IN VIVO CONTROL OF ORAL MUCOSA REGENERATION AFTER FRACTIONAL LASER PHOTOTHERMOLYSIS USING CROSS-POLARIZATION OPTIC COHERENCE TOMOGRAPHY

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N.D. Gladkova, D.Med.Sc., Professor, Head of Task Research Group of Optic Coherence Tomography of Scientific Research Institute of Applied ande Fundametal Medicine[1];
M.M. Karabut, Postgraduate, the Department of Biophysics[2];
E.B. Kiseleva, Junior Research Worker, Task Research Group of Optic Coherence Tomography of Scientific Research Institute of Applied ande Fundametal Medicine[1];
Yu.V. Ostrovskaya, PhD, Tutor, the Department of Dentistry, the Faculty of Doctors' Advanced Training[1];
A.A. Muraev, PhD, Tutor, the Department of Maxillofacial Surgery and Implantology, the Faculty of Doctors' Advanced Training[1];
I.V. Balalaeva, PhD, Head of the Laboratory of Cellular Technologies[2];
F.I. Feldstein, PhD, Vice-president[3]

[1]Nizhny Novgorod State Medical Academy, Minin and Pozharsky Square, 10/1, Nizhny Novgorod, Russian Federation, 603005;
[2]Nizhny Novgorod State University named after N.I. Lobachevsky — National Research University, Gagarin Avenue, 23, Nizhny Novgorod, Russian Federation, 603950;
[3]Dental Photonics Inc., Boston-Providence Highway, Walpole, USA, 1600

The aim of the investigation is to assess the possibilities of cross-polarization optic coherence tomography (CP OCT) for in vivo control of oral soft tissues regeneration after fractional laser photothermolysis.

Materials and Methods. The experiments were carried out on 18 healthy rabbits having undergone in vivo fractional laser photothermolysis, the observation period being 90 days. In the study there was used original laser system by "Dental Photonics Inc." (USA) based on diode laser with wave length of 980 nm and power of up to 20 W. Each column was formed by contact of the tip of 400 micrometers in diameter and gingival tissue within long exposure of 80, 120 and 150 ms. Laser columns were made in rows (2–3 columns in a row) on rabbit's maxilla in an incisor area. For CP OCT-survey of mucosa there was used CP OCT-tomograph "OKT 1300-У" (Institute of Applied Physics, Russian Academy of Sciences, Nizhny Novgorod) equipped by an end endoscopic probe with outside diameter of 2.7 mm. In the device there used probe radiation with wave length of 1300 nm and power of 3 mW.

Results. By measuring the standart deviation of OCT-signal in orthogonal polarization there was stated that in the period between the 5th and the 12th days after fractional laser photothermolysis, the maximum increase of the quantity measured ($p=0.001$) occurs due to the increased collagen synthesis and the formation of new collagen fibers. It is in agreement with morphological study data. By the 28th day the signal intensity in the column area has recovered though not completely ($p=0.001$ compared to the initial level) and has reached the base value by the 90th day ($p=0.31$ compared to the initial one). No signal increase on the 90th day gives the evidence of collagen recovery in the laser damage area with no signs of fibrosis (scarring).

Conclusion. Cross-polarization optic coherence tomography can serve as an effective in vivo control method of oral mucosa regeneration after fractional laser photothermolysis. The criteria of successful gingival healing after photothermolysis are: 1) the presence of layer structure in direct polarization; 2) complete recovery of standart deviation of signal intensity in orthogonal polarization.

Key words: cross-polarization optic coherence tomography, oral mucosa of rabbits, collagen, numerical analysis of OCT-images, fractional laser photothermolysis.

Optic coherence tomography (OCT) in dentistry is traditionally used to diagnose dysplasia of oral mucosa epithelial against the background of benign conditions — leukoplakia and erythroplakia [1, 2]. Many papers demonstrate OCT diagnostic efficiency both on animal models [3], and on a human being [4, 5]. Recently, Nizhny Novgorod researchers have turned their minds on clinical possibilities of the technique modification — cross-polarization OCT (CP OCT) extending the diagnostic potential of traditional OCT [6–8]. *In vivo* CP OCT not only studies microstructural changes of biotissues with spatial resolution of 10–15 micrometer but also gives an insight into the change of collagen fiber structure based on the assessment of its polarization characteristics. CP OCT images are in initial (impinging on tissue) and orthogonal polarization [9, 10]. Publications on CP OCT application in dentistry have demonstrated its high diagnostic efficiency [11–13].

