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Nanocomposite Materials for Cell Growth

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We propose a development of carbon nanotube (CNT)/albumin nanocomposite for 2D and 3D tissue organization by cell growth. The adhesion and proliferation for neuroblastoma and fibroblast cells have been investigated on films based on CNT/bovine serum albumin (BSA) nanocomposite. Single-walled carbon nanotube (SWNT)/BSA composites can be used as a substrate for cell growth of different kind. The layers of nanocomposite properties growing method based on laser radiation action. Investigations of stability, an adhesion and internal structure of layers were performed. Stabilizing properties of the described laser method of manufacture (laser nanoforming) of layers may be associated with the ability to obtain nanotube frame work in composite structure under action of electric field of directed laser radiation. The presence of a such frame creates the conditions for self-assembly of biomedical tissues.

Keywords: Bovine serum albumin, Single-walled carbon nanotube, Layers of nanocomposite, Laser radiation, Cell growth.

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1. INTRODUCTION

Among modern methods of bioengineering and medicine, directed on health protection and improvement quality life of people more attention is paid regeneration of organs and tissues. New an interdisciplinary branch of science and techniques - bioengineering of tissues is an alternative to conventional transplantation of human organs. This area of study has the aim restoration of vital functions by replacing pathologically changed biological tissues, its conservation and operation. In these studies a prominent place occupies the development of new biomaterials - natural stimulants of growth and differentiation of cells in the formation of functional tissues and the establishment of conditions of applicability of biomimetic synthetic 3-D structures [1]. These materials should have characteristics (biodegradability, porosity, size and connectivity of pores, immune response etc.) that are not worse than these of the natural extracellular matrix, which provides formation, seeds, adhesion, proliferation and differentiation of cells [2, 3].

Unique electronic properties, high mechanical strength, excellent flexibility and large specific surface area of CNTs make them suitable for creating novel biocompatible composite materials for tissue engineering [4]. Producing a scaffold material is widely investigated in cell seeding and growing applications [5]. At present, there are a number of works on producing composite materials based on nanotubes for bioengineering, and namely, for bone and cartilaginous tissue regeneration and fibroblast growth investigation [6,7].

For further developments in integrating nanotubes in living tissues, a number of serious problems like improving the biocompatibility and biodegradation of nanotubes as well as producing composites based on protein matrices for 3D tissue regeneration must be solved [8]. New perspectives in this area traditionally orienting to 2-D structures may be associated with the development of new technologies for volume nanocomposites. Analogous 3-D synthetic structure were used successfully in tissue regeneration; their properties should be similar to the natural extracellular matrix.

It has already been established that the action of the electric field of the laser radiation can promote the formation of nanotube skeleton in the structure of volume nanocomposites [9]. The presence of such a framework creates the preconditions for self-assembly of biological tissues. Laboratory experiments on animals have no shown allergic reactions when volume samples of nanocomposites obtained by laser method were injected into a rabbit perichondrium. Replacement of the remote perichondrium segment into implanted sample caused regeneration of the operated tissues and stimulated in it active division of usualy passive cartilage cells (chondrocytes) [10].

Moreover the functionalized CNT is less toxic then pure nanotubes [11-13]. The protein adsorption (natural functionalization of CNT) leads to CNT cytotoxicity decreasing. It was observed the SWNT with BSA and fibrinogen adsorbed molecules have no toxic effect [14]. Therefore, in our previous studies the effect of functionalization of CNTs in the BSA we used [10,15,16].

The aim of this study is the investigation of carbon nanotube/albumin nanocomposite formation for cell seeding and substantiation of laser nanoforming of volume nanocomposites obtained from an water dispersion of albumin with CNT (WDA-CNT) over alternative making methods of such nanocomposites.

2. METHODOLOGY AND EXPERIMENT RESULTS

For investigation conduction there were fabricated WDA from Amresco ® BSA and distilled water. The

