HISTOLOGICAL EXAMINATION OF THE EFFECTS OF LOW-LEVEL LASER THERAPY ON HEALING OF GINGIVA AFTER GINGIVECTOMY IN RATS

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ABSTRACT
Lasers in dentistry find an increasing usage in recent years. The aim of this study was to assess the effects of low-level laser therapy (LLLT) on healing of gingiva after gingivectomy in rats. Forty-two male Wistar rats, 250 g to 300 g in weight, were used in this study. Gingivectomy was made to gingiva of mandibular incisors in all rats. The rats were divided into: group 1 (control group, non-irradiated), and group 2 (experimental group, irradiation-treated). After gingivectomy, a low-level laser therapy (LLLT) was applied to the side of the operation area. Treatment using a GaAlAs laser at a wavelength of 940 nm and a dose of 10 J/cm² began after surgery and was repeated on the 2nd, 4th and 6th day post-surgery. Seven animals from each group were sacrificed on the 7th, 14th and 21st day after surgery. Biopsies were performed for histological analysis. In the laser-treated group, the histopathological findings revealed increased mitotic activity of fibroblasts and collagen synthesis on the 7th day, better formed epithelial layer, mild keratinization, collagen fibers and vascularization on day 14. On the 21st day after surgery, marked collagen fibers and vascularization was assessed according to the control group. The results of this study indicate that low-energy level laser therapy appeared to exert a positive effect in epithelization and wound healing after gingivectomy.

Biotecnol. & Biotechnol. Eq. 2013, 27(5), 4137-4140

Keywords: laser, biostimulation, gingiva, wound healing

Introduction
Following the technological developments in recent years, laser treatments have become quite popular (22). Low-level laser therapy (LLLT) has been used in dentistry for improvement of wound healing (15). Low-level lasers do not cut or ablate the tissues. The basic principle of low-level laser therapy (LLLT) is based on the biostimulation or the biomodulation effect (2, 23) which is due to the ability of irradiation at a specific wavelength to alter cellular behaviour (9, 20). In vitro and in vivo data suggest that LLLT facilitates fibroblast and keratinocyte cell motility (14, 23, 25), collagen synthesis (19), angiogenesis, and growth factor release (21), which lead to increased wound healing.

Wound healing is a complex interaction between many cell types, their cytokines and mediators, and the extracellular matrix. It can be divided into three phases: an inflammatory phase, a proliferative phase, and a remodeling phase. During the inflammatory phase, platelets, neutrophils, macrophages, and lymphocytes migrate to a wound. The proliferative phase shows an increase in fibroblasts and macrophages with a decrease in the acute-phase reactants. And during the final, remodeling phase, fibroblasts assist in the reconstruction of the extracellular matrix and deposit collagen (13). The use of a laser to accelerate the wound healing process has been extensively studied; however, most investigations have been performed on animals dorsal skin (10, 26).

Materials and Methods
Animals
A total of 42 adult male Wistar rats (250 g to 300 g) from the Department of Medical Science Application and Research Centre of Dicle University were used. All animals were provided with commercial rat chow and water ad libitum, and maintained on a 12 h light/12 h dark cycle, at a temperature of 22 °C ± 1 °C. The study was performed in accordance with the Helsinki Declaration and with the permission of the Governmental Animal Protection Committee. The animals had free access to a pellet diet and tap water. All the procedures involved in the experimental protocol were approved by the Animal Ethics Committee of Dicle University (protocol No: 2010/49).

The surgical procedures were performed under ketamine HCl (35 mg/kg) and xylazine (3 mg/kg) based anesthesia. Gingivectomy was made to gingiva of mandibular incisors in
all rats. The rats were divided into: group 1 (control group, non-irradiated), and group 2 (experimental group, irradiation-treated). After gingivectomy, a low-level laser therapy (LLLT) was applied to the side of the operation area.

Irradiation protocol
Following gingivectomy, a GaAlAs laser was used with a 940 nm wavelength which had been previously calibrated by the manufacturer. Each wound of the experimental group animals received 10 J/cm² laser stimulation. The power output was kept constant at 0.1 W in the continuous wave (cw) mode and the spot size was 0.09 cm². The fiber was positioned at a distance of about 5 mm from the wound surface. The irradiation time was 9 s. The control group did not receive any irradiations. The experimental group received the first dose of irradiation 2 h after the surgery and were subsequently irradiated at 2-day intervals following the surgery for a total of four sessions. The efficacy of the laser irradiation protocol was evaluated after a wound healing period of 6 days.

