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Original Article

The Effect of Cold Plasma and Low-Level Laser Therapy on Oral Fibroblast Proliferation

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Abstract

Background: Wound healing is a complex physiological process involving multiple phases and cellular mechanisms that restore damaged tissue. The oral cavity presents unique challenges for wound healing due to the presence of microorganisms and the impact of various diseases and treatments. Recent advancements, including low-level laser therapy (LLLT) and cold plasma, offer promising approaches to enhance wound healing by promoting cell proliferation and reducing inflammation. This study aimed to investigate the effects of cold plasma and low-level 980nm laser on the growth of oral fibroblasts and compare their respective impacts on wound healing.

Methods: Human gingival fibroblasts were divided into nine study groups, including a control group. Two groups were exposed to low-level 980nm diode laser irradiation for 15 and 30 seconds, while six groups received cold plasma irradiation with helium gas at flow rates of 1.85, 2.78 and 5.56 cm3/s for the same durations. Fibroblast proliferation was evaluated on days 1, 3, and 5 after treatment using the MTT assay.

Results: The results showed that on the 5th day after irradiation, 30 seconds of 980 nm laser irradiation significantly increased fibroblast proliferation compared to the other groups. In contrast, 15 seconds of plasma irradiation at a flow rate of 1.85 cm3/s had the least effect on promoting fibroblast proliferation. On the 1st day after radiation, plasma irradiation at flow rates of 2.78 and 5.56 cm3/s exhibited a greater impact on fibroblast proliferation compared to the other five test groups.

Conclusion: The 980nm diode laser demonstrated a greater capacity to enhance the proliferation of oral fibroblasts compared to cold plasma using helium gas.

Keywords: Diode Laser; Fibroblasts; Low-level Laser Therapy; Non-Thermal Atmospheric Pressure Plasma; Wound Healing

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Introduction

The process of Wound healing is a physiological response in which the body repairs and restores damaged tissue (1). There are four overlapping phases involved in the healing of a wound: homeostasis, inflammation, proliferation and regeneration (2). Various cell populations, extracellular matrix, growth factors, and cytokines, are required for the wound healing process (3). Fibroblasts, in particular, play a crucial role in facilitating wound healing by participating in the secretion of growth factors, formation of granulation tissue, and deposition of extracellular matrix (4, 5). Wounds in the oral cavity can be caused by immune system disorders (such as recurrent aphthous ulcers and lichen planus), infections (such as periodontal disease, herpes, and candida), congenital abnormalities (such as ankyloglossia, cleft lip or palate), trauma and other pathological conditions (6). Additionally, certain gingival (7) and periodontal (8) treatments and cancer therapy (9) can induce oral wounds. If left untreated, oral wounds can impair normal mouth function, food intake, and overall health (6).

Low-level laser therapy (LLLT), also known as photobiomodulation (PBM), has been introduced as a potential method to accelerate wound healing (10-13). The disinfection property of laser is advantageous in the oral cavity (14), which is populated by microorganisms that can affect the wound healing process (15). LLLT has been shown to reduce inflammation and edema (16), while promoting stem cell differentiation, cell proliferation (17), and migration (18), particularly in fibroblasts (1). When applied to biological tissue, light can be absorbed, reflected, scattered, or transmitted (19). The red spectrum (600 to 700nm) and near-infrared spectrum (780 to 1100nm) are considered the "optical window" as they penetrate more effectively within this range (20). The chromophores of mitochondria act as photoreceptors, absorbing photons and converting them into energy in the form of ATP (21), or activating signaling molecules such as calcium ions, nitric oxide (NO), and reactive oxygen species (ROS) (17). There is ongoing debate among studies regarding the most effective wavelength for fibroblast proliferation (22-24). However, some studies have found the 980nm wavelength to be effective compared to other diode laser wavelengths (25-29).

Cold plasma, also known as non-thermal atmospheric pressure plasma (NTAPP), shows promise as a tool for wound healing (30). It is also utilized in decontamination, disinfection, blood coagulation (31), and cancer treatment (32). Plasma is a partially ionized gas that represents the fourth state of matter, following solid, liquid, and gas (33). It can be generated in a strong electric field, which renders it hot and composed of reactive molecules, ions, electrons, UV radiation, and excited species (34). However, cold plasma primarily operates at temperatures close to human body temperature (below 400C) and is considered a treatment that does not cause damage (35). Various types of gases can be employed in plasma devices, including N2 (36), helium, argon (37), and even ambient air (38). NTAPP has the ability to induce angiogenesis and epithelization, as well as activate different signaling pathways, growth factors, and cytokines to accelerate wound healing (39).

