HISTOLOGICAL RESPONSE OF ORAL MUCOSA ON FRACTIONAL LASER PHOTOTHERMOLYSIS IN ANIMAL EXPERIMENTS

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The aim of the investigation is to prove the supposition that gingival treated by fractional laser photothermolysis is able to initiate regeneration of gingival tissues due to the stimulation of fibroblasts formation, new collagen and vascular structure growth, and complete gingival healing.

Materials and methods. There has been used an original laser system of "Dental Photonics" based on diode laser with wave length of 980 nm generating radiation, with radiation power being up to 20 Watt to perform fractional laser photothermolysis. Each column of microcoagulation was formed in exposure time of 80, 120 or 150 ms. The experiments have been carried out on 18 healthy rabbits. The animals have undergone in vivo laser treatment, and then have been followed up within 90 days.

Results. A single fractional laser photothermolysis procedure with wave length of 980 nm and optimal pulse length of 150 ms was found to induce oral mucosa regeneration till health tissue structure. In healing process, new tissue is characterized by increased blood supply and fibroblasts concentration with no fibrosis signs.

Conclusion. The obtained data enable to consider fractional laser photothermolysis as prospective technique of the treatment of oral soft tissue diseases.

Key words: oral mucosa, fractional laser photothermolysis, regeneration.

The stimulation of periodontal soft tissues regeneration is a primary task in oral mucosa therapy. To initiate gingival and oral mucosa regeneration, in the present study there was used minimal invasive approach based on fractional laser photothermolysis (FLP) [1, 2]. Microscopic thermal injuries in the form of damage islands surrounded by living tissue contribute to the stimulation of regeneration resulting in complete tissue recovery with no scar formation. FLP is successfully used in tissue renewal, therapy of various skin disorders and retinal diseases [3]. Skin healing with no scars has been stated to occur even if thermal injury area exceeds 25% of untreated surface [4]. The technique has gained popularity due to low tissue damage level, insignificant side effects, and high procedure efficacy.

The similar type of laser exposure has never been used regarding oral soft tissues, whereas gingival recession,

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periodontitis, and gingivitis are common conditions being a real problem for practicing dentists, and declining patients' life quality. High vascularization and intensive gingival metabolism contribute to its quicker healing and recovery compared to skin [5].

The aim of the investigation is to prove the supposition that laser gingival treatment using fractional laser photothermolysis is able to initiate the regeneration of gingival tissues due to the stimulation of fibroblasts formation, new collagen and vascular structures growth, and complete gingival healing.

Materials and Methods. The experiments have been carried out on 18 healthy rabbits (16 male rabbits aged 6–8 months and 2 female rabbits aged 5 months). The animals have undergone in vivo laser exposure, and then have been followed up within 90 days. In the study there has been used an original laser system developed by "Dental Photonics Inc" based on diode laser with wave length of 980 nm generating radiation, with radiation power being up to 20 Watt. Each column was formed when the tip, 400 micrometer in diameter, came in contact with the gingiva (exposure time — 80, 120 and 150 ms). The trial laboratory experiments to form the columns in soft tissues ex vivo have shown maximal aspect ratio of columns (injury depth-to-cross dimension ratio) to be achieved in impulse time of 150 ms. In further increase of impulse time, cross dimension grows faster than the depth, reducing by that aspect ratio.

Laser columns were formed in rows (2–3 columns in a row) on a rabbit's maxilla in incisors area (Fig. 1). For each impulse time at least 2 columns were formed. After the experiment the right column area was fixed in 10% neutral formalin solution, embedded in paraffin, and then serial sections, 3 micrometer in thickness, were made, stained by hematoxylin and eosin to determine the tissue general condition. Frozen sections were made from non-fixed gingival tissue containing the second column, and they were stained by nitroblue tetrazolium to determine tissue viability.

Before laser exposure the animals were anesthetized by Rometar, muscle relaxant, 3 mg/kg. The material sampling was made in 1 h on the 1, 2, 3, 4, 5, 6, 7, 12, 28 and 90 days after the exposure, the animals being euthanized by administration of Zoletil 50, 100 mg/kg.

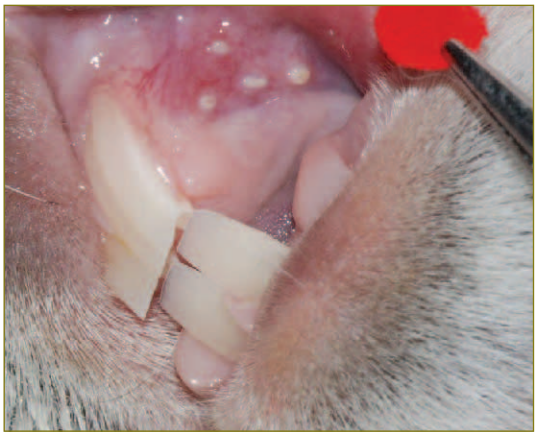

Fig. 1. Laser columns appearance on gingiva of a rabbit's maxilla

When carrying out the investigations, ethical principles were kept inviolate according to European Convention for the protection of vertebrata used for experimental and other scientific purposes (the convention was passed in Strasburg, 18.03.1986, and adopted in Strasburg, 15.06.2006).

