Columbia International Publishing
American Journal of Tissue Engineering and Stem Cell
(2014) Vol. 1 No. 1 pp. 27-38

Research Article

Cell Adhesive Nanocomposite Materials Made of Carbon Nanotube Hybridized with Albumin

Ivan Bobrinetskiy[1*], Alexander Gerasimenko[1], Levan Ichkitidze[1], Ol'ga Khrolova[2], and Vitaly Podgaetsky[1]

Received 9 February 2014; Published online 26 April 2014

Abstract

The authors have developed a biocompatible carbon nanotube (CNT) / albumin nanocomposite for 2D and 3D tissue organization by cell growth. The adhesion and proliferation of neuroblastoma and fibroblast cells have been investigated using films based on CNT / bovine serum albumin (BSA) nanocomposites. Single-walled carbon nanotube (SWNT) / BSA composites could be used as a substrate for growing cells of different types. The properties of bulk nanocomposites (BNCs) produced under laser exposure have been compared with those manufactured using such techniques as heating, ultrasonic treatment and light-emitting diode (LED) effect. Stability, density, hardness and internal structure of BNCs have been analyzed. BNCs made under laser exposure were stable within several years. Density of nanocomposites prepared by all the methods was close to that of water. This fact is explained by high material porosity. Hardness of stable BNCs obtained by laser technique (~ 300 MPa) was significantly higher than that of unstable ones mentioned below. Stabilizing properties of the described laser based nano-manufacturing/forming of BNCs may be associated with the ability to obtain a nanotube scaffold in a composite structure under the action of the electric field of directed laser irradiation. The presence of such a scaffold can enable the self-assembly of biological tissues. Results of the study on the biological properties of the BNCs manufactured by the laser technique have pointed to the possibility of their application in surgical implants.

Keywords: Single-walled Carbon Nanotubes; Bovine Serum Albumin; Laser

1. Introduction

Among the modern methods of bioengineering and medicine focused on human health protection and quality life improvement more attention is paid to regeneration of organs and tissues. The new interdisciplinary branch of science and techniques, i.e. bioengineering of tissues, is an alternative to conventional transplantation of human organs. It aims at restoration of vital functions through

*Corresponding e-mail: vkn@nanotube.ru
1* National Research University of Electronic Technology, Moscow, Russia
2 Scientific-Production Firm "DELTARUS", Dolgoprudnii, Moscow Reg., Russia

replacing pathologically affected biological tissues, their preservation and proper operation. This field of science places the main emphasis on the development of new biomaterials - natural stimulants of cell growth and differentiation under formation of functional tissues, i.e. biomimetic synthetic 3-D structures (Langer and Vacanti, 1993). These materials should have characteristics (biodegradability, porosity, size and connectivity of pores, immune response etc.) that are no worse than those of the natural intercellular matrix, which provides cell formation, seeding, adhesion, proliferation and differentiation (Rice et al., 2005; Ma, 2008).

CNTs, in particular, are some of promising nanotechnology products that can be used in biological engineering as a scaffold material for tissue regeneration. Unique electronic properties, high mechanical strength, excellent flexibility and large specific surface area of nanotubes make them suitable for creating novel cell adhesive composite materials for tissue engineering (Harrison and Atala, 2007).

Scaffold structuring has been widely investigated in cell seeding and growing applications (Dvir, 2010). At present, there are a number of works on producing composite materials based on nanotubes for bioengineering, including bone and cartilage tissue regeneration and fibroblast growth (Correa-Duarte et al., 2004; Zanello et al., 2006; Yuen, et al., 2008; Ageeva et al., 2009).

For further advances in integrating nanotubes into living tissues a number of serious problems such as improving nanotube biocompatibility and biodegradation and producing composites based on protein matrices for 3D tissue regeneration should be solved (Kam et al., 2009). At present, technological progress in many respects depends on the development of nanocomposite materials. Their unique and widely variable properties define their applicability and competitiveness as compared with traditional structural materials, including those designed for biological applications (Elisseeff et al., 2002; Liu and Ma, 2004). New perspectives in this area traditionally orienting to 2-D structures may be associated with the development of new technologies for bulk nanocomposites (BNC). Analogous three-dimensional synthetic structures are successfully used in tissue regeneration; their properties should be similar to the natural intercellular matrix.

