Laser Technology for the Formation of Bioelectronic Nanocomposites Based on Single-Walled Carbon Nanotubes and Proteins with Different Structures, Electrical Conductivity and Biocompatibility

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Abstract: A laser technology for creating nanocomposites from alternating layers of albumin/collagen proteins with two types of single-walled carbon nanotubes (SWCNT) at concentrations of 0.001 and 0.01 wt.% was proposed. For this purpose, a setup with a diode laser (810 nm) and feedback system for controlling the temperature of the area affected by the radiation was developed. Raman spectroscopy was used to determine a decrease in the defectiveness of SWCNT with an increase in their concentration in the nanocomposite due to the formation of branched 3D networks with covalent bonds between nanotubes. It was revealed that adhesion of proteins to branched 3D networks from SWCNT occurred. The specific electrical conductivity of nanocomposites based on large SWCNT was 3.2 and 4.3 S/m compared to that for nanocomposites based on small SWCNT with the same concentrations—1.1 and 1.8 S/m. An increase in the concentration and size of nanotubes provides higher porosity of nanocomposites. For small SWCNT-based nanocomposites, a significant number of mesopores up to 50 nm in size and the largest specific surface area and specific pore volume were found. Nanocomposites with small SWCNT (0.001 wt.%) provided the best cardiac fibroblast viability. Such technology can be potentially used to create bioelectronic components or scaffolds for heart tissue engineering.

Keywords: laser treatment; three-dimensional structure; nanocomposites; proteins; electrical conductivity; bioelectronics
the frame is made, the higher the resolution of the nanocomposite structure [5]. Carbon nanoparticles are promising fillers for creating functional 3D frame nanocomposites [6]. Carbon nanotubes are filamentous structures, to which researchers from different fields draw a lot of attention. This is due to their high mechanical strength, and excellent thermal, optical and electrical properties [7]. Functional nanomaterials based on the carbon nanotubes, including self-organizing ones, are created by functionalizing of nanotubes with various chemical groups and binding them to organic molecules [8–12]. Along with that, nanotubes are able to form frame structures using an electromagnetic radiation field [13]. Laser radiation is the most preferred from these effects. Recently, lasers have been actively used in 3D printers, since it is possible to concentrate laser radiation into a small volume (voxel). Moreover, radiation can be directed with considerable accuracy and high speed to the given area [14]. When the mechanism of interaction of laser radiation with carbon nanotubes is considered, it is known that radiation in a wide spectral range is absorbed by nanotubes, while optical energy is converted into thermal energy [15]. It is known that carbon nanotubes have a large number of defects, mainly vacancy defects that were formed during synthesis [16]. Moreover, nanotubes have high thermal conductivity, however, in the area of defects due to the crystal lattice disruption, the thermal conductivity decreases and hot spots are formed [17]. Such spots are the most probable regions for the bonding of nanotubes to each other into frame structures [18]. In this case, X, Y, and T-shaped nanotube joints are formed. For the first time, nanomaterial with frame morphology of multi-walled carbon nanotubes in phenolic resin was obtained under the action of continuous CO\textsubscript{2} laser radiation [19]. In [20] the possibility of mechanical rotation of CNT was shown upon their excitation by polarized IR radiation propagating along the nanotubes. The inertia moment of the nanotube was 28 GHz, and the centrifugal acceleration on the surface of the tube was \(0.5 \times 10^{12}\, \text{m/s}^2\). This value is 2–5 orders of magnitude more than the fastest centrifuges created. However, due to the small net force on each carbon atom, it is negligible compared to the action of the usual molecular bond force.

Using laser evaporation of a composite target based on nanotubes in toluene matrix, it was possible to immobilize and fix nanotubes with the given network-like morphology on glass substrate [21]. This process was carried out with a pulsed laser with a wavelength of 248 nm, pulse duration of 25 ns, and pulse frequency of 10 Hz. With a gradual increase in the power density of laser radiation acting on CNT, a controlled introduction of defects into their structure was obtained [22]. Defects in CNT were the places where nanotubes were bound into an ordered network.

Recently, more and more studies have aimed to the methods of using frame materials made of carbon nanotubes in biomedical engineering of tissues and organs, as well as bioelectronic interfaces for implantable devices [23]. Such materials should have controlled mechanical properties and biocompatibility [24,25]. Since nanotubes have dimensions close to the ones of the main components of the natural cellular matrix, their mechanical properties are similar to those of the protein structures of a living organism [26]. Nanotubes are able to mimic collagen (the fibrillar protein of the extracellular matrix) due to recurring defects in the structure of the tubes, similar to the structure of collagen [27].

