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Alveolus soft and bone tissue regeneration after laser biomodulation – a histological study

DIANA FLORINA NICA[1)], ELENA RODICA HEREDEA[2)], DARINCA CARMEN MARILENA TODEA[3)]

[1)]*Department of Anesthesiology and Oral Surgery, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

[2)]*Department of Histology, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

[3)]*Department Oral Rehabilitation and Dental Emergencies, Faculty of Dentistry, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*

Abstract

Functional and esthetic recovery of the patient after tooth extraction is a concern in the nowadays-dental medicine. Immediate implant placement in fresh sockets in posterior sides of the jaws is difficult because of the high amount of bone loss and the disparity between the diameter of the alveolus and the implant. The objective is to evaluate the effect of laser biomodulation alveolar socket healing process of healthy patients. A number of 36 molars have been extracted due to advanced caries lesions from the same dental arch but on opposite sites. Laser irradiation was performed on one side after extraction; the other side was used as control. An Epic-X laser diode (Biolase) Indium–Gallium–Arsenide–Phosphorus (In–Ga–As–P) 940 nm was used in a continuous mode, 0.9 W, 36 J for 80 seconds, daily exposure, in the first seven days after extraction. Specimens of soft and hard tissue were surgically incised and removed by a 4.4 mm diameter trepan from the extraction sites, eight weeks after the surgical procedure. The specimens were prepared by use of two staining procedures: Hematoxylin–Eosin (HE) and Mallory's trichrome. The prepared slides were examined under Leica DM750 optical microscope, 5× and 10× magnification. Laser biomodulation therapy accelerates bone formation by increasing osteoblastic activity. The histological study demonstrates early new bone formation, the regeneration effects in fresh intact bony alveolus compared with the soft and bone regeneration level of non-treated fresh alveolus. Laser biomodulation therapy accelerates soft tissue regeneration and bone formation.

Keywords: laser biostimulation therapy, laser diode, histological examination, bone regeneration.

Introduction

Restoring the normal morphology and the function of the dental arch after teeth extraction remains the main goal in dental treatment. In consequence, soft and bone tissue regeneration after tooth extraction represents a permanent challenge in oral and maxillofacial surgery.

Alveolar bone changes after extraction are essential especially when an implant-supported prosthesis is planned.

Periodontal disease, trauma or periapical infections lead to various degree of bone resorption.

The healing process of alveolar bone after tooth extraction has been described in numerous studies in humans [1, 2] and animals [3]. Histological studies demonstrated that the healing of the alveolus is a complex process, divided into five stages. In the first stage of healing, a blood clot fills the alveolus, in the second stage the blood clot is replaced by granulation tissue until the day 5 after extraction. Until day 16 after the moment of extraction, the granulation tissue is replaced by connective tissue, containing fibroblasts, collagen and ground substance. In the fourth stage, the osteoid structure starts to form beginning at the base of the socket and this process continues a period of six weeks until the alveolus is filled with bone trabeculae. In the fifth stage, the socket is covered by epithelial tissue and this process closes at day 35 after extraction. After 10 weeks of healing, the socket is filled with bone [4].

Concurrently with the healing process of the socket, important morphological changes take place at the level of soft overlaying mucosa and underlying bone structure, generating alveolar ridge atrophy [5, 6], reduction of density of the alveolar bone and soft tissue atrophy [7].

The average width reduction reported by Lekovic *et al.* was 4.6 mm measured six month after tooth extraction [8], while Camargo *et al.*, in 2000, reported around 3 mm bone loss [9]. A clinical study led by Schropp *et al.*, in 2003, reported a reduction in width of the alveolar ridges by 50% at 12 months after tooth extraction, which means a bone loss between 5 mm and 7 mm [10]. An extended systematic review conducted by Tan *et al.* showed that the rapid alveolar ridge reduction takes place during the first three to six months followed by gradual reduction in dimensions thereafter [11].

If an implant-supported prosthesis is planned, the primary stability of the implant and a mature soft tissue around it are the main requirements in this kind of rehabilitation.

The effort to accelerate the bone healing and to minimize the alveolar bone changes after extraction, led to a variety of surgical techniques and grafting procedures in the concept of alveolar ridge preservation. Clinical and experimental studies demonstrated that bone repair could be accelerated also through different complementary therapies, such as photobiomodulation [12].