While numerous studies have investigated the effects of LLLT or NTAPP on fibroblast proliferation, there is currently no comparative study between the two. The aim of this study is to examine the impact of LLLT and NTAPP on fibroblast proliferation in order to determine the most effective device for promoting fibroblast proliferation.

Materials and Methods

Cell Culture

The current study was conducted with the approval of the Research Ethics Committee at Islamic Azad University, Dental Branch Tehran, Iran (IR.IAU.DENTAL.REC.1401.041, on 30 March 2022). Human gingival fibroblast cells obtained from the Dentistry Research Institute, Tehran University of Medical Sciences, were cultured (40). The cells were cultivated in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, St. Louis, MO, the USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, the USA), penicillin (100 IU/mL), and streptomycin (100 µg/mL). The cells were incubated under conditions of 5% CO2 and 95% humidity at 370C. For the experiments, cells from the third passage were used. Twenty-four hours prior to laser and plasma treatments, the cultured cells were distributed among six 96-well plates, with an empty well placed on each side between the wells containing

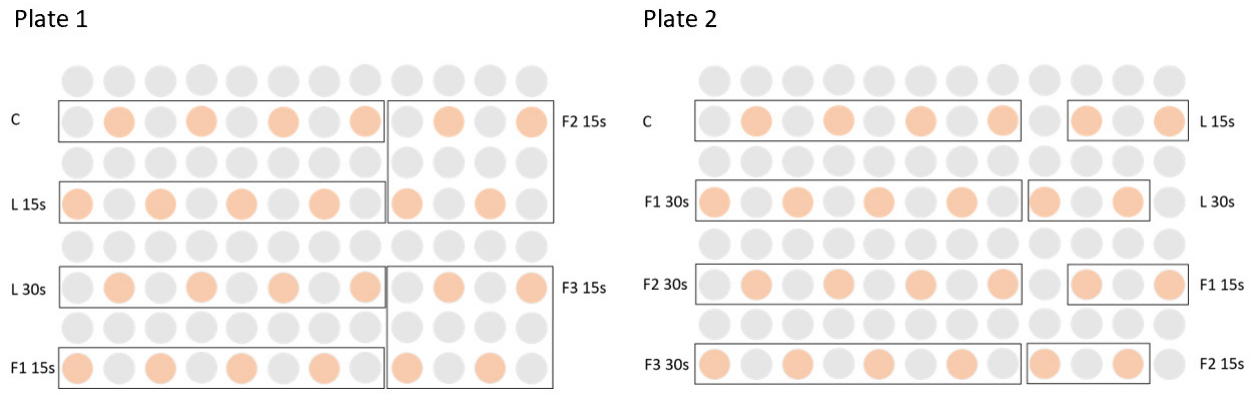

Figure 1. Test groups array in 96-well plates. In each plate, 4 wells were dedicated to control groups. Plate 1 includes one control and five tests (each has 4 wells), and plate 2 includes one control and seven tests (three have four wells and four have two wells). C, Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3, Flow rate 3, 5.56 cm3/s.

fibroblasts. This arrangement was implemented to prevent any potential errors resulting from over-radiation due to the proximity of the wells. Each plate accommodated a total of 24 wells containing fibroblasts. **(Figure 1)**

Plasma and Laser Irradiation

In each 96-well plate, four wells were dedicated to the control group; which received no treatment. Two test groups of cultured cells were exposed to a 980nm diode laser (Wiser, Doctor Smile, Lambda SpA, Italy) with a power density of 400 mW/cm2, energy densities of 3 and 6 J/cm2 and a power of 100 mW. The emission mode was Continuous Wave (CW), with a beam diameter of 0.5 cm2 and the device was kept 1 cm away from the cells. Six test groups were exposed to cold air plasma (Plasmart, Iran), using helium at atmospheric pressure with three different flow rates (1.85, 2.78, and 5.56 cm3/s). The power was 8 W, the frequency was 100 KHz, the irradiation diameter was 2.5mm and the device was kept at a distance of 1 cm from the cells. For both laser and plasma treatments, the test groups were exposed for either 15 or 30 seconds. The following parameters were used in the eight test groups:

L 15s: LLLT, 3 J/cm2; 15s
L 30s: LLLT, 6 J/cm2; 30s
F1 15s: NTAPP, 1.85 cm3/s, 15s
F2 15s: NTAPP, 2.78 cm3/s, 15s
F3 15s: NTAPP, 5.56 cm3/s, 15s
F1 30s: NTAPP, 1.85 cm3/s, 30s
F2 30s: NTAPP, 2.78 cm3/s, 30s
F3 30s: NTAPP, 5.56 cm3/s, 30s

MTT Assay

Cell proliferation was assessed on days 1, 3, and 5 following exposure. To examine cell proliferation, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric (MTT) assay, provided by Sigma (Germany), was performed. To conduct the assay, the culture medium was completely removed from each well, and 100µl of MTT solution (5mg/ml MTT in phosphate buffer saline) was added. The cells were then incubated for 3-4 hours at 370C and %5 CO2. Subsequently, the MTT solution was removed and replaced with 60µl DMSO. After shaking for 15 minutes, the absorbance was measured at 570nm using a microplate Reader from BioTek (USA). The proliferation of the treated groups was reported as a percentage of the controls.

