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Study of the physical and biological properties of nanocomposite materials obtained with laser radiation

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Abstract

The new method of the formation of nanocomposite materials based on carbon nanotubes for the regeneration of connective tissues has been developed.
Aim. Study of the structure, mechanical characteristics and biocompatibility of the obtained materials.
Materials and methods. The experimental samples of nanocomposite materials were based on multi-walled and single-walled carbon nanotubes, the matrix was bovine serum albumin. A layer of liquid dispersion of the components on a silicon substrate or in a container was irradiated with laser radiation to form the solid nanocomposite material. The microstructure of the obtained samples was analyzed with X-ray microtomography, the tensile strength was investigated using a testing machine. Fibroblast cells were incubated with experimental samples for 3, 24, 48, and 72 h and then fixed with glutaraldehyde. Cell growth during incubation with samples was studied using optical and atomic force microscopy.
Results. It was found that a slight decrease in tensile strength and increase in the degree of deformation were observed with an increase in the concentration of carbon nanotubes. At the same time, the mechanical parameters of the samples corresponded to the requirements for materials for the restoration of connective tissue defects. Microscopic studies indicate good adhesion of cells to the nanocomposite material, no toxic effect of the samples on the cells was found. After 3 hours of incubation, the cells had their original rounded shape, after 24 hours of incubation cells began to proliferate on the sample's surface and were spindle-shaped. After 48 and 72 hours, the cells practically formed a monolayer on the surface of the samples.
Conclusion. The results of the study show that the structural and mechanical parameters of the developed nanocomposite materials meet the requirements of biomedicine. It was also shown that nanocomposite materials do not suppress cell growth and can serve as a scaffold for the regeneration of damaged tissues.

Keywords: carbon nanotubes; serum albumin; scaffold; laser structuring; atomic force microscopy; tissue engineering; fibroblasts; connective tissue defects
MeSH terms:
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Исследование физических и биологических свойств нанокомпозитных материалов, полученных с использованием лазерного излучения

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Аннотация

Разработан новый метод формирования нанокомпозитных материалов на основе углеродных нанотрубок для регенерации соединительных тканей организма.

Цель. Исследование структуры, механических характеристик и биосовместимости полученных материалов.

Материалы и методы. Основой экспериментальных образцов являлись многостенные и одностенные углеродные нанотрубки, в качестве матрицы использовался бычий сывороточный альбумин. Слой жидкой дисперсии компонентов на кремниевой подложке или в емкости обрабатывался лазерным излучением с образованием объемного нанокомпозитного материала в твердой фазе. Микроструктура полученных образцов была исследована методом рентгеновской микротомографии, прочность на разрыв исследовалась с помощью испытательной машины. Клетки фибробласты инкубировались с экспериментальными образцами в течение 3, 24, 48 и 72 часов, а затем фиксировались глутаровым альдегидом. Рост клеток во время инкубации с образцами был изучен с помощью оптической и атомно-силовой микроскопии.

Результаты. Установлено, что с увеличением концентрации углеродных нанотрубок наблюдается небольшое снижение прочности и увеличение степени деформации. При этом механические параметры образцов соответствовали требованиям, предъявляемым к материалам для восстановления дефектов соединительных тканей. Микроскопические исследования указывают на высокую степень адгезии в процессе взаимодействия клеток с нанокомпозитным материалом, токсического действия образцов на клетки не было обнаружено. Через 3 часа инкубации клетки имели первоначальную округлую форму. Клетки на образцах после 24 часов инкубации начали распространяться по поверхности образцов и имели веретенообразную форму. Через 48 и 72 часа клетки практически образовывали монослой на поверхности образцов.

Заключение. Результаты исследования показывают, что структурные и механические параметры разработанных нанокомпозитных материалов удовлетворяют требованиям биомедицины. Также было показано, что нанокомпозитные материалы не подавляют рост клеток и могут служить в качестве каркаса для регенерации поврежденных тканей.

