

RESEARCH ARTICLE

Comparison of Adhesion of Blood Components on Root Surfaces treated with Citric Acid, Nd:YAG, Er:YAG, and CO₂ Lasers: An *in vitro* Analysis

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ABSTRACT

Objectives: Erstwhile studies have emphasized the importance of establishing a secure fibrin linkage between the tooth-soft tissue interface for formation of a new connective attachment. Thus, periodontal regeneration is reliant on the constant adhesion, maturation and absorption of fibrin clots to the root surfaces which are compromised periodontally. Improved fibrin clot formation and blood cell attachment is being aimed by modification of the root surfaces with different agents. Limited studies have evaluated the attachment of blood cell component on various laser treated root surfaces individually.

Hence, the aim of this *in vitro* study was to evaluate and compare the adhesion of blood components on the root surfaces treated with citric acid, Nd:YAG, Er:YAG and CO₂ lasers by scanning electron microscopy (SEM).

Materials and methods: The proposed study was conducted on 35 root specimens (5 × 5 × 1 mm) obtained from extracted periodontally compromised permanent teeth. The root specimens were randomly divided in five groups depending upon the type of treatment rendered. Group I: Untreated control group, group II: Citric acid (pH:1), group III: Nd:YAG laser (112.5 m J/pulse), group IV: CO₂ laser (12.5 J/cm²), group V: Er:YAG laser (120 m J). Following the respective treatments, fresh human whole peripheral blood obtained from a healthy donor was applied to the external surface of all root specimens. The specimens were then analysed and scored for the adhesion of the blood components with photomicrographs of SEM.

Results: Statistically significant increase in the adhesion of blood components was seen in all the test groups compared to control group both citric acid and Er:YAG laser showed higher adhesion of blood cells to the root surface than the Nd:YAG laser and CO₂ laser.

Conclusion: Er:YAG laser enhanced the adhesion of blood components over the treated root surfaces. Hence, it can be safely used as a root bio-modifier ensuring stable fibrin linkage to promote periodontal regeneration.

Keywords: Blood, Clot, CO₂ lasers Er:YAG, Fibrin, Nd:YAG.

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INTRODUCTION

The challenge for periodontal therapy is to modify previously diseased root surfaces to make them compatible for new connective tissue and epithelial attachment to promote periodontal regeneration. The stabilization of fibrin network, i.e., formed following scaling and root planing is disturbed by the formation of the smear layer due to the cytotoxic residue from the plaque, calculus and the microorganisms. To achieve this, in several studies various agents have been tested to remove the smear layer and expose more collagen fibers on the root surface.¹⁻⁵ Periodontal therapy includes removal of plaque, calculus and root planing the affected root surface, which usually results in the removal of diseased cementum. Depending on the severity and the depth of the disease on the root surface, this procedure may remove the entire cementum layer, leaving dentin in contact with the periodontium. Few *in vitro*⁶⁻⁹ and *in vivo*^{10,11} studies have emphasized the conditioning of root surfaces and reducing the number of microorganisms by means of different agents adjuvant to manual instrumentation.¹² The ability of lasers to attain smear free root surfaces in conjunction with bactericidal and detoxification effects, makes them suitable alternatives to chemical agents.¹³ Previous studies on CO₂ laser when used at a relatively low energy in a pulsed and/or defocused mode found to be effective on decontamination of root surfaces.^{14,15} Nd:YAG laser treatment has been reported to remove the smear layer without significantly damaging the hard tissue, however it was associated with a considerable rise in intrapulpal and root surface temperature.^{16,17} Investigation on the effect of Er:YAG lasers have shown that this laser may be suitable for root conditioning in periodontal therapy.¹⁸ In addition Er:YAG laser treated surfaces did not interfere with adhesion of blood components.¹⁹

Except for the Er:YAG laser, there are no studies evaluating the adhesion of blood components on CO₂ and Nd:YAG laser treated root surfaces. Further, there is a lack of comparative studies between the three lasers. Hence, the aim of this study was to evaluate and compare the adhesion of blood components according to an *in vitro*