Results. The zones of laser damage were studied on histological preparations of two types of staining. The effect of immediate laser exposure on tissue was identified more clearly on the preparations stained by nitroblue tetrazolium. A column was seen as the thermal injury area of epithelium and submucosa with no viability. Nitroblue tetrazolium staining fixes enzymatic oxidative activity inhibition enabling to measure column size to high precision. The columns' width varied from 0.4 to 0.7 mm, and the depth — from 1.2 to 1.8 mm, the maximum depth being reached at impulse time of 150 ms (Fig. 2, *a*).

The preparations stained by hematoxylin and eosin immediately after the exposure of impulse time of any duration (80–150 ms) had damaged epithelium, completely disorganized collagen, no signs of tissue viability in the columns area.

The healing of incised wounds is known to have the following sequentially overlapping stages: inflammation, proliferation, and regeneration [6]. The healing of laser injury has the similar stages [7].

Inflammatory stage of healing. Inflammatory stage of healing starts 1 day after the exposure in all laser pulse durations (80–150 ms). The mass of inflammatory cell infiltrate that enhances the disorganization of damaged collagen is proportional to the duration of laser pulse, i.e. maximum number of neutrophils is seen 1 day after the exposure, when pulse time is 150 ms (Fig. 3, *c*). The peak of inflammatory cell activity is on the 2–4 day, and after that the intensity of cell infiltration is slowly decreasing reaching the basal level on the 12 day. On the 28 day there is no cell infiltration, though the stroma is slightly edematous. By the 90 day there are no signs of inflammation.

Proliferating stage of healing. The first signs of epithelial regeneration, such as basal cell activity and epithelial intussusception into column area are revealed 2 days after the exposure in all laser pulse durations. These facts suggest the minimal risk of contamination, any discharges or macroscopic scabs when FLP is used as a treatment modality. On the 5–7 day there is epithelial reactive hypertrophy that is indicative of rapid proliferation of basal and spinous layer of epithelium. By the 12 day, in 150 ms, new epithelium is formed (Fig. 3, *c*) with the signs of para- and hyperkeratosis. Histological study revealed functional hyperactivity of epithelial cells including intensive synthesis of keratohyalin and keratin resulting in para- and hyperkeratosis. Moreover, on 7–12 day after the exposure, there are poorly expressed signs of dyskeratosis and spongiosis. New epithelium is completely formed on the 28 day after the procedure. Connective tissue regeneration begins with hyperproliferation of fibroblasts taking part in new collagen production and the formation of a new network of thin-walled blood vessels: if pulse time is 80 ms — on the 3rd day after the exposure, in 120 ms — on 4 day, and in 150 ms — on 5 day (Fig. 3, *b*). On the 12 day new collagen is formed on all the levels of structural organization, though

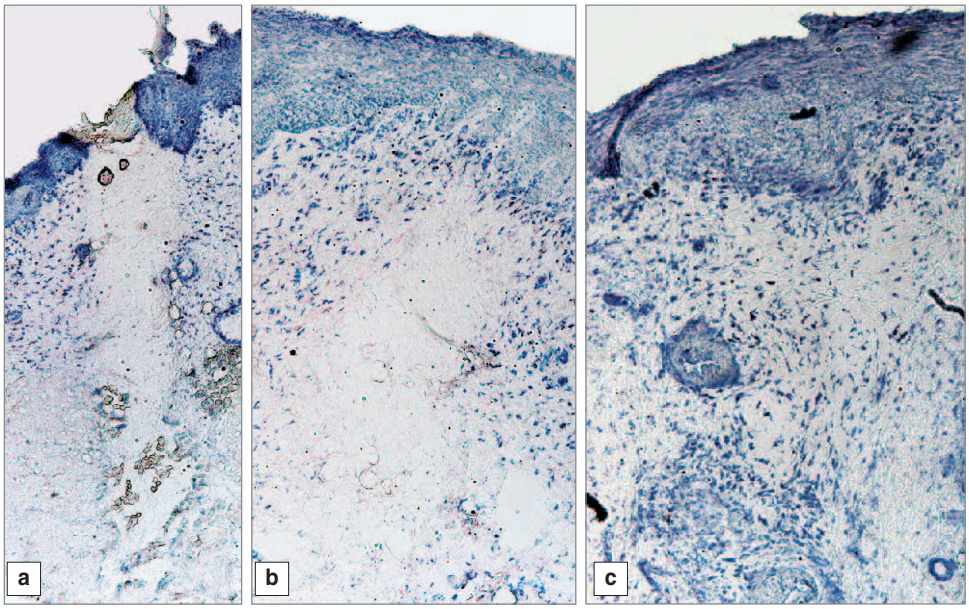

Fig. 2. Tissue devitalizing in laser column area and its revitalizing after a single procedure of fractional laser photothermolysis, with pulse time of 150 ms: *a* — 1 h after the exposure; *b* — 1 day after the exposure; *c* — 7 days after the exposure. Staining — by nitroblue tetrazolium. Column size on Fig. *a* — 0.5 x 1.3 mm; image size — 0.95 x 1.6 mm