Ключевые слова: углеродные нанотрубки; сывороточный альбумин; каркас; лазерное структурирование; атомно-силовая микроскопия; тканевая инженерия; фибробласты; дефекты соединительной ткани

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List of abbreviations

AFM – atomic force microscopy
CNT – carbon nanotubes
MWCNT – multi-walled carbon nanotubes
SWCNT – single-walled carbon nanotubes

Today one of the most rapidly developing areas of biomedicine is biophotonics. Laser radiation is widely used for theranostics of a wide range of diseases. A large number of biocompatible materials, in particular, scaffolds for tissue engineering, can be obtained with the technology of laser polymerization [1]. To increase the similarity of such materials to the natural extracellular matrix and thereby improve the proliferation of cells in their volume, various nanoparticles can be embedded in them [2].

Among the requirements for such materials, the most important are biocompatibility, biodegradability, the presence of the pore system, good mechanical properties and acceptable surface structure [3]. The most widely used materials which meet these requirements are natural or synthetic polymers and their combinations [4–6]. However, low mechanical strength and insufficient biocompatibility limit their use [7].

Reinforcing components can be added to polymers in order to make their mechanical characteristics better – nanoparticles of metals, carbon (nanodiamonds, nanotubes, nanofibers, graphene and its derivatives) [8–10]. The addition of carbon nanotubes (CNT) to biocompatible polymers, alongside increasing the strength, also promotes the formation of surface unevennesses, which improves the adhesion of cells to the material [11–13]. CNT are electrically conductive; thereby they can be located in the volume of the material under the influence of laser radiation in the required manner [14].

In this work, the characteristics of a nanocomposite material produced by laser treating of water dispersions of single-walled (SWCNT) and multi-walled (MWCNT) carbon nanotubes with albumin were investigated. The structure was analyzed with X-ray microtomography, and the tensile strength was investigated using a testing machine. Biocompatibility with fibroblasts was investigated in vitro using microscopic methods. The resulting nanocomposite materials can be used as scaffolds for the regeneration of damaged tissues.

MATERIALS AND METHODS

The experimental samples were based on CNT (Russia). The matrix material – bovine serum albumin (Germany) – was chosen for its ability to bind tissues well during laser welding using albumin as solder [15].

As the first operation, the aqueous dispersion of CNT was created with a magnetic mixer ELMI MS-01 (ELMI, Latvia). The concentration of CNT in the samples was 0.1 and 0.01 wt. %, the concentration of albumin was 25 wt. %. For obtaining more uniform dispersion, the CNT were further processed by a submersible ultrasonic homogenizer Sonicator Q700 (Qsonica, USA). At the next stage, bovine serum albumin was dissolved in the dispersion, after that dispersion was mixed completely with a magnetic mixer and an ultrasonic bath Sapphire (Sapphire, Russia). At the last stage, the dispersion was sprayed over a silicon base or poured in a clean container and irradiated with a laser until the solid film was formed. It took a layer of dispersion several (5-10) seconds to be completely dried. The dispersion in the container was treated with a laser setup for several (7–8) minutes until a rubbery state was achieved. Irradiation was performed using a continuous-wave semiconductor laser LSP (IPG Photonics Corporation, Russia) with a wavelength of 810 nm. The radiation power was 5.5 W, the beam profile was Gaussian and 10 mm in diameter. After laser irradiation, the final sample had the form of a scaffold of CNT inside the albumin matrix. The microstructure of the samples of nanocomposite material was investigated by X-ray microtomography using Skyscan 1174 (Bruker, USA) X-ray tomography. The following parameters were selected: voltage at the X-ray tube cathode was 26 kV; current at the cathode of the X-ray tube was 400 mA; rotation step was 0.15 (~ 4000 shadow projections); the spatial resolution was 12 microns.

To study the mechanical properties, the dumbbell-shaped samples were prepared. The samples had dimensions of 3.5×3.5×1.4 mm. The distance between the grips was 2.5 mm, the loading rate was 1 mm/min, and the maximum load was 50 N. The samples were tested on Instron 3343 (Instron, USA) universal testing machine.

In vitro studies with the cell culture were performed using fibroblasts. The cells were incubated with experimental samples for 3, 24, 48, and 72 h and then fixed with glutaraldehyde for microscopic studies. The changes in cells spreading and morphology during incubation of them with samples of nanocomposite material were studied using optical and atomic force (AFM) microscopy. Optical microscope Biolam M-1 (LOMO, Russia) was used for optical microscopy, and Bruker Dimension Icon (Bruker, USA) was used for AFM.