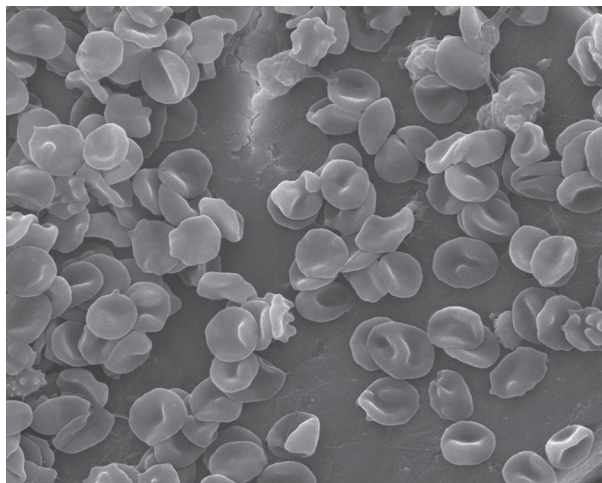


Fig. 1: Control specimen: Tooth root showing sparse, widely spaced network of fibrin strands over a smear layer with single/isolated clumps of erythrocytes. A few isolated activated platelets are also observed

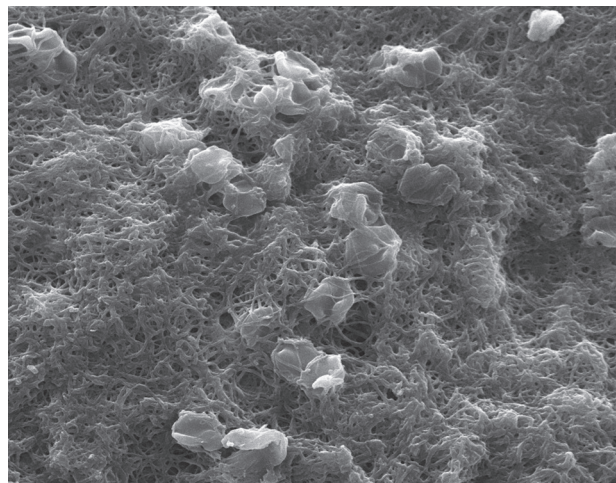


Fig. 2: Treated with citric acid: Tooth root surface treated with citric acid showed an extensive fibrin meshwork at the base beneath which a large number of concealed erythrocytes are present. A few entrapped erythrocytes are seen at the surface

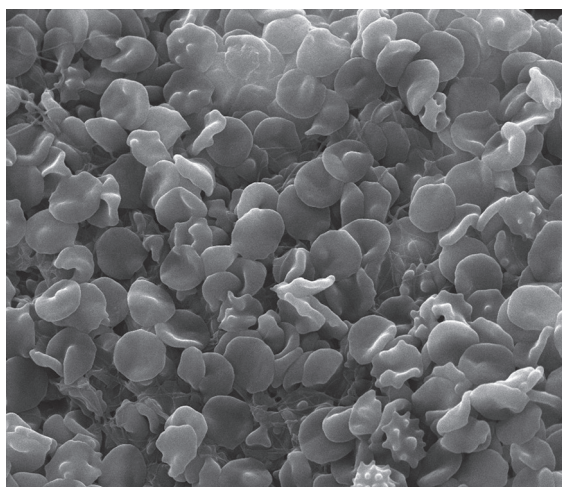


Fig. 3: Treated with Er:YAG laser: Root surface covered by a dense layer of erythrocytes and a few activated platelets on the underlying masked fibrin network

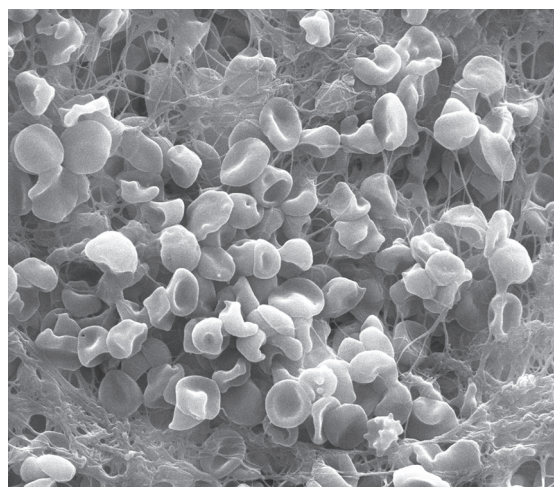


Fig. 4: Treated with Nd:YAG laser: Nd:YAG laser treated samples revealed a moderate and spread out fibrin network with majority of erythrocytes distributed irregularly on the surface. Some erythrocytes are entrapped within the fibrin network. A few isolated activated platelets are seen