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics version 27.0.1 (SPSS, Inc., Chicago, IL, USA). The normal distribution of the data was confirmed using the Shapiro–Wilks test. Cell viability was calculated separately for each dose using a one-way ANOVA test, which indicated a significant difference between the study groups and the control group on each day ($P<0.05$). As the data exhibited equal dispersion across all experimental days, as confirmed by the Homogeneity of Variances Test, pairwise comparisons were performed using Tukey's Test ($\alpha=0.05$). The results of the analysis were summarized in graphs using GraphPad Prism 9.

Results

The growth and proliferation differences between the control group and the test groups were examined on the first, third, and fifth days. Although laser or plasma radiation did not induce cell death, the effect of radiation on increasing fibroblast proliferation was generally insignificant in most of the test groups. On the first day, the control group exhibited significantly higher proliferation compared to the L 30s, F1 15s, F2 15s, F3 15s, and F3 30s groups. On the third day, the control group had significantly higher proliferation than the F1 15s and F2 15s groups. On the fifth day, the control group had significantly lower proliferation than the L 30s group, but still higher proliferation than the F1 15s group. **(Figure 2)**

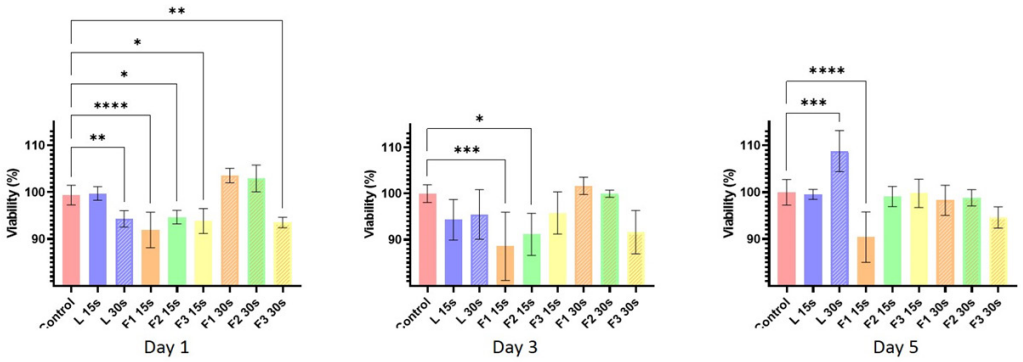

Figure 2. Comparison of the control group with other test groups. On the first day, the control group exhibited significantly greater proliferation compared to the four groups. On the third day, the control group maintained its significant proliferation over only two of those four groups. Finally, on the fifth day, the control group demonstrated significantly lower proliferation than the L 30s group, while still exhibiting higher proliferation than the F1 15s group. C, Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3, Flow rate 3, 5.56 cm3/s; *:P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001.

On the first day, the L 15s group exhibited significantly higher proliferation compared to the L 30s, F1 15s, F2 15s, F3 15s and F3 30s groups. On the third day, there were no significant differences observed when comparing the L 15s group to other groups. On the fifth day, the L 15s group demonstrated significantly lower proliferation than the L 30s group, while still exhibiting higher proliferation than the F1 15s group. **(Figure 3)**