Histological procedures
Seven animals from each group were euthanized on the 7th, 14th and 21st day after the surgery, by cardiac puncture under the intraperitoneal anesthesia with Ketamine HCl (35 mg/kg) and xylazine (3 mg/kg). Biopsies were performed for histological analysis.

The specimens were fixed in 10% neutral formalin solution for 24 h, and decalcified in 5% formic acid. The sections were dehydrated by a step-wise application of 70% and 100% (v/v) ethanol solutions, and embedded into paraffin (Histosec, Merck). The blocks were cut into 5 µm sections. Two sections of each wound were randomly selected and stained with hematoxylin and eosin (H&E) and Masson’s Tripple (MT) for light microscopy evaluation. The microphotographs were taken with a Nikon Eclipse E400 light microscope (Japan).

Results and Discussion
In previous studies conducted by Damante et al. (2, 3), laser was applied at 48 h intervals for four sessions, and it was suggested that daily treatment with LLLT is required to achieve maximal benefit. Similarly, in the present study, with the aim to achieve maximal benefit, four sessions of laser therapy were performed on days 0, 2, 4 and 6 after gingivectomy.

On day 7 following gingivectomy, the comparison of the histological findings demonstrated that, in the group which received laser therapy, fibroblast activity and collagen fibre formation were better expressed than in the control group (Fig. 1). Furthermore, while the epithelium was not evident in the control group, epithelium was observed to have become evident in the group that received laser therapy. In the group irradiated with laser, blood capillaries were greater in number, and fibroblast activity and collagen fibre formation were more regular. It was also observed that the contact between the teeth and the connective epithelium was stronger and of a more regular structure.

On day 14 following gingivectomy, the comparison of the histological findings demonstrated that, in the control group epithelium had started to form, while in the group that received laser therapy, the epithelium was better developed, with a greater number of layers and even slight keratinization (Fig. 2). In the control group, the number of fibroblasts and collagen production were moderate, whereas in the group that received laser therapy, both the fibroblast number and collagen production were greater. Furthermore, in the group irradiated with laser, there was well-developed capillary vascularization.

On day 21 following gingivectomy, the histological findings demonstrated that, in the control group, the epithelium was stratified, with slight keratinization and microscopic papilla slightly visible (Fig. 3A). In the group that received laser therapy, the stratified structure of the epithelium was more clear. The microscopic papilla were deeper and epithelial keratinization was well-developed. Furthermore, in the group irradiated with laser, collagen fibre formation and vascularization were more evident, and the blood capillaries were of a wider diameter (Fig. 3B).

It is well known that important factors in the effectiveness of LLLT include dose, wavelength and the amount of energy applied (12). The wavelength used in this study was 940 nm, and the total applied energy for each session was 10 J/cm². The biological effectiveness of laser irradiation at a wavelength of 940 nm is not well documented in the literature. For this reason, we aimed to investigate the effects of LLLT (at a 940 nm wavelength and a dose of 10 J/cm²) on gingivectomy wound healing in rats.

Al-Watban et al. (1) found that irradiation with a wavelength of 633 nm at a dose of 10 J/cm² elicited the most beneficial effects on wound healing in diabetic mice. Güngörmüş et al. (7) also concluded that low-level laser therapy (808 nm wavelength at a dose of 10 J/cm²) can elicit beneficial effects on diabetic wound healing when used at two-day rather than five-day intervals. Kawalec et al. (11) showed that treatment with a 980 nm GaAlAs laser at 5 W (18 J/cm²) every 2 days enhanced the wound healing in diabetic mice. In our study, we found that LLLT (with a 940 nm GaAlAs at 0.1 W and a dose of 10 J/cm²) elicited a positive effect on gingivectomy wound healing in rats.

Wound healing following laser therapy depends mostly on the activity of fibroblasts, keratinocytes and immune cells (4, 8). Therefore, during the first few days post-surgery, while fibroblasts continue to proliferate, epithelial cells start to migrate to the borders of the lesion, and to form the new epithelium (16, 24). It is known that fibroblasts play a significant role in the healing of tissue trauma, including surgical wounds, as well as in epithelization and collagen synthesis (8). Our findings that fibroblast activity and collagen fibre formation were greater in the LLLT group suggest that irradiation with laser light has a positive effect on wound healing.