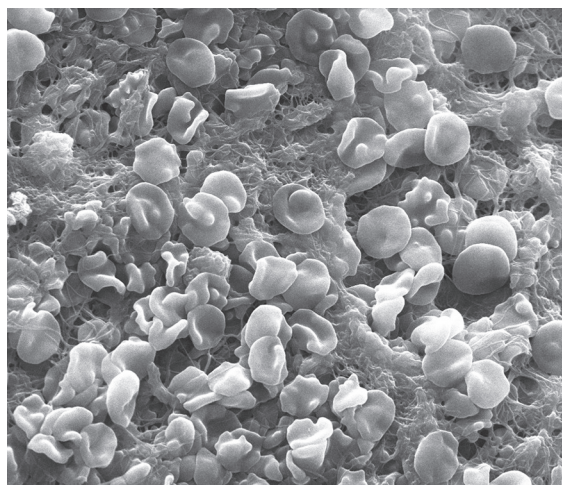


Fig. 5: Treated with CO₂ laser: CO₂ laser treated samples were covered by a moderate and relatively dense network with a few single/isolated clumps of erythrocytes on the surface. Some erythrocytes are entrapped within the fibrin network. A large number of activated platelets can be observed

screening model proposed by Baker et al,²⁰ on root surfaces treated with citric acid and irradiated with Nd:YAG, Er:YAG and CO₂ lasers (Figs 1 to 5).

MATERIALS AND METHODS

The proposed *in vitro* study was carried out on non-carious periodontally compromised extracted permanent tooth's root specimens. Teeth with cervical abrasion/erosion, any abnormalities, restorations or root fractures were excluded from the study.

Sample Preparation

All extracted teeth were scrubbed lightly with a tooth brush and rinsed in running water to remove any adherent blood, saliva and soft debris. The root surfaces were

then scaled with ultrasonic scaler (Dentsply Cavitron Bobcat Pro) and root planned with appropriate hand curettes (Gracey curette no: 1 to 14) until all the visible calculus was removed to obtain a smooth hard surface. All the treatment procedures were carried out by a single operator. Root specimens of $5 \times 5 \times 1$ mm were obtained from the prepared teeth by using the following procedure; an individual tooth was sectioned at the cemento-enamel junction and at the apical third of the root by using a diamond disk mounted on a contra-angled handpiece. The obtained root section was longitudinally split into two halves and the pulp contents were removed. It was then reduced to the predetermined dimension and stored in phosphate buffered saline (pH 7.4) until further treatment was carried out. All the root specimens were sterilized in an autoclave prior to their utilization in the study. A total of 35 root specimens were randomly divided into five groups depending on the type of treatment rendered to them.

Treatment

Group I (Control): Specimens that were not subjected to any treatment.

Group II: Specimens were treated by saturated solution of citric acid pH1 that was topically applied for 2 to 3 minutes.

Group III: Specimens were subjected to Nd:YAG laser (Quanta Ray PIV, Spectra Physics, California) treatment with energy settings of 112.5 mJ/pulse using pulsed mode at 10 pulses per second in order to reduce the alterations on the root surface.²¹ The root specimens were mounted perpendicular to the laser beam by using double sided tapes which were changed for each specimen to avoid cross contamination of bacteria. The distance between the laser beam and the tooth specimen was standardized to 5 cm and the angulation was fixed to 90°.

Group IV: Specimens were subjected to CO₂ laser (CO₂ laser surgical system PCO 15-B, LDL, China) treatment at super pulse mode with pulses of 0.99 seconds at intervals of 0.02 seconds. The laser treatment was done with energy settings of 0.5 W for 5 seconds which equals to an energy density of 12.5 J/cm². The distance between the laser tip and the surface of the specimen was standardized to 3.5 cm.

Group V: Specimens were subjected to Er:YAG laser (Fotona Er fidelis, Fotona, Slovenia, EU) treatment with energy settings of 120 mJ using super short pulse mode at 9 pulses per second for 5 seconds. The laser had a spot size of 5 mm. The distance between the laser tip and the surface of the specimen was standardized to 1 cm.

