Journal of Lasers in Medical Sciences

J Lasers Med Sci 2020 Winter;11(1):74-80

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.15171/jlms.2020.13

Comparative Evaluation of the Effects of CO_2 and Er:YAG Lasers on Smear Layer Removal and Blood Cell Attachment to Tooth Root Surfaces

Narges Naghsh[1], Reza Birang[1*], Fahimeh Shafiei[2], Fatemeh Ghorbani[3], Norbert Gutknecht[4], Jaber Yaghini[1]

[1]Dental Implant Research Center, Department of Periodontology, Dental Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Department of Operative and Aesthetic Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3]Department of Periodontology, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran
[4]Department of Conservative Dentistry, Rwth Hospital, Aachen, Germany

***Correspondence to**
Reza Birang, School of dentistry, Isfahan University of medical sciences, Hezar jrib street, Isfahan, Iran.
Tel: +98913-116-1003
and +9831-37925525
Email: birang@dnt.mui.ac.ir

Published online January 18, 2020

Abstract

Introduction: The tooth root surfaces are modified by different agents for better removal of the smear layer, the formation of fibrin clots, and the attachment of blood cells. This in vitro study compared the removal of the smear layer, the formation of fibrin clots and the attachment of blood cells after exposing periodontally compromised root surfaces to ER:YAG and CO_2 laser beams.

Methods: Eighteen dentin block samples were prepared from freshly extracted periodontally compromised teeth that were deemed hopeless, and they were divided into 3 groups: exposed to Er:YAG laser beams, exposed to CO_2 laser beams, and the control group. The samples were evaluated using scanning electron microscopy and micrographs were taken. Smear layer removal and blood cell attachment were scored. Data were analyzed using Kruskal-Wallis and Mann-Whitney tests.

Results: In the Er:YAG laser group, the smear layer was removed completely. In the specimens exposed to blood, better fibrin clot formation and blood cell attachment were observed in the Er:YAG laser group. In the CO_2 laser group, the smear layer was also removed; however, there were no significant differences between the CO_2 laser and control groups in fibrin clot formation and blood cell attachment.

Conclusion: The application of the Er:YAG laser to the root dentin appears to result in the formation of a suitable surface for fibrin clot formation and blood cell attachment. Further clinical studies are necessary to support these results.

Keywords: CO_2 laser; Er:YAG laser; Root planing.

Introduction

Gingival recession is defined as the apical displacement of the gingival margin from the cementoenamel junction (CEJ), exposing the root surface to the oral environment, which causes esthetic problems and tooth hypersensitivity.[1] It is a challenge for periodontal therapy to cover denuded root surfaces; therefore, various techniques have been developed for root coverage. Studies have shown gingival recession results in diseased cementum and smear layers (in the denuded root surface), preventing cell attachment and causing inappropriate periodontal healing. The formation of the smear layer after scaling and root planing (SRP) interferes with the stabilization of the fibrin network due to cytotoxic residues from microorganisms, plaque and calculi present in the structure of the smear layer.[2]

The removal of calculi by hand instruments is time-consuming and has limitations because the root anatomy and pocket depth favor the formation of a smear layer, inhibiting cell re-attachment and serving as a reservoir for microbial growth.[3] To improve root surface debridement, the root surfaces are conditioned chemically to remove the smear layer and improve their biocompatibility. Conditioning of the root surface improves clinical outcomes. Root surface conditioners include citric acid,[4] tetracycline HCI,[5] EDTA,[6] phosphoric acids[7], hydrogen peroxide, enamel matrix proteins,[8] recombinant human growth factors, platelet-rich plasma[9] and dentin bonding conditioners.[10]

Currently, there is an ever-increasing interest in the application of lasers as an adjunct to periodontal treatment. Studies on the effects of lasers on root surface conditioning have shown successful smear layer removal. Thermal and photo-disruptive effects of laser beams result in the elimination of periodontopathic bacteria,[11,12] which might help improve the outcomes of periodontal

Please cite this article as follows: Naghsh N, Birang R, Shafiei F, Ghorbani F, Gutknecht N, Yaghini J. Comparative evaluation of the effects of CO2 and Er:YAG lasers on smear layer removal and blood cell attachment to tooth root surfaces. *J Lasers Med Sci.* 2020;11(1):74-80. doi:10.15171/jlms.2020.13.

