In Vitro Study of Er:YAG and Er, Cr:YSGG Laser Irradiation on Human Gingival Fibroblast Cell Line

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Abstract- The ultimate goal of the periodontal treatments is a regeneration of periodontium. Recently, laser irradiations are commonly used to improve wound repair. Because of many controversies about the effects of laser on soft tissue regeneration, more in vitro studies are still needed. The aim of the present in vitro study was to compare the effects of different doses of Er:YAG (erbium-doped:yttrium, aluminum, garnet) and Er, Cr:YSGG (erbium, chromium-doped: yttrium, scandium, gallium, garnet) laser treatment on human gingival fibroblasts (HGF) proliferation. In this randomized single-blind controlled in vitro trial, HGF cells were irradiated using Er:YAG and Er, Cr:YSGG laser for 10 and 30 seconds or remained unexposed as a control group. After a culture period of 24 and 48 hours, HGF cell proliferation was evaluated by MTT assay. The data were subjected to one-sided analysis of variance and Tukey multiple comparison tests. Our results showed Er:YAG application for 10 and 30 seconds as well as Er, Cr:YSGG irradiation for 10 and 30 seconds induced statistically significant (P<0.05) proliferation of HGF cells as compared with the control at 24 hours up to 18.39%, 26.22%, 21.21%, and 17.06% respectively. In 48 hour incubations, Er:YAG and Er, Cr:YSGG irradiation for 10 and 30 seconds significantly increased cellular proliferation up to 22.9%, 32.24%, 30.52% and 30.02% respectively (P<0.05). This study demonstrates that Er:YAG and Er, Cr:YSGG laser significantly increased HGF cell proliferation compared to the control specimens. This higher proliferation can lead to increased wound repair in clinical conditions.

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Keywords: Fibroblast; Proliferation; Lasers

Introduction

The main objective of periodontal treatment is to complete reconstruction of damaged tissues and regeneration of lost periodontium (1). The periodontal wound healing is a process that requires coordination of four soft and hard tissues containing gingival connective tissue, periodontal ligament tissue, Cementum and bone (2). Gingival fibroblasts represent the predominant cell type in healthy gingival connective tissue and play a key role in the development of gingival apparatus and repair of gingival connective tissue. The main role of fibroblasts is to synthesize extracellular matrix components such as various types of collagen, glycoproteins, and proteoglycans in connective tissue. In addition, fibroblasts can completely remodel the collagens during the repair of connective tissue (3). Treatment of periodontal pockets with laser radiation has been considered as an adjunct to non-surgical periodontal treatment. In addition, it has been shown that lasers can facilitate adhesion of fibroblasts to root surfaces (4). Moreover, lasers can enhance DNA synthesis, collagen, and procollagen production and increase the rate of cell proliferation (5).

The lasers that show the most promise for hard and soft tissue surgery are the erbium: yttrium-aluminum-garnet (Er:YAG) (2940 nm wavelength) and erbium, chromium: yttrium-scandium-gallium-garnet (Er, Cr:YSGG) (2790 nm wavelength) (6). Moreover, the healing of tissues incised by the laser is comparable to

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steel scalpel incision (7). As regards the effects of laser depend on various parameters such as the wavelength, output power and exposure time (7,8), the aim of this study was to analyze the effects of different doses of Er, Cr:YSGG laser and Er:YAG laser on human gingival fibroblast proliferation.

Materials and Methods

Cell culture

The HGF cell line (NCBI: C-165) was obtained from the National Cell Bank of Iran (Pasteur Institute of Iran, Tehran). The cells were grown in DMEM medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% antibiotic-antimycotic (Gibco, Grand Island, NY, USA) in 5% CO2 at 37 ° C incubator.

Laser irradiation treatment

The cells were seeded triplicate into 24-well culture plates at a density of 5000 cells per well in complete medium. 24 hours after cultivation (half confluence), first and second group of cells (6 cultured wells) were exposed to Er:YAG laser irradiation (2940 nm wavelength, 1W power output, 10Hz frequency, 100mj energy) by using Smart 2940D Plus instrument (Deka Laser, Florence, Italy) and Er, Cr:YSGG laser (2780 nm wavelength, 0.5W power output, 10Hz frequency, 150mj energy) by using Biolase Technologies instrument (San Clemente, CA, USA) for 10 seconds, respectively. This process was repeated for the third and fourth group, but for 30 seconds exposure time. Cultured cells without any radiation treatment (unexposed) considered as control group.

