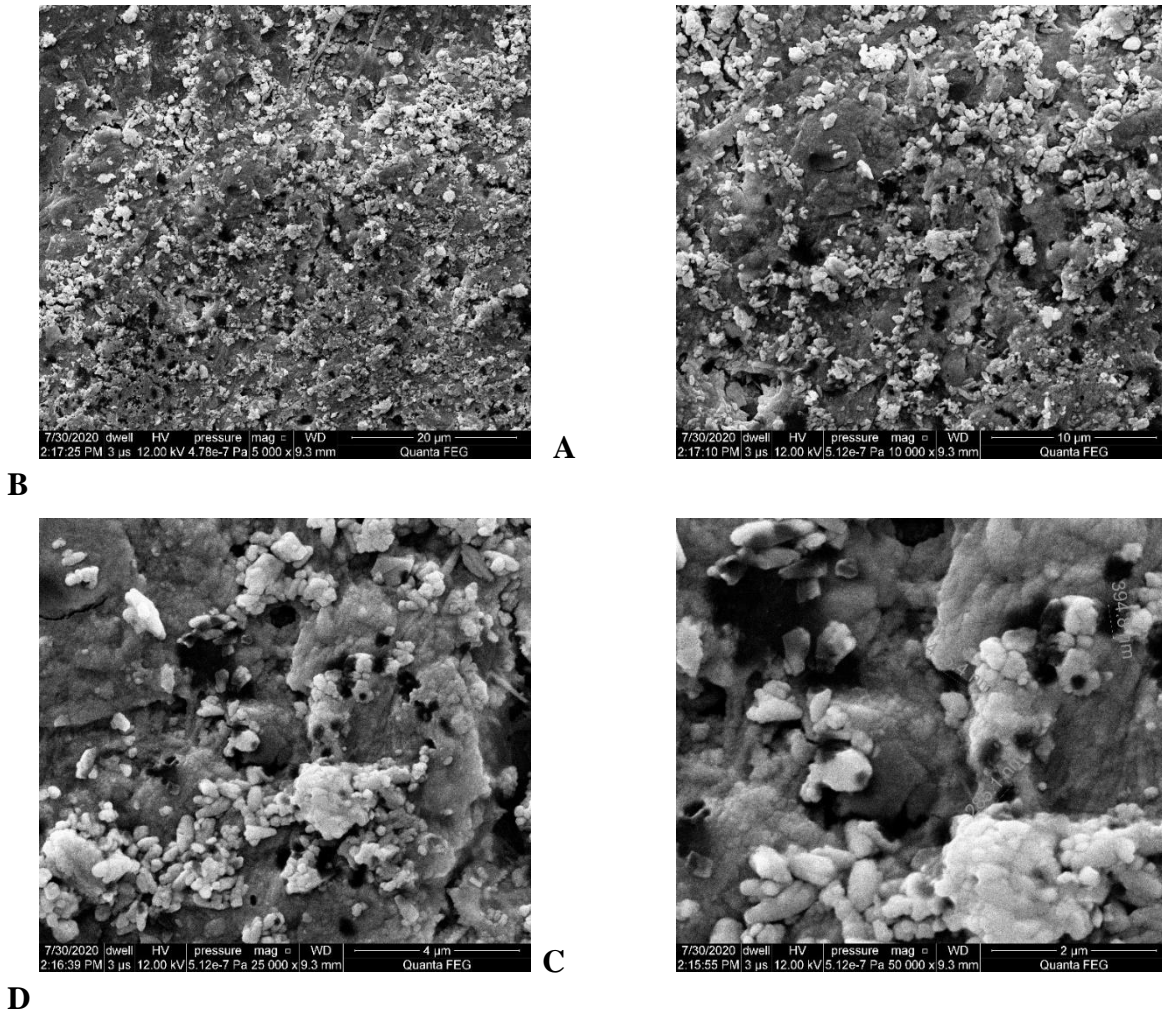


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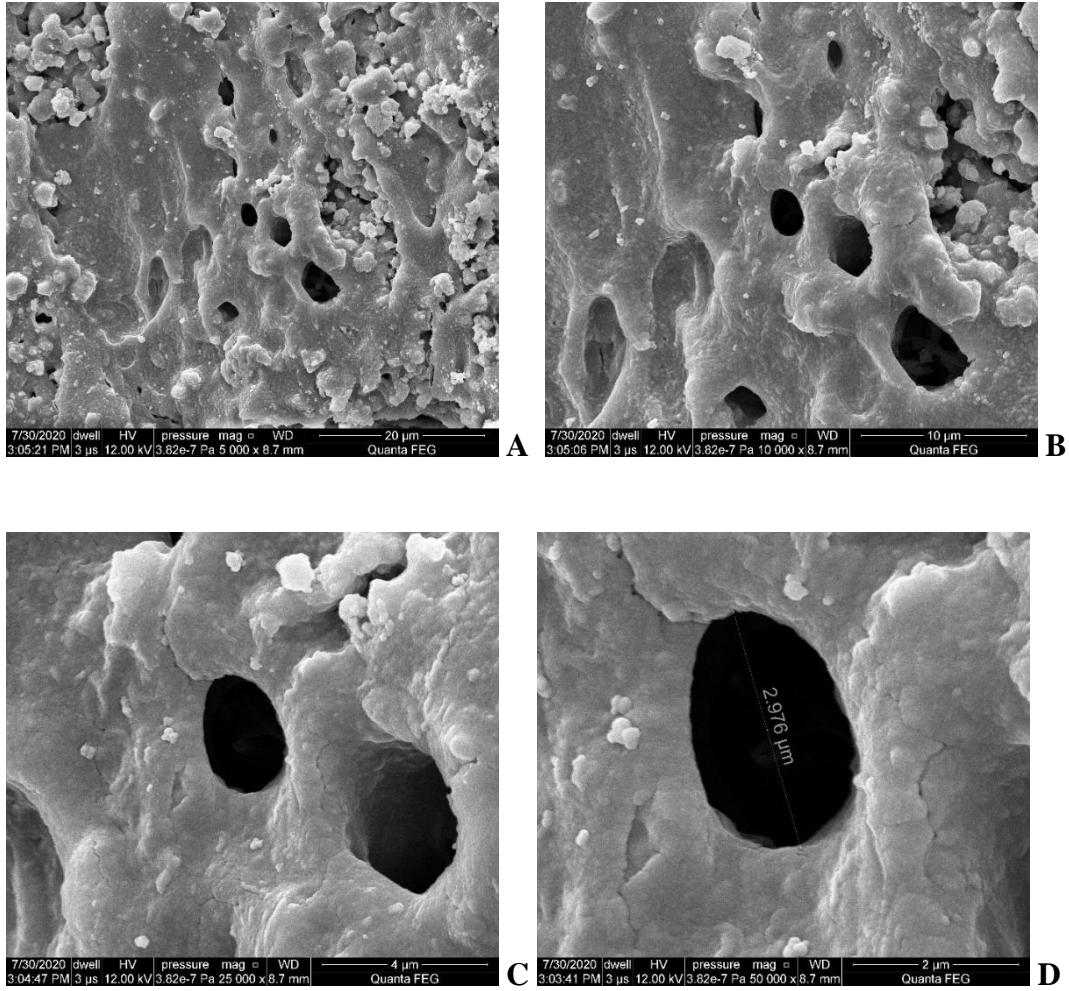