For contacts: Gladkova Nataliya Dorofeevna, tel.: 8(831)465-59-04; e-mail: natalia.gladkova@gmail.com

In recent years fraction laser photothermolysis (FLP) is used in clinical practice in ophthalmology and dermatology, aiming at biotissue regeneration stimulation. FLP results in isolated thermal micro-wounds surrounded by the areas of vitalized tissue [14–17]. The biotissue damage and FLP side effects are minimal. Based on the experience of medical use of skin and retina FLP, for the first time we have used FLP to study a regenerative reaction of oral soft tissues [18–20].

In damage and further regeneration of oral mucosa in FLP, the crucial components determining the healing process are epithelium and collagen fibers of connective tissue stroma [21, 22]. In the present study the surveillance of their damage and recovery has been carried out using *in vivo* CP OCT. The modification of the original technique of quantitative estimation of OCT signal in cross-polarization CP OCT-image [23] was used for the first time, by its standard deviation that contributes to information content increase.

The aim of the investigation is to assess the possibilities of cross-polarization optic coherence tomography for *in vivo* control of oral soft tissues regeneration after fractional laser photothermolysis.

Materials and Methods. The experiments were carried out on 18 healthy rabbits (16 male rabbits aged 6–8 months and 2 female rabbits aged 5 months). The animals underwent life-time fractional laser photothermolysis, and the follow-up period was 90 days.

In the study there was used original laser system by “Dental Photonics Inc.” (USA) based on diode laser with wave length of 980 nm generating radiation up to 20 W. Each column was formed by contact of the tip of 400 micrometers in diameter and gingival tissue within long exposure of 80, 120 and 150 millisecond. The performed preliminary laboratory experiments on forming the columns in soft tissues *ex vivo* showed the maximum aspect ratio of columns (between damage depth and transverse size) to be reached in mode of 150 ms. Further increase of exposure time causes more rapid growth of column transverse size, by that reducing the aspect ratio. Laser columns were made in rows (2–3 columns in a row) on rabbit’s maxilla in an incisor area, the distance between the columns centers being 1.2±0.2 mm; the distance of the columns from incisors or lower teeth being 3.0±0.5 mm; columns orientation — perpendicular to the surface; force of pressure — 20 g; cap’s diameter — 400 micrometer. In each mode at least 2 columns were formed (Fig. 1, *c*).

When performing researches, there were strictly observed ethical principles established by European Convention for the Protection of vertebrates. The convention is used for experimental and other research purposes (it was passed in Strasbourg on the 18th of March 1986, and in 2006, 15th of June was adopted in Strasbourg). Before laser treatment the animals were anesthetized with muscle relaxant Rometar intramuscularly, in dose of 3 mg/kg.

For CP OCT survey of mucosa there was used CP OCT-tomograph “OKT 1300-У”. The device is developed in Institute of Applied Physics, Russian Academy of Sciences (Nizhny Novgorod, Russia) and equipped by an end endoscopic probe with outside diameter of 2.7 mm. Probe radiation with wave length of 1300 nm and power of 3 mW are used in the device. It has two channels and demonstrates simultaneously two conjugated images (Fig. 1, *b*): the lower — in co-polarization polarization, the upper — in cross-polarization, with strict compatibility of spatial arrangement of image elements. Every CP OCT-image registered within 2 s has the following characteristics: size 200x512 px, 2x2.3 mm, depth resolution — 15 micrometer in free space, lateral resolution — 25 micrometer [10].

Results and Discussion.

In vivo study of oral mucosa microdamages formed in FLP on experimental animals using CP OCT technique. CP OCT of rabbits’ maxilla, 3 mm to the left and to the right of the incisors, at first was performed before the laser action (Fig. 1, *a, b*). CP OCT with high resolution visualizes the tissue structure and enables to assess the thickness and degree of epidermidalization, the density and thickness of connective tissue stroma, and consider the presence and location of glands and vessels in stroma, i.e. the structures having an impact on the depth and character of laser effect on tissue.

FLP results in microtraumas of epithelium and underlying connective tissue stroma that immediately have an effect on their optic (dissipative and depolarizating) properties. Micro-columns were studied by CP OCT technique straight after FLP of healthy oral mucosa of the rabbits. CP OCT is