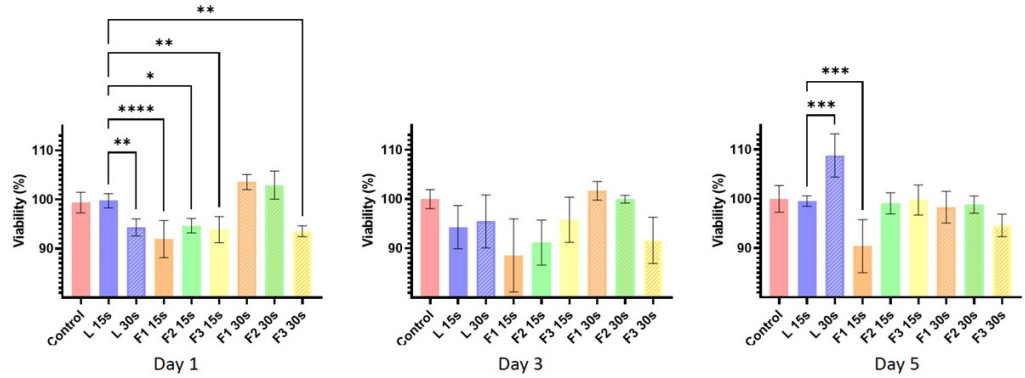

Figure 3. Comparison of the L15s group with other groups. On the first day, the L 15s group exhibited significantly higher proliferation than the five groups. However, on the third day, the difference in growth compared to the other groups was no longer significant. On the fifth day, the L 15s group demonstrated significantly lower proliferation than the L 30s group but still exhibited higher proliferation than the F1 15s group. C, Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3, Flow rate 3, 5.56 cm3/s; *: P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001.

On the first day, the L 30s group exhibited significantly lower proliferation than the L 15s, F1 30s, F2 30s and control groups. On the third day, there were no significant differences in proliferation compared to the other groups. However, on the fifth day, the L 30s group caused significantly higher proliferation than all other groups.**(Figure 4)**

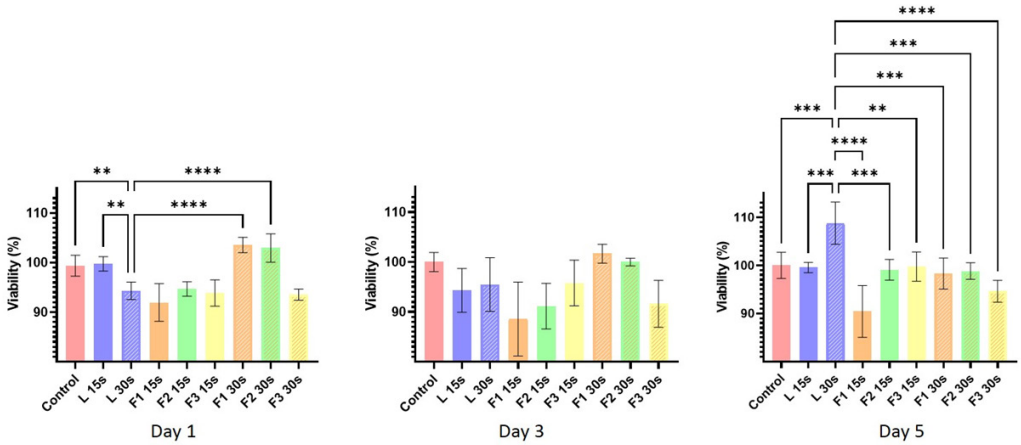

Figure 4. Comparison of the L30s group with other groups. On the first day, the L 30s group demonstrated significantly lower fibroblast proliferation than the control group and three others. This relationship remained on the third day but was not statistically significant. However, on the fifth day, the L 30s group exhibited a significant increase in fibroblast proliferation compared to all other groups. C, Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3, Flow rate 3, 5.56 cm3/s; *: P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001.

On the first day, the F1 15s group exhibited significantly lower proliferation compared to the L 15s, F1 30s, F2 30s and control groups. On the third day, the F1 15s group caused significantly lower proliferation compared to the F1 30s, F2 30s and control groups. On the fifth day, the F1 15s group demonstrated significantly lower proliferation compared to all other groups, except for the F3 30s group. **(Figure 5)**