therapy. It has been shown that different laser systems can be used to remove the smear layer, including CO_2, Nd:YAG, diode and Er:YAG lasers. The Er:YAG laser beams can also remove calculus and a superficial layer of infected cementum with no detrimental thermal effects. The effects of this laser system on periodontally involved root surfaces have been investigated in vitro[13,14] and in vivo.[15] The CO_2 laser beams exhibit excellent absorption in water and result in surface conditioning of the root and the internal side of the periodontal flap during surgery.[16] In an animal model, the periodontal tissues were regenerated after conditioning of the root surface and the vaporization of periodontal pocket soft tissue with CO_2 laser beams.[17] It is obvious that healing after SRP is caused by epithelial attachment. The first step in the wound healing process is the formation of a fibrin clot between the root surface and gingival connective tissue. In some studies, blood was applied after SRP on the root surface to mimic periodontal surgery, which resulted in the blood component attachment.

Cekici et al reported that the application of Er:YAG laser beams to the root dentin appears to result in the formation of a suitable surface for the formation of fibrin clots and the attachment of blood cells.[18] However, Theodoro et al compared Er:YAG and diode lasers and reported no significant differences in the adhesion of blood components in comparison to the control group.[19] Crespi et al reported that the CO_2 laser is a useful tool for the conditioning of the root surface and increasing fibroblast attachment to root surfaces.[20] In another study, they showed that CO_2 laser beams resulted in predictable clinical improvements when used as an adjunct to traditional periodontal surgery.[21] Therefore, there is no consistent information available on the effect of laser beams on root conditioning.

This study was undertaken to compare the efficacy of Er:YAG (2.94 μm) and CO_2 (10.6 μm) laser beams in removing the smear layer and forming a fibrin network on root surfaces.

Materials and Methods

Sample Preparation

Eighteen human single-rooted teeth with periodontal problems were extracted from non-smoking patients referring to the Periodontology Clinic, Faculty of Dentistry, Isfahan University of Medical Sciences. The extracted teeth had severe periodontal disease and were deemed hopeless. The teeth had no restorations and caries. After extraction, the teeth were cleaned in distilled water and stored in phosphate-buffered saline (PBS) solution at pH=7.4 until they were used for the purpose of the study. Then SRP was performed with manual instruments (Gracey curettes no. 5/6, Hu Friedy Co., Chicago, IL, USA) in all the samples until a smooth root surface was achieved. The tooth crowns were removed with a high-speed cylindrical bur under water irrigation. Then the roots were cut using a sterile disk at 2 mm apical to CEJ up to 4 mm above the apex. From each tooth, two dentin blocks, approximately 4×4×1 mm in size, were prepared.

Treatment Procedure

Two samples from the same mesial or distal surfaces were kept in an identified bottle containing PBS. The bottles were randomly divided into three groups for the following treatments:

Group A (GA): irradiation with Er:YAG laser beams (2.94 μm,120 mJ, air and water,15 s, 20 Hz,7-8 mm perpendicularly)

Group B (GB): irradiation with CO_2 laser beams (10.6 μm, 4 s, 5 cm, 3 W)

Group C (GC): (control group) SRP with curettes

Preparation of Root Blocks

Immediately after the application of laser beams, fresh human whole peripheral blood from a healthy donor was applied to the external root surfaces of 6 blocks in all the three experimental groups. The blood was allowed to clot on the root blocks for 20 minutes in a humidified chamber at 37°C. Subsequently, the root blocks were rinsed three times for 5 minutes in PBS in small Petri dishes using gentle swirling motions on a rotating table-top shaker at low speed. These root surface samples were used to evaluate the attachment of blood cells and the formation of fibrin networks. The 6 remaining samples in each experimental group were used to evaluate the removal of the smear layer from the root surface. The samples were then fixed in 2.5% glutaraldehyde in PBS for 30 minutes and rinsed again as described above. The samples underwent a dehydration procedure in a series of graded ethanol: 25%, 50%, 75%, 95% and three exchanges of 100%. The samples were then dried at room temperature. The blocks were mounted on aluminum stubs and stored and desiccated at room temperature for 3 days.[10]

Scanning Electron Microscopy Observations

Subsequently, the samples in all the groups were fixed in 2.5% glutaraldehyde in a phosphate-buffered solution (pH = 7.3) for 24 hours, followed by washing three times for 10 minutes in the phosphate buffer. Then the samples were dehydrated in a graded series of aqueous ethanol solutions (70%, 85%, 95% and 100% ethanol) for 10 minutes each, followed by drying overnight at room temperature. The samples were mounted on aluminum stubs and sputter-coated with a gold-palladium alloy under vacuum for 120 seconds. A representative photomicrograph was obtained from all the samples at ×3500 under a scanning electron microscope (SEM). The photomicrographs were used to score the adhesion of blood components (BCA) and the smear layer removal (SLR).

