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Development of carbon nanotube-based biointerfaces for seamless blood vessel reconstruction

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The aim of this study is to develop a carbon nanotube-based nanocomposite material that enables the restoration of the integrity of blood vessels damaged during anastomosis procedures, using laser irradiation without disrupting blood flow. In most cases, traditional methods for restoring the integrity of blood vessels such as sutures, adhesives, or electrocautery are inefficient, technically challenging, or may cause further damage to the surrounding tissues. The proposed approach, which combines laser biophotonics with nanomaterials science, enables treatment of vascular defects with diverse geometries, minimizes mechanical contact with biological tissues, and accelerates the healing and regeneration process of the damaged tissue. The core of the method is a nanocomposite material composed of: water; single-walled carbon nanotubes at a concentration of 0.001 to 0.1 wt.%; indocyanine green as a chromophore at a concentration of 0.01 wt.%; sodium cholate as a surfactant at a concentration of 0.004 wt.%; and proteins collagen and bovine serum albumin at concentrations of 12 wt.% and 25 wt.%, respectively. Under controlled laser irradiation with a wavelength of 810 nm and a maintained temperature of 57 °C, the carbon nanotubes interconnect to form a robust scaffold within the protein matrix. In vivo and in vitro experiments on porcine, bovine, and ovine aortas demonstrated the formation of a bond with a tensile strength exceeding 1.5 MPa, without disrupting blood flow, causing material leakage into surrounding healthy tissues, or inducing thrombosis.

Keywords: laser surgery, blood vessels, biopolymers, single-wall carbon nanotubes.

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Introduction

Restoring the integrity of blood vessels remains one of the key problems of modern vascular surgery, since rupture or lesion of the vascular wall results in critical hemodynamic disorders and requires immediate intervention. Despite the fact that conventional suture anastomosis is the gold standard of therapy, it is inevitably accompanied by mechanical tissue injury, formation of an inflammatory response, and an increased risk of stenosis, especially in small-diameter vessels. These limitations stimulate the search for minimally invasive technologies capable of providing gentle, predictable and at the same time durable vascular repair.

One of such technologies is laser soldering of tissues — a photothermal junction method based on heating a biological solder with laser radiation. The principle of the method is that, under the influence of a laser, the solder protein structures denature and form a homogeneous layer connecting the edges of the vascular wall without using the sutures. Numerous studies have proven that laser soldering can reduce surgical trauma, shorten the duration of surgery, and achieve a neat and airtight joint [1–7].

However, such joints are still not enough strong for the technology to have a widespread clinical use: the most advanced formulations of albumin solders reinforced with chromophore (indocyanine green) to localize radiation rarely provide a leakage pressure higher than 300−400,mm Hg (≈ 40−53,kPa) [1,2] and are still inferior to suture technology in terms of mechanical strength. Significant progress has been achieved with the use of bioresorbable scaffolds that prevent solder runoff and ensure uniform heat distribution; the scaffolds based on polycaprolactone and polylactic-glycolic acid allowed to obtain a leakage pressure of 400−500,mm Hg (≈ 53−67,kPa) [8–11].

Despite the variety of approaches, modern biomaterials are limited in their ability to provide both a strong mechanical joint and a controlled heat transfer. Most

solders are based on protein matrices, which do not have sufficient structural rigidity and have poor distribution of thermal energy thus forming the overheated and local tissue destruction areas.

In recent years, modifications of the solder protein matrix have been actively investigated to improve the mechanical and adhesive properties [12–16]. For example, it has been shown that the introduction of polyethylene glycol (PEG) into a formulation based on bovine serum albumin (BSA) resulted in higher strength of the joint at 23 %−28 % due to the formation of cross-links and creation of a hydrated stabilizing shell [17]. An alternative direction is creation of composite hydrogel systems combining biopolymers (carboxymethyl chitosan) with photothermal agents. Such hydrogels not only provide a strong and adhesive wound covering but also demonstrate a pronounced antibacterial activity and the ability to accelerate collagen remodeling in combination with laser treatment [17,18]. However, the proposed systems are still not capable of forming a reinforced structure that could withstand prolonged hemodynamic loads.