Depending on the type of biological tissues being restored, multi-walled, single-walled carbon nanotubes (SWCNT) and network-like structures are used in polymer or biopolymer matrices of polycaprolactone, chitosan, polylactide, hydroxyapatite, etc. [28–32]. The use of frame structures from SWCNT in bioelectronics and engineering of biological tissues improves cell population kinetics: their adhesion, proliferation and differentiation [18]. This is especially important for the engineering of cardiac tissue, since nanotubes provide the electrical conductivity of the material they fill in, while the heart generates electric current, and the heart tissue allows it to pass [33,34]. Thus, implants for stimulating and restoring the structure of the heart tissue must have electrical conductivity [35]. The electrical conductivity of implants plays an important role in the survival of the seeded cells and the efficient signal transmission between two adjacent cells [36]. In addition,
nanotubes have high strength, which is important for the restoration of cardiac tissue under stress [37].

Matrix materials can also be chosen to improve characteristics of 3D structures. Studies indicate that using albumin and collagen proteins for these purposes is promising. Albumin molecule has two modifications of the isomer, which are an ellipsoid of revolution and an irregular triangular prism [38]. Albumin is capable of cross-linking under the action of laser radiation using amino acid residues on the outer surface of the molecule [16,39]. This indicates the possibility of creating nanocomposite globules with SWCNT. Albumin is widely used to create implants that interact with blood because it inhibits platelet adhesion and prevents thrombus formation [40]. Collagen is the main protein in the connective tissue produced by fibroblasts. In case of damage to biological tissue, collagen is involved in the restoration and renewal of cells, i.e., it serves as a reserve of amino acids [41]. Fibrillar collagen molecules have a similar structure to SWCNT, especially thin ones. The addition of collagen to implants ensures cell proliferation [42]. The surface of collagen filaments promotes the adhesion of cells and molecules, for example, growth factors, due to the presence of a large number of ligands and functional groups [43,44].

We have previously demonstrated methods of laser formation of SWCNT frame structures in biopolymer matrices [18,45]. The mechanisms of SWCNT binding to each other [18] and to amino acid residues of proteins [16,46] were determined. When forming frame materials for tissue engineering, it is very important to control their structure, namely porosity. In case of using pulsed laser radiation to create 3D structures, it is necessary to be guided by nonlinear mechanisms of laser pulses absorption in media based on nanotubes [47]. It is known that when laser pulses hit carbon nanotubes, electromagnetic radiation is absorbed and its energy is converted into heat, which provokes the appearance of the vapor shell around SWCNT and their bundles. It promotes the formation of pores in the material. Such materials must be porous to allow the proliferation of cells, nerve fibers and blood vessels during implantation. However, the porosity can be influenced by the concentration of nanotubes as well as their type [48].

In this work, the structure and properties of frame nanocomposites of albumin and collagen proteins were investigated depending on the type and concentration of SWCNT. Such nanocomposites were created by laser action on a water-protein dispersion with carbon nanotubes. Previous studies on the interaction of SWCNTs with albumin showed that laser irradiation of water-albumin dispersion leads to the chemical reaction between negatively charged amino acid residues Asp and Glu and nanotubes and, accordingly, to the formation of covalent bonds between them [16]. The structure of the nanocomposite, represented by alternating layers of CNTs with albumin and collagen, was selected based on the simulation results. Such a structure is energetically favorable. It is characterized by high stability and is achieved in a short period of time [17]. The results of the interaction of the nanocomposite components with each other are shown, as well as the analysis of the internal micro- and nanostructure of nanocomposites. Effects of the SWCNT type and concentration on the electrical conductivity of nanocomposites and the proliferation of heart cells in their structure are presented. The investigated properties of nanocomposites indicate the potential of their application as electrically conductive interfaces.

2. Materials and Methods

2.1. Composition of Nanocomposites

Two types of single-wall carbon nanotubes were used to fabricate nanocomposites. They differed in the method of synthesis and, accordingly, in morphology. Nanotubes of the first type (SWCNT1) were synthesized by the electric arc method on a Ni/Y catalyst, purified in air with washing with HCl, and functionalized with carboxyl groups in an HNO3/H2SO4 mixture, with washing until neutral. The average nanotube diameter was 1.4–1.8 nm, the length was ~0.3–0.8 µm, and the specific surface area of the product was 400 m²/g. The purity of SWCNT1 was 97%. Nanotubes of the second type (SWCNT2) were synthesized by gas-phase synthesis and purified similarly to the first type of SWCNT1.
The average diameter was \( \sim 2\text{–}3.5 \) nm, their length was more than \( 5\text{–}10 \) \( \mu \)m, and the specific surface area was \( 420 \) \( m^2/g \). The purity of SWCNT2 was 91%.

As a matrix for nanocomposites, proteins were used: powders of lyophilized bovine serum albumin (99% purity, BioClot, Germany) and bovine collagen Type II (99% purity, MakMedi Ltd., Moscow, Russia).