BCA Scoring

0: Absence of fibrin network

1: Scarce fibrin network and/or blood
2: Moderate fibrin network and a moderate quantity of blood cells
3: Dense fibrin network and trapped blood cells.[22]

SLR Scoring

1: No smear layer and open dentinal tubules
2: No smear layer and partially open dentinal tubules
3: No smear layer and obliterated dentinal tubules
4: Moderate smear layer and open dentinal tubules
5: Moderate smear layer and partially open dentinal tubules
6: Heavy smear layer and open dentinal tubules
7: Heavy smear layer and partially open dentinal tubules[19]

Data were analyzed with SPSS using Kruskal-Wallis and Mann-Whitney tests.

Results

SEM Analysis

Group A (the Er:YAG laser): The majority of the samples exhibited no smear layer, with partially open dentinal tubules (4 samples, score 2); one sample exhibited no smear layer, with open dentinal tubules (score 1), and one sample exhibited a moderate smear layer, with open dentinal tubules (score 4) (Figure 1.a_1-a_2).

Most of the photomicrographs (5 samples) exhibited the adhesion of blood components (score 3), characterized by a dense fibrin network and trapped blood cells, and one sample exhibited a moderate fibrin network and a moderate quantity of blood cells (score 2) (Figure 2.a_1-a_2).

Group B (CO_2 laser): The majority of the samples exhibited no smear layer, with partially open dentinal tubules (4 samples, score 2); one sample exhibited no smear layer and open dentinal tubules (score 1), and one sample exhibited a heavy smear layer, with partially open dentinal tubules (score 7) (Figure 1.b_1-b_2).

The majority of the photomicrographs (5 samples) exhibited the adhesion of blood components (score 1), characterized by a scarce fibrin network and/or blood, and one sample exhibited a moderate fibrin network and a moderate quantity of blood cells (score 2) (Figure 2.b_1-b_2).

Group C (untreated/control): The samples exhibited a moderate smear layer, with partially open dentinal tubules (score 5, 3 samples) and a heavy smear layer, with partially open dentinal tubules (score 7, 3 other samples) (Figure 1.c_1-c_2).

In this group, two samples exhibited no fibrin network (score 0); two samples exhibited a moderate fibrin network and a moderate quantity of blood cells (score 2); one sample exhibited a scarce fibrin network and/or blood (score 1); and one sample exhibited a dense fibrin network and trapped blood cells (score 3) (Figure 2.c_1-c_2).

Statistical Results

Tables 1 and 2 show the frequencies of the SLR scores and the BCA scores in groups A, B and C respectively. The application of the non-parametric Kruskal-Wallis test showed significant differences between the groups in SLR ($P=0.007$) and BCA scores ($P=0.009$). When the groups were compared with the use of the Mann-Whitney test following the analysis of SLR scoring, groups A and B exhibited lower scores compared to group C, which was statistically significant. However, the difference between groups A and B was not significant (Table 3).When BCA scoring was analyzed, group A exhibited significantly higher scores compared to groups C and B; however, there was no statistically significant difference between groups B and C (Table 4).

