Comparing the effects of manual, ultrasonic & Er:YAG laser treatment

An in-vitro study on chronical periodontitis patients

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The elimination of calculus and bacterial micro-flora has been a time-tested modality in treating chronic periodontitis. To date, several approaches have been introduced to achieve a complete elimination of calculus, plaque and necrotic cementum. Hand and ultrasonic instrumentation has long been considered as the most effective and convenient method of plaque and calculus removal. These conventional treatments, however, leave the root surface covered with a smear layer that contains germs and bacterial endotoxins. Also, with ultrasonic instrumentation, more damaged and rougher surfaces have been seen.1, 2

Of late, research on the use of different lasers for calculus removal such as CO₂, Nd:YAG and Er:YAG has been conducted. Of these, the Er:YAG laser is believed to be the most effective due to its absorption capacity by water. It induces root surface changes which are more biocompatible for soft tissue attachment and thus improves the treatment outcome of periodontal disease.3, 4 Thus the aim of the present study was to analyse the effects of the Er:YAG laser as compared to hand and ultrasonic scaling on fibroblast attachment to periodontal diseased root surfaces.

Materials and Methods

Patients with chronic periodontitis reporting to the M.A. Rangoonwala College of Dental Sciences and Research Centre in Pune were selected for the study. The patients included in the study were non-smokers, systemically healthy and were of age ≥ 35 years. Patients selected presented with at least one periodontal involved single-rooted teeth indicated for extraction. 15 such teeth extracted from different selected patients were used in the study. Patients with a history of antibiotic treatment in the past four months were excluded from the study.

Immediately after extraction, blood, saliva and soft-tissue debris were removed by light scrubbing with a sterile scrub and by rinsing with sterile saline solution. Two specimens were obtained from each tooth by cutting with a sterile diamond disk running at low speed with sterile water coolant.

The coronal sectioning was done 1 mm below the CEJ and the apical sectioning was done 4 mm from the root apex. Longitudinal buccolingual sectioning was
done to expose the pulpal wall and to obtain two specimens from each root. To avoid contamination from the pulp, the pulpal wall was separated from the remaining outer portion of root dentin using a bur running at low speed. A total of 30 specimens thus obtained from all selected teeth were randomly assigned to three groups:

- **Group A** (n = 10 treated with hand scaling)
- **Group B** (n = 10 treated with ultrasonic)
- **Group C** (n = 10 treated with Er:YAG laser)

Specimens of Group A were hand scaled using Gracey curettes 1–2, 3–4, 5–6, 7–8 until all the visible calculus was removed. Specimens of Group B were scaled with ultrasonic for 60 sec until all the visible calculus was removed.

Specimens of Group C were treated with an Er:YAG laser system (wavelength = 2.94 µm, Fotona, Slovenia) used at 160 mJ/pulse at 10 Hz, equivalent to the energy densities of 94 J/cm² per pulse. The laser was used in contact mode under water irrigation. A laser sapphire tip was used in a parallel direction along the root surface with an angulation of 20 degree for 40 sec for each sample. Root specimens were then placed in a petri dish containing anti-bacterial and anti-fungal solution to avoid contamination for 1 hour. Specimens were then thoroughly rinsed in Dulbecco’s Phosphate Buffered Saline and covered with 2 ml fibroblast L929 suspension. Cell culturing was done at 37 degrees in a humidified atmosphere of 95 % air and 5 % CO₂ for 3 days.

5 ml of cell suspension was seeded into the tissue culture containing root samples and incubated for 3 days. At the end, the cells were rinsed with Dulbecco’s Buffered Saline (DPBS) and fixed by DPBS solution containing 4 % glutaraldehyde. Fixed samples dehydrated by passing through ethanol/water solution were immersed in hexamethyldisilazane for 30 min to complete the dehydration. Dehydrated cells were sputtered with gold and were observed by scanning electron microscopy to view fibroblast attachment to the root surfaces of the specimens.