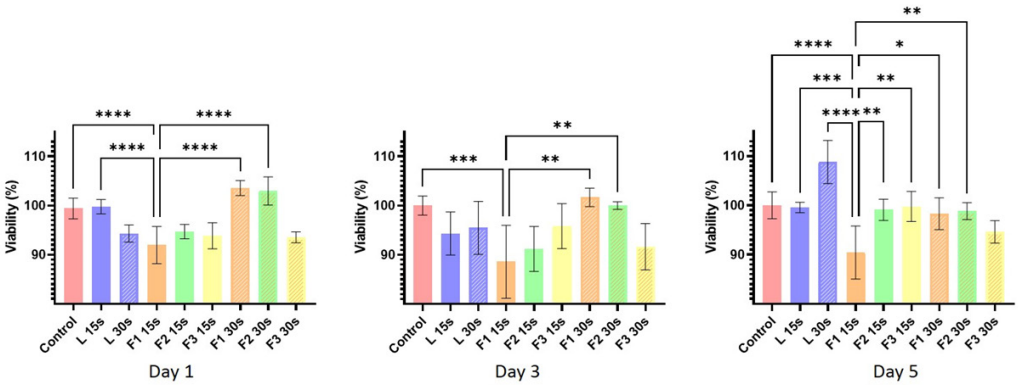

Figure 5. Comparison of the F1 15s group with other groups. On the first day, the F1 15s group exhibited significantly lower proliferation compared to the four groups. On the third day, the F1 15s group maintained significantly lower proliferation in three out of the four groups. On the fifth day, the F1 15s group showed significantly lower proliferation compared to all groups, except for the F3 30s group. Additionally, the F1 15s group caused a significantly lower increase in fibroblast proliferation compared to the control group on all three days. C, Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3, Flow rate 3, 5.56 cm3/s; *: P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001.

On the first day, the F2 15s group caused significantly less proliferation than the L 15s, F1 30s, F2 30s and control groups. On the third day, F2 15s group showed significantly less proliferation compared to the control and F1 30s groups. On the fifth day, it exhibited significantly less proliferation than the L 30s group, but demonstrated higher proliferation than the F1 15s group. **(Figure 6)**

On the first day, it showed significantly less proliferation compared to the L 15s, F1 30s, F2 30s and control groups. On the third day, there was no significant difference in proliferation compared to the other groups. On the fifth day, it caused significantly less proliferation than the L 30s group but demonstrated higher proliferation than the F1 15s group. **(Figure 7)**

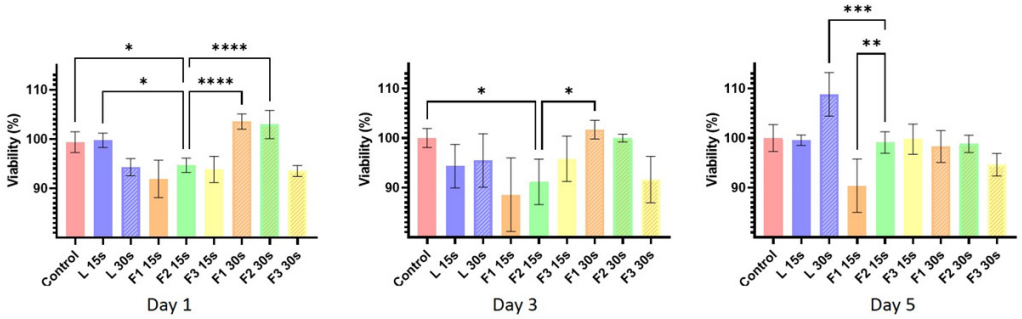

Figure 6. Comparison of the F2 15s group with other groups. On the first day, the F2 15s group demonstrated significantly lower fibroblast proliferation compared to the four groups. On the third day, there was significantly less proliferation observed in two out of the four groups. On the fifth day, it showed significantly less proliferation than the L 30s group but demonstrated higher proliferation than the F1 15s group. C, Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3, Flow rate 3, 5.56 cm3/s; *: P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001.

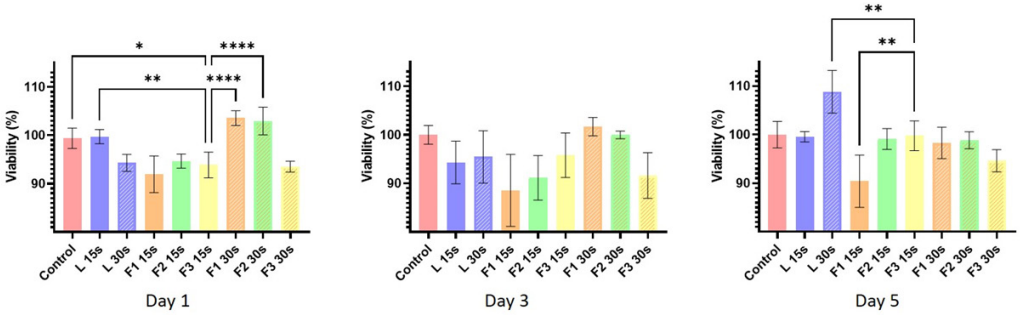

Figure 7. Comparison of the F3 15s group with other groups. On the first day, F3 15s group exhibited significantly less proliferation compared to the four groups. However, on the third day, it no longer showed a significant difference in proliferation compared to the other groups. On the fifth day, it demonstrated significantly less proliferation than the L 30s group but showed higher proliferation than the F1 15s group. C, Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3, Flow rate 3, 5.56 cm3/s; *: P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001.