RESULTS

3D visualization of the internal structure of nanocomposite material samples is shown in Fig. 1. The results of the complex analysis of samples show that

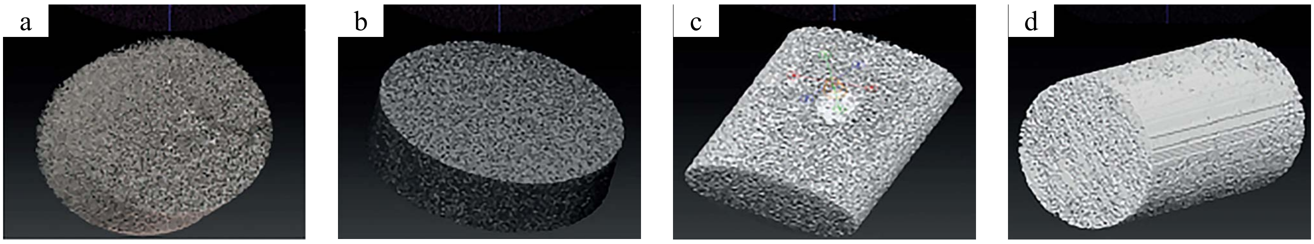

FIG. 1. 3D visualization of samples of nanocomposite material. a – sample with SWCNT (0.01 wt %), b – sample with SWCNT (0.1 wt %), c – sample with MWCNT (0.01 wt %), d – sample with MWCNT (0.1 wt %).
РИС. 1. 3D-визуализация образцов нанокомпозитного материала. a – образец с одностенными углеродными нанотрубками (0,01 мас.%), b – образец с одностенными углеродными нанотрубками (0,1 мас.%), c – образец с многостенными углеродными нанотрубками (0,01 мас.%), d – образец с многостенными углеродными нанотрубками (0,1 мас.%).

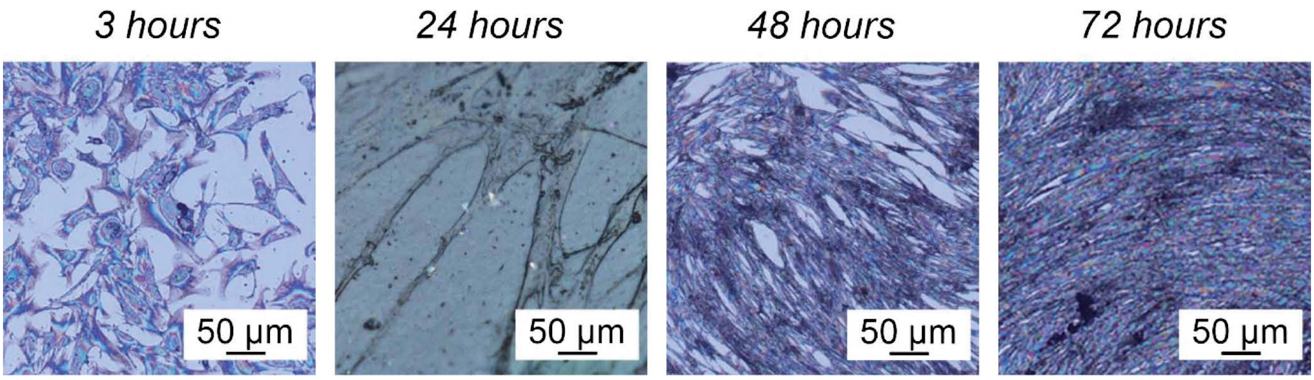

FIG. 2. Optical microscopy images of the nanocomposite material based on MWCNT with fibroblasts. Magnification: ×10.
РИС. 2. Изображения нанокомпозитного материала на основе многостенных углеродных нанотрубок с фибробластами, полученные методом оптической микроскопии. Увеличение: ×10.

with an increase in concentration from 0.01 to 0.1%, porosity increases. All samples had the same amount of open pores. The sample with a lower concentration of SWCNT (0.01 wt. %) had a homogeneous structure with a number of small pores with a diameter of about 0.4 mm and micropores with a diameter of 65 μm. Sample with 0.1 wt. % SWCNT had a homogeneous structure with micropores 43 μm in diameter. Sample with 0.01 wt. % MWCNT had a series of micropores with an average diameter of 37 μm. Sample with 0.1 wt. % MWCNT contained micropores with an average diameter of 49 μm.

