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Approbation of the technology of laser soft tissue reconstruction using laser speckle contrast imaging *in vivo*

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The aim of the work is the approbation of the developed technology of laser reconstruction of soft tissues on laboratory rabbits *in vivo*. The technology of tissues laser repair includes application of laser biopolymer solder based on bovine serum albumin, single-walled carbon nanotubes and chromophore — indocyanine green to the area of dissection and subsequent irradiation with laser radiation with a wavelength of 810 nm. The laser system is equipped with temperature feedback, allowing continuous monitoring and temperature control in the area of laser suture formation. During the operation and before the day of withdrawal by the method of laser speckle-contrast imaging the study of microcirculation recovery of blood flow in the area of laser connection of biological tissues was carried out. After the experiments on each day of withdrawal, histological and immunohistochemical studies of laser sutures were performed to assess the effectiveness of tissue healing and the degree of scarring, as well as toxicological studies of lymph nodes adjacent to the laser repair area. As a result of the studies, it was found that the developed technology does not disrupt blood flow and allows to create sutures without rough scar formation.

Keywords: laser surgery, speckle contrast imaging, biopolymers, single-walled carbon nanotubes.

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Introduction

One of the key stages of surgical interventions is the joining of dissected sections of biological tissues. Needles and suture materials are traditionally used to restore the integrity of such dissections. The suture method is characterized by simplicity and relative accessibility. However, despite these benefits, there are significant disadvantages of this approach, such as additional injury to the wound, possibility of immune reaction and the formation of rough scars. A modern alternative is a "seamless" joint that avoids making punctures of biological tissues, but uses adhesives instead, as well as electric welding of tissues.

Adhesive technologies ensure creation of airtight sutures with minimal scarring, however, they often suffer from insufficient strength. Cyanoacrylates have the highest strength among adhesive methods, but their high toxicity may cause inflammatory and allergic reactions, as well as neurotoxicity and mutagenicity [1]. Fibrin glue is used as an alternative to cyanoacrylates. Fibrin does not cause inflammatory reactions or toxic tissue necrosis, however, its mechanical properties are often insufficient for use in conditions requiring high strength. Moreover, there is a risk of infections transmission through the blood. Some dangers today are also associated with a growing number of cases of acquired immunodeficiency syndrome (AIDS) and bovine spongiform encephalopathy (BSE). One of the ways to tackle these problems is to isolate fibrinogen from the patient's own blood. This approach avoids the risk of infections transmission, but requires at least two days of preparation, which may postpone the surgery procedure [2].

When using electrical, ultrasonic and radiofrequency welding of biological tissues, there is a risk of side electromechanical effects with an adverse impact on the surrounding cells. These methods, however, have limited

use in practice. Ultrasonic welding, for example, is characterized by a narrow heat treatment zone, which can lead to local overheating and tissue damage. Studies show that the joints obtained using these methods have a strength which is not sufficient enough to meet the clinical requirements, which may cause complications in the healing process and the connective tissues not functioning properly [3].

The most promising method of non-contact tissue repair is laser repair of biological tissues. The laser repair technology is based on applying bioorganic protein solder to the damage area and further irradiation with laser until the protein denatures and changes the molecular structure of both, the main components of the extracellular matrix of connective tissue, which form connecting bridges, and the components of bioorganic solder.

At the first stage of laser exposure, the triple helices inside the collagen molecules are unwound due to the hydrolysis reaction of hydrogen bonds, and the collagen fibers on the ends of the incision are untangled. Further laser exposure leads to fiber intertwining and interdigitation across the incision. As a result, fusion occurs either between the cut ends of the collagen fibers or between their parallel faces. Also, during laser irradiation, new chemical bonds are formed: covalent crosslinking at the soldering site and non-covalent interactions between unwound collagen strands on both sides of the suture. After cooling, non-covalent interactions between the resulting hardened (denatured) solder and collagen matrices in the tissue ensure the active proliferation of cellular structures [4,5].

This method may ensure waterproofing of the affected area, airtightness of the wound, and also sealing small vessels and shortening the duration of bleeding. In addition, there is no need to compress the tissue with sutures, which reduces the risk of marginal necrosis and promotes tissue healing without the formation of coarse scars. Because laser methods are non-contact, the likelihood of wound infection is minimized [6–8].

The key drawbacks of laser exposure are excessive thermal damage to surrounding tissues and low penetration depth of laser radiation, resulting in a tensile strength of welds that is inferior to the traditional suture method using suture material [9].

The depth of penetration of laser radiation and the degree of thermal damage to biological tissues when exposed to laser radiation depend on several key parameters, including laser power, components of the solder used, duration and method of exposure, as well as final resulting temperature that can be attained in the tissues. Semiconductor laser systems with wavelengths in the near infrared range have become the most widespread in the laser soldering of biological tissues (800–1100 nm). Semiconductor systems are smaller in size and consume significantly less energy than other laser technologies. However, the use of diode lasers has certain limitations, since the peak output power of radiation is significantly lower than that of CO_2 and Nd : YAG-lasers [10,11]. This disadvantage is minimized due to bioorganic solders and dyes that increase the absorbency of tissues [12].