It has already been determined that the action of the electric field of the laser irradiation can promote the formation of nanotube skeleton in the structure of volume nanocomposites (Podgaetskii et al., 2007). The presence of such a scaffold creates preconditions for self-assembly of biological tissues. Self-organization of the tissue structure which occurs without human intervention is formed and maintained, as a rule, by weak non-covalent (eg., hydrogen or ionic) bonds. Such self-assembly of biological macromolecules is realized in nature, for example, in phospholipids - major components of the plasma cell membranes (Whitesides and Grzybowski, 2002).

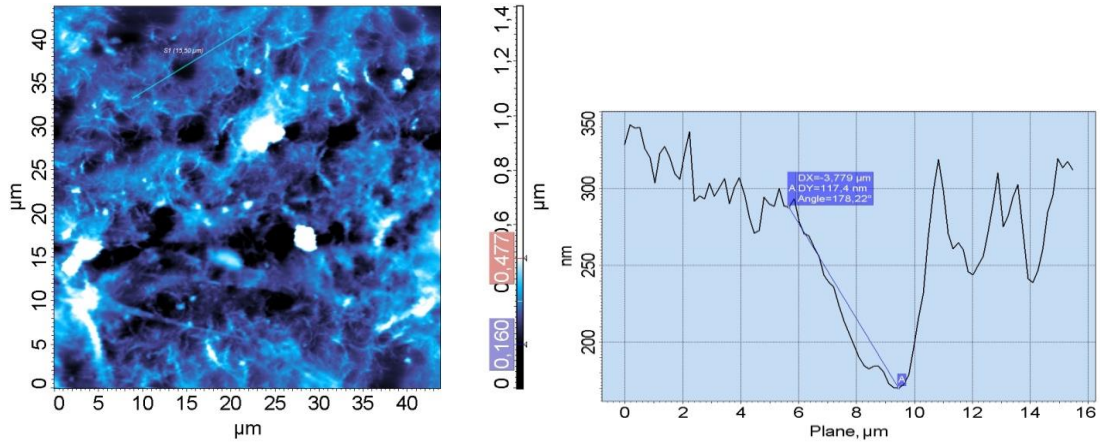
Biological laboratory experiments on animals revealed no allergic reactions when bulk samples of nanocomposites obtained by laser technique were injected into a rabbit perichondrium. This effect was attributed to the presence of albumin. Albumin is one of the most important transport proteins which regulates metabolism functions during cell growth and tissue regeneration. Also, the albumin molecules can serve as a surfactant to form a biocompatible composite of carbon nanotubes. Replacement of a removed perichondrium segment by an implanted sample caused the regeneration of the operated tissues and stimulated the active division of usually passive cartilage

cells (chondrocytes) in it (Bagratashvili et al., 2006; Ageeva et al., 2010). Thus, the use of BNC may enable the conditions for functional biotissue formation similar to the effect of the biological matrix. The aim of this study is to prove the advantages of laser nanoforming of bulk nanotube/albumin nanocomposites based on aqueous dispersion of albumin with CNT (WDA-CNT) as compared with alternative methods of producing such nanocomposites. During the experiments the properties of BCNs fabricated by laser irradiation were compared with those of bulk nanocomposites manufactured by thermal and ultrasonic methods, as well as LED and Hg lamp irradiation.

2. Materials and Methods

2.1 Albumin/nanotube dispersions

In our studies WDA containing bovine serum albumin (BSA) manufactured by Amresco® and distilled water were used. The liquid dispersions were prepared by a mechanical mixing method using a magnetic mixer at a temperature of 20-23 °C (for t = 1-2 hours). The approximate level of dispersion quality was evaluated by transparency degree and the more precise one was measured using an absorption spectrum in the UV spectra region where BSA absorption bonds were located. The samples usually stored in a refrigerator at T = 5-10 °C for 20-30 days showed no changes in their parameters. In contrast to the WDA used, aqueous dispersions of human serum albumin decomposed within 15-20 days as evidenced by the smell of hydrogen sulfide. This could suggest a break of disulfide bonds in an albumin molecule, i.e. protein denaturation.