Bone autografts and bone substitutes are widely used in order to restore the alveolus bone after extraction. The gold standard for bone grafting remains the autologous bone due to the content of osteogenic cells and bone matrix

proteins, osteoinductive and osteoconductive properties [13]. The morbidity of the donor site and the difficulties in bone harvesting could limit these procedures in some cases.

Photochemical interactions between laser light energy and targeted tissues mean biomodulation. Its efficiency is based on the theory of chromophores, which absorb energy from light by biochemical cellular interactions. The photochemical process is at the basis of laser photobiomodulation photodynamic therapy and fluorescence diagnostic [14].

All these three effects are already demonstrated in *in vitro* experiments.

The effect of laser photobiomodulation has been already studied on biological cells, such as fibroblasts, endothelial cells, osteoblasts [15–18]. Several mechanisms are believed to support the beneficial wound-healing responses, including light absorption by mitochondrial enzymes, photoactivation of calcium channels with accumulation of intracellular calcium and cellular proliferation, singlet oxygen production, photon absorption by cytochromes in the mitochondrial respiratory chain [16]. Photobiomodulation also increases osteoblastic activities, vascularization, number of collagen fibers and their alignments [19, 20]. Laser photobiomodulation or low-level laser therapy is a form of therapy, which involves the application of a directional, monochromatic, coherent and non-ionizing electromagnetic radiation to injuries and lesions to stimulate healing [21, 22].

It operates in a wavelength range of 630–980 nm with typical irradiance of 5 mW/cm^2 to 5 W/cm^2 and generated by devices with power from 1 mW up to 10 W, continuous or pulsed waves. Hemoglobin and melanin are the main absorbers for these wavelengths. Light is not well absorbed by the oral mucosa in comparison with muscles, which have the greatest absorption [23].

Aim

The aim of the study was to determine how photobiomodulation could be beneficial in the complex process of soft and bone tissue healing after tooth extraction. The histological study aimed at assessing the degree of inflammation, new vascular and collagen fibers formation, osteoblastic activity by comparison the irradiated group with the control group.

Patients, Materials and Methods

An informed consent was obtained from all patients before their participation in the study. The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania.

A number of 18 human healthy subjects, 10 males and eight females, aged 22–63 years with indications for bilateral molar extraction, entered the study. The extractions have been indicated because of extensive coronary destruction by decay process.

The primary selection point was based on the absence of any local or systemic disease and the necessity of implant insertion as part of the prosthetic plan at the level of extraction sites.

The excluding criteria were: poor oral hygiene, smokers, pregnancy, immunological deficiencies, radio- and chemotherapy in patient's medical history, periodontal disease. The local including criteria are as follows: the necessity of extraction of two teeth on the same arch but on opposite sites in the same surgical intervention and the integrity of the socket walls.

The surgical protocol started with a professional cleaning followed by oral rinse with 0.2% Chlorhexidine solution. Local anesthesia was performed using Articaine solution with adrenaline 1:200 000. Non-traumatic extractions were performed, in the same surgical session, using piezosurgery, root separation and progressive enlargement of the alveolus.

The laser irradiation was performed on one side after extraction; the other side was used as control. An Epic-X laser diode (Biolase) Indium–Gallium–Arsenide–Phosphorus (In–Ga–As–P) 940 nm was used in a continuous mode, 0.9 W, 36 J, for 80 seconds. Irradiation was performed in the non-contact mode, 1 mm from the probe, buccal/lingual, immediate after extraction and 24, 48, 72, 96 hours and in day 7 after extraction. Specimens of soft and hard tissue were surgically taken by a 4.4 mm diameter trepan from the extraction site, eight weeks after the surgical removal of the tooth. The samples were stored in 10% neutral buffered formalin solution and embedded in paraffin and sent for histopathological examination. The samples were stained with Hematoxylin–Eosin (HE) and Mallory's trichrome. The probes were analyzed on Leica DM750 optical microscope, 5× and 10× magnification.

Results

Multiple HE and Mallory's trichrome-stained cross-sections of light-treated and untreated gingival tissue and bone tissue have been examined. The following parameters were analyzed: new bone formation, connective tissue, inflammatory cells.