The use of bioorganic compounds in laser tissue repair has significantly increased the strength of laser sutures, reduced the risk of thermal tissue necrosis, and accelerated the recovery process. Blood protein „serum albumin“ has become the most widely used as the basis of bioorganic solders. An aqueous suspension of albumin provides primary bonding of the damage edges, turning into a solid (diverse) nanocomposite during thermal denaturation under the action of laser radiation. In order to focus the laser effect in the dissection area and prevent thermal necrosis of the surrounding tissues that were not the target, a chromophore is added to the solder that absorbs the laser radiation wavelength. The most successful combination in the laser repair of biological tissues is the use of the cyanine dye called ”indocyanine green” (ICG) in conjunction with a semiconductor laser with a wavelength of 810 nm [13]. ICG is a proprietary non-toxic fluorescent iodide dye with rapid liver clearance [14]. The spread of this combination is due to the fact that the peak absorption of this bioorganic dye occurs at 800 nm, which is consistent with the laser radiation wavelength.

Despite significant advances in laser repair of biological tissues, the technology still has significant limitations and has not been widely used in clinical practice.

In previous studies [15,16], the authors revealed that the addition of single-walled carbon nanotubes in low concentrations (up to 0.1 wt.%) to the composition of biopolymer solder leads to the formation of a durable nanocomposite material consisting of a reinforcing frame based on interconnected carbon nanotubes in a protein matrix. The resulting nanocomposite makes it possible to increase the strength of laser suture by two orders of magnitude compared to laser suture formed using solder without using single-walled carbon nanotubes [17]. Also, to prevent overheating, the designed solder should be used together with a laser system based on a 810 nm wavelength semiconductor laser with temperature feedback, which allows continuous monitoring and temperature control in the area of laser suture formation, ensuring high precision of thermoregulation, and contributes to improving the quality of compounds.

To demonstrate the efficiency of the new technology, in this work, an *in vivo* study was conducted on laboratory rabbits. During the surgery and before the day of removal, laser speckle contrast imaging was used to study the recovery of microcirculation of blood flow in the area where the biological tissues had been repaired by laser. After the experiments, histological and microscopic examinations of the laser sutures were performed to assess the effectiveness of tissue healing and the degree of scarring, as well as toxicological examinations of the lymph nodes adjacent to the laser repair zone.

Materials and methods

This section highlights in details the bioorganic solder preparation technique, the new system for laser repair of soft tissues using an adaptive thermal stabilization system, the device for monitoring blood flow using speckle contrast imaging, the procedure for laser repair surgery, as well as methodological approaches to histological studies.

0.1. Biopolymer solder

The biopolymer solder applied on the area of a laser suture was itself a solution of the single-walled carbon nanotubes (SWCNT, OOO „Uglerod ChG“, Chernogolovka, Russia) of the bovine serum albumin (BioClot GmbH, Aidenbach, Germany) and indocyanine green in distilled water. To acquire hydrophilic properties, single-walled carbon nanotubes were functionalized with carboxyl groups. Bovine serum albumin is the main protein component of solder, which has high biocompatibility and does not cause immune reactions when injected into the human body. Exposed to laser radiation, albumin denatures and coagulates, forming a strong adhesive protein matrix. This matrix connects the edges of the wound, creating conditions for tissue regeneration. Albumin gradually resolves without formation of toxic decomposition products, minimizing the risk of prolonged inflammatory reactions and promoting wound healing without formation of coarse scars.

Indocyanine green — is a fluorescent dye with a high absorption coefficient in the near-infrared region of the spectrum (about 800 nm), which is consistent with the selected laser wavelength. By absorbing laser radiation, green indocyanine converts light energy into heat, providing local controlled heating in the area where the solder has been applied. This leads to denaturation of albumin without overheating the surrounding tissues, reducing the risk of thermal damage to healthy skin areas due to an accurate and selective effect of indocyanine green.

Single-walled carbon nanotubes provide additional mechanical strength and elasticity to the solder, which is critical for the soft tissues exposed to dynamic loads. Due to reinforcement of the solder with nanotubes the durability and stability of the joint is provided, reducing the risk of wound edges diverging.

The biopolymer solder was prepared in three stages. At the first stage, a homogeneous suspension of single-walled carbon nanotubes was prepared at a concentration of 0.1 wt.% in distilled water using an immersion ultrasonic homogenizer (Qsonica Sonicator Q700, Qsonica, Newtown, USA) at a power of 40 W during 45 min. Next, the dye — indocyanine green at a concentration of 0.1 wt.% and the protein — bovine serum albumin at a concentration of 25 wt.% were added to the aqueous suspension of nanoparticles under continuous mechanical stirring. After that the biopolymer solder was moved to the ultrasound bath (OOO „SAPFIR+“, Moscow, Russia) filled with ice for 30 min until the protein is fully dissolved.

0.2. Laser system for repair of soft tissues

The laser system for soft tissue repair consists of three modules: optoelectronic, temperature and control module enclosed in a single body and a surgical terminal device (laser pencil) connected to the body via an optical fiber (Fig.1).

The optoelectronic module was based on a semiconductor laser based on a GaAs diode, designed to generate continuous laser radiation with parameters: wavelength — 810 nm, maximum output power of laser radiation — 5W. A collimator with a focal distance of $f = 10.99$ mm was used to form a plane-parallel laser beam. The diameter of the incident laser beam was ~ 2 mm. The surgical area was illuminated by a pilot red light laser with a wavelength of 650 nm.

In the area of laser joint formation temperature was measured and controlled by means of a temperature module. On the dissection surface the temperature was read contactless using an infrared temperature sensor. The angle of the sensor's field of view is approximately 35° horizontally and 25° vertically, allowing to determine the temperature in the area and highlighting the heated area itself. The set temperature was maintained using temperature feedback implemented by means of a proportional-integral-differential (PID) controller. The PID controller continuously reduces the current supplied by the power source to the laser diode as the set temperature is reached. The temperature measurement error was 0.5 °C, which is an acceptable accuracy for working with biological tissues.