MTT assay

The HGF cell proliferation and viability were evaluated 24 and 48 hours after exposure to laser treatments using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma, Germany) colorimetric assay. Briefly, the cells were incubated with culture medium containing 0.5 mg/ml MTT for 3 hours. Only viable cells with a normal functional mitochondrial enzyme (succinate dehydrogenase) can alter water-soluble MTT dye to insoluble purple formazan product. After completion incubation, the medium was aspirated, and the reduced formazan was dissolved in a dimethyl sulfoxide (DMSO) solvent. Then 100 µl of the purple solution in each well was transferred (triplicate) to 96-well ELISA plate, and the spectrophotometric absorbance (optical density) of each well was measured using an ELISA reader instrument (Anthos 2020, Salzburg, Austria) at 570 nm with 620 nm as a reference.

Statistical analysis

Results were presented as mean±SE of three replicate cultures from three independent experiments. Multiple comparisons were carried out using one-way analysis of variance (ANOVA) followed by a Tukey–Kramer post hoc analysis using Graph-Pad Prism software (version 6).The P value less than 0.05 ($P < 0.05$) was considered significant and determined by an asterisk in the corresponding figures.

Results

For the MTT assay, in either time of the experiment (24 and 48 hours), the percentage of viability in each experimental group (Mean ±SE) compared to control group (viability 100%) is summarized in table 1. According to the figure 1 (A), Er:YAG irradiation at 30 and 10 seconds, increased statistically significant cell viability compared with the control group in values of 26.22% and 18.39%, respectively ($P < 0.05$).

Table 1. Percentage of HGF cell viability (% viability ±SE, n=9), 24 and 48 hours after laser irradiation treatment, compared to control (unexposed) cells. Values are presented as mean±standard deviation

	Control	Er:YAG		Er, Cr:YSGG	
		10s	30s	10s	30s
24 hours	100	118.39±0.45	126.22±0.33	121.21 ± 1.8	117.06 ± 3.8
48 hours	100	122.90±0.75	132.24 ± 0.37	130.53 ± 1.30	130.03 ± 1.76

Also Er, Cr:YSGG irradiation at 30 and 10 seconds induced cell proliferation significantly compared with the control group in values of 17.06% and 21.21%, respectively ($P<0.05$). Moreover, Er: YAG irradiation at

30 seconds, increased cell proliferation significantly more than Er, Cr:YSGG (P<0.05). Figure 1 (B), shows the percentage of HGF cell viability after laser irradiation at 48 hours incubation. According to the figure 2 both Er:YAG and Er, Cr:YSGG lasers increased cell proliferation significantly compared with the control group at 10 and 30 seconds (P<0.05).

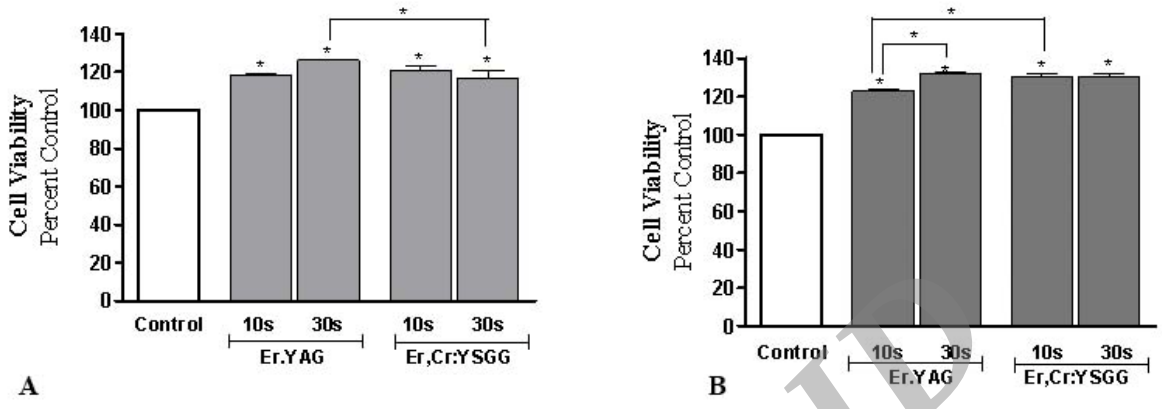

Figure 1. The effect of ER.YAG and Er, Cr:YSGG laser irradiation in two different exposure time (10 and 30 seconds) on HGF cell proliferation, 24 (A) and 48 (B) hours after treatment. The results are presented as mean ± SE (n = 9, *P < 0.05) compared with control group (100% viability)