Table summarizes the results of studies of tensile strength and elongation. It was found that with the increase in CNT concentration, there was a slight decrease in tensile strength (3% for samples with SWCNT and 5% for samples with MWCNT) and an increase in the degree of deformation (12% for samples with SWCNT and 15% for samples with MWCNT).

Optical microscopy made it possible to obtain images of the distribution of the cells along the surface after incubation with experimental samples (Fig. 2).

After 3 hours of incubation, the cells had their original rounded shape and were practically not distributed over the surface of the samples (Fig. 2a). The cells were up to 50 μm in size with short processes up to 10 μm

Table. **Mechanical characteristics of samples of nanocomposite materials**
Таблица. **Механические характеристики образцов нанокомпозитных материалов**

Sample / Образец	Average tensile strength, MPa / Средняя прочность на разрыв, МПа	Average relative extension, % / Среднее относительное удлинение, %
SWCNT (0.01 wt %) Одностенные углеродные нанотрубки (0,01 мас.%)	3.7	12.2
SWCNT (0.1 wt %) Одностенные углеродные нанотрубки (0,1 мас.%)	3.6	13.7
MWCNT (0.01 wt %) Многостенные углеродные нанотрубки (0,01 мас.%)	3.8	11.7
MWCNT (0.1 wt %) Многостенные углеродные нанотрубки (0,1 мас.%)	3.6	13.5

Note / Примечание. SWCNT – single-walled carbon nanotubes; MWCNT – multi-walled carbon nanotubes.

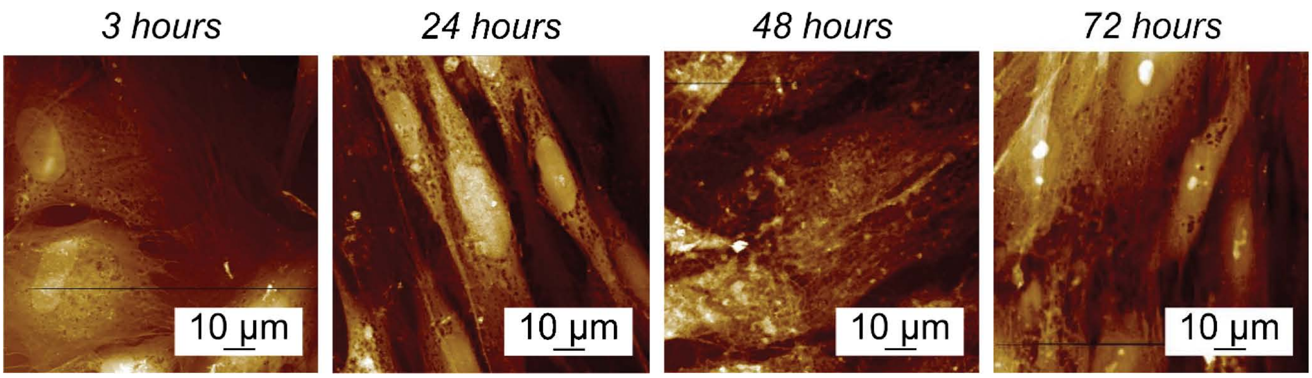

FIG. 3. Atomic force microscopy images of the nanocomposite material based on SWCNT with fibroblasts.
РИС. 3. Изображения нанокомпозитного материала на основе одностенных углеродных нанотрубок с фибробластами, полученные с помощью атомно-силовой микроскопии.

in length, more actively occupied more inhomogeneous areas of the samples.

The cells on the samples after 24 hours of incubation had a spindle-shaped shape with increased proliferation over the surface of the samples (Fig. 2b). The cell layer was denser than the cell layer after 3 hours of incubation.

After 48 and 72 hours, the cells practically formed a monolayer on the surface of the samples (Fig. 2c, d), the shape of the fibroblasts became more elongated in comparison with the previous periods.