The histological examination of the irradiated oral mucosa showed acanthosis in the photobiostimulated area comparative with non-treated soft tissue alveolus, where the epithelium was ulcerated on 50% of the width. A parallel disposal of the collagen fibers related with the surface, lower quantity of inflammatory infiltrate under the epithelium were found on biomodulation exposed healing soft tissue. The laser-stimulated epithelium presented an enhancement in fibrogenesis and vascularization. Spongiosis, an abnormal accumulation of fluid in the epidermis, persisting inflammatory infiltrate were found in non-stimulated epithelium (Figures 1 and 2).

The collagen deposition was examined by Mallory's trichrome staining. Laser-stimulated soft tissue presented an enhancement in the number of collagen fibers, a compact and a mature alignment compared with the non-irradiated soft tissue. The non-stimulated soft tissue presented a poor arrangement of collagen fibers (Figures 3 and 4).

The histological examination of the hard tissue found new bone and osteoid tissue in much higher quantity and placed just under the epithelium in the photobiomodulation alveolus comparative with the control sockets. Osteocytes were found in a higher number comparative with control alveolus. Bony tissue in the stimulated sites was with 10–25% more than in the control sites. Osteoblasts in the stimulated alveolus are found in a number with 25% until 50% higher than in the control sites (Figures 5 and 6).

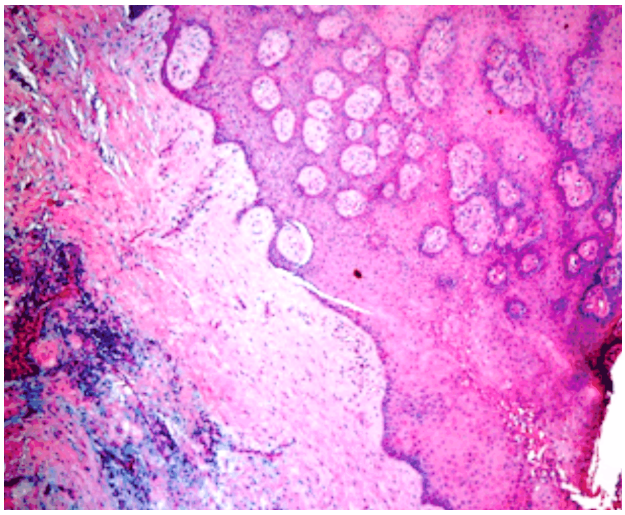

Figure 1 – *Image of complete epithelization of the biostimulated alveolus soft tissue (HE staining, ×100).*

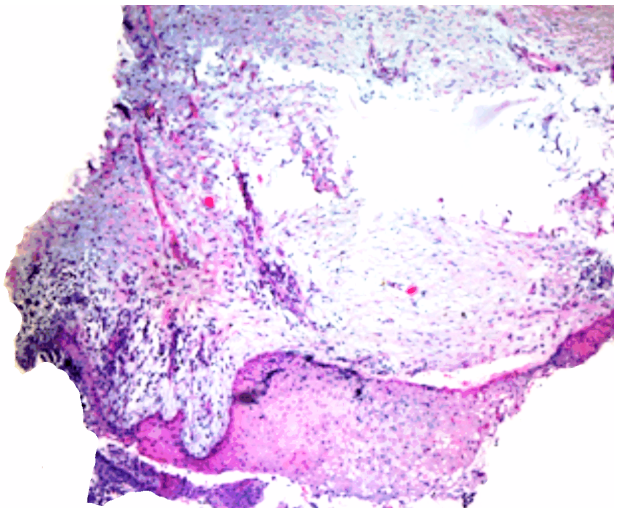

Figure 2 – *Image of ulcerated alveolus soft tissue control site (HE staining, ×100).*