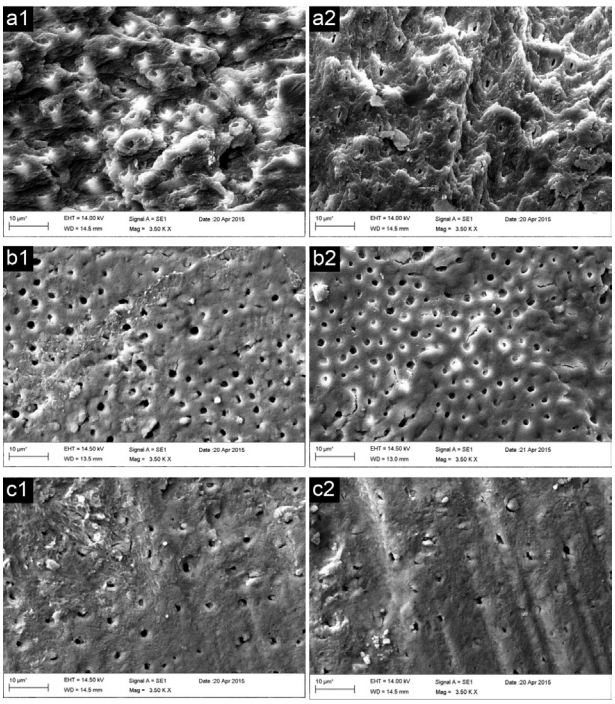

Figure 1. Photomicrographs of Root Surfaces After Treatment.
Group A (treated with the Er:YAG laser): a1) The smear layer was removed completely and dentinal tubules were open: Score 1; a2) The smear layer was removed completely and dentinal tubules were partially open: Score 2.
Group B (treated with the CO2 laser): b1) The smear layer was removed completely and dentinal tubules were open: Score 1. b2) The smear layer was removed completely and dentinal tubules were partially open: Score 2.
Group C (the control group): c1) There was a moderate smear layer and dentinal tubules were partially open: Score 5. c2) There was a heavy smear layer and dentinal tubules were partially open: Score 7.

Discussion

Gingival re-attachment on tooth surfaces is considered an important concern in periodontal treatment. This randomized, controlled, double-blind clinical trial was designed to compare root surface conditioning and blood cell attachment to these surfaces after the application of 2 different laser systems. Considering the ever-increasing interest in the application of lasers in dentistry, this in vitro study was undertaken to evaluate the effects of two different lasers. Data from the present study indicated that

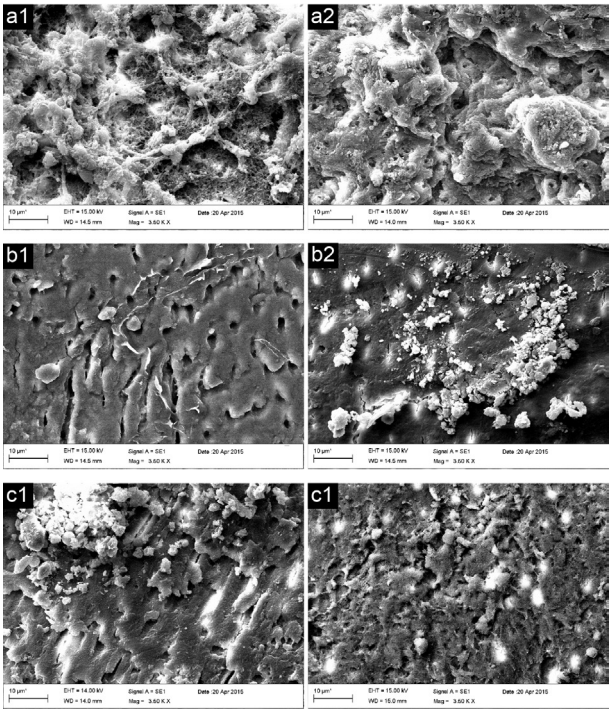

Figure 2. Photomicrographs of Root Surfaces After Fibrin Clot Formation on Dentin.
Group A (treated with the Er:YAG laser: a1) There were dense fibrin networks and trapped blood cells: Score 3. a2) There were moderate fibrin networks and a moderate quantity of blood cells: Score 2.
Group B (treated with the CO2 laser): b1) The smear layer was removed completely and dentinal tubules were open: Score 1. b2) There were moderate fibrin networks and a moderate quantity of blood cells: Score 2.
Group C (the control group): c1) There were moderate fibrin networks and a moderate quantity of blood cells: Score 2. c2) There were dense fibrin networks and trapped blood cells: Score 3.

Table 1. Frequencies of SLR Scores in the Study Groups (n=6)

Scores	A	B	C
1	1	1	0
2	4	4	0
3	0	0	0
4	1	0	0
5	0	0	3
6	0	0	0
7	0	1	3

Table 2. Frequencies of BCA Scores in the Study groups (n=6)

Scores	A	B	C
0	0	0	2
1	0	5	1
2	1	1	2
3	5	0	1

Nd:YAG laser beams positively affected the attachment of fibrin networks to tooth surfaces.

In this study, the tooth surfaces prepared by different lasers were compared through SEM. According to several studies, it appears it is safe to apply Er:YAG laser beams

Table 3. Median Values and the Comparison of SLR Scores Between Groups

Groups	Median	*P* Value
A	2	A and C (*P*=0.002) A and B (*P*=0.937)
B	2	B and C (*P*=0.026)
C	6	-

Table 4. Median Values and the Comparison of BCA Scores Between Groups

Groups	Median	*P* Value
A	3	A and B (*P*=0.002) A and C (*P*=0.026)
B	1	B and C (*P*=0.818)
C	1.5	-

to tooth hard structures, with no damage to surrounding areas.[23] Irradiation of root surfaces with Er:YAG laser beams resulted in irregular surfaces with no cracks and smear layers, leading to the conclusion that this laser is suitable for resin restorations.[24]