In a clinical study conducted in humans by Özçelik et al. (17), it was determined that, following gingivectomy, the complete healing of regions irradiated with laser occurred within 18 to 21 days, while the healing of the regions that were not irradiated with laser and maintained for control purposes took 19 to 24 days. Based on these findings, the researchers suggested that laser therapy after gingivectomy
Fig. 1. Histological appearance of rat gingiva samples taken on day 7 after gingivectomy. G7: control group, non-irradiated (A); LG7: experimental group, irradiation-treated (B). Arrow: gingival epithelium; C: collagen; cp: capillary; arrowhead: fibroblast activity; T: tooth. Haematoxylin and eosin staining; scale bar = 50 µm.

Fig. 2. Histological appearance of rat gingiva samples taken on day 14 after gingivectomy. G14: control group, non-irradiated (A); LG14: experimental group, irradiation-treated (B). Arrow: gingival epithelium; C: collagen; cp: capillary; arrowhead: fibroblast activity; m: microscopic papilla; T: tooth. Haematoxylin and eosin staining; scale bar = 50 µm.

Fig. 3. Histological appearance of rat gingiva samples taken on day 21 after gingivectomy. G21: control group, non-irradiated (A); LG21: experimental group, irradiation-treated (B). Arrow: gingival epithelium; C: collagen; cp: capillary; arrowhead: fibroblast activity; m: microscopic papilla; T: tooth. Haematoxylin and eosin staining; scale bar = 50 µm.
and gingivoplasty operations would increase epithelization, and thus, enable a more rapid healing.

In agreement with the results of Özçelik et al. (17), our study based on histological assessment in rats demonstrated that the group which received LLLT after gingivectomy, showed more evident collagen fibre formation and re-vascularization on day 21 after gingivectomy, compared to the control group. Furthermore, the stratification and keratinization of the epithelium occurred within a shorter period of time in the group that received laser therapy.

According to Fahimipour et al. (6), on the 7th and 14th day, the fibroblasts and new blood vessel counts and collagen density fibers in laser-treated groups were also significantly higher than those in the control groups. In line with this report, on the 7th, 14th and 21st day after surgery, we detected an increase in the mitotic activity of fibroblasts in the non-irradiated group as compared to the group irradiated with a 940 nm GaAlAs at 0.1 W and a dose of 10 J/cm².

The histological examination performed in the present study showed that healing was had already begun on day 7 after gingivectomy, continued on day 14 after gingivectomy, and was completed by day 21 after gingivectomy. Notably, healing was observed to have occurred much more rapidly in the group that received laser therapy.

Wound healing following gingivectomy is similar to wound healing in other parts of the body. However, in the dentogingival junction, the interaction of the epithelium and connective tissue with the mineralized (hard) tissue is different. The present study revealed that, in the trial group which received laser therapy, fibroblast activity in this interaction area was greater, and thus, produced a stronger junction (Fig. 1B). This finding was considered significant, since the integrity of the dentogingival junction is fundamental to the periodontal ligament and the alveolar bone lying beneath.

Another important effect of LLLT on wound healing is the increase in the revascularization rate, as it is known that successful wound healing following periodontal surgery is strongly influenced by the revascularization rate (5). In the present histological study, it was determined that revascularization was better and more evident in the group that received laser therapy, as compared to the control group. Therefore, it could be suggested that laser therapy increases revascularization in the wound site after gingivectomy, and induces a positive effect on wound healing.

Conclusions

Based on the findings obtained in the present study, it could be suggested that the use of LLLT following gingivectomy has a stimulatory effect and increases the fibroblast activity and collagen fibre formation. Laser therapy affects wound healing positively by enabling a more rapid epithelization and by increasing revascularization. It was determined that in the trial group, which received laser therapy, fibroblast activity in the dentogingival junction was at a higher level, and resulted in a stronger junction. This finding was considered to be significant.

However, as different energy doses, strengths, wavelengths, frequencies and laser application modes have been used in previous studies, it is complicated to interpret the obtained results. Further studies are needed to produce standardized results.

Acknowledgements

This study was supported by Dicle University Scientific Research Projects Coordination Office (11-DH-67).

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