For Er:YAG and CO₂ lasers, tooth root specimens were placed in a separate well of a sterilized microtiter plate to avoid cross contamination. The laser tips were held at a constant angle of 90° and owing to its small size of focus tip of about 5 mm, it was used in an overlapping sweeping motion to cover the entire treatment area.²²

During the laser treatment, protective barrier like gloves, mouth mask and eye wear were used by both the operator and the assistants. Sterile tweezers were used to handle the root specimen during laser treatment.

Preparation of Tooth Root Specimens for Adhesion of Blood Components

After carrying out respective treatments, the tooth root specimens were placed groupwise in separate sterile petri dishes. An informed consent to participate in the study was obtained from the healthy blood donor. Fresh human whole peripheral blood (5 ml) was obtained from a donor and applied to the individual root specimens using needle and syringe. The blood was allowed to clot onto the root blocks for 20 minutes in a humidified chamber at 37 °C. Root specimens were then rinsed three times for 5 minutes in phosphate-buffered saline. Washes and rinses of the root blocks were carried out in small Petri dishes with gentle swirling motion using a rotating table-top shaker at low speed.¹⁹

Preparation of Tooth Root Specimens for Scanning Electron Microscopy

Immediately after rinsing, the blocks were fixed in 1% formaldehyde in phosphate-buffered saline for 15 minutes. After three 5-minute phosphate-buffered saline rinses, the blocks were incubated for 10 minutes in 0.02 M glycine in phosphate-buffered saline, and then rinsed again, as previously described. The samples were post-fixed in 2.5% glutaraldehyde in phosphate-buffered saline for 30 minutes and rinsed once more, as described above. The samples were dehydrated through a graded ethanol series: 25, 50, 75, 95% and three exchanges of 100% for 20 minutes at each concentration. The root specimens were allowed to dry completely, mounted and sputter coated with 200 Å of gold palladium.²³ All the specimens were examined under scanning electron microscope (JSM-840A Scanning Electron Microscope, JEOL-Japan) and photographed at 2000× magnification.

The photomicrographs obtained were analyzed and scored for the adhesion of blood components in all the study groups. The scoring was performed by a single calibrated operator.

The "blood components adhesion" rating system was as follows:

- Absence of fibrin network and blood cells
- Scarce fibrin network and/or blood cells
- Moderate fibrin network and moderate quantity of blood cells.
- Dense fibrin network and trapped blood cells.

Statistical Analysis

Descriptive analysis was done and a graph was plotted to evaluate and compare the blood adhesion in different groups of root treatments.

RESULTS

Group I: Out of 7 untreated controls, 4 root specimens exhibited a scarce fibrin network and/or blood cells and were scored 1 while 3 root specimens presented moderate fibrin network with moderate blood cells and were scored as 2.

Group II: Among the 7 citric acid treated root specimens, only 1 specimen showed moderate fibrin network with moderate blood cells (Score: 2), rest of the specimens showed dense fibrin network with entrapped blood cells. Although the number of blood cells seen was relatively less in number, considering the proven fact that it is the fibrin network that entraps red blood cells, platelets and plasma to create blood clot, a score of 3 was given.

Groups III and IV: All the specimens from Nd:YAG and CO₂ laser treated groups exhibited moderate fibrin network and moderate quantity of blood cells and was scored as 2.

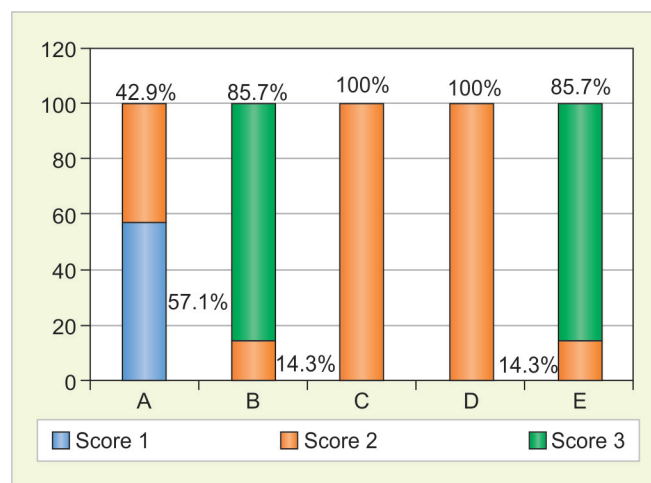
Group V: Out of the 7 Er:YAG laser treated root specimens, 6 specimens exhibited a dense layer of blood cells positioned over fibrin network and it was scored 3. Only 1 specimen which presented moderate blood cells with moderate fibrin network was scored 2.