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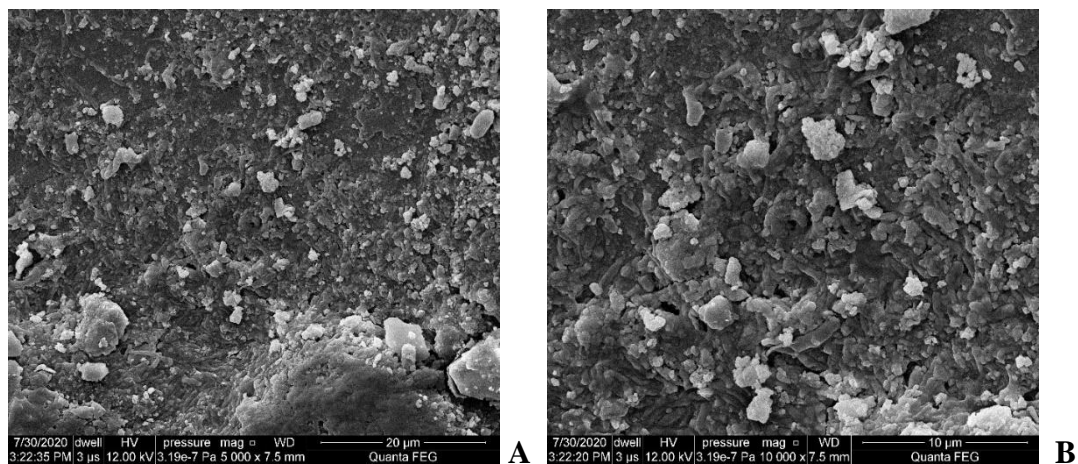
## Figures