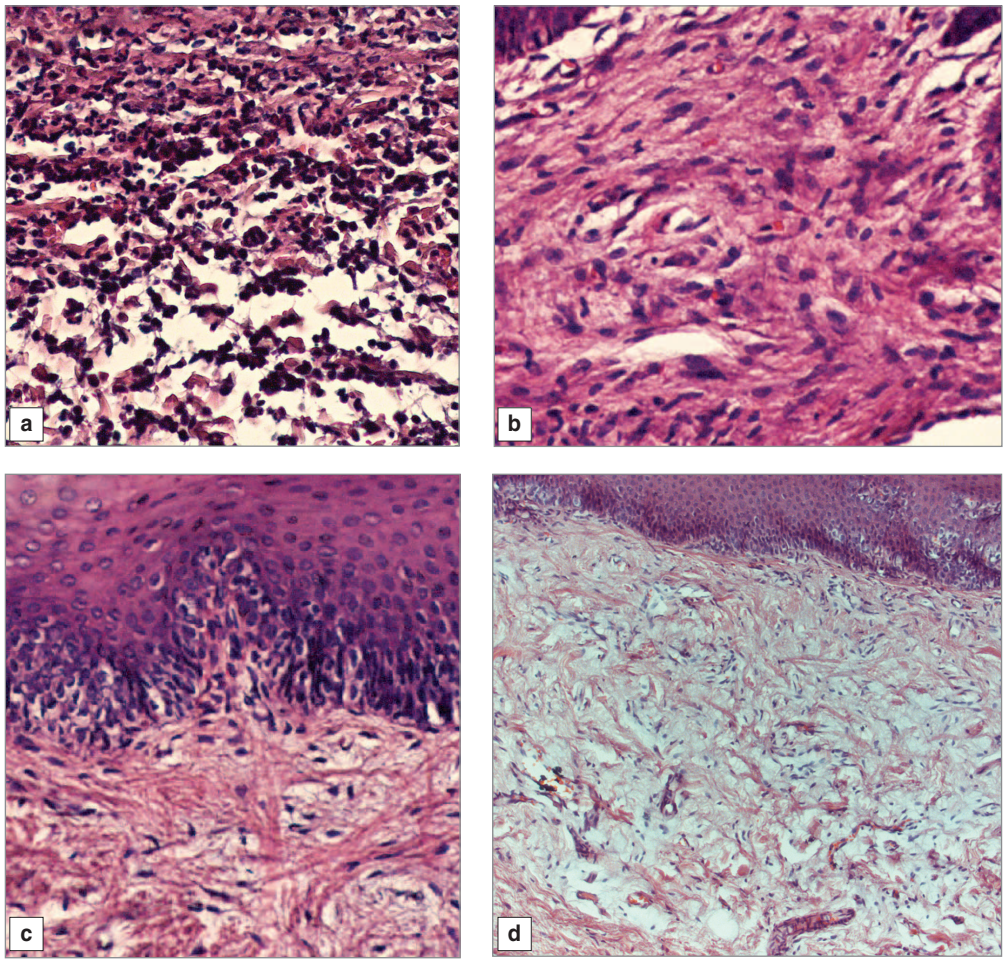

Fig. 3. The healing of laser column after a single procedure of fractional laser photothermolysis, with pulse time of 150 ms: *a* — 1 day after the exposure (infiltration stage — massive inflammatory cell infiltrate); *b* — 5 days after the exposure (proliferative stage — fibroblasts activation and formation of thin-walled blood vessels); *c* — 12 days after the exposure (stage of proliferation and regeneration — new epithelium and new collagen fibers; their orientation in subepithelial layer has no yet ordered horizontal structure); *d* — 90 days after the exposure (regeneration stage of healing — collagen is completely formed). Staining — by hematoxylin and eosin. Image size — 0.30x0.30 mm

its orientation in subepithelial layer has no ordered horizontal structure yet (Fig. 3, *c*). And new connective tissue contains newly formed thin-walled blood vessels.

Regeneration stage of healing. On 12 and even 28 day after laser exposure, in 150 ms of pulse time, collagen fibers located immediately below the epithelium still have no regular orientation (Fig. 3, *c*) corresponding to the state before the laser exposure. However, on 90 day collagen of connective tissue appears to be completely formed (Fig. 3, *d*).

Thus, the data obtained are strong evidences of the fact that on 28 day of follow up the tissue structure after a single procedure of FLP is regenerated, and though there is a slight increase of a number of small vessels in subepithelial layer and slight edema, no signs of scarring are seen. On the 90 day tissue structure is completely restored: there are no signs of dyskeratosis and spongiosis in epithelium, as well as there are no signs of scarring in connective tissue. Knowing the mechanisms and analysis of the healing time after laser exposure enables to state that a repeated fractional treatment is to be a good reason to perform not earlier than a week after the first procedure to avoid complications related to incomplete healing of epithelium and connective tissue [8].

Conclusion. A single fractional treatment by laser photothermolysis induces oral mucosa regeneration that permits to consider the technique as prospective method of treatment of oral soft tissues diseases.

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