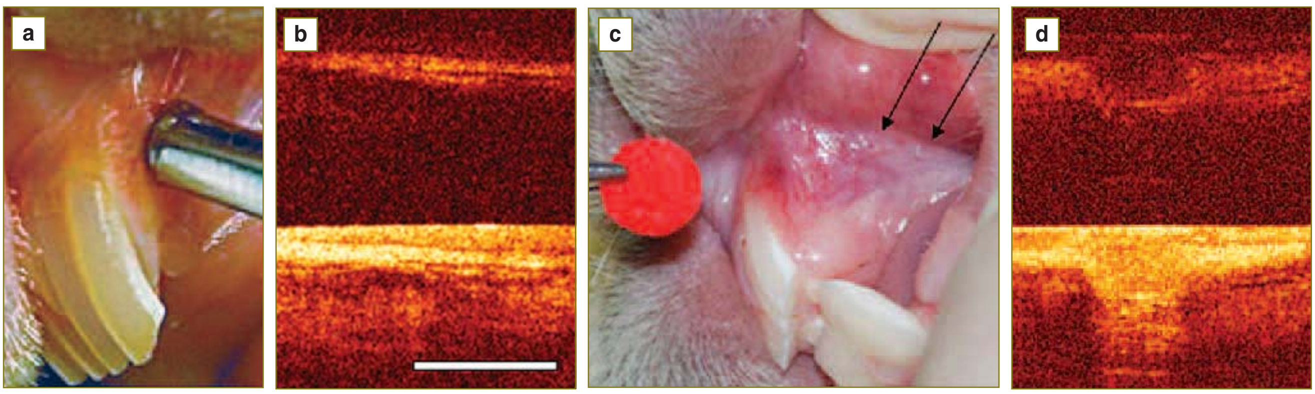

Fig. 1. CP OCT study of rabbit oral mucosa: before FLP — photo (*a*), CP OCT-image (*b*); after column formation in the mode of 150 ms — photo (*c*) and CP OCT-image (*d*). Hereinafter bars 1 mm

used for this purpose because collagens of stromal tissue depolarize light in a greater degree than other mucosal components.

CP OCT-image of maxillar mucosa before the columns formed (Fig. 1, *b*) demonstrates high-contrast structural image with intense signal from stroma in co- and cross-polarization due to organized collagen depolarizing the probe radiation to a great degree. FLP column formed in the mode of 150 ms (Fig. 1, *c*) is seen in cross-polarization CP OCT-image (Fig. 1, *d*) as the area with deformation structure, and sharply reduced signal. The column has vertical orientation. In co-polarization the signal from epithelium in column's place is intense.

The signal in co-polarization is known to be the result of back scatter of probe radiation and depends on physical parameters of scattering particles, the density of their packing and optic properties. In column's place there is the area thermo-damage causing partial epithelial necrosis and stroma collagen denaturation. Therefore, all initial properties of scattering particles change significantly, and it is demonstrated by the enhancement of OCT signal from epithelium in direct polarization.

One ought to bear in mind analyzing the signal on CP OCT-image in cross-polarization that a signal appears if in the tissue there are local, random anisotropic micro-irregularities that can depolarize probe radiation. The particles with the diameter being considerably larger than the wave length of probe radiation, and aspect ratio being over 1.5, can be effective depolarizers in case the difference of refraction index between a particle and its medium is not great [9]. Normally in cross-polarization numerous collagen fibers of connective tissue stroma prove to be the areas with high intensity signal. After column formation the intensity of signal in cross-polarization down due to disorganization and coagulation of collagen fibers (Fig. 1, *d*).

Thus, CP OCT image with high resolution in real time demonstrates the tissue structure and its functional state. CP OCT use can be useful when choosing optimal modes and techniques (re-courses) of laser treatment, as CP OCT reflects objectively the scope and degree of laser damage of oral mucosa, enables to observe further processes followed by the laser action, as well as long-term FLP effects.

In vivo study of the healing processes of oral mucosa soft tissues microdamages in FLP on experimental animals using CP OCT technique. Healing of incisions is known to pass through the consecutive overlapping stages: inflammation, proliferation, and regeneration [15]. The healing of laser damage has the same phases [24, 25]. Early morphological studies carried out by our group have showed the inflammatory phase of healing including cell infiltration (in its turn it initiates disorganization of damaged collagen fibers) to start in 24 h after FLP in all modes (80–150 ms). Inflammatory cell activity maximum is on the 2nd–4th days, and then the intensity of cellular infiltration is gradually decreases and terminates on the 12th day. On the 28th day there is no cellular infiltration, though slight stroma edema persists. By the 90th day there are no inflammatory signs.

The first signs of epithelial regeneration appear 2 days after FLP in all modes. On the 5–7th day there will be reactive epithelial hypertrophy giving evidence of rapid proliferation of basal and spinous layer of epithelium. By the 12th day increased functional activity of epithelial cells is observed including intensive keratohyaline and keratine synthesis resulting in para- and hyperkeratosis. Moreover, on the 7–12th day in new epithelium there are subtle sings of dyskeratosis and spongiosis. New epithelium forms completely on the 28th day.