For accurate measurement, the temperature sensor is positioned directly above the laser beam in the surgical terminal device. The surgical terminal device is connected to the body by means of optical fiber with a diameter of 600 μm.

The laser system is controlled using software installed on a personal computer connected via Bluetooth.

0.3. Procedure of laser repair of soft tissues

Laser soldering was used to restore the skin integrity of laboratory animals — chinchilla rabbits. The experiment *in vivo* was conducted according to a strict protocol at I.M. Sechenov First Moscow State Medical University under the supervision of the Ethics Committee. Rabbits of this breed are the most commonly used animals for the tissue regeneration surgery. In this regard, a large number of papers have been published on the peculiarities of the physiology and anatomy of pre- and postoperative rabbit care. The surgery surface area allows making the entire scheduled volume of surgical procedures. Laser restoration of biological tissues was performed on 28 animals, 4 individuals for each 24h of breeding (1, 3, 5, 10, 30, 60, 90). Also, traditional sutures were made on 7 animals using a needle and thread as a control group. This value is the minimum sufficient value to obtain statistically significant

Figure 1. Approbation of laser repair of soft tissues *in vivo*: marking of dissections (*a*), application of biopolymer solder (*b*), laser irradiation of the dissected area (*c*).

results, allowing you to exclude random factors and obtain reliable data. The sex of the animal did not matter.

On the back of each laboratory animal, 3 linear incisions with a length of 1 cm and 2 U-shaped incisions with a length of 7 cm were formed. The hair on the withers was removed and the marks were made to determine the location of incisions. Right before the procedure, rabbits were injected intramuscularly with ZOLETIL 100 (VIRBAC, Carrault, France). The dosage for short-term general anesthesia for minor surgical procedures was 10 mg/kg. With intramuscular injection, loss of rectifying reflexes occurred 3–6 min after administration. A surgical scalpel was used for making the incisions. One of the most common suture materials, Prolene 5.0 (Ethicon Inc., Bridgewater, USA), was used to make the control sutures.

To form laser sutures the solder with a size of 10 μl was applied to the wound area and the suture area was continuously scanned by the laser system. Rate of a laser suture formation — 1 cm/min. Laser irradiation temperature was 50 °C. Power of laser irradiation varied up to 2 W. The process of skin repair is shown in Fig. 1.

0.4. Laser speckle-contrast imaging system

Laser speckle-contrast imaging is based on the analysis of temporal fluctuations of speckle patterns that occur when biological tissues are illuminated by coherent laser radiation. A speckle pattern is itself a random interference pattern, resulting from the backscattering of coherent light on micro-heterogeneities inside an object, such as red blood cells in blood vessels. In static media, the speckle pattern remains unchanged over time. However, the movement of scattering particles, for example, red blood cells, leads to a change in the phases of scattered waves and, as a result, to fluctuations in the intensity of the speckle pattern. By analyzing the degree of blurring of the speckle pattern on a series of images or on an image with a certain exposure, it is possible to quantify the speed of movement of particles inside the tissue [18].

An experimental setup has been developed to implement the laser speckle contrast imaging method. The 802 nm wavelength laser was used as the source of radiation. To ensure uniform illumination of the area under study, the laser radiation is scattered using a light diffuser. The radiation receiver was a monochrome digital camera CS505MU (DCC3260M, 2448 × 2048 pixels with a pixel size of 3.45 pixels, Thorlabs, Inc., Newton, USA). 150 frames were recorded for each study area on each individual day. This number of frames was required for the subsequent averaging of speckle images over several frames to reduce the noise, as well as to eliminate potential artifacts during recording caused by sudden movements, breathing, etc. The exposure time of the camera in all experiments was set to 10 ms. This parameter was chosen based on a compromise between the sensitivity to rapid fluctuations of the speckle pattern and the noise level of the image. A film polarizer was installed in front of the camera lens, oriented perpendicular to the axis of polarization of the incident radiation. This made it possible to filter out the radiation mirrored from the surface of the object, which retained its polarization, and to let through the depolarized light scattered inside the tissue, which carries information about the movement of red blood cells. To reduce the influence of background lighting and increase the signal-to-noise ratio, a FB800-10 bandpass optical filter (Thorlabs, Inc., Newton, USA) with a central wavelength of 800 nm and a half-width of 10 nm was placed in front of the camera.

When a biological tissue is exposed to laser radiation, light is scattered by moving red blood cells inside the vessels. As a result of the interference of scattered waves, a dynamic speckle pattern is formed, which is recorded by the camera. The speckle contrast parameter is determined by the formula

$$K = \frac{\sigma}{\langle I \rangle}, \tag{1}$$

where σ — standard deviation of intensity, $\langle I \rangle$ — average value of intensity found in a grazing window of 5 × 5 pixels. To reduce noise, the final speckle pattern was obtained by averaging 20 consecutive frames. The images from the camera were saved using ThorCam program and processed using an application independently developed in Octave. To prevent the artifacts associated with breathing and other movements of the animal, the area around the examined sutures was carefully fixed with a metal ring. The effort was chosen to be minimal in order to minimize the possible effect on blood flow in the studied area. The setup is shown in Fig. 2.