(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). The film has pores 40-130 nm deep.

In our cell growth experiments we used 2.5 mg of 99.5 mass % single-walled carbon nanotube (SWCNT) provided by A.V. Krestinin (Institute of Problems of Chemical Physics of RAS, Moscow, Russia). SWCNTs were placed into WDA (10 mg of BSA in 5 mL of water) and dispersed in a Branson B300 (Branson Ultrasonics, Danbury, CT, USA) ultrasonic bath (34 kHz, 50 W) for 10 hours. Cover-slips of 24 x 24 mm in size and 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 into an ultrasonic bath. About 25 μL of WDA with SWCNT were applied onto the surface with a microdispenser and a thin film designed to cover the whole slip surface was made by the rod-

coating method. Then the film was dried for 15 minutes at 40 °C. To improve the film adhesion the structure was annealed in the air at a temperature of 150 °C for 2 min. In common, the thickness was about 70 nm with a lot of pores (**Fig. 1**).

In our further experiments we used WDAs at a concentration of 25 wt. % BSA with inserted multi-walled carbon nanotubes (MWCNT), type "Taunit" (Tkachev et at., 2007), at a concentration C_1 = 0.1-0.3 wt. % or SWCNT at a concentration $C_2 \leq 0.1$ wt. % produced by the methods described by Golikov et al. (2006) (SWCNT I) and Shofner et al. (2006) (SWCNT II). The ultrasonic dispersion method appeared to be the most applicable for the preparation of a WDA-CNT structure (WDA-CNT). Dispersion process which took place in an ultrasonic bath with a capacity of 2.8 l at a frequency of 35 kHz, with a heater power of ~ 0.3 kW, lasted ~ 1 hour. To increase the albumin solubility the dispersion was kept under heating at 40 °C. Besides, the composition quality was estimated based on the degree of fluid transparency and WDA absorption spectra.

Thermal stability of WDA-CNT (C_1 = 0.1 wt. %) placed in a test tube ~ 15 cm^3 in volume was analyzed by means of incubation at a temperature of 40 °C for about 80 hours. The tubes with the same WDA-CNT were also stored at a room temperature for about 950 hours.

2.2 2D Cell Culture

The authors have developed the method for the cell growth on the SWCNT / BSA composite film and studied the above interaction and behavior using AFM methods. To analyze the cell growth on nanotubes 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 line was cultured in Eagle DMEM medium for three days. After growing the cells were fixed in glutaraldehyde.

2.3 The photostability test

In order to determine the photostability of WDA-MWCNTs with 0.1 wt. % of the "Taunit" type they were exposed by turns either to light-emitting diodes (LEDs) that emitted within three different (UV, visible and near-IR) spectral regions or to a UV Hg arc lamp (power N = 125 W). For that purpose, about 20 ml of liquid were placed into a glass conical flask with the capacity of 50 cm^3. Light was directed to the bottom of the flask. The distance from the light source to the bottom of the flask was ~ 1 cm. Emission spectra maxima were λ_{max} = 365 nm (UV-LED, N = 30 mW), λ_{max}= 630 nm (red-LED, N = 65 mW),), λ_{max}= 810 nm (IR-LED, N = 80 mW).

The spectra of irradiated WDA-MWCNT were recorded using the spectrophotometer SF-26 for 50-60 hours, every ~ 10 hours. As shown in **Fig. 2**a, irradiation of liquids generally caused a slight monotonous increase in their optical density, which may be associated with a variation in the degree of aggregation of the nanotubes in the fluid. As shown in **Fig. 2**b, the change in the optical density (increasing for UV and red LEDs and decreasing for IR LED) at an equal irradiation dose of 0.6 kJ can be attributed to the power variation.