Laser beams initially vaporize water and other hydrated organic components of the tissue, during which the internal pressure increases in the tissue until explosive destruction of inorganic substances occurs, leading to the formation of hydrokinetic forces that can quickly ablate the tooth hard structures. This ablation mechanism is referred to as the hydrokinetic system.[25] The use of Er:YAG laser beams with air/water spray resulted in no pulpal inflammation compared to conventional methods.[26] Several studies have suggested that the initial healing processes such as the absorption and adhesion of blood components along with a fibrin clot adhering to the blood clot on the root surface are crucial determinants of the repair process between the gingival flap and the root surface.[2,27] Based on current concepts, the control of adhesion of fibrin clots to the root surface in reconstructive periodontal therapy is vital to the success of periodontal treatment.[10]

Rahimi and Babazade in a review article explained the application of Er:YAG laser and compared it with Diode laser for periodontal plastic surgery. They emphasized the significant effect of Er:YAG on forming a denseer fibrin network with blood cells attached to it and its superior efficacy rather than diode laser.[28]

When SLR scoring was analyzed, the Er:YAG laser-treated group exhibited smear layer removal with a course and irregular surface and open dentinal tubules, consistent with the results of a study by Cekici et al.[18] In relation to blood cell attachment and fibrin network formation, the samples in the Er:YAG laser-treated group exhibited a higher adhesion of blood components compared to the control and CO_2 laser groups (Figures 1 and 2 and Table 1), consistent with the results of a study by Oliveira et al.[29] There might be several explanations for this effect. A plausible explanation is that the formation of the smear layer, which consists of cytotoxic residues from microorganisms, plaque or calculi, can interfere with the

stabilization of the fibrin network,[2,22] and the presence of endotoxins and bacteria might inhibit the adhesion of plasma proteins to the root surface.[29] The Er:YAG laser has exhibited high bactericidal effects even at low energy levels. The Er:YAG laser parameters used in this study may have resulted in a decrease in bacterial counts as proposed by Ando et al., who demonstrated significant in vitro decreases in *Porphyromonas gingivalis* colonies.[30] Such a bactericidal effect increases the attachment of the fibrin network to the root surface. Poormoradi et al also evaluated root surface conditioning by the Er,Cr:YSGG laser and concluded that the laser improved the mean root coverage and the percentage of complete root coverage with the subepithelial connective tissue graft; however, these changes were not significant.[31] The application of Er:YAG laser beams results in the removal of mineralized tissues and creates holes and bumps, resulting in irregular root surfaces and a larger exposure area of collagen fibers. The exposure of collagen fibers facilitates the adhesion and formation of primary homeostatic buffers through the adhesion of plaque to the exposed collagen fibers.[19] On the other hand, an increase in root surface roughness with the application of the Er:YAG laser might result in a possible increase in bacterial plaque retention. Further in vitro and clinical studies are necessary to evaluate the possible risk of bacterial colonization on the Er:YAG laser-irradiated root surfaces in relation to the maintenance of a long-term periodontal regeneration treatment. Er:YAG laser energy is highly absorbed by water. Water is effective in cleaning the surface and decreasing thermal effects. The power settings and the use of water as a coolant during Er:YAG laser irradiation should be controlled to avoid deleterious effects on the irradiated tissues.[32,33]

In relation to the morphology of root surfaces irradiated with Er:YAG laser beams, there was a significant difference compared to the control and CO_2 groups. The laser-irradiated root surfaces in the Er:YAG laser group were more irregular and rough without the smear layer, consistent with the results of several studies on the topographical and morphological features of Er:YAG laser-treated root surfaces.[3,13,14,18,19,29,34] These morphological features can be attributed to the high interaction of the laser with mineralized tissues after its energy absorbed by the water present in the mineralized tissue is released through micro-explosions, a process referred to as explosive ablation. According to some researchers, Er:YAG laser-treated samples might also facilitate the adhesion of blood components through an increase in the physical retention of the fibrin clot on the root surface. In addition, the laser beams might play a role in cell retention; however, this surface roughness might not be important in the adhesion of blood components. Some studies have shown that polymorphonuclear leukocytes have a tendency to adhere to rough surfaces rather than smooth surfaces due to functional differences of these cells,[35] which might explain the results in the Er:YAG laser-treated groups with higher adhesion of blood cells, probably red and white blood cells. Atteya et al studied Laser-Assisted New Attachment Procedure by the Nd:YAG laser and explained the significance of forming the fibrin clot in closing mini-flaps and keeping the sulcus sealed against bacterial penetration as well as avoiding the development of epithelium down into the sulcus. Although we used Er:YAG with the aim of root conditioning, maintaining the fibrin clot was a common goal in both studies.[36]