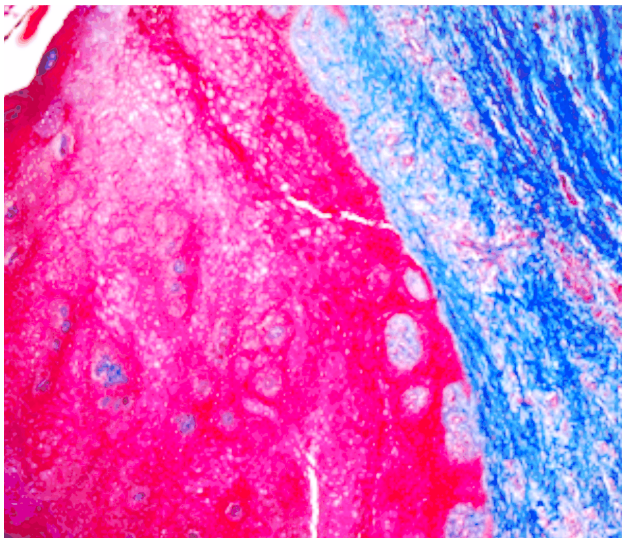

Figure 3 – *Higher number of collagen fibers with mature arrangement of the biostimulated alveolus soft tissue (Mallory's trichrome staining, ×50).*

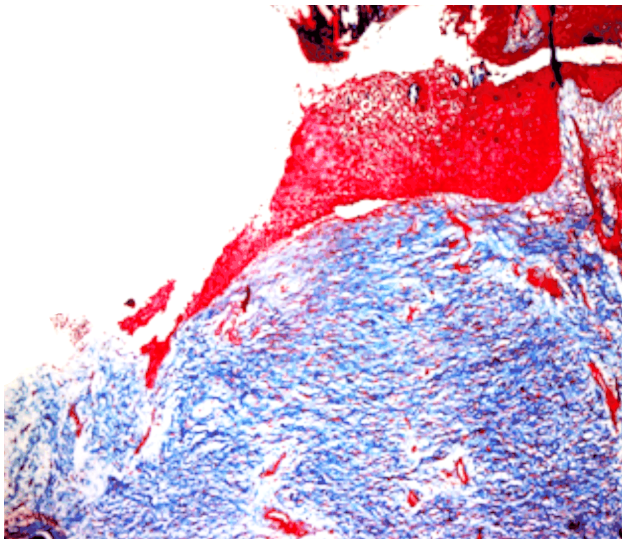

Figure 4 – *Less number of collagen fibers with immature arrangement in the ulcerated epithelium of control site (Mallory's trichrome staining, ×50).*

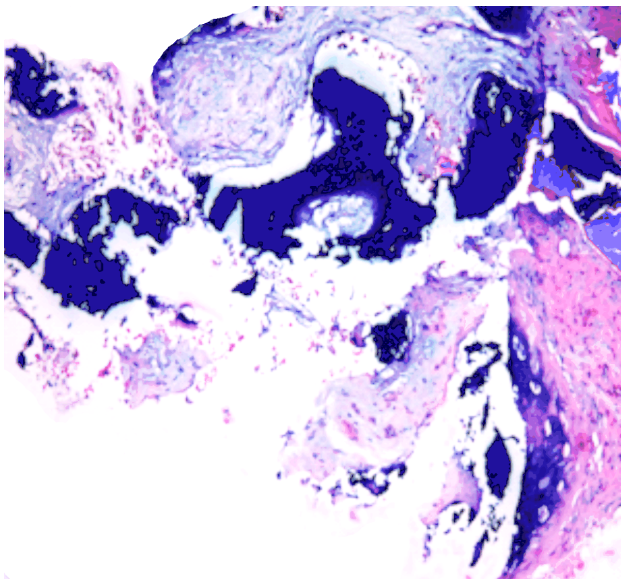

Figure 5 – *Fibrous connective tissue with cancellous bone of the biostimulated alveolus (HE staining, ×50).*

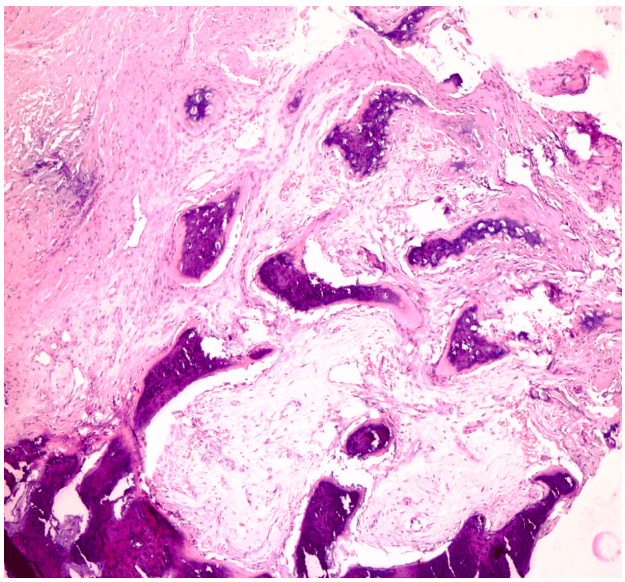

Figure 6 – *New bone formation just under the epithelium of the biostimulated alveolus (HE staining, ×50).*

Discussions

The aim of the study was to evaluate the effect of a 940 nm laser diode applied in a continuous and non-contact mode at the level of the fresh socket.