In this regard, there is a growing interest in nanocomposite materials that can combine the advantages of biocompatible polymers with the functionality of nanostructured components. To date, systems have been developed that integrate particles with various roles: e.g., nanoplasmonic particles (such as gold nanorods) provide efficient and selective absorption of laser radiation in the translucent range, creating a localized heating, while luminescent nanothermometers (e.g., based on BiVO4:Nd3+) provide a real-time non-contact temperature monitoring reducing the risk of thermal damage to tissues [19]. However, despite high precision of the process control, these nanoparticles perform mainly auxiliary functions and practically do not affect the mechanical strength of the final joint. As a result, the strength of the joint remains limited because of the properties of protein or polymer matrix.

Of particular interest are single-walled carbon nanotubes (SWCNT), which combine several key properties: exceptionally high mechanical strength, thermal conductivity, and the ability to form ordered reinforcing nets inside biopolymer matrices [20]. Due to their ability to absorb radiation in the near-infrared range, SWCNT also act as an effective photothermal agent, ensuring uniform heat distribution in the joint area. Thus, the addition of SWCNT enhances the strength of the composite, since nanotubes participate in the process of controlled heating. Although carbon nanotubes are widely used in biomedicine, their potential as a basis for nanocomposite biointerfaces designed for laser reconstruction of blood vessels has not been properly studied so far.

The purpose of this work was to develop nanocomposite biointerfaces based on carbon nanotubes for a non-suture restoration of blood vessels and to study the proposed technology in *ex vivo* and *in vivo* conditions.

1. Materials and methods

Section 1 gives a detailed description of method for fabricating the nanocomposite biointerfaces based on carbon nanotubes, as well as a system for laser restoration of blood vessels using temperature feedback, method for laser restoration *ex vivo* on vessels of large and small cattle and *in vivo* — on laboratory rabbits, as well as methodological approaches to conducting strength tests.

1.1. Nanocomposite biointerface

To form a laser joint in a tissue, a biopolymer nanocomposite system — a biointerface was developed, which is itself a multicomponent dispersed medium including single-walled carbon nanotubes (SWCNT, LLC „Carbon CHG“, Chernogolovka, Russia), BSA (BioClot GmbH, Eidenbach, Germany), type II collagen (Sigma Aldrich, St. Louis, Missouri, USA) and indocyanine green (Sigma Aldrich, St. Louis, Missouri, USA) dispersed in distilled water.

BSA served as a structure-forming component, forming the protein matrix during local heating. Exposed to laser radiation, albumin and collagen were denatured, forming a strong and elastic matrix connecting the edges of the wound and creating a scaffold for tissue regeneration. The protein components gradually resolved without forming toxic products, which minimized the risk of an inflammatory reaction.

Collagen increased the viscosity of initial dispersed medium, preventing the components draining from the tissue surface and being washed out by the bloodstream, ensuring stable retention of the biointerface in the suture area, and also improved the system's adhesive properties promoting close contact with tissues and uniform distribution of active components over the laser exposure area.

Exposed to laser radiation SWCNT functionalized by carboxyl groups were bound to the protein matrix of albumin and collagen forming strong structural chains. This interaction resulted in higher mechanical strength of the formed joint by about an order of magnitude, increasing its stability and reducing the risk of separation of the wound edges.

Indocyanine green was used as a fluorescent dye with a high absorption coefficient in the near-infrared region (~ 800 nm). Absorption of laser radiation by the dye provided local and controlled heating of the biointerface, causing selective denaturation of protein components and binding of nanotubes to the matrix without damaging surrounding tissues.

The initial dispersion medium was prepared in several stages. First, a homogeneous suspension of single-walled carbon nanotubes (SWCNT) 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. Then, with continuous mechanical stirring, green indocyanine

(0.1 wt.%), bovine serum albumin (25 wt.%) and type II collagen (12 wt.%) were introduced. After mixing, the biointerface was soaked in an ultrasonic bath (LLC „SAPPHIRE+“, Moscow, Russia) filled with ice for 30 min until the protein components were completely dissolved and a homogeneous viscous dispersion was formed.

To increase the stability of dispersions and prevent nanotube sedimentation, a second biointerface was developed with the addition of a surfactant — sodium cholate($C_{24}H_{39}NaO_5$ xH_2O, BioXtra, $\geq 99\,\%$, Sigma Aldrich, St.-Luis, Missouri, USA) — in the ratio of 1 : 9 (nanotubes : surfactant). This formulation ensured formation of homogeneous and dispersed systems resistant to sedimentation even with a 10-fold decrease in SWCNT concentration, while mechanical and adhesive properties of the initial dispersed medium necessary to form a durable laser suture being preserved.