Connective tissue regeneration starts with an increased proliferation of fibroblasts participating in new collagen fibers production and the formation of new thin-walled vessels network on the 3rd–5th day after the manipulation. From the 5th to 12th days the most intensive process of collagen fibers formation occurs. On the 12th day new collagen fibers form on all levels of structural organization, and it is proved by polarization properties (picrosirius red staining), however, even on the 28th day after FLP in 150 ms mode, the fibers orientation in subepithelial layer is not horizontal, as it is in norm. And on the 90th day collagen fibers of the connective tissue are indistinguishable from initial ones [21, 22].

In the present investigation there have been distinguished the following characteristics of CP OCT images of columns after laser treatment and their healing dynamics (Fig. 2):

in 1 day after FLP: co-polarization imaging — damaged layer structure; cross-polarization — the absence or OCT

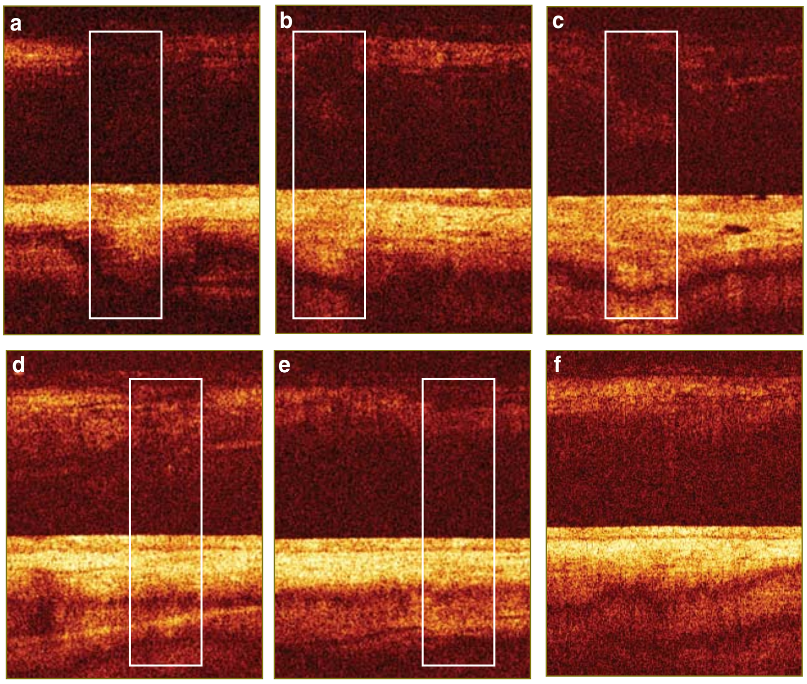

Fig. 2. CP OCT study of the dynamics of columns healing after FLP: *a* — immediately after the exposure; *b* — in one day; c — after 5 days; *d* — after 12 days; *e* — after 28 days; *f* — after 90 days. Rectangles mark columns area

signal intensity decrease in the columns area (Fig. 2, *a,b*);

on the 5th day after FLP: co-polarization — the start of layer structure recovery; cross-polarization — the presence of a slight signal from stroma in the columns area, though there is still no-signal zone сигнала (Fig. 2, *c*);

on the 12th day after FLP: co-polarization — almost complete recovery of layer structure; cross-polarization — signal intensity from stroma is not equal to its initial level (Fig. 2, *d*);

on the 28th day after FLP: co-polarization — layer structure recovery; cross-polarization — the column zone with a weak signal is hardly perceptible to the eye (Fig. 2, *e*);

on the 90th day after: co-polarization — layer structure recovery; cross-polarization — no zone with a weak signal (Fig. 2, f).

Quantitative assay of OCT-signal in cross-polarization images to assess the healing processes of oral mucosa soft tissues microdamages in FLP on experimental animals using CP OCT technique. For objectivization of visual criteria of CP OCT-images there has been performed quantitative analysis of OCT-signal in cross-polarization images.

Intensity or light intensity of a signal in co- and cross-polarization is frequently used to characterize OCT-images. However, average signal intensity being a statistic value of the first order [6, 7] is known to be quite dependent on device parameters, and non-informative constant component of a signal in particular, while standard deviation (SD) of OCT signal from an average value (a statistic value of the second order) is less dependent (if it is higher than the noise level at least by several decibel). The use of standard deviation of a signal has been introduced in some papers [25–27]. In contrast to the most imaging technologies with low noise level, OCT is based on interferential speckles and always has significant (up to 100%) spatial modulations of speckle structures. The presence of speckle modulation makes SD a good tool for signal intensity measurement, especially in non-homogenous areas with dark spots (e.g. Fig. 1, *b* — upper image). These dark areas can significantly reduce an average brightness, but they introduce the less error, and the error is less dependent on the marquee size.