Although the application of laser beams did result in irregularities on the root surface, there were no carbonizations, craters, cracks or fractures, consistent with the results of similar studies.[13,37] Various studies have shown that temperature rise with the application of Er:YAG laser beams using different laser powers does not exceed 3°C which is below the safe temperature threshold (5°C) reported by Zach and Cohen.[38] The SEM evaluation of surfaces exposed to Er:YAG laser beams showed an irregular and retentive pattern with a scaly appearance, consistent with previous results.[39,40]

In the CO_2 laser group, thermal side effects such as melting, crack formation and fissures were detected. The mechanism of the action of the CO_2 laser on the target tissue is the conversion of laser energy into heat. Various studies have demonstrated that CO_2 laser beams with different parameters result in the vaporization of water and dental organic components, creating irregularities, fissures and melting areas. In fact, cracks result from the contraction of the tissue after loss of water and the collagen matrix. Due to the presence of melted areas that might affect the adhesion of fibrin, it appears that CO_2 laser irradiation is not a good choice for surface treatment.[41] In the present study, in the CO_2 group, the smear layer was removed and dentinal tubules were relatively open, but smooth surfaces exhibited decreased fibrin attachment.

Conclusion

Er:YAG laser beams prepare a better surface for the attachment of fibrin, and the CO_2 laser is not recommended due to its hazardous effects and lower fibrin attachment; however, further studies are necessary. Therefore, the Er:YAG laser can be considered an alternative technique for surface treatment and might be as safe as conventional techniques. Furthermore, the CO_2 laser has some thermal side effects, making it inappropriate to this end. Further studies are necessary to evaluate the characteristics of laser-irradiated surfaces to determine appropriate parameters for the attachment of fibrin.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of the article.

Ethical Considerations

The protocols were reviewed in the research ethics

committee of Isfahan University of Medical Sciences.

Acknowledgments

This study was performed with the financial support of Dental Implants Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

