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Nanomaterials based on carbon framework for cells stimulation

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Abstract. The work is devoted to the technology of formation and study of physical and chemical characteristics and biocompatibility of nanomaterials based on hybrid structures of carbon nanotubes and reduced graphene oxide, obtained by spray deposition and laser processing with nanosecond pulsed laser of 1064 nm wavelength, for bioelectronics applications. The formation of a connected structure between the components was proved with scanning electron microscopy, the optimal power of laser processing was found as 0.07 W to obtain the largest number of connections between carbon nanotubes and reduced graphene oxide, thanks to which high electrical conductivity was achieved. The experiments on electrical stimulation in vitro were provided with the developed setup based on a culture plate, an impulse generator and electrodes connected to samples. Cells seeded on the obtained structures with electrical stimulation demonstrate better proliferation and monolayer forming compared to the control sample. Thus, the developed structures can be successfully used as part of various bioelectronic devices for improved tissue recovery using electrical stimulation.

Keywords: carbon nanotubes, reduced graphene oxide, bioelectronics, fibroblasts, electrical stimulation

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Наноматериалы на основе углеродного каркаса для стимуляции клеток

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Аннотация. Работа посвящена технологии формирования и исследованию наноматериалов на основе гибридных структур углеродных нанотрубок и восстановленного оксида графена, полученных методом напыления и лазерной обработки наносекундным импульсным лазером с длиной волны 1064 нм для применения в биоэлектронике. Доказано образование связанной структуры между компонентами, благодаря чему достигается высокая электропроводность. Эксперименты по электростимуляции in vitro показали, что клетки на структурированных лазером участках в совокупности с электростимуляцией демонстрируют лучшую пролиферацию и формирование монослоя по сравнению с контрольным образцом. Таким образом, разработанные структуры могут быть успешно использованы для создания чипов, стимулирующих ионные процессы жизнедеятельности клеток биологических тканей в составе различных биоэлектронных устройств.

Ключевые слова: углеродные нанотрубки, восстановленный оксид графена, биоэлектроника, фибробласты, электростимуляция

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Introduction

Currently, such field of science as bioelectronics is actively developing. Bioelectronic devices can read signals and stimulate heart, nerve and other tissue regeneration through improved cell proliferation under the influence of electrical impulses [1, 2]. The electrical stimulation changes the transmembrane resting potential of the cells, which has a significant effect on cell functionality and cellular metabolism [3]. An important component of bioelectronic devices are electrically conductive biocompatible interfaces, which conduct efficiently ionic and electronic currents between soft biological tissues and electronic pulse generators [4].

Carbon nanomaterials are promising for flexible bioelectronics applications because they can conduct electrical signals with high resolution and signal-to-noise ratio [5]. These materials have high mechanical flexibility, stability, and tunable properties depending on their functionalization and treatment [6]. It is important to reveal patterns of structuring carbon nanotube systems to form chips that stimulate ionic processes in living cells and biological tissues. In our work we obtained and studied samples of carbon framework nanomaterials used as materials for stimulating connective tissue cells.

Materials and Methods

The process of forming was as follows. As the first step, homogeneous dispersed media were formed with single-wall carbon nanotubes (SWCNT), reduced graphene oxide (rGO), and combination of them to form hybrid structures. Then, up to 50 layers of these dispersions were applied layer by layer onto the surface of clean silicon wafers using the spray deposition method. After that, samples were irradiated with laser using the developed system shown on Fig. 1, *a*, based on a pulsed ytterbium laser with a wavelength of 1064 nm with moving the laser beam along a predetermined path over a sample; under the action of laser radiation, the liquid component evaporated and the nanotubes were oriented and connected for electrical conductivity increasing.

The obtained samples are shown on Fig. 1, *b*. The micro- and nanoarchitecture of the layers of the samples were evaluated with a scanning electron microscope.

Experiments were carried out on electrical stimulation of connective cells (fibroblasts) on the obtained samples with stimulation device based on a culture plate and electrodes connected to the samples. The cells were cultivated the first 24 hours without electrical stimulation, then stimulation was started and continued for 48 hours, after that the cells were stained with Hoechst 33342 and observed with a fluorescence microscope.