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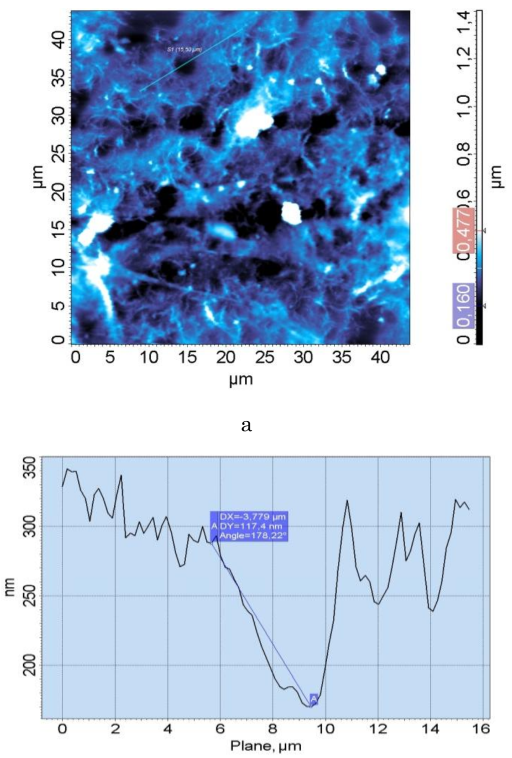
liquid dispersions prepared by mechanical mixing method on magnetic mixer at temperature 20-23 °C during the time t = 1-2 hours. The compounds can survive in refrigerator at T = 5-10 °C in 20-30 days without its parameter changes.

For cell growth experiments we used 2.5 mg of 99.5 mass % SWCNT provided by A.V. Krestinin (Institute of Problems of Chemical Physics of RAS, Moscow, Russia). SWCNT's were placed into WDA (10 mg of BSA in 5 mL of water) and dispersed in a Branson B300 ultrasonic bath (34 kHz, 50 W) for 10 hours. Cover-slips of 24 x 24 mm in size, 0.13 – 0.17 mm thick were preliminary mechanically washed with cotton in 2-propanol, then they were kept in 2-propanol for 15 minutes and placed in ultrasonic bath. About 25 μL of WDA with SWCNT were applied onto this surface with a micro-dispenser and a thin film was made to cover the whole slip surface by rod-coating method. Then the film dried for 15 minutes at 40 °C. To improve the film adhesion the substrate was annealed in the air at a temperature of 150 °C for 2 min. On the fig. 1 shows a typical AFM image of the film obtained from the WDA-CNT. Its thickness was 70 nm and had many pores.



a

b

Fig. 1 – AFM of carbon nanotubes/albumin film formed after dropping and drying from a WDA with SWCNT (a) and a cross section (b). Film has pores of varying depth 40-130 nm

To form the WDA-CNT, we performed the preliminary procedures:

- the BSA powder was dissolved in distilled water. The obtained dispersion WDA with concentration 25 wt % BSA was dispersed in a magnetic stirrer for 1-2 h and in the ultrasonic bath at ≤ 40°C for 1 h, decanted, and filtered.
- powders of SWCNT with the concentration 0.1 wt % were added into the WDA.
- obtained dispersion WDA-SWCNT (25 wt % BSA+0.1 wt % SWCNT) were treated similarly to point 1.
- dispersion WDA-SWCNT deposited on the substrate.

In this study, we used the methods for obtaining bulk CNM based BSA and MWCNT, which are described in [10,17], where nanotechnology and laser technology are used in combination. During preparation of the samples, numerous technological parameters were varied including the power density, the duration time of laser irradiation, parameters of preparation of aqueous solutions BSA+MWCNT (the duration time of mixing, the power level and duration time of ultrasonic treatment, etc.), the MWCNT concentration level, temperature, and the extra drying duration time.

The prepared suspensions were irradiated using a diode laser (the wavelength of the radiation 970 nm, the radiation power changes in the interval 0÷20 W) until the water evaporation and the layer formation with varying the consistency from rubber-like to a solid state. Later they were dried in air at 30-35 °C. The indication of sufficiently high quality of the final product was the absence of whitish areas of denatured albumin in it. Bulk nanocomposites were prepared by the identical method and measured different characteristics (density, hardness, tensile strength, etc.). Volume dispersion BSA/SWCNT for typical samples was 2.5-3 cm^3, and after preparation its volume and mass is reduced to 1.5-2 times. For their a density (~ 1.2 g/cm^3), a hardness (~ 300 MPa), a tensile strength (~about 30 MPa) have the same order, which have been implemented for bulk nanocomposites based dispersion BSA / MSWCNT previously investigated in [9,10,15,16].

Layers obtained with the use of laser irradiation behave as bulk nanocomposites [15,16]: when stored in normal conditions they retain their shape and strength during the year or longer. Layer dried without laser irradiation via > 15 h disintegrate and become powder.

In this study we developed the method for cells growth on SWCNT/BSA composite film and investigated their interaction and behavior with making use of AFM methods. For cell growth on nanotube study we have cultured the neuroblastoma cell line Neuro-2a and Normal human embryo fibroblast (HEF) cells from Tissue Culture Collection of D.I. Ivanovsky Institute of Virology.