2.2. Technique for the Manufacture of Dispersed Media and the Formation of Nanocomposites

Initially, homogeneous dispersed medium (dispersion) was prepared with one of the SWCNT types and one of the proteins in water. Tridistilled water was used as a solvent for dispersed media. The dispersed medium in the liquid state served as ink for the fabrication of a three-dimensional structure of nanocomposites. To prepare the dispersed medium, SWCNT1 or SWCNT2 were added to water. The final concentration of CNTs in the samples was 0.01 or 0.001 wt.%. These concentrations were chosen to provide a compromise between the biocompatibility of nanocomposites and their structural and electrophysical properties [47].

Initially, the aqueous dispersion of SWCNT was subjected to powerful ultrasonic action of a probe homogenizer for 45 min at a power of 40 W. This was necessary to break the van der Waals forces between SWCNT in bundles. As a result, the dispersions mainly contained isolated nanotubes. Next, albumin or collagen powder was immediately added to the aqueous dispersion of nanotubes, since proteins acted as surfactants for SWCNT. In dispersions, it was possible to reach a maximum concentration of albumin \( \sim 20 \) wt.%, and collagen \( \sim 1 \) wt.%. After that, the composition was dispersed in an ultrasonic bath at a lower power \(<10 \) W) for 60 min. Thus, the structure of protein molecules was not damaged.

To form nanocomposites in a three-dimensional form, the obtained disperse medium in a liquid state was applied in layers. The layers of SWCNT1/SWCNT2 with albumin alternated with layers with collagen. Each layer of the dispersion was transformed from liquid to solid state under the action of laser radiation using the developed laser setup (Figure 1). After drying the dispersion layer, the volume of dispersion necessary for the formation of the next layer was poured on it, after which laser treatment was performed again. Physical mixture of components without laser treatment does not promote the formation of a bonded nanotubes framework; this does not ensure the formation of nanocomposites. Exposure to electromagnetic laser radiation is necessary for the formation of strong electrically conductive nanocomposites [16,47]. The setup made it possible to apply uniform layers of dispersion (1) in a container (2) by an extrusion method using a nozzle (3) connected to a syringe (4). The positioning of the head (5) with the nozzle (3) was carried out using the movement system based on stepper motors of a three-coordinate system (X, Y, Z) on a massive bed. The movement accuracy reached 0.1 \( \mu \)m at a speed of 0.01 to 5 m/s. The head movement trajectory (5) was set using a computer 3D model. Moreover, an optical fiber (6) with a collimator (7) was connected to the head (5). Thus, the setup made it possible to move the laser radiation along a given trajectory along the deposited layers of dispersion (1) in the container (2). However, in this experiment, we used unfocused radiation (8), which moved along the vertical axis depending on the number of deposited layers. Plane-parallel radiation covered the entire area of the dispersion in the container (2). For this purpose, a diode laser (9) with a wavelength in the near IR range of 810 nm was used. The spatial profile of the laser radiation had a Gaussian shape. The setup contained a 630 nm pilot laser to illuminate the main IR radiation. The radiation power was varied from 0.1 to 3 W depending on the temperature of the layers. Temperature detection was carried out using an IR sensor (10) connected to a microcontroller (11), which changed the current supplied to the laser diode (12) in real time. Such construction makes it possible to control the heating of the liquid to the desired temperature, excluding the destruction of the protein structure. The creation of nanocomposites took place in the temperature range 40–55 °C. A heating table was used to preheat the dispersion (13).
ble to control the heating of the liquid to the desired temperature, excluding the destruction of an electron column of 200 kV. For this purpose, SWCNT were applied to specialized copper meshes.

2.3. Techniques for Studying the Structure of Nanocomposites

The structural features of protein molecules and nanotubes in nanocomposites were described by Raman spectroscopy using an inVia Qontor confocal Raman microscope (Renshaw, Pliezhausen, Germany). The nanocomposites were excited by laser radiation with a wavelength of 785 nm. The spectrum was recorded with a resolution of 4 cm$^{-1}$, averaged over 200 scans, and subjected to inverse Fourier transform.

To study the structure and analyze the porosity of nanocomposites, the method of X-ray microtomography (MicroCT) was used with the Skyscan 1174 complex (Bruker, Kontich, Belgium). The X-ray source voltage did not exceed 24 kV. The current at the cathode was selected to achieve the average value of the X-ray beam intensity in the range of 30–50% of the maximum (380 mA).

The study of the specific surface area and volume of nanoscale pores in nanocomposites was carried out using the method of low-temperature nitrogen porosimetry using the Sorbtometer-M complex (ZAO Katakon, Novosibirsk, Russia).

To study the morphological features of SWCNT1/SWCNT2, transmission electron microscopy (TEM) was used with a JEOL JEM-2100Plus complex at an accelerating voltage of an electron column of 200 kV. For this purpose, SWCNT were applied to specialized copper meshes.