The grown cell cells were seen in more detail using an atomic force microscope (Fig. 3). Areas of size 90 μm were examined. AFM images made it possible to clearly define changes in cell morphology.

AFM makes it possible to analyze the structure of the surface and its microrelief with high resolution – down to the nanometer scale. The samples were analyzed using the semi-contact method, often used for biological samples. NanoScope Analysis software was used for processing the resulting images. Cell nuclei and nucleoli were observed in the centre of each cell, up to 3 in each nucleus, as well as cell processes that attached to the sample. It was seen more cells after 72 hours of incubation compared to the previous periods.

DISCUSSION

Damages of connective tissue are now extremely common among people of both young and old age. At the same time, the regenerative capacity of such tissues is limited, so they, as a rule, are replaced by autografts or artificial materials. Transplantation of an autograft into the damaged tissue site leads to additional surgical intervention, while artificial materials disintegrate over time and can damage the surrounding healthy tissues. Thus, the field of tissue engineering is extremely promising, since it allows one to overcome the mentioned disadvantages.

Bioactive nanomaterials can also be used as coatings for artificial structures, increasing their biocompatibility. A key factor in successful tissue regeneration is an effective combination of scaffold materials and patient's cells. A number of requirements are imposed on the materials of the scaffolds, one of them is porosity. The main task of porosity in scaffolds is a fairly accurate mimicking of the natural extracellular matrix, consisting of collagen, elastin and other components. In this case, the inner surface of the pores should facilitate the adhesion of cells to it and the parts of the porous network should communicate with each other. The pores in the scaffolds are also necessary for the transport of nutrients to the cells and the disposal of their waste products. The porosity of the developed samples of the nanocomposite material estimated from the tomographic images is sufficient for effective tissue growth on them.

To ensure the ability of tissues to withstand the load during regeneration and to adequately support proliferating cells, it is necessary for tissue-engineered scaffolds to have a certain strength. The tensile strength of the native soft connective tissues is about 3 MPa [16]. Thus, the tensile strength of the obtained samples exceeds the strength of native tissues. The tensile strength of the scaffold is inversely related to the porosity, while the type of nanotubes did not significantly affect the mechanical properties of the nanocomposite material samples.

Cell proliferation is one of the main indicators of the efficiency of the developed scaffold. The obtained images of fibroblasts incubated with samples of nanocomposite material indicated good adhesion of cells to the material. No toxic effect of the sample on the cells was found at all the cultivation times, their morphology corresponded to the morphology of cells in the control samples, the cells in the process of growth had the correct size and shape.

CONCLUSION

Nanocomposite materials based on SWCNT and MWCNT has been developed. Material can be obtained in a form of a film on a base or in a form of volume samples in a container. The formation of nanocomposite materials is carried out by treating the water dispersion of albumin and CNT with laser radiation of the following parameters: wavelength of 810 nm, a laser beam diameter of 10 mm, a power of 5.5 W, irradiation time of 5–10 seconds (for samples on a base) or for 7–8 minutes (for samples made in a container). The final sample had the scaffold of CNT structured with a laser inside the albumin matrix. This scaffold was electrically conductive and had high mechanical strength. Biocompatibility *in vitro* was proven by results of AFM and optical microscopy of fibroblasts cultured with experimental samples. Periods of incubation were 3, 24, 48, and 72 hours. No toxic effect of the sample on the cells was found, cells formed a monolayer on the surface of the samples. The research results show that the structural and mechanical parameters of the samples of nanocomposite materials make them promising for use as scaffolds for the regeneration of damaged tissues.

AUTHOR CONTRIBUTION

Alexander Yu. Gerasimenko developed the concept and the plan of scientific work, Uliana E. Kurilova and Alexander Yu. Gerasimenko were responsible for obtaining and interpreting data, preparing materials for publication. All authors approved the final version of the publication.

ВКЛАД АВТОРОВ

А.Ю. Герасименко разработал концепцию и план представленного исследования. У.Е. Курилова и А.Ю. Герасименко отвечали за получение и интерпретацию данных, подготовку материалов к публикации. Все авторы утвердили окончательную версию публикации.

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