**Results**

Fibroblast morphology on all treated surfaces was observed and was found to be different following different treatment modalities. In the Groups treated with hand scaling and ultrasonic scaling, scattered flat and healthy fibroblasts with a low number of lamellipodia and attachment extensions into the wavy surfaces, covered with smear layer, were observed. In the Er:YAG laser group, the treated surfaces were observed to be covered by a confluent monolayer of flat, spindle-shaped fibroblasts, which were firmly attached to the root surface by means of many lamellipodia and attachment extensions.

**Discussion**

In periodontal treatment, mechanical removal of plaque and calculus is mandatory to control and prevent inflammatory processes. When ultrasonic and hand instrumentation were compared in clinical studies results showed reductions in probing depth and bleeding on probing. However, mechanical instrumentation leaves the root surfaces covered with smear layer that obliterates the orifices of the dentinal tubules and contains germs, bacterial endotoxins and residual contaminated root cementum that hampers good periodontal healing and regeneration of connective tissue attachment.

Er:YAG laser, however, is shown to induce a root surface that has better biocompatibility for soft-tissue attachment. It removes lipopolysaccharides, calculus, smear layer and cementum, providing high bactericidal potential at a low energy level on the root-infected dentin layer. In the present study we found...
that the fibroblasts were tightly attached to specimens treated with Er:YAG laser as compared to specimens treated with hand and ultrasonic instruments.

Frentzen et al.\textsuperscript{10}, in a histologic study compared the effects of Er:YAG instrumentation of diseased root surfaces to mechanical removal of plaque and calculus with ultrasonic instrumentation. The results showed that ultrasonic debridement resulted in a smooth surface covered by a smear layer\textsuperscript{11} containing remnants of dental debris, contaminated root cementum, bacterial endotoxin and subgingival plaque\textsuperscript{12, 13} whereas Er:YAG laser irradiation induced a glazed microstructure presenting a rough aspect to the root surface.

Babay\textsuperscript{14} evaluated fibroblast attachment to periodontal involved root surfaces, which were either root planed with curette, ultrasonic scaler or acid chelated by different agents such as citric acid, tetracycline hydrochloride or EDTA to produce different surface textures. The results demonstrated that there was a significantly greater number of fibroblasts attached to specimens treated with citric acid, tetracycline, and EDTA than those scaled only, which means that fibroblasts were more likely to attach to rough-surfaced than to smooth-surfaced specimens.

Er:YAG laser induced a homogenous roughness to the root surfaces\textsuperscript{15-17}; this morphological roughness of lased surfaces enhances the adhesion and proliferation of fibroblasts, which are present in higher numbers than those of the ultrasonically treated specimens. This surface transformation obtained by the Er:YAG laser probably exposes chemical root substances that are highly selective for chemotaxis to fibroblasts.\textsuperscript{18}

It has been suggested that the biochemical modifications of the root surface induced by the use of an Er:YAG laser are responsible for an increase in fibroblast attachment. These modifications could be either a direct consequence of root conditioning by the exposure of some of the extracellular matrix constituents acting on the attachment mechanism of fibroblasts or an indirect effect of biochemical factors, from increased fixation on the demineralised root surface. The results of the present study concurred with the previous studies.\textsuperscript{19}

Study results were similar to those obtained by Feist et al.\textsuperscript{20}, who studied fibroblast adhesion and growth or cultured human gingival fibroblasts on root surfaces treated by both Er:YAG laser and curette. He found that fibroblasts adhered to and grew on all treated surfaces, but the group lased at 60 mJ/pulse, 10 Hz, presented a significantly higher cell count than the other groups.

**Conclusion**

Thus the present study suggests that laser treatment could be an important and useful tool to induce a modification of root surface morphology with a complete elimination of the presence of the smear layer, improving fibroblast attachment. Future extensive and well-controlled studies are needed to confirm this hypothesis.

*Editorial note: A list of references is available from the publisher.*

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