2.4. Procedure for Measuring Conductivity

The electrical conductivity of the nanocomposites was measured using a two-probe method. High accuracy of resistivity measurements was achieved by giving the nanocomposites a flat parallelepiped form with a uniform thickness. The dimensions of the samples were 5 mm $\times$ 5 mm. For this, the PM5 station (Cascade Microtech, Beaverton, OR, USA) was used, connected to a 34401A multimeter (Keysight Technologies Inc., Santa Rosa, CA, USA). The measurement was carried out at least 5 times, and the average value was calculated. Then, their specific conductivity was calculated as the reciprocal of resistance, taking into account the geometric dimensions of the nanocomposites.

2.5. Techniques for In Vitro Research

To study the biocompatibility of the composite layers, cardiac fibroblasts purchased from the National Research Center for Epidemiology and Microbiology of the Russian Federation Ministry of Health were used. The cell concentration was $\sim$4 $\times$ 103 cells/mL. Cell cultivation was carried out in a CO$_2$ incubator at 37 °C in DMEM medium (87%) supplemented with fetal serum (13%). The MIT assay was used to assess cell viability [49].

Figure 1. Setup for creating nanocomposites from layers of dispersions of albumin and collagen with SWCNT1/SWCNT2.
Cell morphology was assessed by fluorescence microscopy. The cells were stained with ethidium bromide and scanned on a BX-43 microscope (Olympus, Tokyo, Japan) using a FITC filter. For in vitro experiments, several alternating layers of nanocomposite on a cover glass were prepared. A cover glass with layers of proteins and without SWCNT was used as a control.

3. Results and Discussion

3.1. SWCNT Structure and Appearance of Nanocomposites

TEM imaging of the nanotubes showed that bundles of SWCNT1 in aqueous dispersion were separated into isolated nanotubes by ultrasound (Figure 2a). Prolonged ultrasonic treatment of the aqueous dispersion of SWCNT2 led to their separation into isolated nanotubes to a lesser extent. Figure 2 shows that SWCNT2 clumped in bundles under the action of van der Waals forces. The excess of the dimensions (length and diameter) of SWCNT2 over the dimensions of SWCNT1 is also noticeable. The thickness of a few bundles from SWCNT1 is 3–6 nm, while that from SWCNT2 was much larger, but did not exceed 30 nm. The appearances of the nanocomposites obtained using the SWCNT1/SWCNT2 are shown in Figure 2c,d, respectively.

![Figure 2. TEM images of SWCNT1 (a) and SWCNT2 (b) (green arrows indicate isolated nanotubes, yellow arrows indicate their bundles) and the appearances of the nanocomposites obtained with SWCNT1 (c) and SWCNT2 (d).](image)

3.2. Raman Spectroscopy of Nanocomposites

The structure of SWCNT and proteins in nanocomposites were studied by Raman spectroscopy. (Figure 3). The presence of a “hump” in the spectra is explained by the contribution of the proteins fluorescence that occurs when they are treated with radiation in the visible region of the spectrum. The spectra of nanocomposites contain peaks characteristic of SWCNT: RBM-band (110–160 cm\(^{-1}\)), D-band (~1311 cm\(^{-1}\)), and 2D-band (~2590 cm\(^{-1}\)), the intensity of which is in relation to G-band (~1590 cm\(^{-1}\)) indicates the absence of damage in the structure of nanotubes (twisting, fractures, etc.). The splitting of the G-band corresponds to the manifestation of the semiconducting properties of nanotubes. The degree of defectiveness is different for the two types of nanotubes. It is known that the bands’s intensity ratio \(I_D/I_G\) makes it possible to estimate the defectiveness of carbon nanotubes. Defectiveness is understood as a wide range of disturbances in the structure of the nanotubes surface, from vacancies of carbon atoms to chemical interaction with other substances. In the case of the nanocomposite based on SWCNT1 with a concentration of 0.001 wt.% an \(I_D/I_G\) ratio of ~0.129 was obtained (Figure 3a). This value is presumably associated with the interaction of nanotubes with proteins during the creation of nanocomposites.
Figure 3. Raman spectra of SWCNT1 (a,b) and SWCNT2-based (c,d) nanocomposites with nanotube concentrations of 0.001 (a,c) and 0.01 (b,d) wt.%. 