References

1. Cortellini P, Bissada NF. Mucogingival conditions in the natural dentition: Narrative review, case definitions, and diagnostic considerations. *J Periodontol.* 2018;89 Suppl 1: S204-13. doi: 10.1002/JPER.16-0671.
2. Polson AM, Proye MP. Fibrin linkage: a precursor for new attachment. *J Periodontol.*1983;54:141–7. doi:10.1902/jop.1983.54.3.141
3. Moghare Abed A, Tawakkoli M, Dehchenari MA, Gutknecht N, Mir M. A comparative SEM study between hand instrument and Er:YAG laser scaling and root planing. *Lasers Med Sci*. 2007;22(1):25-9. doi:10.1007/s10103-006-0413-y
4. Caffesse RG, De LaRosa M, Garza M, Munne-Travers A, Mondragon JC, Weltman R. Citric acid demineralization and subepithelial connective tissue grafts. *J Periodontol.* 2000;71(4):568–572. doi:10.1902/jop.2000.71.4.568
5. Bouchard P. Nilveus R. Etienne D. Clinical evaluation of tetracycline HCl conditioning in the treatment of gingival recessions: a comparative study. *J Periodontol.* 1997;68(3):262–269. doi:10.1902/jop.1997.68.3.262
6. Bittencourt S, Ribeiro Edel P, Sallum EA, Sallum AW, Nociti FH Jr, Casati MZ. Root surface biomodification with EDTA for the treatment of gingival recession with a semilunar coronally repositioned flap. *J Periodontol.* 2007;78(9):1695–1701. doi:10.1902/jop.2007.060507
7. Héritier M. Effects of phosphoric acid on root dentin surface: a scanning and transmission electron microscopic study. *J Periodontal Res*. 1984;19(2):168-76. doi:10.1111/j.1600-0765.1984.tb00805.x
8. Sallum EA, Casati MZ, Caffesse RG, Funis LP, Nociti JFH. Sallum AW. Coronally positioned flap with or without enamel matrix protein derivative for the treatment of gingival recessions. *Am J Dent*. 2003;16:287-91.
9. Lacoste E, Martineau I, Gagnon G. Platelet concentrates: effects of calcium and thrombin on endothelial cell proliferation and growth factor release. *J Periodontol.* 2003;74(10(:1498–1507. doi:10.1902/jop.2003.74.10.1498
10. Baker PJ, Rotch HA, Trombelli L, Wikesjo¨ UME. An in vitro screening model to evaluate root conditioning protocols for periodontal regenerative procedures. *J Periodontol.* 2000;71(7(:1139-43. doi:10.1902/jop.2000.71.7.1139
11. BemHatit Y, Blum R, Severin C, Maquin M, Jabro MH. The effects of a pulsed Nd:YAG laser on subgingival bacterial flora and on cementum: An in vivo study. *J Clin Laser Surg Med*. 1996;14(3):137–43. doi:10.1089/clm.1996.14.137.
12. Moritz A, Schoop U, Goharkhay K, Schauer P, Doertbudak O, Wernisch J, et al. Treatment of periodontal pockets with a diode laser. *Lasers Surg Med*. 1998;22(5):302–11. doi: 10.1002/(sici)1096-9101(1998)22:5<302::aid-lsm7>3.0.co;2-t
13. Aoki A, Miura M, Akiyama F, Nakagawa N, Tanaka J, Oda S, et al. In vitro evaluation of Er:YAG laser scaling of subgingival calculus in comparison with ultrasonic scaling. *J Periodont Res*. 2000;35(5):266-77. doi:10.1034/j.1600-0765.2000.035005266.x
14. Sasaki KM, Aoki A, Masuno H, Ichinose S, Yamada S, Ishikawa I. Compositional analysis of root cementum and dentin after Er:YAG laser irradiation compared with CO_2 laser and intact roots using fourier transformed infrared spectroscopy. *J Periodontal Res.* 2002;37(1):50–9. doi:10.1034/j.1600-0765.2002.00657.x
15. Schwarz F, Sculean A, Berakdar M, Georg T, Reich E, Becker J. Periodontal treatment with an Er:YAG laser or scaling and root planing. A 2-year follow-up split-mouth study. *J Periodontol*. 2003;74(5):590-6. doi:10.1902/jop.2003.74.5.590
16. Shariati S, Pogrel MA, Marshall GW Jr, White JM. Structural changes in dentin induced by high energy, continuous wave carbon dioxide laser. *Lasers Surg Med*. 1993;13(5):543-7. doi:10.1002/lsm.1900130508
17. Barone A, Covani U, Crespi R, Romanos GE. Root surface morphological changes after focused versus defocused CO2 laser irradiation: a scanning electron microscopy analysis. *J Periodontol.* 2002;73(4):370-3. doi:10.1902/jop.2002.73.4.370
18. Cekici A, Maden I, Yildiz S, San T, Isik G. Evaluation of blood cell attachment on Er:YAG laser applied root surface using scanning electron microscopy. *Int J Med Sci* 2013; 10(5):560-66. doi:10.7150/ijms.5233
19. Theodoro LH, Sampaio JE, Haypek P, Bachmann L, Zezell DM, Garcia VG. Effect of Er:YAG and Diode lasers on the adhesion of blood components and on the morphology of irradiated root surfaces. *J Periodontal Res*. 2006;41(5):381–90. doi:10.1111/j.1600-0765.2005.00846.x
20. Barone A, Covani U, Crespi R, Romanos GE. Root surface morphological changes after focused versus defocused CO2 laser irradiation: a scanning electron microscopy analysis. *J Periodontol*. 2002;73(4):370-3. doi:10.1902/jop.2002.73.4.370
21. Crespi R, Barone A, Covani U, Ciaglia RN, Romanos GE. Effects of CO2 laser treatment on fibroblast attachment to root surfaces. A scanning electron microscopy analysis. *J Periodontol.* 2002 ;73(11):1308-12. doi:10.1902/jop.2002.73.11.1308
22. Polson AM, Frederick GT, Ladenheim S, Hanes PJ. The production of a root surface smear layer by instrumentation and its removal by citric acid. *J Periodontol.* 1984;55(8):443–6. doi:10.1902/jop.1984.55.8.443
23. Ramos ACB, Esteves-Oliveira M, Arana-Chavez VE, de Paula Eduardo C. Adhesives bonded to erbium:yttrium aluminum- garnet laser-irradiated dentin: transmission electron microscopy, scanning electron microscopy and tensile bond strength analysis. *Lasers Med Sci*. 2010;25(2):181–9. doi: 10.1007/s10103-008-0600-0
24. Botta SB, da Ana PA, Zezell DM, Powers JM, Matos AB. Adhesion after erbium, chromium: yttrium-scandium gallium- garnet laser application at three different irradiation conditions. *Laser Med Sci*. 2009;24(1):67–73. doi:10.1007/s10103-007-0521-3
25. Freiberg RJ, Cozean CD. Pulsed erbium laser ablation of hard dental tissue: the effects of atomized water spray versus water surface film. *Proc SPIE*. 2002;46(10):74–84. doi:10.1117/12.469306