This study underlines the positive effect of laser diode on regeneration of the complex soft and bony tissues of the fresh alveolus. The histological examination demonstrated a faster healing process both on soft and hard tissue with a higher number of collagen fibers with a mature arrangement, lower inflammatory infiltrate, new bony tissue in a higher quantity and a larger amount of bony cells. The laser-treated sockets have showed a large number of new blood vessels and an ample infiltration of osteoblasts, pointed out that the laser diode stimulates cell differentiation into osteoblasts and endothelial. At the level of non-irradiated sockets (control group), the bone was poor in new bone with a less number of osteocytes and osteoblasts.

The prosthetic standard rehabilitation in case of a single missing molar is mainly performed by implant therapy. Consequently, developing methods to increase the healing process of the socket mean, for the patient, a faster morphological, functional and esthetic rehabilitation. Placing implants after a period of eight weeks (as we performed in our study) from the surgical time of extraction represents part of the early implant placement protocol which ensure the availability of soft tissue for primary healing and bone stability [11]. Our study demonstrates an increased healing process both on soft and hard tissue after photobiostimulation, which enables early implant placement with a suitable soft and hard tissue around implant.

Our findings are in accordance with other studies.

The effect of photobiomodulation has been already applied and studied both in general medicine and dentistry. A faster healing after laser therapy has been proved in surgical scars due to the capacity of the near infrared wavelength to penetrate and to be absorbed through the entire papillary dermis [24–26]. Recently, Mamalis *et al.* has demonstrated that the light-emitting diode red and infrared can inhibit proliferation of skin fibroblasts and migration speed [27].

The influence of photobiomodulation on the bone regeneration process has been proven in several studies. Bosco *et al.* studied the effects of biostimulation in bone formation treating critical size calvarian defects in rats. Its conclusions were: new bone formation at the edges of surgical defect compared with no bone formation in control group, the collagen fibers of the connective tissue presented more organized than the control group, areas of osteoid matrix with large number of osteoblasts comparing with the control group [28]. The collagen fibers have an important role in bone regeneration and the mature arrangement of the fibers after laser stimulation demonstrates the direct effect on extracellular matrix formation [29].

Because the angiogenesis represents the main key in the process of bone regeneration, the valuation of growth factors level after photobiomodulation is very important. Laser photobiomodulation promotes secretion of growth factors during the bone regeneration process. In 2016, de Freitas & Hamblin demonstrated the promoting effect of laser biomodulation on various growth factors [30]. The expression of transforming growth factor-beta (TGF-β), a strong stimulator of collagen production was assessed in a recent study conducted by Tim *et al.* [31]. The study of Park *et al.* demonstrated that the highest expressions of representative osteogenic and growth factor genes and proteins could be obtained after five minutes of laser irradiation with a 980-nm Ga–aluminum (Al)–As. They have concluded that bone healing, in its early stages, is beneficial and this effect is irradiation depends of the time of exposure [32].

Conclusions

This study demonstrated that laser biomodulation improves soft tissue and bone regeneration after teeth extractions. This protocol could be introduced as a part of therapy after extraction alone or in combination with other regeneration techniques. Extraction cases in patients associated with comorbidities, especially diseases treated with antiresorptive and antiangiogenic drugs, where a close assisted healing is required.

Conflict of interests

The authors declare that they have no conflict of interests.

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Corresponding author
Darinca Carmen Marilena Todea, Professor, DMD, PhD, Department of Oral Rehabilitation and Dental Emergencies, Faculty of Dentistry, "Victor Babeş" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timişoara, Romania; Phone +40744–505 503, e-mails: todea.darinca@umft.ro, carmen.todea@gmail.com

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