For the spectrum of the nanocomposite based on SWCNT1 (0.01 wt.%), a decrease in defectiveness to \( I_D/I_G \sim 0.116 \) was obtained (Figure 3b), since an increase in the concentration of SWCNT leads to an increase in the probability of the contacts formation between nanotubes in defect areas. When laser irradiated, SWCNT’s defects behave like chemically active hotspots due to a decrease in thermal conductivity. For the spectrum of the SWCNT2-based nanocomposite (0.001 wt.%), the intensity ratio of the \( I_D/I_G \) bands for the sample was \( \sim 0.076 \), which is lower in comparison with the SWCNT1 nanocomposite (0.001 wt.%). Since SWCNT2 initially had greater defects, the number of defects decreased during the formation of a nanocomposite with SWCNT branched 3D networks. For the spectrum of the SWCNT2-based nanocomposite (0.01 wt.%), the intensity ratio of the \( I_D/I_G \) modes was minimal, \( \sim 0.035 \). The smallest defectiveness of the SWCNT2-based nanocomposite (0.01 wt.%) can be explained by the formation of branched 3D networks from isolated nanotubes and their bundles.

Figure 3c,d shows that the radial RBM-band (110–160 cm\(^{-1}\)) is represented by several peaks, which indicates the presence of SWCNT2 of different diameters in the nanocomposite. SWCNT1-based nanocomposites had a narrow RBM-band with a high intensity, which is associated with a small spread of nanotube diameters.

The spectrum line in the interval between the radial and D bands passes well above the zero mark, which indicates the presence of proteins in the composite. Under the influence of laser radiation, by means of which the nanocomposites were obtained, the proteins albumin and collagen underwent denaturation (by heating the dispersion with laser radiation above 55 °C). The degree of damage to the proteins structure can be assessed by the presence of peaks in the amide group: the presence of a weak peak at 1656 cm\(^{-1}\) (Amide I, presence of C=O bonds) indicates damage in the tertiary/secondary structure of proteins. The Amide II band is weakly manifested in the Raman spectrum and, therefore,
is not used for analyzing the structure of proteins. The Amide III band (about 1300 cm\(^{-1}\)) coincides with an intense peak in the D-mode region, which makes it difficult to conclude about damage in the secondary and tertiary structure of the protein (alpha-helix and beta-structure). A small sharp peak at about 1000 cm\(^{-1}\) indicates the presence of the amino acid phenylalanine, according to which the obtained spectra were normalized. There are practically no pronounced peaks at about 1010 cm\(^{-1}\), which are responsible for the presence of van der Waals interactions between side groups of amino acids in proteins. At the same time, there are few pronounced peaks in the range of 500–550 cm\(^{-1}\) in the Raman spectrum, which complicates the identification of the presence of disulfide bridges in protein molecules that hold the molecule in the form of a spiral; a weak peak relative to the peaks of carbon nanotubes at 859 cm\(^{-1}\) is noticeable, which indicates the presence of the amino acid tyrosine in the nanocomposites. The Amide II band (parallel/non-parallel beta structures) is practically not manifested in the spectra around 1550 cm\(^{-1}\), since it cannot be observed in the absence of resonant excitation. Figure 3a,b shows a peak at about 2922 cm\(^{-1}\), which indicates the presence of partially intact bonds in amino acids.

### 3.3. MicroCT of Nanocomposites

To study the microstructure of nanocomposites using X-ray tomography, the samples were fixed on object tables made of a polymer transparent for X-ray radiation. The scanning parameters were selected in accordance with the density and geometric dimensions of the nanocomposites. The rotation step was 0.1° when the sample was rotated 360° around its axis. This corresponded to ~4000 shadow projections and a spatial resolution of up to 10 µm. Figure 4 shows shadow projections and 3D views of nanocomposites from different types of nanotubes and with different concentrations. The analysis of projections and 3D views shows that nanocomposites take an even shape in the process of layer-by-layer formation. The middle part of all samples is lower in level than the peripheral region of the nanocomposites. This is due to the Gaussian shape of the laser beam with a maximum radiation intensity in the central region. To visualize the internal structure of the nanocomposites, visual sections were made with a step of 100 µm along the vertical axis of the sample. The main sections of the nanocomposite at distances of 0.5, 1.3 and 1.7 mm from the bottom point of the sample are shown in Figure 4. Each section is accompanied by three views—front view, top view and side view.

When analyzing the images of sections of the SWCNT1-based nanocomposite with a concentration of 0.001 wt.%, it was determined that the average pore size was ~48 µm (Figure 4a). Almost the entire sample volume was homogeneous with a uniform pore distribution. Several single pores were observed with size of ~80 µm. With an increase in the concentration of SWCNT1 to 0.01 wt.% in dispersion to obtain a nanocomposite, a significant increase in pores with a size of 100–200 µm was obtained. In this case, the average pore size was ~86 µm (Figure 4b). In the case of using carbon nanotubes with a large diameter and length (SWCNT2) with a concentration of 0.001 wt.% as a filler of the nanocomposite, a significant porosity with a large pore size—200–280 µm was obtained (Figure 4c). In this case, the average pore size was ~101 µm. An increase in the concentration of SWCNT2 to 0.01 wt.% in nanocomposite contributed to an increase in the number of pores; however, their diameter decreased to 150–200 µm (Figure 4d). At the same time, the number of large pores increased and the average pore size increased to ~73 µm. A decrease in the structure density and an increase in the number of pores were observed at the edges of all samples of nanocomposites. Most likely, a more uniform central part with fine pores was obtained due to the nonuniform front of the distribution of thermal heating by the Gaussian profile of laser radiation.
Figure 4. Shadow projections, 3D view and sections at depths of 0.5, 1.3 and 1.7 mm along the vertical axis from the bottom point of nanocomposites based on SWCNT1 (a,b) and SWCNT2 (c,d) with nanotube concentrations of 0.001 (a,c) and 0.01 (b,d) wt.%.