1.2. Laser system for the formation of biointerfaces

To create a nanocomposite biointerface, a small-size laser system was used that combined generation of radiation, temperature control, and a control system in it. The major units were located in a common housing and worked together with a surgical tip connected via a 600 μm diameter fiber optic waveguide.

The basis of the emitting module was a GaAs diode laser, which generated continuous radiation with a wavelength of 810 nm and a maximum power of 5 W. A collimator with a focal length of 10.99 mm provided a stable plane-parallel beam with a diameter of about 2 mm. An auxiliary red beam (650 nm) was used for visual positioning.

Thermal regime was monitored by a non-contact infrared sensor located along the axis of the laser beam in the tip. The sensor's wide field of view made it possible to determine the maximum temperature in the work area. The required temperature was maintained by feedback from a PID controller that automatically corrected the radiation power. The measurement accuracy was 0.5 °C.

The complex was operated and the parameters were changed on a PC connected to the device via Bluetooth.

1.3. Laser restoration of blood vessels *ex vivo*

Laser restoration of blood vessels *ex vivo* was aimed at estimating the strength of formation of CNT nanocomposite biointerfaces and determining the effect of initial bio-solder on the quality of the photothermal joint. Fragments of the aorta of a domestic bull were used for experiments. A total of 240 samples were used (20 samples for each bio-solder concentration). The vascular tissue was previously cleaned of fat and connective tissue, after which the fragments were washed with saline solution and standardized in width and length, ensuring comparability of subsequent mechanical tests.

To model the defect, a longitudinal section with a length of 1 cm was created on each sample. The incision lines were straightened and fixed on a substrate so that the edges of the dissected area fit tightly together before forming the biointerface. The sample was irradiated by a point method: by moving the applicator in 2 mm increments, the laser was applied to individual points along the joint line. The duration of a one-point exposure was 3–5 s, after which the applicator shifted to an adjacent area. If necessary, one or two repeated passes were performed at the same points to fully seal the applied material.

The temperature of the affected area was controlled by an integrated infrared sensor located along the axis of the laser beam. During the recovery process, an operating temperature of 55 °C was maintained, sufficient for thermal denaturation of the protein components of solder and formation of a strong joint but not causing deep coagulation lesion to adjacent tissues.

The procedure was considered completed when there were clear signs that the joint had been formed: compact sealing, uniform darkening or slight shrinkage of the biointerface material. On average, recovery of one sample took 60–120 s, depending on the thickness of the vascular wall. After completion of laser exposure, the samples were kept at room temperature 10–15 min to stabilize the structure of the formed joint and further prepare for mechanical testing.

1.4. Laser restoration of blood vessels *in vivo*

The testing of laser technology *in vivo* was carried out on rabbits of the Soviet Chinchilla breed. A total of 24 animals were involved in the experiment: 12 in the control and experimental groups (4 individuals for each day of breeding). Laser restoration of the vascular wall *in vivo* was performed after a full-fledged standard surgical preparation, including anesthetic support, creation of surgical access and preparation of the abdominal aorta for laser treatment. Before the intervention, the animals were subjected to inhalation anesthesia (veterinary isoflurane), which ensures stable analgesia and lack of physical activity throughout the procedure. After reaching the required depth of anesthesia, the skin in surgical field was shaved and treated with an antiseptic, after which the animal was placed on the operating table in a supine position with limbs fixed to prevent displacement.

Median laparotomy was performed with layered dissection of the skin, subcutaneous tissue, and musculoaponeurotic layer. The abdominal organs were carefully separated using soft retractors to ensure safe access to the infrarenal aorta. The vessel was isolated throughout the required segment, avoiding rough tension and traumatization of the surrounding tissues. After visualization of the vessel, soft vascular clamps were applied to its proximal and distal sections, which provided a temporary cessation of blood flow and allowed safe operation with a model aortic wall defect.