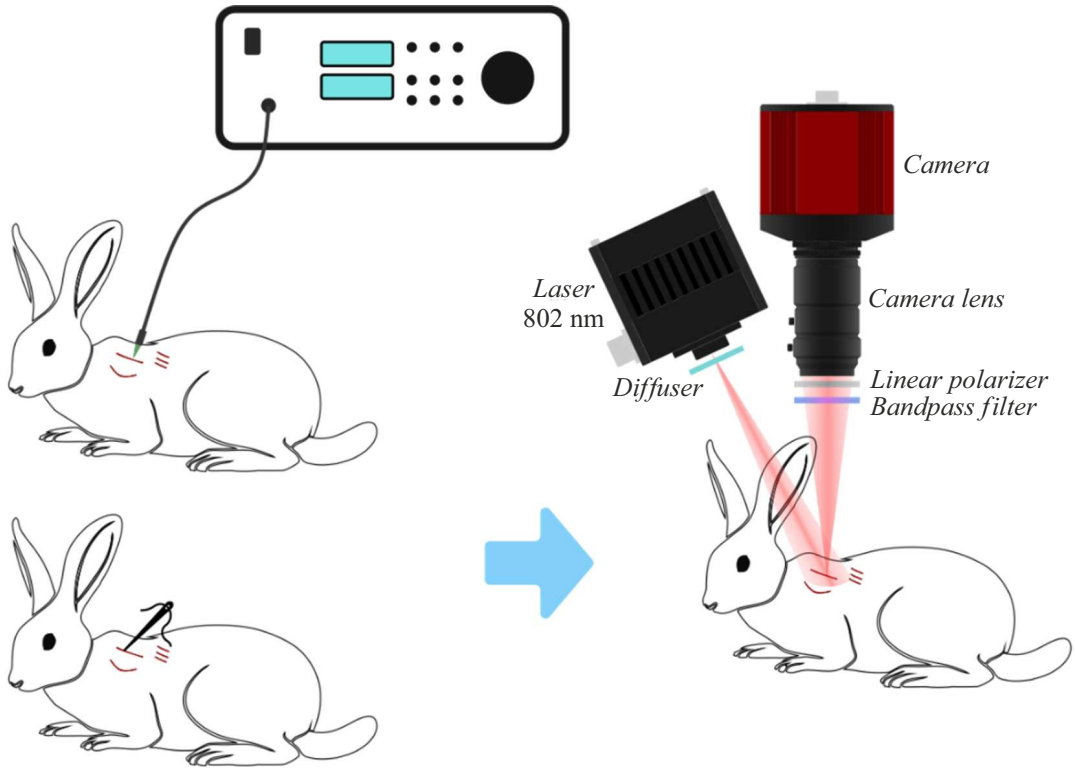

Figure 2. Experimental setup for comparing the blood flow in laser and traditional sutures using the method of laser speckle contrast imaging.

0.5. Histological studies upon completion of laser repair

Skin areas from the incision area and subsequent laser repair of soft tissues, as well as lymph nodes, were taken from all laboratory animals and fixed by immersion in neutral buffered formalin (Biovitrum, Moscow, Russia) for 24 hours. The samples were washed, dehydrated, placed in paraffin wax, and sections with a thickness of 5 μm were made using microtome Thermo Scientific HM 325 (Thermo Scientific, Waltham, Germany). The sections of tissue were placed on adhesive silane slides and stained with hematoxylin and eosin preparations; also an immunohistochemical (IHC) reaction to Ki-67c protein by staining with hematoxylin was checked.

To make the IHC reaction, the antigens were unmasked in an acetate buffer for half an hour at a temperature of 98 °C. Next, endogenous peroxidase was blocked and sections were incubated in bovine serum albumin in a thermostat at 37 °C during 20 min. Primary recombinant mouse monoclonal antibodies anti-Ki-67 were used (incubation 3 h at a temperature of 37 °C) diluted 1 : 300, as well as secondary monoclonal recombinant goat antibodies to mouse immunoglobulins (incubation 1 h at a temperature of 37 ifx17xeC). Secondary antibodies were conjugated with horseradish peroxidase. Diaminobenzidine (DAB) was used as a chromogen. The finishing was done using Carazzi hematoxylin. The sections were placed into a mount media. The images were obtained using Zeiss AxioImager A1 microscope and an Axiocam 305 color camera (Zeiss, Germany).

The spatial parameters of the scar were calculated using specialized microscopy measurements program ZEN (Zeiss, Jena, Germany) by method of morphometry after calibration with a micrometer glass. Ki-67 + -cells were counted in relation to the total number of cells in program QuPath (Queen's University, Belfast, UK) using StarDist kernel segmentation script with automatic clustering by color.

1. Experimental results and discussion

1.1. Comparison of blood flow after suture and laser methods of biological tissues repair

Figure 3 shows a comparison of speckle-patterns in the area of sutures made by traditional methods (needles and threads) and laser repair. The data analysis on a daily basis allows making conclusions about peculiarities of each method.

At the initial stage (day 0), there is a significant violation of perfusion in the periarticular region when using traditional methods for both types of dissections. Areas colored blue indicate a decrease in vascular microcirculation, which may be due to soft tissue injury during making the sutures. In some cases of laser recovery, despite surgical intervention, blood flow appears to be more uniform.