The quantitative parameters of the internal nanocomposites microstructure are shown in Table 1. The table shows the results of the quantitative analysis for nanocomposites, which was performed for three-dimensional models of each of the samples. Samples’s preparation using SWCNT2 with concentrations of 0.001 and 0.01 wt.% was accompanied by a large number of pores and their sizes. Most likely, this depended on the large size of SWCNT2, as well as their bundles, into which nanotubes in a protein dispersion were stuck together due to van der Waals forces. The SWCNT2 consisted mostly of bundles versus SWCNT1. For composites based on both types of SWCNT, it was found that an increase in the concentration of nanotubes entails an increase in the total pore volume by 17 and 9%, respectively for SWCNT1 and SWCNT2. In this case, the fraction of open pores from the entire sample volume for nanocomposites with two concentrations of SWCNT1 exceeded this value for samples with SWCNT2. This is probably due to the orientation of SWCNT1, which protruded beyond the boundaries of the nanocomposite. Since nanotubes of the first type were in an isolated form, it is easier to control them by laser radiation. In case of long bundles, this process is difficult. As a result, the percentage of open pores was lower for SWCNT2-based nanocomposites. The presence of open pores in tissue-engineered constructions is important for the initiation of the neoinnervation and neovascularization processes of during implantation.
3.4. Mesoporosity of Nanocomposites and Their Specific Surface and Specific Pore Volume

It has been established that nanoscale pores in biomedical materials can allow drugs with a small molecular weight (molecule size 1–2 nm) and proteins (2–10 nm) to diffuse slowly [50,51]. In case of micron-sized pores, drugs and proteins spread much faster. In addition, nanoscale pores can be used to control the biodegradation of an implant, since they significantly increase its surface area [52,53]. There are also studies where nanoporous scaffolds that mimic the architecture of the native extracellular matrix can remain unnoticed by the body’s immune system [54].

To study the distribution of nanoscale pores, the most homogeneous fragment with a volume of ~1 mm$^3$ was isolated from the nanocomposites. This fragment was selected based on microCT studies. Figure 5a shows nitrogen adsorption isotherms at 77 K in nanocomposites based on both types of SWCNT and both concentrations. All isotherms had capillary-condensation hysteresis, which indicates the presence of through mesopores in the samples. The size distributions of nanoscale pores, the specific surface area and the specific pore volume were calculated using the Barrett–Joyner–Halenda (BJH) method [55]. The mesopore size distribution is shown in Figure 5b.

The values of specific surface area, specific volume and distribution of pore diameters in nanocomposites are shown in Table 2. The nanocomposite with a lower concentration of SWCNT1 (0.001 wt.%) showed the largest specific surface area and specific pore volume. The nanocomposite based on SWCNT2 (0.001 wt.%) also demonstrated a high specific surface area. An increase in the concentration of both types of nanotubes leads to a decrease in the specific surface area and specific pore volume. This may be due to the oversaturation of nanocomposites with tubes. The formation of mesopores can be primarily associated with the formation of inhomogeneities around the nanotubes of their bundles during the absorption of laser radiation by the nanotubes and the conversion of its energy into...
heat. We have previously demonstrated a similar effect when exposed to pulsed laser radiation on biopolymer dispersions of SWCNT [47]. From the dependences of the pore size distribution presented in Figure 5b, it can be seen that the curves are polymodal. The largest scatter of pore sizes was obtained for nanocomposites based on SWCNT1 (0.001 wt.%). However, the majority of mesopores in these nanocomposites were in the ranges of 5–20 and 30–50 nm (Table 2). An increase in concentration to 0.01 wt.% promoted a decrease in the specific surface area and pore volume in nanocomposites based on SWCNT1 and SWCNT2. Moreover, an increase in the size of the nanotubes themselves and their bundles affected the decrease in the specific surface area and pore volume. The sample based on large SWCNT2 and their bundles had pores with a size close to 100 nm. Such pores cannot be called mesopores, since their sizes were mainly in the ranges of 85–100 and 95–100 nm.