A standard defect was formed by longitudinal dissection of the anterior wall of a 1 cm long vessel. The edges of the incision were carefully adapted and fixed in an anatomically correct position, after which a thin layer of biological formulation containing 25 wt.% BSA, 0.1 wt.% indocyanine green, 0.01 wt.% SWCNT and 0.09 wt.% sodium cholate was applied to the lesion area and uniformly distributed over its entire surface.

Laser restoration of the blood vessel was performed using a point technique, sequentially moving the working tip along the joint line with an interval of 1–2 mm. Each point was exposed to laser radiation for 3–5 s, providing controlled local thermal sealing of the biointerface. The temperature in the exposure area during *in vivo* procedure was maintained at 5 °C lower than in *ex vivo* conditions, due to heat transfer and maintenance of the body's physiological homeostasis.

After the laser restoration was completed, the vascular clamps were removed and the vessel tightness and patency under natural blood flow were monitored. The animals were given a single prophylactic dose of unfractionated heparin (40 U/kg), after which the wound was sutured in layers. Anticoagulants were not administered in the postoperative period due to their poor tolerance by rodents. Further, observations were carried out for 7, 14 and 21 days, followed by the removal of fragments of the aorta for mechanical and histological assessment of the formed joint.

1.5. Mechanical testing of blood vessels

The strength characteristics of the restored vascular samples were evaluated both for models *ex vivo* and for samples obtained *in vivo* after laser restoration. After completing the relevant stages of the experiment, the vessel fragments were extracted and prepared according to a single protocol. The tissue segments were washed with saline solution, excess surrounding tissue was removed, and length was standardized to ensure the same fixation conditions in the rupture system.

For the tests, a dynamometric installation UGO BASILE (Italy) was used, which allows recording the rupture force with high accuracy. The vascular sample was fixed between two clamps in such a way that the line of the restored joint (laser or suture) was located strictly in the center of the working area. Tension was applied gradually, at a constant rate of deformation, until the joint was completely destroyed. During the tests, the maximum loads at which the rupture of the repaired section occurred were automatically registered.

For *ex vivo* samples, mechanical tests were performed after completion of the stabilization exposure (10–15 min) necessary for the biointerface to be structurally completed. For *in vivo* samples, testing was performed on days 7, 14, and 21 after surgery, which made it possible to assess the dynamics of strength recovery during regeneration. In both models, the nature of the fracture was taken into account: along the joint line, along the edge of the biointerface, or along the unchanged vascular wall.

To rule out the dehydration effects, the tests were carried out at room temperature, and the samples were moistened with saline solution immediately before being fixed in the setup.

2. Experimental results and discussion

2.1. Selection of optimal components of biointerfaces *ex vivo*

Prior to the experimental testing of the laser blood vessel restoration technology in animals, a series of experiments were conducted on aortic tissue *ex vivo* to select the most optimal components of the dispersed medium for the formation of a biointerface. The first group of formulations of dispersed media included: 25 wt.% BSA, 12 wt.% collagen, 0.1 wt.% indocyanine green and various concentrations of SWCNT (0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 wt.%). Studies of SWCNT-based media dispersibility demonstrate that the addition of surfactants will achieve a uniform distribution of nanoparticles throughout the medium, which enhances its optical properties [21], and therefore a second group of formulations has been proposed, including the addition of sodium cholate as a surfactant in the ratio of 9:1 to carbon nanotubes; the selected concentrations of carbon nanotubes were 0.001, 0.002, 0.004, 0.006, 0.008, and 0.01 wt.%.

Figure 1 illustrates mechanical characteristics of laser reconstruction of the bovine aorta using both groups of biointerface formulations, compared.

In experiments *ex vivo*, it was found that dispersions without surfactants exhibit limited stability: during sample preparation, an uneven distribution of nanotubes was noted, which is consistent with known data on the tendency of SWCNT to agglomerate in aqueous solutions without stabilizers. Visually, this was observed as a presence of local darkening zones and heterogeneity of viscosity when applying the formulation to a fragment of the aorta. In *ex vivo* tests, the second series of formulations demonstrated more predictable and stable suture formation. The dispersions were applied evenly, there were no visual signs of aggregation, and the photothermal sealing under the action of laser radiation was more uniform along the entire exposure line.