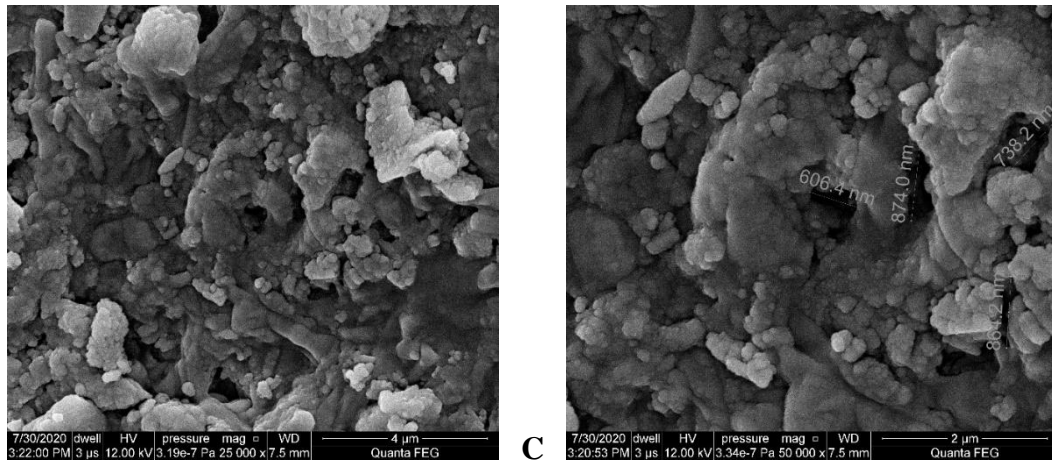
**Figure 1. Surface morphology of root surface after scaling and root planing in control group under FESEM (A) At 5000X (B) At 10000X (C) At 25000X (D) At 50000X**



**Figure 2. Surface morphology of root surface after Irradiated with Er-YAG under FESEM (A) At 5000X (B) At 10000X (C) At 25000X (D) At 50000X**