Table 2. Specific surface area, specific volume and pore size distribution in nanocomposites.

| Type and Concentration of Nanotubes in Nanocomposites | Specific Surface (m²/g) | Specific Volume of Mesopores (mL/g) | Mesopore Size Ranges (nm) |
|------------------------------------------------------|-------------------------|-------------------------------------|--------------------------|
| SWCNT1 (0.001 wt.%)                                  | 8.4 ± 0.2               | 1.8 ± 0.1                           | 5–20                     |
|                                                      |                         |                                     | 30–50                    |
|                                                      |                         |                                     | 50–100                   |
| SWCNT1 (0.01 wt.%)                                   | 6.7 ± 0.2               | 1.5 ± 0.1                           | 10–20                    |
|                                                      |                         |                                     | 45–70                    |
| SWCNT2 (0.001 wt.%)                                  | 6.9 ± 0.3               | 1.6 ± 0.2                           | 85–100                   |
| SWCNT2 (0.01 wt.%)                                   | 3.8 ± 0.3               | 0.9 ± 0.2                           | 38–55                    |
|                                                      |                         |                                     | 95–100                   |

3.5. Electrical Conductivity of Nanocomposites

The study of electrical conductivity made it possible to determine the features of the nanocomposites structure, namely the formation of branched 3D networks from SWCNT in the protein matrix. It is known that SWCNT under the action of laser radiation in a dielectric matrix (proteins) creates electrically conductive 3D networks with percolation nodes [47]. 3D networks of nanotubes are formed due to the formation of covalent bonds in defective regions of nanotubes that are in close proximity.

Figure 6 shows the conductivity data for nanocomposites with different compositions. The diagram shows that an increase in the SWCNT concentration from 0.001 to 0.01 wt.% leads to an increase in the specific electrical conductivity of nanocomposites from 1.1 ± 0.05 to 3.2 ± 0.1 S/m. The increase in electrical conductivity depends on an increase in the number of X- and Y-shaped percolation nodes. Consequently, higher concentration of nanotubes increases the probability of binding of defective nanotubes regions located at a distance of 1.38 to 1.55 Å from each other. In this regard, Figure 6 shows regularity in the excess of electrical conductivity in nanocomposites with SWCNT2 compared to nanocomposites with SWCNT1. From the analysis of the Raman spectra, it was found that the defectiveness in the nanocomposites with SWCNT1 is higher than in the samples with SWCNT2. Most likely, this is due to the fact that the defective regions of SWCNT1 formed chemical bonds to a greater extent with protein molecules in nanocomposites than with each other. While in nanocomposites with branched 3D networks of SWCNT2, their defectiveness is much lower. Most likely, SWCNT2 formed covalent bonds with each other, increasing the electrical conductivity of nanocomposites to 3.2 ± 0.3 and 4.3 ± 0.2 S/m for 0.001 and 0.01 wt.%, respectively. However, the lower stability of SWCNT2 in a water-protein dispersion led to a larger error in measuring the electrical conductivity compared to nanocomposites based on more stable and homogeneous dispersion with SWCNT1. It is known that the electrical conductivity of the native myocardium varies in the range of 0.03–0.6 S/m [56]. Therefore, the implantable part of the device for electrical stimulation of the myocardium or restoration of its structure must have comparable or higher electrical
conductivity values. Thus, nanocomposites created from both types of SWCNT with both concentrations are suitable for such a task.

**Figure 6.** Specific electrical conductivity of nanocomposites.

### 3.6. Cell Viability on Nanocomposites

Fibroblasts are one of the important types of heart cells. They are responsible for the production of proteins that make up the extracellular matrix, which supports all other cells in the heart and is responsible for the elasticity of the heart tissue, as well as the ability to withstand stress. Fibroblasts express a wide range of growth factors and cytokines [57]. A number of studies indicate the possibility of electromechanical interaction of fibroblasts with neighboring myocytes, which can potentially be used for therapeutic purposes [58]. Thus, the compatibility of the cardiac tissue regeneration material with fibroblasts is an important requirement. In this regard, a comparative analysis of the viability of heart fibroblasts in nanocomposites based on SWCNT1 (0.001/0.01 wt.%) and SWCNT2 (0.001/0.01 wt.%) with control was carried out. The nanocomposites were formed in layers of a flat shape on a cover slip. Next, MTT assay and fluorescence microscopy of fibroblasts were performed (Figure 7).