The introduction of surfactants (sodium cholate) made it possible to increase the stability and uniformity of the nanotube dispersion, which, in turn, led to a decline in the optimal concentration of SWCNT in the biointerface by an order of magnitude from 0.1 wt.% to 0.01 wt.%. At the same time, the mechanical strength of the formed joint was not only preserved, but also improved compared to formulations without surfactants, which proves the effectiveness of the proposed approach to dispersion and reinforcement of the protein matrix.

A further increase in the concentration of nanotubes, despite the theoretical growth of strength, would lead to a degradation of the composite's rheological properties, complicating its clinical use. In accordance with the

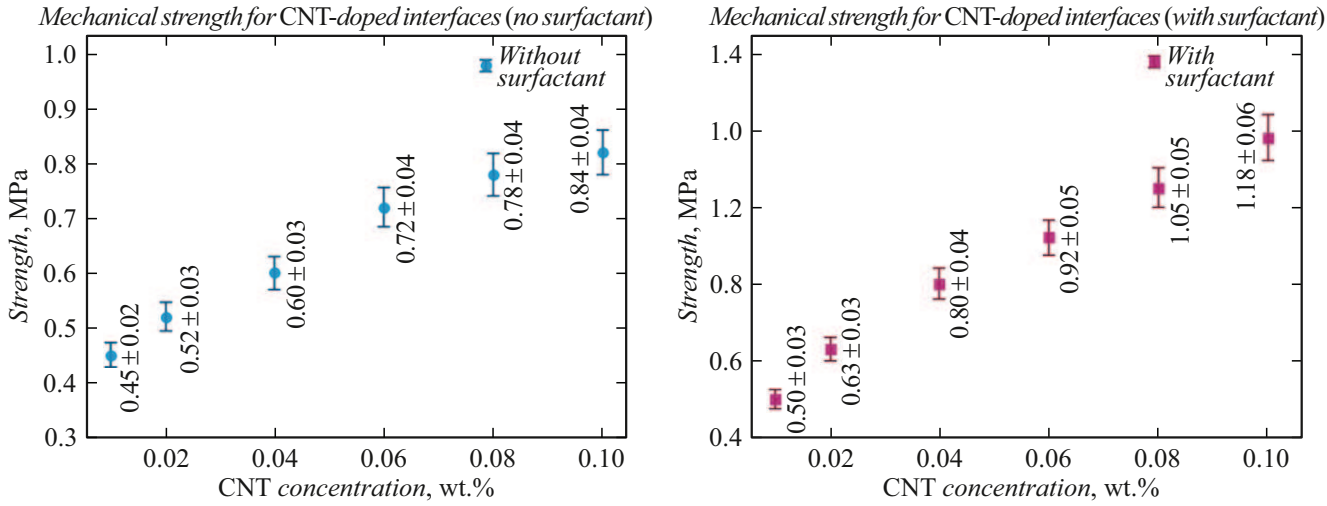

Figure 1. Approximated curves of the laser aortic restoration strength SWCNT concentration in the biointerface: without addition of surfactants (*a*), mechanical strength for CNT-doped interfaces with surfactants (*b*).

principle of minimizing the content of synthetic component (ALARA — As Low As Reasonably Achievable), it was important to demonstrate the effectiveness of technology at the lowest achievable concentrations, which reduces potential long-term risks without loss of functionality.

Based on a comprehensive assessment of dispersibility, ease of application, uniformity of photothermal sealing and strength characteristics, it was found that the following biointerface formulation is optimal for subsequent *in vivo* studies: 25 wt.% BSA, 12 wt.% collagen, 0.1 wt.% indocyanine green, 0.01 wt.% SWCNT, 0.09 wt.% sodium cholate.

2.2. Laser restoration of blood vessels *in vivo*

The second stage of the study included experimental approbation of the newly developed technology of vascular wall laser restoration *in vivo*. The purpose of this stage was to evaluate the clinical applicability of the improved biointerface formulation found in a series of *ex vivo* tests, as well as to determine the strength, morphological and functional characteristics of the formed joint in the healing process.

In all series of tests, including the control group with classical suturing and the laser vascular wall repair group, there was no mortality in animals; in all animals of both groups, the restored aortic segment remained completely permeable at all follow-up periods (days 7, 14, and 21). In all series of tests, including the control group with classical suturing and the laser vascular wall repair group, there was no mortality in animals; in all animals of both groups, the restored aortic segment remained completely permeable at all follow-up periods (days 7, 14, and 21).