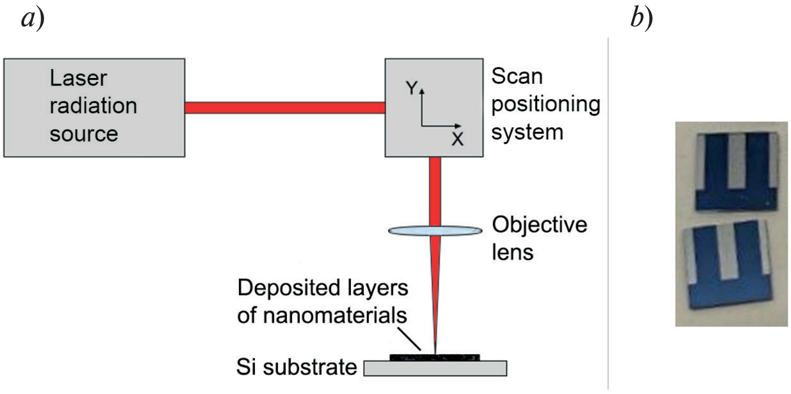

Fig. 1. The scheme of system for laser irradiation of samples (*a*), appearance of obtained samples (*b*)

Results and Discussion

It was found that carbon framework nanomaterials after laser structuring with a power of ≥ 0.07 W are characterized by the formation of bonds between components and branched structure due to photon-phonon interaction (Fig. 2, *a*, *b*). A further increase in the processing power leads to gradual destruction of the structure and the formation of areas with amorphous carbon.

The electrical conductivity of the obtained samples increased with increasing laser processing power, which is associated with the formation of a greater number of bonds between the nanotubes, the highest value obtained was 37.84 ± 1.16 mSm for irradiation power of 0.07 W (the initial value without radiation was 13.07 ± 0.71 mSm). With a further increase in power, the electrical conductivity decreases, which can be explained by the formation of an amorphous phase, which conducts the electrical signal poorly. Thus, by choosing the processing power, materials with different conductivities can be obtained depending on the proposed application.

To test the effectiveness of the developed samples for cell proliferation, electrical stimulation experiments were carried out (Fig. 2, *c*–*e*). Structured and unstructured areas for experimental sample (Fig. 2, *d*) are marked on Fig. 2, *e*.

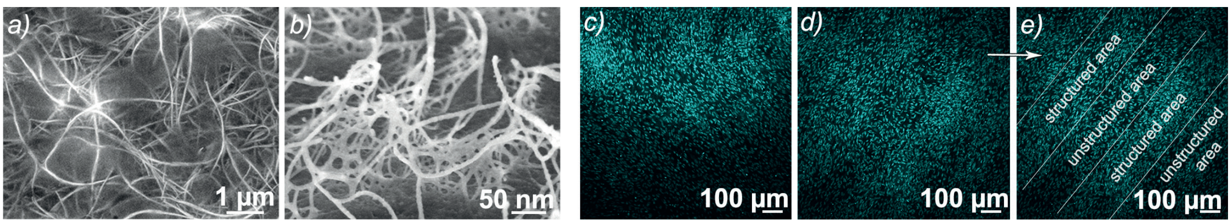

Fig. 2. Results of experiments with obtained samples: SEM images before (*a*) and after (*b*) laser irradiation, fluorescence images of cells on samples: control – cover glass (*c*), experimental sample with structuring and cells stimulation (*d*) and its areas visualization (*e*)

According to microscopy data, connective tissue cells formed a monolayer during cultivation. It is seen that areas structured with laser radiation demonstrate better proliferation of cells on these surfaces compared to unstructured areas. An increase in the cell number was obtained by 4.3 times for cells compared to normal conditions on control samples. The combined effect of the conductive framework and electrical stimulation shows active proliferation associated with electrical conductivity and suitable surface structure for cell adhesion. The morphology of cultured cells does not differ from the control sample, indicating the absence of toxicity.

Conclusion

The method for creating biocompatible nanomaterials from carbon nanomaterial has been developed. The binding of nanotubes together occurs after pulsed laser irradiation in the near-infrared region of the spectrum (1064 nm) with radiation power of 0.07 W. The resulting structures with SWCNT and rGO after laser irradiation were characterized by more than twofold increase in electrical conductivity compared to the original samples. The results of experiments on electrical stimulation of cells showed an increase of cell proliferation on obtained samples. The developed materials can be successfully used for various bioelectronic applications, including electrical stimulation for more effective cultivation for different types of cells, for wearable devices, which record and stimulate cells activity and many others.

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