**Figure 7.** Diagram of MTT assay and fluorescence microscopy images of heart fibroblasts on nanocomposites based on SWCNT1 (0.001 wt.%) (a) SWCNT1 (0.01 wt.%) (b) SWCNT2 (0.001 wt.%) (c) and SWCNT2 (0.01 wt.%) (d) relative to control (cover slip) (e) after 72 h of incubation.
After 72 h of incubation, the following results were obtained. The best fibroblast survival was observed on SWCNT1 nanocomposites (0.001 wt.%). The increase in concentration led to a decrease in the viability of fibroblasts. For both samples, the viability of fibroblasts was 14 and 6% higher than on the control. The situation with nanocomposites based on SWCNT2 (0.001/0.01 wt.%) was different. Cell viability decreased compared to samples from SWCNT1, but for SWCNT2 (0.001 wt.%), it exceeded by 4% than in control. The viability of fibroblasts on SWCNT2 nanocomposites with an increased concentration of 0.01 wt.% was at the control level, and in some cases lower. The high viability on the SWCNT1 samples can be explained by highest purity of this type tubes and their homogeneous distribution over the volume of nanocomposites. The images of fluorescence microscopy show that the cells on the nanocomposites were inhomogeneously distributed in height and filled the entire porous volume of the nanocomposites after 72 h. Therefore, some fibroblasts were not in focus of the objective of the fluorescence microscope. The cells on the control were evenly distributed over the coverslip area and, accordingly, in height. Therefore, their fluorescence image was clearer with visualization of nuclei. Fibroblasts on nanocomposites formed groups (Figure 7a–d). The cells were close to each other in these groups. The control fibroblasts were at some distance from each other. The close arrangement of cells in groups promotes the formation of tissue monolayer, as seen in Figure 7a. The cells were structured in rows and were ready to form a tissue monolayer on the SWCNT1 sample (0.001 wt.%). Fluorescence microscopy images confirmed the results of MTT assay. Thus, using MTT assay and fluorescence microscopy, the compatibility of cardiac fibroblasts with nanocomposites based on SWCNT1 (0.001/0.01 wt.%) and SWCNT2 (0.001/0.01 wt.%) was demonstrated.

4. Conclusions

A laser technology for creating nanocomposites from alternating layers of albumin and collagen with SWCNT was developed. For this purpose, a setup with a diode laser (810 nm) and temperature feedback system was used. This was necessary to control the temperature of the area affected by the radiation. Nanocomposites of two types SWCNT1 and SWCNT2 with different morphologies and concentrations of 0.001 and 0.01 wt.% were created. SWCNT1 had small dimensions (diameter and length) and were represented mainly in the form of isolated nanotubes. The diameter and length of SWCNT2 were larger, while they were mainly presented in the form of bundles. Nanocomposites were created by applying alternating layers of aqueous dispersions with albumin/collagen proteins and nanotubes, followed by laser irradiation using the developed device with temperature control. Using Raman spectroscopy, it was determined that the initially more defective SWCNT2 inside the nanocomposite showed the least defectiveness. The decrease in defectiveness is explained by the formation of branched 3D networks from nanotubes with covalent bonds between carbon atoms in the defective regions. An increase in the concentration of SWCNT2 to 0.01 wt.% provided a decrease in defectiveness from $I_D/I_G \sim 0.076$ to $I_D/I_G \sim 0.035$. It was revealed that during the manufacture of nanocomposites, SWCNT absorbed laser radiation, thereby creating thermal heating of proteins. As the temperature rised, the number of damage to the weak bonds of the tertiary proteins structure increased, which allows the molecule to change its conformation. This process promoted the adhesion of proteins to branched 3D networks of SWCNT in the nanocomposite. The results of Raman spectroscopy were confirmed by the values of the electrical conductivity. The electrical conductivity of nanocomposites based on large SWCNT2 nanotubes at concentrations of 0.001 and 0.01 wt.% was 3.2 and 4.3 S/m compared to the specific electrical conductivity for nanocomposites based on SWCNT1 with the same concentrations—1.1 and 1.8 S/m. The created nanocomposites of both types of SWCNT with both concentrations are suitable for the creation of electrically conductive interfaces of stimulating devices or tissue-engineered structures for myocardial restoration (myocardial conductivity 0.03–0.6 S/m). High porosity values were obtained for all types of nanocomposites. Higher concentration of nanotubes provided an increase in the porosity of nanocomposites. For nanocomposites
based on SWCNT1, the porosity was 44–61% compared to 68–77% for SWCNT2. At the same time, the morphology of isolated SWCNT1 provided a large proportion of open pores. A significant number of mesopores were found in nanocomposites, especially for samples based on SWCNT1. For this type of nanocomposites, the largest specific surface area and specific volume of mesopores with sizes up to 50 nm were obtained. The presence of nanotubes in materials can provide adsorption of nanoscale drugs molecules and biomolecules. Nanocomposites ensured the viability of heart fibroblasts in their structure. The best biocompatibility was observed for SWCNT1-based nanocomposites with a low concentration (0.001 wt.%). Thus, nanocomposites based on SWCNT1 with low concentration (0.001 wt.%) can be used for implantable devices or materials. At the same time, nanocomposites based on SWCNT2 with a concentration of 0.01 wt.% will be the optimal choice for skin devices with high electrical conductivity.

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