The morphometry revealed marked differences between the control group, which used classical suture repair, and the group of laser restoration using a biointerface. In the control group, by day 21, a decrease in the luminal diameter to ∼ 83,% of the intact segment value was observed,

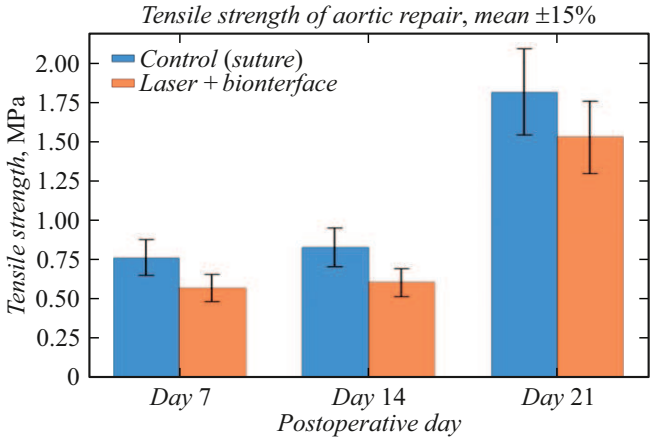

Figure 2. Comparison of the strength characteristics of blood vessel repair in the control group (traditional sutures) and experimental group where a laser restoration was used.

which indicated the onset of moderate stenosis. The probable cause may be scarring and a pronounced reactive inflammatory process around the suture. In contrast, in the laser restoration group, the diameter of the restored vessel made ∼ 94 %–96 % of the initial diameter. This means that the lumen is almost completely preserved and there is no formation of dense scar tissue that can lead to stenosis. These data confirm that the developed biointerface provides a milder effect on the vascular wall, reduces the degree of mechanical injury and provides tissue remodeling in a more physiological way.

Strength tests allowed to quantify the progress of restoration of the vascular wall's mechanical properties after making a laser joint in tissue using a biointerface (Fig. 2).

At early periods (7-th day) the strength of the laser joint was 0.58 MPa. Department of Topographic Anatomy and Operative Surgery of N.V. Sklifosovskiy Institute of

Clinical Medicine, Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University), By day 21, the joint strength increased by more than 2.5 times reaching as high as 1.55–1.60 MPa. This brings the mechanical characteristics of the restored segment closer to the control group and demonstrates pronounced maturation of the collagen matrix.

There were no statistically significant differences between the groups (according to ANOVA) in the later stages, which underlines the comparability of the restoration processes and confirms the effectiveness of the laser joint method. The observed increase in strength indicates gradual integration of the biointerface into the vascular wall structure and the formation of a stable connective tissue scaffold.

Conclusion

As a result of the research, a new type of nanocomposite biointerfaces based on SWCNT has been developed, designed for a laser non-suture restoration of blood vessels. It has been shown that the introduction of SWCNT into a protein-polymer matrix makes it possible to form a reinforced structure that raises the mechanical strength of a laser joint and improves thermal distribution in the affected area. Due to the added surfactants (sodium cholate) high dispersion stability and uniformity of photothermal sealing was ensured making it possible to reduce the required concentration of nanotubes by an order of magnitude without compromising mechanical characteristics.

Experiments *ex vivo* have demonstrated that an improved biointerface formulation ensures formation of a strong and homogeneous joint of the vascular wall, and the mechanical strength of laser joints rises with higher nanotubes concentrations, reaching maximum values at their content of 0.01 wt.%. According to the *In vivo* studies, the new technology has proved to be safe, ensuring complete patency of the aorta at all follow-up periods and being accompanied by minimal adhesions compared to the conventional suture techniques.

Morphological studies demonstrated a more physiological remodeling of the vascular wall during laser repair: the vessel lumen remained 94 %–96 % of the initial diameter, which significantly reduced the risk of stenosis. Strength tests showed a significant increase in the mechanical strength of the joint in the healing dynamics — from 0.58 MPa on day 7 to 1.55–1.60 MPa on day 21, which was comparable with the indicators of the control group.

The results obtained confirm the prospects of using CNT nanocomposite biointerfaces for minimally invasive vascular repair. The technology provides stable formation of a strong joint, minimizes tissue lesion, and demonstrates the potential for its further improvement and subsequent deployment for clinical use.

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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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