From the photomicrographs, it was possible to identify 2 blood components, i.e, erythrocytes which were identified based on their biconcave cell morphology and activated platelets were recognized by their spheroid shape with small bulbous protrusions typically distributed over the entire platelet surface.

Individual intergroup comparison revealed that the root specimens from all the test groups showed significantly higher adhesion of blood cells as compared to the control group. When compared between the test groups, citric acid and Er:YAG treated root specimens showed the highest blood adhesion compared to the Nd:YAG and CO₂ laser treated groups (Graph 1).

DISCUSSION

Considering the increasingly developing interest in dental laser applications, various researches are being



Graph 1: Descriptive analysis of the treated root surfaces which demonstrates that group II and V showed the highest blood adhesion compared to the control group and groups III and IV

conducted to validate the use of lasers in all the branches of dentistry. The laser has widened its array of applications in the field of periodontics starting right from the debridement of gingival tissue of periodontal pockets,²⁴ to their application for calculus removal and biostimulation effect on cells.²⁵

This *in vitro* study was an attempt to evaluate the adhesion of blood components on laser treated root surfaces as compared to that promoted by conventional methods. Citric acid is one of the oldest chemical conditioning agents that has been widely researched. Nd:YAG, Er:YAG and CO₂ lasers are the most commonly used lasers that are also readily available for clinical practice.

The energy density chosen for each laser was the minimum energy that showed significant bactericidal effect on periodontopathic bacteria based on the results of previously conducted pilot study. The use of pulse delivery mode of all three lasers had the advantage of lasing intensity that was significantly greater in magnitude than that of a continuously operating laser²⁶ and prevent unnecessary damage; it facilitates the target tissue to cool between successive pulses.²⁷ Due to the lack of provision to set uniform laser parameters for all the three lasers, different pulsed modes were used according to their suitability for the study and their availability in the laser apparatus.

The results of the present study showed that, citric acid treated root surfaces promoted a dense and stable fibrin network as compared to the control group which is concordance with previous studies.^{28,29} Acid treated root surfaces produce a 4 μm demineralized surface zone consisting of exposed collagen fibrils. There is an attachment interaction between the exposed collagen and fibrin network via arcade like formations.²⁸

Er:YAG laser treated root surfaces also promoted a satisfactory adhesion of blood components equivalent to

that of citric acid. This can be correlated to the findings of Israel et al who demonstrated the changes in surface topography and surface texture of specimens treated with Er:YAG laser at energy densities of 20 and 60 J/cm² were similar to those following an acid-etching technique, i.e., removal of the smear layer and exposure of the collagen matrix.¹¹ In our study, energy densities as low as 120 m J (0.51J/cm²) produced favorable results which may be attributed to the use of pulsed mode which significantly produces greater laser intensity. It has also been demonstrated that Er:YAG treated root surfaces show a roughness of 20 to 25 µm which may have facilitated physical retention of the fibrin clot.³⁰

Nd:YAG and CO₂ laser treated root surfaces supported a superior adhesion of blood components as compared to the control group but it was significantly lower than the citric acid and Er:YAG laser treated root surfaces. Spencer et al characterized the morphologic and chemical alterations in root surfaces exposed to Nd:YAG and CO₂ lasers by using Fourier Transform Infrared photoacoustic spectroscopy. A substantial reduction in the protein content and a relative reduction in the protein/mineral ratio limited to the superficial layer representing lasers potential to ablate the organic material on the tooth surface was observed. The potential surface contamination caused by the interaction between protein and mineral component of the tooth and by product produced by CO₂ laser (cyanate & cyanamide) and Nd:YAG laser (cyanamide and ammonium) treatment also played a critical role in affecting cellular viability and attachment to laser treated root surfaces.^{31,32}

Hence, from the results of this study, it can be concluded that laser treated root surfaces are biocompatible to promote adhesion of blood components. Among the Nd:YAG, CO₂ and Er:YAG lasers, Er:YAG was found to be the most suitable laser treatment option as it produces minimally altered root surfaces and in conjunction, it does not interfere with adhesion of blood components. Further clinical trials are necessary to determine the reliability of the obtained *in vitro* results.

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