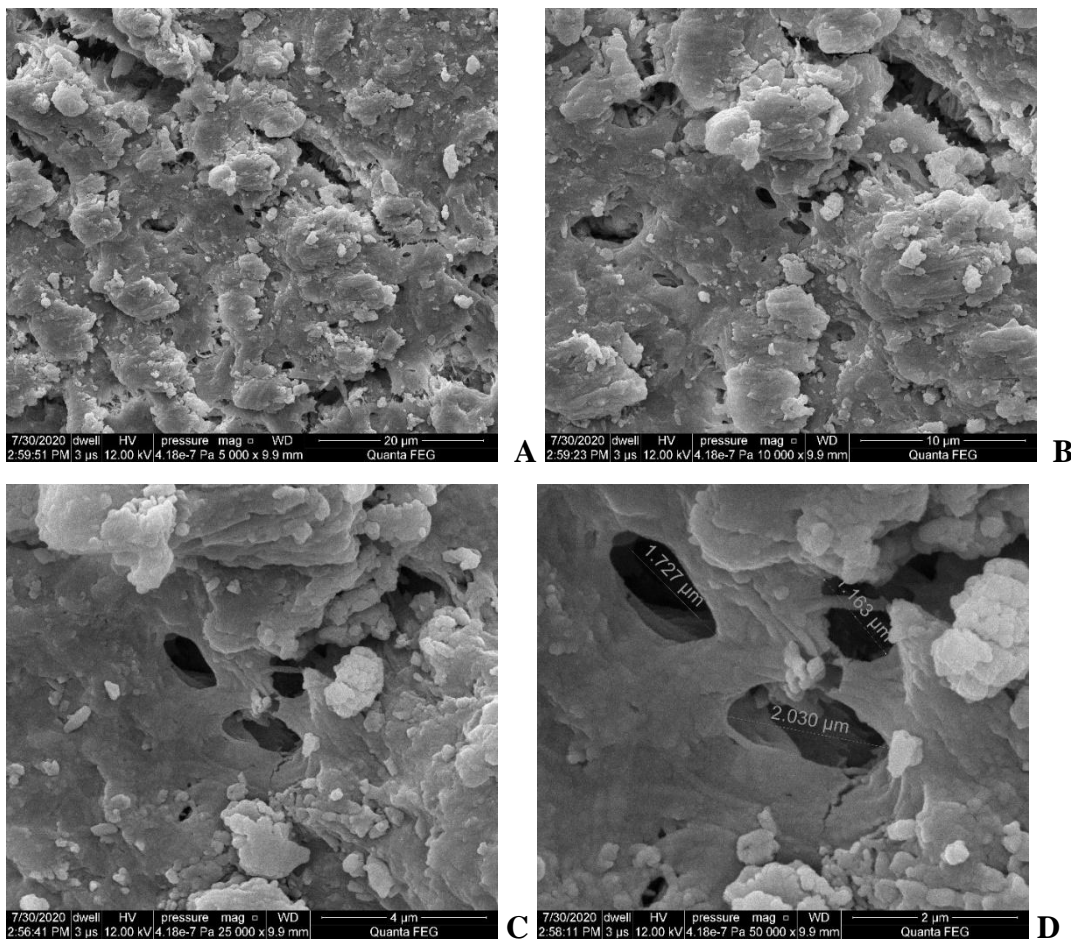






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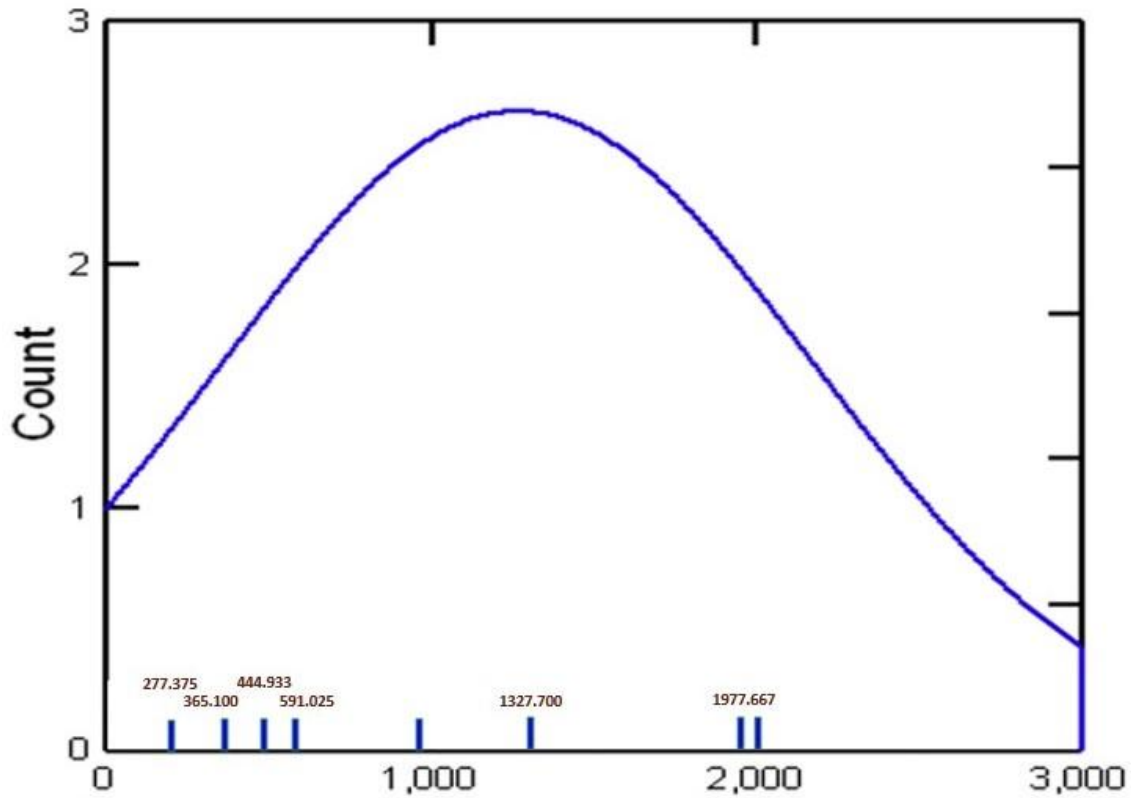
**Figure 3. Surface morphology of root surface after treated by photodynamic therapy under FESEM (A) At 5000X (B) At 10000X (C) At 25000X (D) At 50000X**