On the first day, the F1 30s group caused significantly more proliferation compared to L 30s, F1 15s, F2 15s, F3 15s and F3 30s groups. On the third day, it significantly increased proliferation more than two groups: F1 15s and F2 15s. On the fifth day, it exhibited significantly less proliferation than the L 30s group but showed higher proliferation than the F1 15s group. **(Figure 8)**

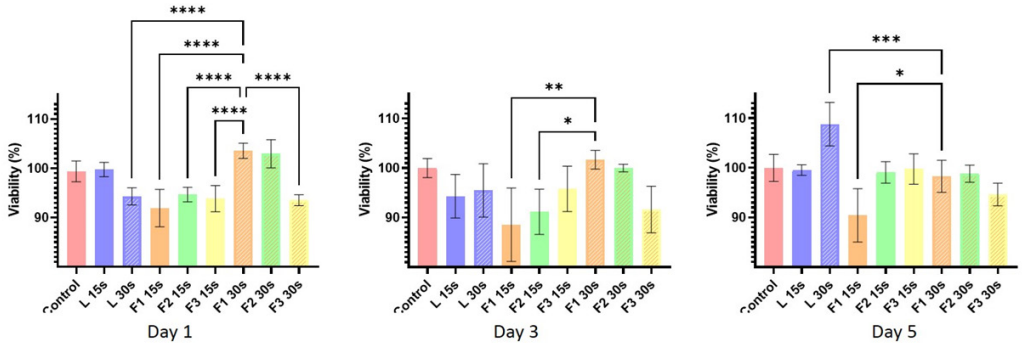

Figure 8. Comparison of the F1 30s group with other groups. On the first day, the F1 30s group exhibited significantly more proliferation compared to the five groups. On the third day, only two out of the five groups showed a significant increase in proliferation. On the fifth day, it demonstrated significantly less proliferation than the L 30s group but show higher proliferation than the F1 15s group. C, Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3,Flow rate 3, 5.56 cm3/s; *: P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001.

On the first day, the F2 30s group exhibited significantly more proliferation compared to the L 30s, F1 15s, F2 15s, F3 15s and F3 30s groups. On the third day, it showed a significant increase in proliferation compared to the F1 15s group. On the fifth day, it demonstrated significantly less proliferation than the L 30s group but exhibited higher proliferation than the F1 15s group. **(Figure 9)**

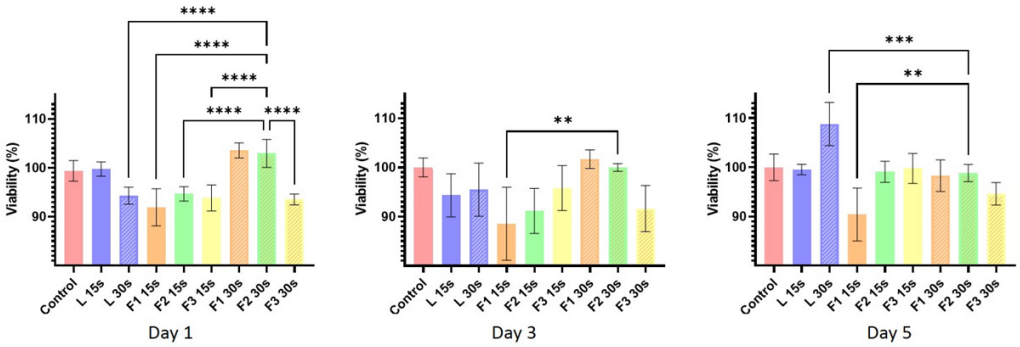

Figure 9. Comparison of the F2 30s group with other groups. On the first day, the F2 30s group exhibited significantly more proliferation compared to the five groups. On the third day, only one out of the five groups showed a significant increase in proliferation. On the fifth day, it demonstrated significantly less proliferation than the L 30s group but showed higher proliferation than the F1 15s group. C, Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3, Flow rate 3, 5.56 cm3/s; *: P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001.

On the first day, the F3 30s group exhibited significantly less proliferation compared to the L 15s, F1 30s, F2 30s and control groups. On the third day, there was no significant difference in proliferation compared to the other groups. On the fifth day, it showed a significantly lower proliferation compared to the L 30s group. **(Figure 10)**