The cell cultured for three days in Eagle DMEM medium. After growth the cells fixed in glutaraldehyde. All measurements were done on the AFM microscopes Solver-PRO (NTMDT, Russia) and Centaur HR (Nano Scan Technology, Russia). Topography was measured in semi-contact mode with NSG30 series cantilevers (NTMDT, Russia). Because of cell height that was as high as 5 μm we used both topography and phase shift contrast to visualize simultaneously cell body and nanotube film (fig 2a, b). Phase of the probe oscillation provide information about the fine structure of the surface, and, indirectly, about the different mechanical properties in the presence of multi-component systems on the surface. These data indicated that there is a uniform film of carbon nanotubes, despite the presence of topographical differences.

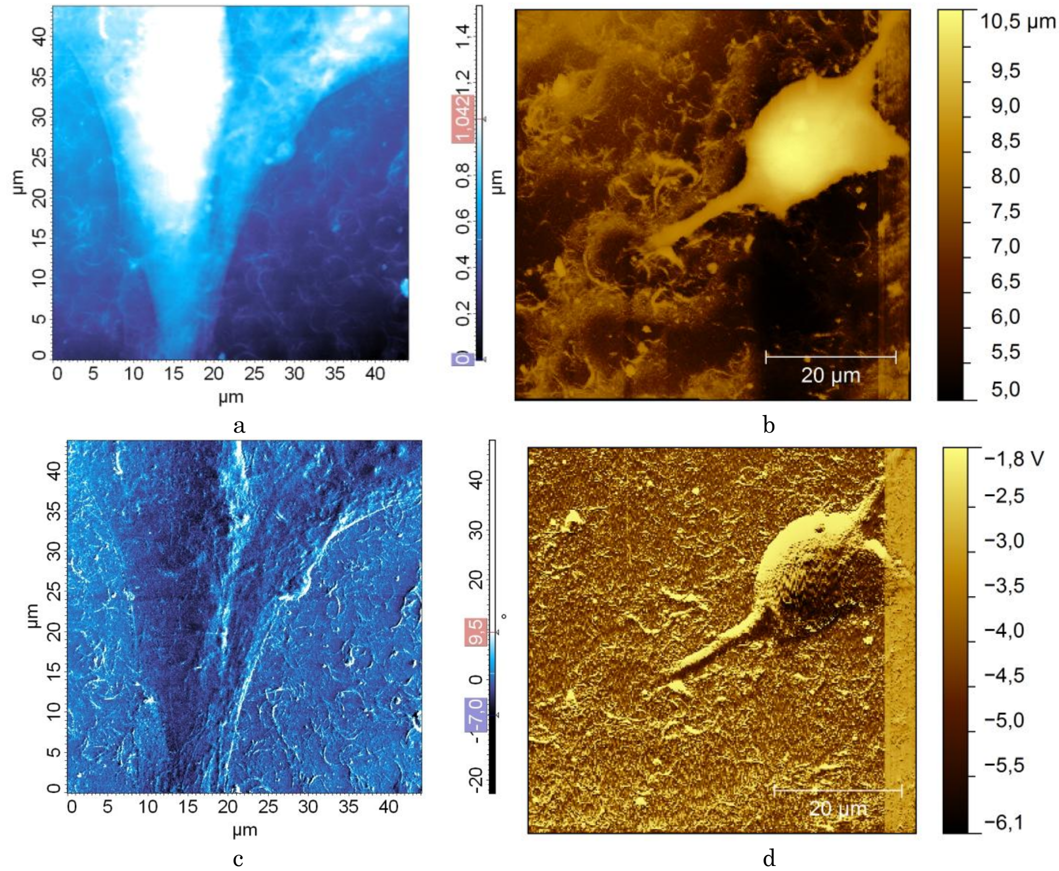


Fig. 2 – AFM image of cell cultured on SWNT/BSA films: a, c– height contrast, b, d –phase shift contrast for HEF (a, b) and Neuro-2a (c, d) cell line respectively

It was also demonstrated that the cell has rather good adhesion that determines the SWNT/BSA mechanical properties similar to the properties of the surface. Phase contrast demonstrates intersection of nanotube from composite and the cell membrane providing better cell adhesion to substrate.

The proliferation was defined by means of a modified MTT assay. For this purpose, the Eagle medium with 10% FBS was removed after electrical stimulation, the cover-slips were moved to a clean plate, where 1mL of the Eagle medium and 200 µL of MTT solution (with the initial concentration of 5mg/mL) were added. The cover- slips were incubated in the thermostat with CO2 at 37°C for 4 hours. Then the medium with MTT removed and 1 mL of DMSO was added into each well to dissolve MTT-formazan reduced by the cells. Cell precipitates with MTT-formazan on glass samples were resuspended for 5 min and the solution absorbance was measured by a Titertek Multiscan Plus photometer (Flow Laboratories, Helsinki, Finland) on a 492 nm wavelength. To achieve that, 1 mL of DMSO with the dissolved formazan was transferred to a 96-well plate, with a 100 µL per well dosage. A pure cover glass was used as a control. The typical value for proliferation factor for VNC film was found to be 1±0.2.