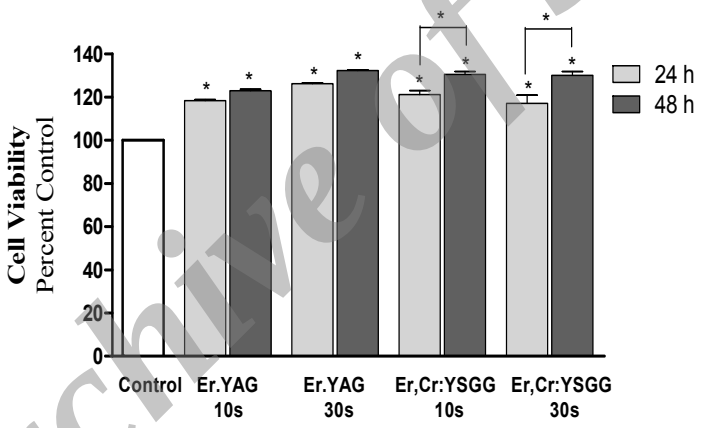

Figure 2. Comparison of laser exposed HGF cell viability, 24 and 48 hours after treatment. The results are presented as mean ± SE (n = 9, *P < 0.05) compared with control group (100% viability)

Er:YAG irradiation at 30 seconds, increased cell viability significantly more than 10 seconds and also Er, Cr:YSGG irradiation at 30 seconds, increased cell viability significantly more than 10 seconds (P<0.05). Moreover, Er, Cr:YSGG irradiation at 10 and 30 seconds, increased cell proliferation significantly more than Er:YAG (P<0.05). As shown in Figure 2, Er, Cr:YSGG laser-treated cells at 10 and 30 seconds showed a significantly higher cell-density number after 48 hours incubation compared to 24 hours incubation (Time dependency effect).

Discussion

Many studies have reported that low energy lasers promote wound healing (9). Low-level lasers enhance wound healing by increasing cell proliferation (10), promoting collagen synthesis and accelerating the formation of granulation tissue (11,12).

Several studies have shown that low energy laser irradiation can enhance HGF proliferation rate as shown in this paper (5,10,13). However, conflicting results have also been reported by Camaco *et al.,* who showed no significant difference in cell proliferation between the

control group and the test group using As-Ga-Al laser (14). In addition, Abergel *et al.,* demonstrated that in fibroblast cultures, He-Ne, and Ga-Al-As lasers enhance procollagen production but had no effect on proliferation (15). Some discrepancies in results may be due to differences in the type of laser irradiation, exposure time, energy density, and the wavelength and cell types.

Stimulation of fibroblast proliferation by low-level laser might be associated with the release of specific growth factors from fibroblasts (16). Laser irradiation can stimulate the autocrine production of basic fibroblast growth factor (bFGF) from fibroblasts (17). This should be considered as one of the important regulatory pathways to accelerate wound healing after Er:YAG laser irradiation. Furthermore, Pourzarandian *et al.,* demonstrated that Er:YAG laser irradiation stimulated the proliferation of human fibroblasts through the production of platelet-derived growth factor (PDGF) (18). However Er:YAG laser irradiation appears to apply its stimulative action on gingival fibroblasts proliferation through the production of PGE2 via the expression of COX-2 (19).

When irradiated, fibroblasts were polarized, forming bundles in different directions (10,20,21). However, laser irradiation is reported to promote human skin fibroblasts proliferation after 24 and 48 h (22). Study of laser irradiation with 2 J/cm showed remarkable accumulation of fibrillar material in the outer part of the fibroblast cytoplasm (23). These findings are also confirmed by Karu *et al.*, who reported increased HeLa cell adhesion following laser irradiation (24).

Our investigation has shown that both Er:YAG and Er, Cr:YSGG lasers have significantly increased human gingival fibroblasts proliferation compared with the control group at 10 and 30 seconds, which can be related to clinically accelerated healing. We believe that our results might help clinicians to choice the most proper laser parameters in various clinical conditions. However, more studies are needed to evaluate the clinical effectiveness of low-level laser.

In this in vitro study, HGF proliferation was enhanced by Er:YAG and Er, Cr:YSGG irradiation. It can be associated with accelerated wound healing in clinical conditions. However, further studies need to be considered to clarify the mechanisms behind this acceleration. Moreover, clinical investigations are required to assess whether low-level laser might be beneficial in periodontal treatment.

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