26. Oelgiesser D, Blasbalg J, Ben-Amar A. Cavity preparation by Er:YAG laser on pulpal temperature rise. *Am J Dent*. 2003;16(2):96–8.
27. Wikesjo UME, Nilve us RE, Selvig KN. Significance of early healing events on periodontal repair: a review. *J Periodontol*. 1992;63(3):158–65. doi:10.1902/jop.1992.63.3.158
28. Rahimi H, Babazade H. Application of the Erbium laser in periodontal plastic surgery: literature review. *Annals of Dental Specialty*. 2018; 6(1): 83-88.
29. 29.Oliveira GJ, Theodoro LH, Marcantonio Junior E, Sampaio JE, Marcantonio RA. Effect of Er,Cr:YSGG and Er:YAG laser irradiation on the adhesion of blood components on the root surface and on root morphology. *Braz Oral Res*. 2012;26(3):256–62. doi:10.1590/s1806-83242012000300012
30. Ando Y, Aoki A, Watanabe H, Ishikawa I. Bactericidal effect of erbium YAG laser on periodontopathic bacteria. *Lasers Surg Med*. 1996;19(2):190–200. doi:10.10.1002/(SICI)1096-9101(1996)19:2<190::AID-LSM11>3.0.CO;2-B
31. Poormoradi B, Torkzaban P, Gholami L, Hooshyarfard A, Farhadian M. Effect of Er,Cr (YSGG laser root conditioning on the success of root coverage with subepithelial connective tissue graft): a randomized clinical trial with a 6-month follow-up. *J Dent (Tehran)*. 2018;15(4):230-239.
32. Bachmann L, Dielbolder R, Hibst R, Zezell DM. Changes in chemical composition and collagen structure of dentine tissue after erbium laser irradiation. *Spectrochim Acta A Mol Biomol Spectrosc*. 2005;61(11-12):2634–39. doi:10.1016/j.saa.2004.09.026
33. Birang R, Yaghini J, Nasri N, Noordeh N, Iranmanesh P,Saeidi A, et al. Comparison of Er:YAG laser and ultrasonic Scaler in the treatment of moderate chronic periodontitis: A randomized clinical trial. *J Lasers Med Sci*. 2017; 8 (1):51-55. doi: 10.15171/jlms.2017
34. Schoop U, Moritz A, Kluger WM et al. Changes in root surface morphology and fibroblast adherence after Er:YAG laser irradiation. *J Oral Laser Applications* 2001;2:83-93.
35. Eriksson C, Lausmaa J, Nygren H. Interactions between human whole blood and modified TiO2-surfaces: Influence of surface topography and oxide thickness on leukocyte adhesion and activation. *Biomaterials* 2001;22(14):1987–96. doi:10.1016/S0142-9612(00)00382-3
36. Atteya A, Shafei R, Jefri A, Alhindi M. Laser assisted new attachment procedure-LANAP. *The Egyptian Journal of Hospital Medicine*. 2017;69(1): 1641-1645.
37. Folwaczny M, Mehl A, Haffner C, Benz C, Hickel R. Root substance removal with Er:YAG laser radiation at different parameters using a new delivery system. *J Periodontol.* 2000;71(2):147–55. doi:10.1902/jop.2000.71.2.147
38. Zach L, Cohen C. Pulp response to externally applied heat. *Oral Surg Oral Med Oral Pathol*.1965;19:515-30. doi:10.1016/0030-4220(65)90015-0
39. Nokhbatolfoghahaie H, Chiniforush N, Shahabi S, Monzavi A. SEM evaluation of tooth surface irradiated by different parameters of Er:YAG laser. *J Lasers Med Sci*. 2012;3(2):51-5.
40. Marracini TM, Bachmann L, Widgor HA, Walsh JT, Stabholtz, Zezell DM. Morphological evaluation of enamel and dentin irradiated with 9.6 μm and 2.94 μm Er:YAG lasers. *Laser Phys* 2005; 11:551-5. doi:10.1002/lapl.200510040
41. Nomelini SMB, Gabriel AES, Marchesan MA, SousaNeto MD, Silva-Sousa YTC. Ultrastructural analysis of radicular dentine surface submitted to CO_2 laser at different parameters. *Microsc Research Tech* 2009; 72:737-43. doi:10.1002/jemt.20723.