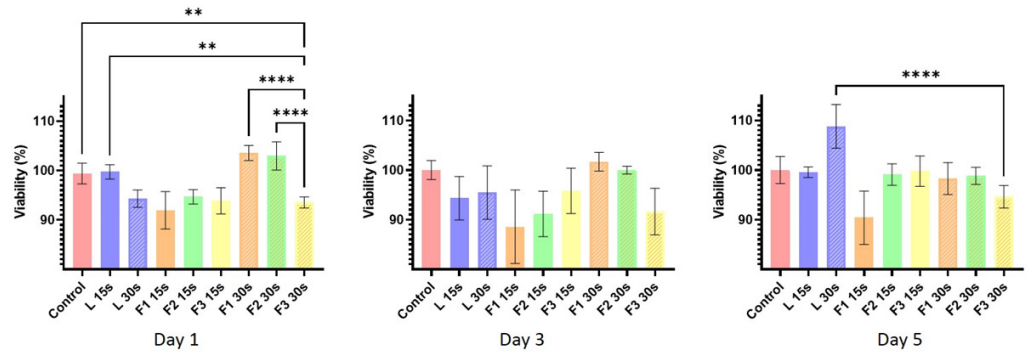

Figure 10. Comparison of the F3 30s group with other groups. On the first day, the F3 30s group exhibited a significant decrease compared to the four groups. On the third day, there was no significant difference in proliferation compared to the other groups. However, on the fifth day, it showed significantly lower proliferation compared to the L 30s group. C,-Control; L, Laser; F1, Flow rate 1, 1.85cm3/s; F2, Flow rate 2, 2.78cm3/s; F3, Flow rate 3, 5.56 cm3/s; *: P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001.

Discussion

This study aimed to investigate the effects of 980 nm laser radiation and helium cold plasma, with three different flows for 15 or 30 seconds, on fibroblast proliferation. The results of the MTT test revealed that after five days, 980nm laser exposure for 30 seconds had the most significant impact on the proliferation of human gingival fibroblast cells compared to other groups.

On the first day, the proliferation rate of the L 30s group, following 30 seconds of laser exposure, was significantly lower than that of the control group and some other test groups. However, the fibroblast proliferation gradually increased over time, and by the fifth day, it surpassed all other groups significantly.

In a study by Sterczała et al. (22), where 980nm laser was irradiated on fibroblasts for 32 seconds, no significant results were achieved. This lack of significance could be attributed to the short follow-up time. Their study examined the proliferation and survival rate of fibroblasts only up to three days after irradiation, whereas our study evaluated the effects up to five days after irradiation. It is worth mentioning that our study did not observe a significant difference in the growth of the L 30s group compared to other groups on the third day after radiation, similar to the findings of Sterczała's study.

Furthermore, on the first, third, and fifth days after irradiation, 15 seconds of laser irradiation with an energy density of 3 J/cm2 did not demonstrate a significant difference in growth compared to the control group. In a study by Etemadi et al. (25), the same laser device was used, but with a different energy density (4 J/cm2), power density, and exposure time (16s). They found that only on the first day after irradiation, fibroblast proliferation was significantly higher than the control group, but there was no significant difference on the third and fifth days.

Studies have been conducted to determine the most effective low-power laser wavelength for promoting the proliferation and growth of fibroblasts (24, 41-44). However, these studies have yielded inconsistent results. Some studies have shown that irradiated fibroblasts do not exhibit significant differences in growth compared to the control group, or even exhibit reduced growth (22, 25, 45). In our study, the L 15s group also exhibited lower growth compared to the control group. Huang et al. (46) attributed the reduced growth of fibroblasts to a lack of understanding regarding the parameters that effectively enhance fibroblast growth, such as power density, wavelength, and duration of radiation. They also emphasized the importance of comprehending the biochemical mechanisms underlying fibroblast proliferation in order to select appropriate parameters. It is crucial that the laser wavelength falls within the range of 650 and 1200 nm, known as the optical window, for effective treatment (47).

According to the Arndt-Schulz law, an energy density of 0.05 to 10 J/cm2 promotes cell proliferation, while an energy density exceeding 50 J/cm2 triggers apoptotic processes (48, 49). In other words, the therapeutic window for laser treatment lies between 0.05 and 10 J/cm2 (50). Additionally, energy density is a critical factor for determining the low-power laser dose, which is calculated by multiplying time (s) and power (w) and dividing the result by the area (cm2) according to the relevant physical formula. However, in the biological context, these three parameters do not necessarily exhibit a linear relationship. For example, doubling the time and halving the power would result in the same energy density, but the biological response would not be identical (46). Since all the aforementioned limits have been observed in our study, the cause of insufficient growth in the L 15s group remains unclear and warrants further investigation.