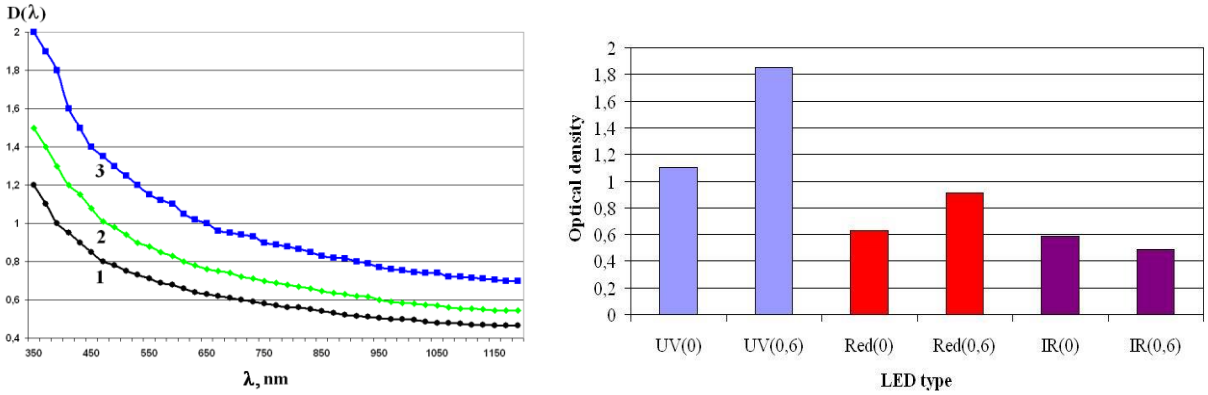
2.4. Atomic-force microscopy

All measurements were made using AFM microscopes Solver-PRO (NTMDT, Russia) and Centaur HR (Nano Scan Technology, Russia). Topography was measured in a semi-contact mode by NSG30 series cantilevers (NTMDT, Russia).

3. Results

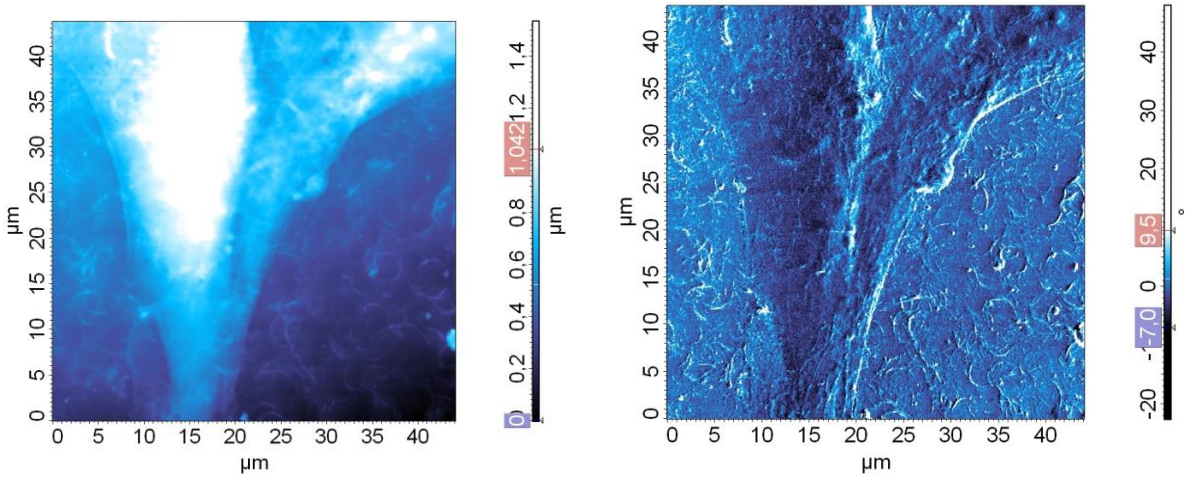
3.1 Studies on adhesion of SWNT/BSA films for different cell line growth

Since the cell height was equal to 5 μm both the topography and phase shift contrast were used to simultaneously visualize the cell body and the nanotube film (**Fig. 3**). The phase of the probe oscillation provides the data on the fine structure of the surface as well as indirect information on different mechanical properties in the presence of multi-component systems on the surface. From these data it can be concluded that there is a uniform film of carbon nanotubes despite the presence of topographical differences. It was also demonstrated that the cell had a rather good adhesion indicating that the SWNT / BSA mechanical properties were similar to those of the surface.