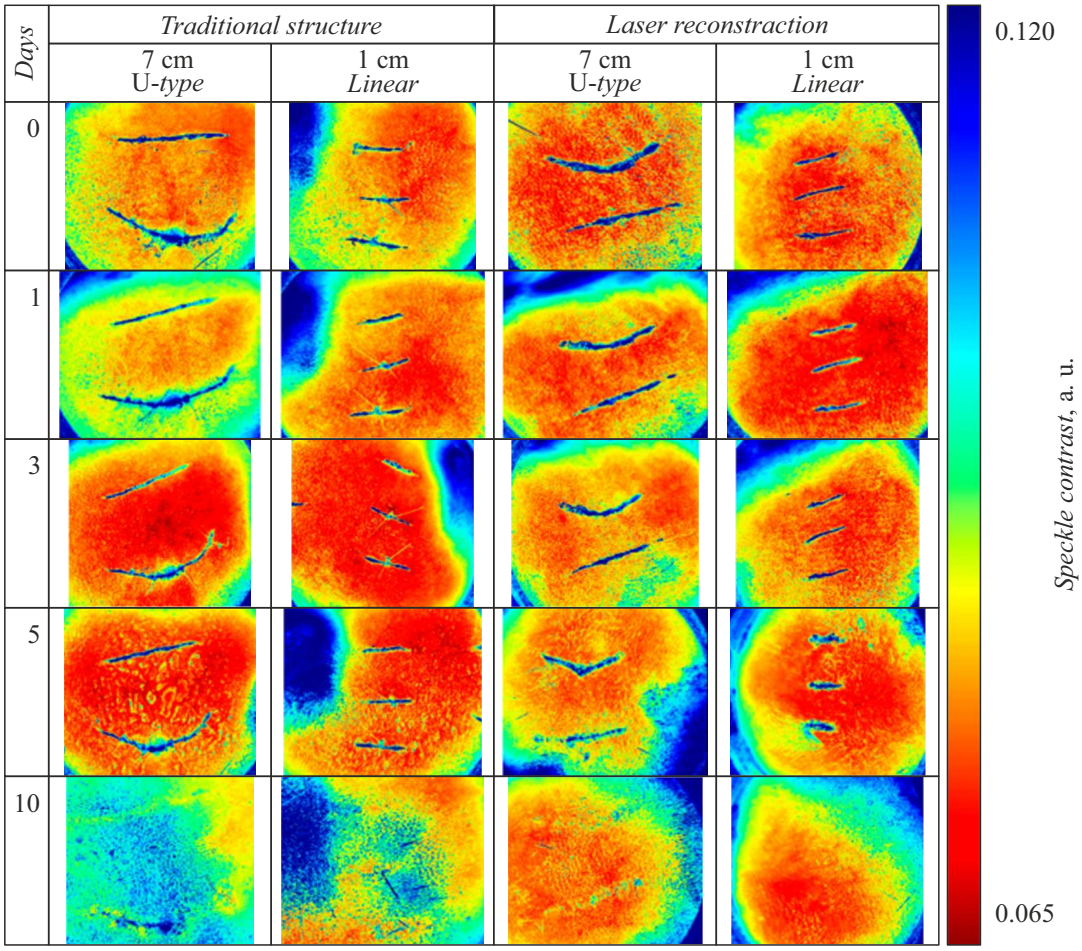

Figure 3. Comparison of the blood flow images in sutures formed by the traditional (suture) method and laser repair methods: immediately during surgery and 1, 3, 5 and 10 days after surgery.

On the first day after surgery, there is a decrease in perfusion when using traditional sutures; however, areas with insufficient microcirculation remain, especially in the suture area. On the contrary, laser repair shows almost complete recovery of blood flow, especially in the area of the linear suture (1 cm), where a more uniform distribution of hemodynamic parameters is observed.

On the third day, perfusion continues to be improved if traditional sutures are used, although some areas still show significant microcirculation impairment. In some cases of laser repair, there are practically no areas with impaired blood flow, which indicates high effectiveness of this method in the context of angiogenesis and revascularization. A similar pattern is also observed on the 5th day.

On the tenth day after surgery, a decrease in peripheral perfusion around the suture is observed in the group of sutures made by traditional way. This may be due to local tissue reactions to the presence of a foreign material (threads), which leads to a vascular response directly in the suture area and a decrease in hemodynamic parameters in the surrounding tissues. This effect may indicate the development of fibrosis or scarring, where the tissues become less elastic and less susceptible to normal blood circulation.

The results obtained are confirmed by a comparative analysis of the dynamics of blood flow changes in the groups with traditional and laser sutures in the area of suture formation, as well as in the surrounding non-injured tissues, using the average speckle contrast value. For U- sutures 6 areas were selected, and for non-linear — 9 (Fig. 4, 5). Since the speckle contrast parameter is inversely proportional to the blood flow rate, a higher speckle contrast value corresponds to a decrease in perfusion. On the tenth day after the surgery procedure, as a result of the active regeneration process, the suture geometry was not traceable.

1.2. Assessment of the dynamics of laser suture healing

The dynamics of regeneration of biological tissues after laser repair of dissections was studied on sections stained with hematoxylin (Fig. 6). In total two groups of laser joints formed on each laboratory animal were studied: U- sutures 7 cm long and linear sutures 1 cm long.