For Neuro-2a it should be noted there is a small tails for nerve cell that can be attributed to axons (fig 2 c, d). More over the arborisation (or axon terminals formation) in cell physiology is attributed to synapse formation. The images depict the rather good adhesion of cells to SWCNT/BSA composite. Moreover the phase contrast shows nanotubes from composite not only under the cell body but on them too that makes the connection more stable. Nevertheless for 2D composite organization only one cell monolayer can be produced. For 3D tissue organization the volume nanocomposite material should be developed.

3. DISCUSS OF REZULTS

Natural extracellular environment that consists of proteins, such as laminin, fibronectin, and collagen allow cell growth. Synthetic conducting polymers such as PEDOT and polyaniline can be used to create electrically active substrates, but are less suitable for fixing the cells in comparison to natural proteins. SWCNT / BSA composites can be used as a substrate for cell growth, as well as for the organization of the electrical connection of cells to the substrate. The cell can be cultivated at such composites film in natural environment of protein and the SWCNT provide skeleton for better cell adhesion. But at film conditions the cell can make only monolayer as shown in our experiments. For 3D or 2-D tissue organization we developed the method for nanotube/albumin volume nanocomposite formation by laser irradiation.

Experiment data point to the advantages of laser manufacturing method of volume nanocomposite materials. Nanocomposites obtained by laser nanoforming maintain stability, according to our observations, in the next five years. It should be noted that for the solution a denaturation temperature of pure bovine serum albumin is of 60-70 ^oC, but layers nanocomposites obtained with using laser, are much more stable and at more high temperature - to 200 ^oC.

The high strength and an adhesion properties and durability of the studied layers obtained by laser irradiation, can be defined in the emergence of volume nanotube framework created by the electric field of a directed laser radiation. According to [18], CNT's can be oriented (perpendicular to the substrate surface) electric field of $C0_2$ laser with N = 30 W at an electric field of light E_1 = 10-20 kV/m. When power density diode lasers used in the present study (10-20 W/cm2) E_1 = 5-10 kV/m. In these conditions, the orientation of CNT's, which created conditions for the appearance in the layers of the nanocmposite BSA/SWCNT nanocarcass seems likely. The high strength and an adhesion properties of nanocomposites can also be explained by the appearance inside their nanotube skeleton, which can be created by an electric field of laser radiation.

4. CONCLUSIONS

Thus, we have developed an adhesive structure based on carbon nanotubes and BSA composite. As AFM results demonstrates the structure provides a good contact with culturing cells because of spatial nanotube organization in albumin matrix. This structure provides both porous environment for better cell adhesion and biocompatibility and nanotube fixation provided by protein.

The laser nanoforming of volume nanocomposites from aqueous-albumin dispersion of carbon nanotubes allocated among different methods of preparing such composites ability to obtain stable materials with high mechanical properties. Orientation of carbon nanotubes by the electric field of a directed laser radiation creates conditions for nanotube nanocomposites framework receiving that is able to cause a more effective self-organization of cellular material than in the case of planar structures of CNT's, in which the rise, development and branching of the living nerve, bone and stem cells observe [19].

Distinctive features of the laser method of manufacture of nanocomposite materials are also remote and disinfecting ability of high-power optical radiation.

It is interesting to note that the use of laser radiation in the experiments described above for the irradiation of aqueous dispersions of several types of cellulose and synthanol with the CNT's does not result in volume nanocomposites. A negative result was also obtained by using an alcohol-albumin dispersion with CNT's.

Biocompatibility of received volume nanocomposites is largely determined by the properties of high soluble globular protein albumin, which carries out the transport function in many living organisms [20]. We suggest that there is no any internalization mechanism of SWCNT / BSA into the cells because of strong coupling between nanotube and substrate. This suggestion is proved by strong adhesion of cell that can be obtained from atomic-force microscopy and proliferation factor equal to normal cell growth. Complex formation ((functionalization) CNT and albumin can significantly expand the scope of biomedical application of investigated volume nanocomposites as the toxicological properties of nanotubes in this case are improving [21].

The investigations of stability, strength, an adhesion, internal structure and the biocompatibility of nanocomposites obtained from nanotube and albumin indicate the possibility of their use as biomedical materials for 2D and 3D tissue organization.

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