(a) (b)

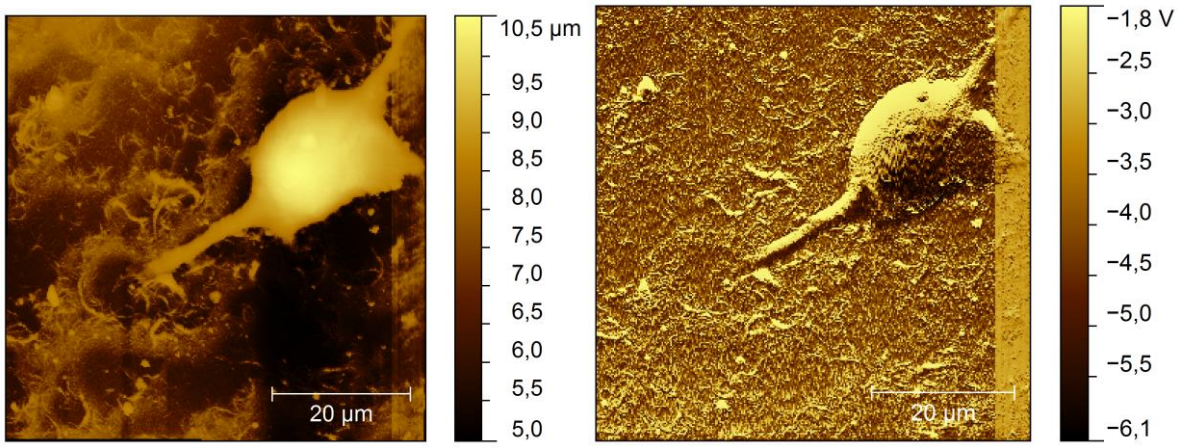
Fig. 2. Absorption spectra of the water D(λ) of the 25 wt. % aqueous dispersions of bovine serum albumin with multiwalled nanotubes "Taunit" at concentration of 0.1 %. The spectra were obtained after irradiation of liquid by red light-emitting diodes at time intervals of 0 (1), 33 (2), и 63 (3) hours (a). Absorbance D0 (λ) and D0.6 of a liquid dispersion layer for different LEDs irradiation at doses of 0 and 0.6 kJ, respectively (b). The thickness of the liquid layer is 1 cm



(a) (b)

Fig. 3. AFM image (a) and phase shift contrast (b) for HEF cultured on SWNT/BSA films

In case of Neuro-2a it should be noted that there is a small tail of a nerve cell that can be attributed to axons (**Fig. 4**). Moreover, the arborisation (or axon terminals formation) in cell physiology is attributed to synapse formation. The images depict the rather good adhesion of cells to SWNT / BSA composite. Besides, the phase contrast shows nanotubes from the composite not only under the cell body but on it as well, which makes the connection more stable. Nevertheless, for 2D composite organization only a one-cell monolayer can be produced. For 3D tissue organization the bulk nanocomposite material should be developed.



(a) (b)

Fig. 4. AFM image for Neuro-2a cell cultured on SWNT/BSA film: a – height contrast, b –phase shift contrast

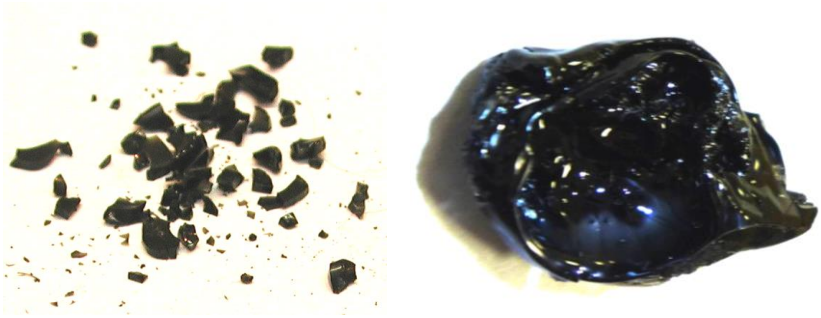
3.2 Bulk Nanocomposites

In the first series of experiments the thermal stability and photostability of VNCs made from 25 % WDA with MWCNTs "Taunit" at a nanotube concentration of 0.1 wt. % were investigated. The fluid samples with mass of 1 g were placed in glass tubes 10 mm in diameter and the liquid was added