In terms of intracellular mechanisms, low-power laser photons are absorbed by mitochondria, leading to an increase in the production of adenosine triphosphate (ATP) (51). Additionally, low-power laser irradiation induces the generation of reactive oxygen species (ROS). Low levels of ROS promote cell proliferation and maintain energy density within the therapeutic window while keeping ROS production in check. Furthermore, ROS activation of the transcription factor NF-κB regulates DNA transcription and cell survival (19). Low-power lasers have also been found to effectively enhance collagen production (52).
The current study aimed to investigate the effect of a 980nm laser and different flows of cold plasma devices using helium gas on the proliferation capacity of fibroblasts. Under the same experimental conditions, the F1 15s group exhibited significantly lower growth than all other groups on the fifth day after irradiation. Bourdens et al. (53) irradiated human skin fibroblasts with plasma generated by helium gas for 3 minutes. Their findings indicated that while plasma did not induce cell death, it caused cell cycle arrest by upregulating p53/p21 and inducing DNA damage. Additionally, the fibroblasts exhibited an aging phenotype characterized by increased expression of p16, β-galactosidase associated with aging, and secretion of pro-inflammatory cytokines, which is known as the age-associated secretory phenotype (SASP). Although the duration of plasma irradiation in our study differed from that of Bourden's study, examining the aforementioned factors, may help elucidate the reasons behind the

reduced growth observed in the F1 15s group.

Similar to the laser device, the plasma device generates ROS, as well as active nitrogen species (RNS) and acidic conditions in aqueous solutions (54). Schmidt et al. (55) investigated the effect of plasma on primary skin fibroblast cells in mice. Their analysis of intracellular mechanisms revealed that plasma induces alteration in the phosphorylation of signaling molecules, including focal adhesion kinase and paxillin α, which are involved in adhesion-associated complexes. Furthermore, plasma affects cell surface adhesion receptors such as integrinα5β1 and syndecan 4, as well as structural proteins like vinculin, talin, and actin, thereby promoting fibroblast migration and expansion. By increasing ROS levels, plasma enhances tissue oxygenation and skin perfusion during wound healing.

In our study, most of the plasma groups did not exhibit significant differences in growth compared to the control group. These findings align with the results obtained by Lopes et al. (56). They investigated the impact of cold plasma irradiation using argon gas for 30 and 60 seconds on the growth of skin fibroblasts at three and five days post-irradiation, and observed no significant difference between the control and experimental groups. However, it is important to note that, unlike the F1 15s group in our study, their study did not observe reduced growth compared to the control group.

While our study did not observe significant growth of gingival fibroblasts following plasma irradiation, there are studies that support the effect of plasma in promoting fibroblast proliferation (57-60). Plasma generated with helium gas has been shown to enhance the production of granular layer and keratin in the epidermal layer of mouse skin, as well as stimulate the production of type 1 and 3 collagen and alpha smooth muscle actin (61). Additionally, it can upregulate the expression of PPARγ, which is a nuclear receptor involved in modulating inflammatory responses (62). Kwon et al. (63) by irradiating human gingival fibroblasts with plasma for 1, 2, and 4 minutes, discovered that plasma can increase the mRNA expression of growth factors in human gingival fibroblasts. They reported that over the course of a five-day follow-up period, the number of fibroblasts did not increase significantly, but observed that the fibroblasts became more elongated.

Due to the conflicting results in the existing studies, conducting further experiments would contribute to expanding our knowledge regarding the capabilities of laser and plasma therapies, as well as enhancing the validity of the results obtained. Additionally, incorporating long-term follow-up assessments of fibroblasts may yield different outcomes and provide valuable insights. Given the significant role of ROS and RNS in fibroblast proliferation, it is crucial to measure and evaluate these factors in future investigations. By addressing these considerations, we can gain a more comprehensive understanding of the effects of laser and plasma therapies on cellular behavior.

Conclusion

In conclusion, we demonstrated that a 30s radiation of 980nm low-level laser on gingival fibroblasts yields a more pronounced effect on proliferation compared to both a 15s low-level laser radiation and a helium gas plasma radiation for 15 and 30s. Furthermore, our findings indicate that a 15s plasma irradiation with a flow rate of 1.85cm3/s significantly diminishes the increase in fibroblast proliferation. Although initial observations suggested that a 30s plasma irradiation with flow rates of 2.78 and 5.56cm3/s increases fibroblast proliferation on the first day post-irradiation, this increase was found to be statistically insignificant when compared to the control group. Moreover, as time progressed, the increase in proliferation was not statistically significant on the third and fifth days after radiation.

Ethics approval

The current study was conducted with the approval of the Research Ethics Committee at Islamic Azad University, Dental Branch Tehran, Iran (IR.IAU.DENTAL.REC.1401.041, at 30 March 2022).

Declarations

The authors have no relevant financial or non-financial interests to disclose.

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Conflicts of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Availability of data and material

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Authors' contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Helia Sharif. The first draft of the manuscript was written by Helia Sharif, and Shabnam Aghayan commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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