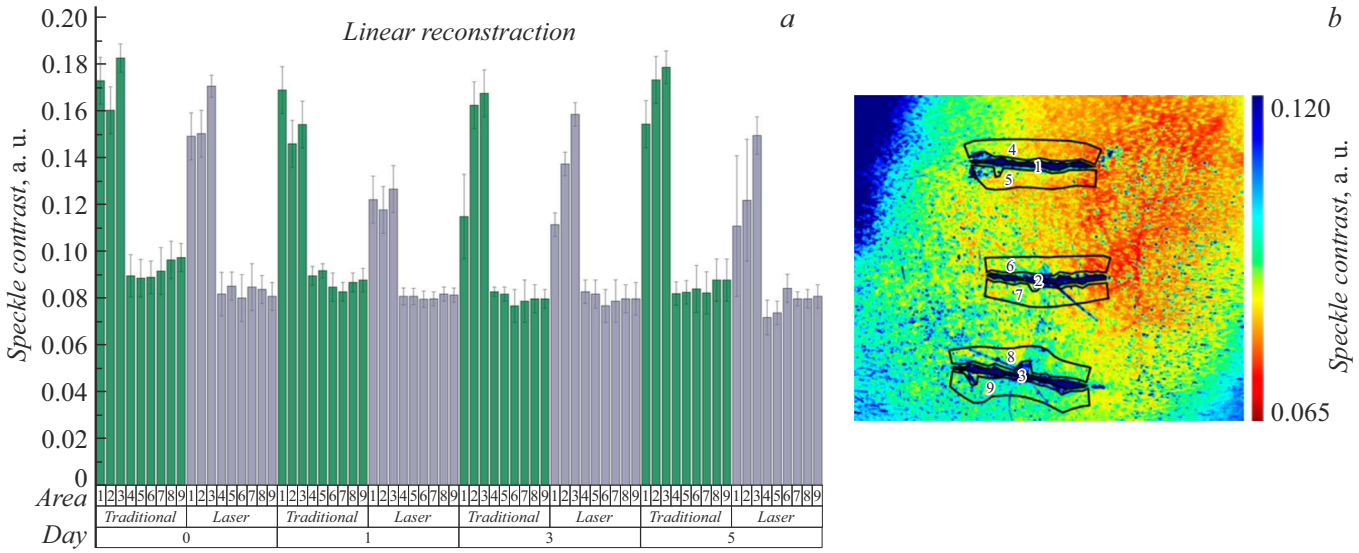

Figure 4. Progress of the blood flow recovery in traditional and laser sutures of linear type (*a*); *b* — highlighted are the areas corresponding to the areas in the curves (with a traditional suture as an example).

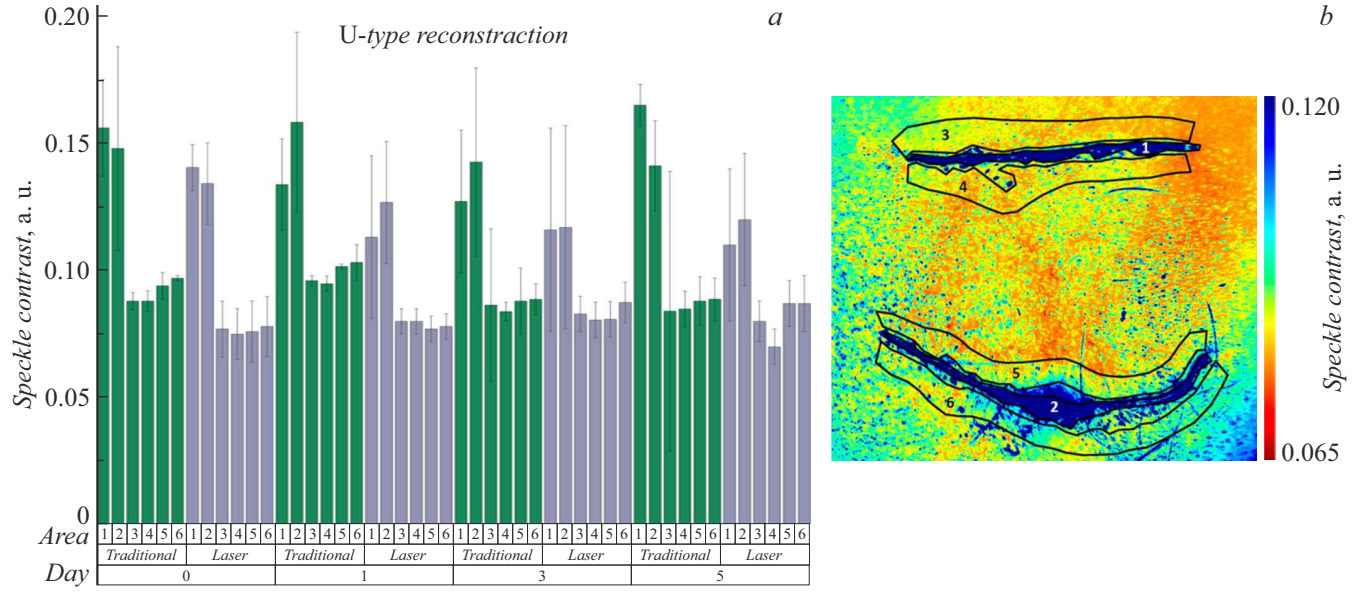

Figure 5. Progress of the blood flow recovery in traditional and laser sutures of U- type (*a*); *b* — highlighted are the areas corresponding to the areas in the curves (with a traditional suture as an example).