On each CP OCT-image there has been measured the of OCT signal SD in cross-polarization in column area (Fig. 3, *b*) and in the same area — before column formation — the initial, control value (Fig. 3, *a*). SD has been measured in marquee, as we suppose homogeneous distribution of collagen fibers in stroma in health and none. By means of quantitative assessment of the OCT signal SD there have been distinguished 6 groups of columns by the healing period (See the Table), and the results have confirmed the main stages of mucosa regeneration: the period of increased collagen synthesis and the formation of new collagen fibers is 5–12 days, and a complete recovery of collagen fiber structure — by the 90th day after FLP (Fig. 4).

Thus, there has been developed a *in vivo* method of tissue reparation control after FLP using CP OCT with quantitative assessment of OCT-signal. Between the 5th and the 12th days after FLP, OCT-signal in cross-polarization has been stated to increase to the utmost that corresponds to the most intensive collagen recovery (p=0.001). It is in agreement with morphological data obtained by our group. By the 28th day after FLP the signal intensity in cross-polarization in the column area has recovered though not completely (p=0.001 compared to the initial level) and has reached the base value by the 90th day (p=0.31 compared to the initial one). No signal increase on the 90th day gives

The results of quantitative assessment of standard deviation of the OCT signal intensity in CP OCT cross-polarization (n=15)

Norm (n)	0 days (the moment of columns formation)	5 days	12 days	28 days	90 days
6,02±0,12	3,82±0,11	3,88±0,06	5,00±0,15	5,16±0,14	5,86±0,29
			p^*_{5-12}=0,001	p^*_{12-28}=0,23	p^*_{28-90}=0,038
				p^*_{H-28}=0,001	p^*_{H-90}=0,31

* p — statistical significance of differences between the groups of interest according to Student one-dimension t-criterion

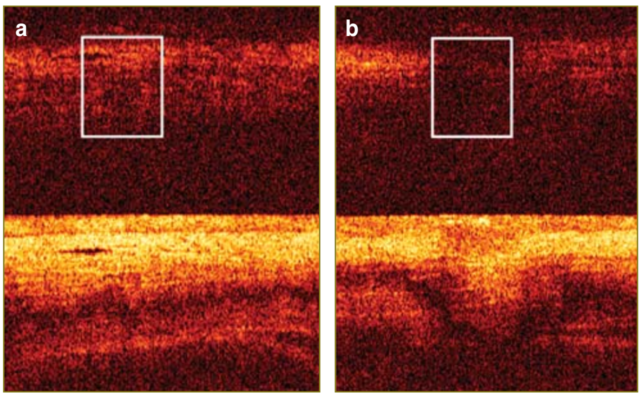

Fig. 3. The selection of the area to measure standard deviation of the OCT signal intensity in cross-polarization CP OCT-image of rabbit gingiva before (*a*) and after (*b*) column formation

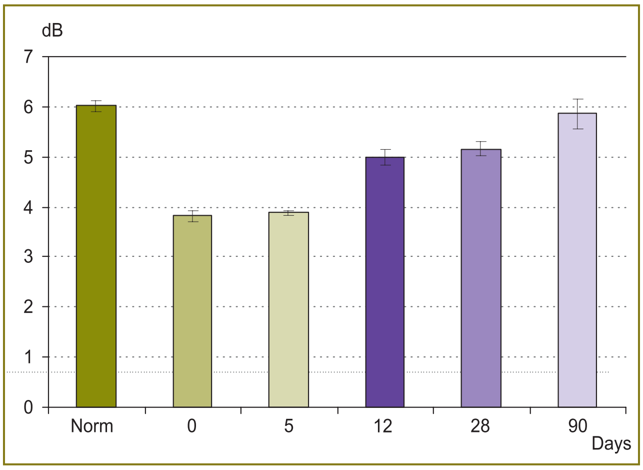

Fig. 4. Mean values of standard deviation of the OCT signal intensity in cross-polarization in dynamics of laser columns healing from the moment of formation (0 days) to 90 days

the evidence of collagen recovery in the laser damage area with no signs of fibrosis (scarring).

Conclusion. Cross-polarization optic coherence tomography can serve as an effective *in vivo* control method of oral mucosa regeneration after fractional laser photothermolysis. The criteria of successful gingival healing after photothermolysis are 1) the presence of layer structure in co-polarization image; 2) complete recovery of standart deviation of signal intensity in cross-polarization image.

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