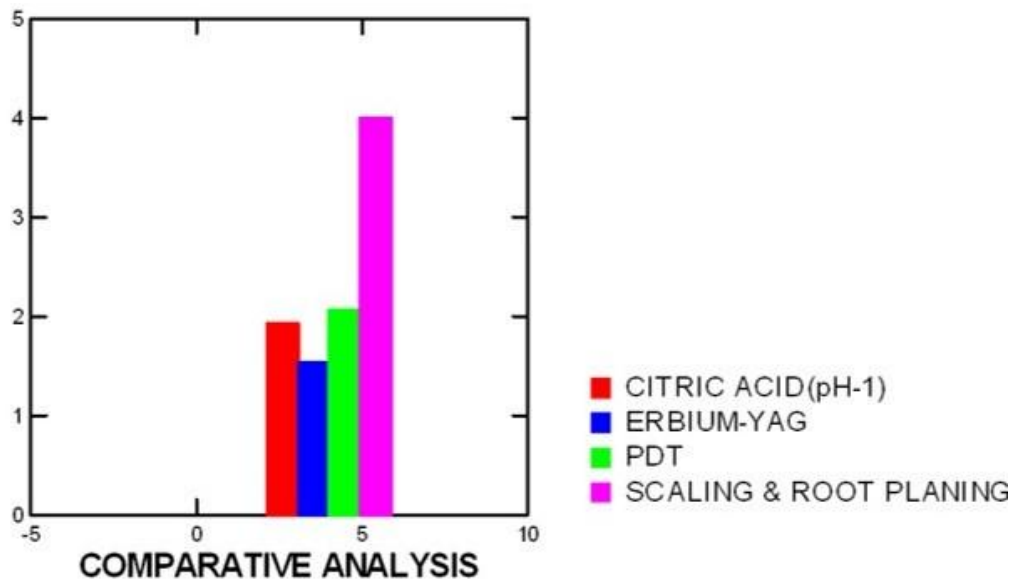
**Figure 4. Surface morphology of root surface after treated by Citric acid under FESEM (A) At 5000X (B) At 10000X (C) At 25000X (D) At 50000X**

**GRAPHS**

**Graph A: - Comparative graphical presentation for diameter of dentinal tubules between PDT, Er-YAG, Citric acid & control groups**



**Graph B: - Comparative graphical presentation for presence of smear layer between PDT, Er-YAG, Citric acid & control groups**



**TABLES:**



**Table 1. Comparison of mean diameter of dentinal tubules**

	<b>PDT</b>	<b>Er-YAG</b>	<b>CITRIC ACID</b>
<b>Location or Mean (mu)</b>	<b>591.025</b>	<b>1977.667</b>	<b>1327.700</b>
<b>Lilliefors Probability (p value)</b>	<b>0.041</b>	<b>0.05</b>	<b>0.66</b>

**Table 2. Comparative analysis of smear layer removal**

	<b>P-Values</b>
<b>PDT</b>	<b>1.92</b>
<b>Er-YAG</b>	<b>0.34</b>
<b>CITRIC ACID</b>	<b>0.79</b>