On the first day after surgery, hypertrophic-normotrophic scar type is observed in both groups of laser sutures. During this period, the peak stage of inflammation is noted. As a result of the injury, the epidermis, basement membranes, as well as papillary and reticular layers of the dermis are affected, which is accompanied by damage to the hair follicles. Microscopic examination shows the presence of granulation tissue, which is a characteristic feature of active healing. Moderate lymphocytic infiltration indicates an ongoing inflammatory process. There are also full-blooded and dilated capillaries, which indicates increased blood flow in the suture area and active restoration of the vascular tree. The active proliferation of connective tissue in the papillary and especially in the reticular layer of the dermis is an evidence of the repair process aimed at recovery of damaged tissues.

On the third day after surgery, the proliferation stage begins in the group with linear laser sutures, which is an important stage of healing. In this period, the granulation tissue is fully formed, which serves the basis for further recovery of damaged structures. Lymphocytic infiltration persists, indicating an ongoing inflammatory process. However, at this stage, a significant accumulation of fibroblasts is already recorded, which indicates the beginning of active

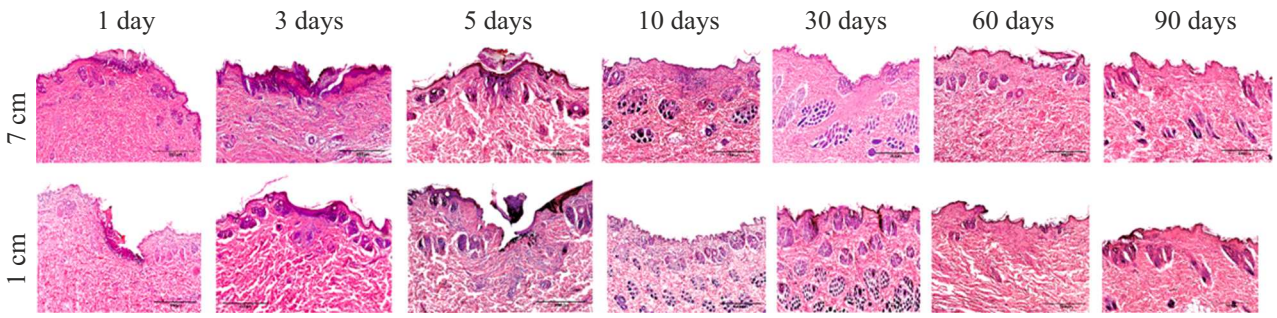

Figure 6. Progress of scarring of the damaged skin area in group 7 cm (U–type laser suture) and 1 cm (linear laser suture) after 1, 3, 7, 10, 30, 60 and 90 days of surgery.

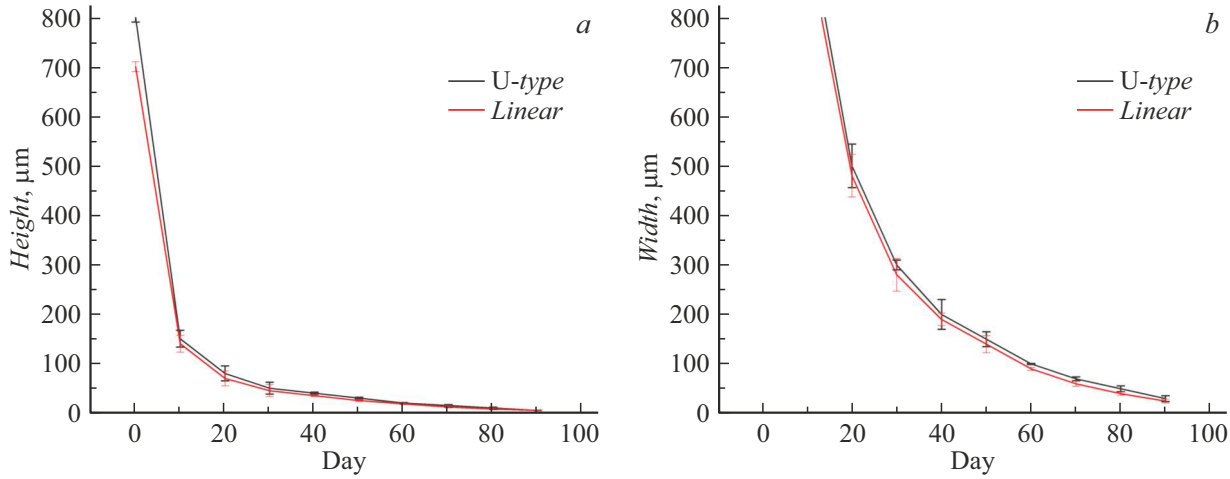

Figure 7. Progress of scarring: height of scar (*a*); width of scar (*b*).

fibroplasia, a process associated with the formation of connective tissue and the recovery of skin structures. An important aspect is the recovery of the skin appendages, which indicates the active migration of keratinocytes to the site of damage. These cells play a key role in the regeneration of the epidermis and restoration of the protective function of the skin. The basement membranes also begins to form, which is obligatory for the normal functioning of the skin. The fullness of capillaries in the suture area indicates increased vascularization and active blood supply, which contributes to the delivery of essential nutrients and oxygen to the healed tissues. The proliferation stage is also observed in the group with U- shaped laser sutures, during which the full formation of granulation tissue occurs. Lymphocytic infiltration remains pronounced, and accumulation of fibroblasts is observed, indicating active healing processes.

On the fifth day after surgery, the end of the proliferation stage and transition to the epithelialization stage were observed in both groups. During this period, a significant reduction of the wound occurs, which indicates a high level of activity of the healing processes. The replacement of granulation tissue with epithelium indicates successful recovery of the skin structures. The formation of the basement membrane is an important step ensuring the normal functioning and protective properties of the skin. The active development of skin appendages, including the end of the formation of hair follicles, indicates the recovery of the skin functionality. The presence of hair growth activation at this stage confirms restoration of normal hair growth cycle and restoration of dermal structures. The keratinization process is also active, which indicates formation of a protective barrier on the surface of the skin. The migration of melanocytes responsible for pigmentation additionally highlights the active regeneration processes. At the same time, there is a decrease in lymphocytic infiltration, which indicates a decrease in the inflammatory process and a transition to a more stable tissue condition.

On the tenth day, both groups demonstrate the completion of the processes of epithelialization and wound reduction, which is an evidence of a high level of healing. Nevertheless, the group of laser linear sutures shows more pronounced signs of recovery with moderate lymphocytic infiltration and active blood supply, whereas the group with U--type sutures is characterized by residual effects of exposure and rare presence of fibroblasts and lymphocytes. The formation of the epidermis and hair follicles has been completed, which confirms successful recovery of the

superficial skin structures. On the 60th day, normalization of the epidermis structure is observed. After 90 days, there are no deviations from healthy tissue.

Figure 7 shows the progress of reducing the size of scars in length and width during 90 days.

1.3. Immunohistochemical analysis.

Immunohistochemical reactions to Ki-67 protein showed pronounced proliferative activity in the epidermis at the edges of the wound surface at early stages, their number was maximal in the first days after removal of inflammation, then it continuously decreased during the healing process (Fig. 8).

1.4. Toxicological analysis of lymph nodes

A histological examination of the lymph nodes was performed to study the toxicity of the solder components, since changes in the structure of the lymph nodes and their functions may indicate an organism's response to the nanoparticles. In addition, the lymph nodes are the most common area of nanoparticles accumulation.

As a result of the study, it was noted that the lymph nodes morphologically do not show any abnormalities and tissue changes after experimental exposure. Absence of signs of artifact particles and macrophage activity (Fig. 9).

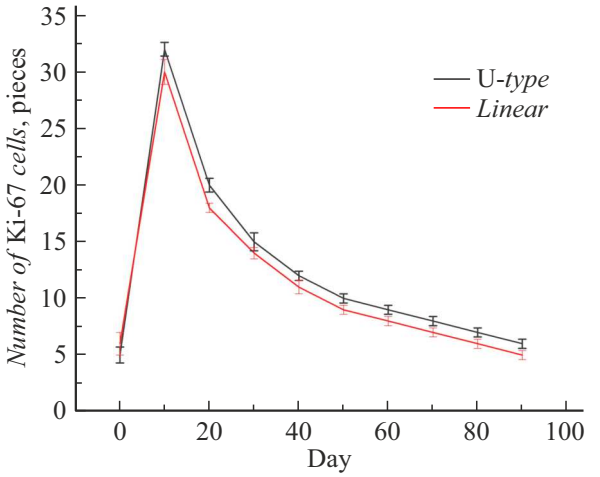

Figure 8. Progress of laser sutures proliferation (by the number Ki67- of positive cells) in the wound surface location.

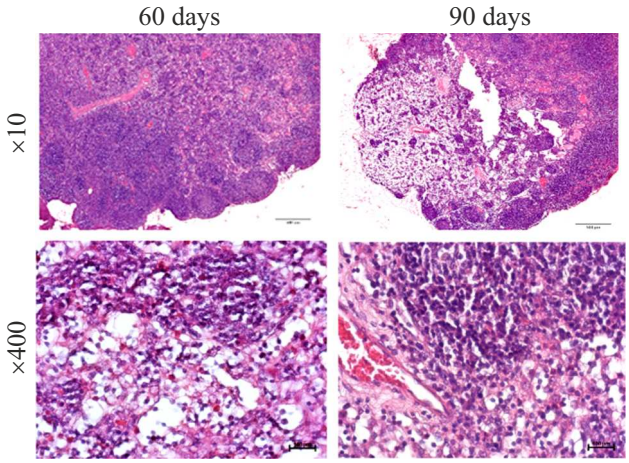

Figure 9. Microphotographs of lymph nodes at 60-th (×10, ×400) and 90-th (×10, ×400) day of study.

Conclusion

The study demonstrated high efficiency of the developed technology of soft tissue repair by laser *in vivo* in laboratory rabbits. The use of laser biopolymer solder based on bovine serum albumin, single-walled carbon nanotubes and the indocyanine green chromophore, combined with laser irradiation with a wavelength of 810 nm, demonstrated an accelerated and uniform restoration of the blood flow microcirculation in the area where the laser suture was made. The results obtained by laser speckle contrast imaging showed that the laser repair method is superior to traditional suturing methods, especially on the third and fifth days after surgery, demonstrating higher healing efficiency. Histological and immunohistochemical studies confirmed the accelerated onset of the proliferative phase and epithelialization of the wound surface, which indicates a high-quality tissue regeneration process without formation of a rough scar. The absence of morphological changes and signs of toxicity in the lymph nodes adjacent to the laser exposure area indicates that the new technology is safe and doesn't result in any negative effects on the surrounding tissues and organs.

Thus, laser repair of soft tissues using the developed biopolymer solder and controlled laser irradiation is a promising area in surgery, providing accelerated wound healing, minimizing scarring and maintaining normal microcirculation.

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Compliance with ethical standards

The experiment *in vivo* was conducted according to the strict protocol of the Ethics Committee of I.M. Sechenov First Moscow State Medical University.

Conflict of interest

The authors declare that they have no conflict of interest.

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