up to a height of 5 cm, then the tubes were kept in an incubator at a temperature of 50 °C for 50-60 hours. At the end of this period hardened composite samples were arisen from the tube bottom for 1-2 hours. After moving off the tubes and cooling them in the air during a short period of time (from ten minutes to several hours) a decay of obtained products into separate small fragments took place (**Fig. 5**a). This phenomenon resembled the decomposition of dried albumin dispersion (without nanotubes) into flakes accompanied by a conformational change in the albumin molecule (wedge dehydration) (Belova and Potekhin, 2003). The same results were observed when WDA with MWCNTs "Taunit" was processed in an ultrasonic bath at a temperature of 40-50 °C for ~ 10 hours.

In the other series of experiments the WDA with MWCNTs "Taunit" was irradiated by the above LEDs and Hg lamp. The exposure time was 53 - 63 hours for LED and ~ 50 hours for a Hg lamp. Fluid samples with a mass of 20 g were placed into glass tubes with a cross-section of 4 cm^2 and were irradiated from the top. The irradiation source was located at a distance of 50 mm from the surface of the liquid. After irradiation solid hardened composite samples were removed from the vessels and kept in the air. The final result of these series of experiments was as follows: nanocomposites decayed into fragments in a relatively short time as in the first experimental series.

Coherent irradiation of WDA with MWCNTs "Taunit" with C_1 = 0.3 wt. % was conducted using an irradiation device completed with a laser light source. Two continuous diode lasers (λ_{gen}= 810 and 970 nm) with optic-fiber output of radiation power N ≤ 20 W were applied. In addition, the devices were equipped with low-power green or red solid-state lasers which were used to target the laser beam on the surface of the liquid. Targeting was performed so that the light spot diameter was approximately the same as the internal diameter of the vessel with the liquid.



(a) (b)

Fig. 5. The appearance of fragments of broken composites after thermostatting or sonication or irradiation by light diodes (a) and samples of nanocomposites obtained by laser method (b)

The device was also completed with laboratory equipment including a tripod with a push-in clip to fix the optical fiber and thermocouples and a table with a glass stand for the irradiated samples kept in glass beakers ~ 20 mm in diameter. To control the temperature of the irradiated dispersions a remote infrared thermometer "Optris MiniSight" was used in addition to an electronic temperature meter (multimeter DT 832).

Laser radiation was directed from the top along the axis of the glass and passed to the end of the evaporating liquid. As a result, homogeneous samples of bulk nanocomposites of black color were

formed at the bottom of the glass (**Fig. 5**b). For comparison **Fig. 5**a shows the fragments of broken composites after red light diode irradiation. The samples had a homogeneous staining and did not contain whitish areas of denatured albumin. Hardness (see below) of the samples depended on the radiation intensity and duration. The mass of the product was 20-70 % less than that of the initial dispersion.

BNCs stability obtained under the action of laser radiation was significantly higher than that of the unstable bulk nanocomposites obtained in the previous series of experiments (dispersion thermal drying, effects of dispersion ultrasound and irradiation exposure by LEDs within a wide range of the spectrum and by an UV Hg lamp). In that case, appearance and other properties of bulk nanocomposites remained unchanged for several years. Cold-storage (in an ordinary refrigerator) is preferable because of the possible impact of abnormal flora on their properties as they contain such a biological component as albumin in their composition.

To study the density ρ of the investigated composites the samples were broken into small fragments with linear dimensions of 3-4 mm, in 10-15 pieces for each experiment. Further the samples were immersed in a measuring cylinder half-filled with gasoline of high purity for the measurement of the volume. The volume of the samples was calculated using a measuring scale.

Table 1 shows the ρ values of composites for which the confidence intervals correspond to the confidence coefficient $\alpha = 0.9$ in comparison with the data on other materials, such as crystallized albumin hydrate, iron, aluminum and polymethylmethacrylate PMMA. As it seen from **Table 1**, the density values are close for all investigated composites and a little higher than the density of albumin hydrate and PMMA, but more than twice less in relation to that of aluminum, iron and aluminum.

Table 1 Density and hardness of materials

Materials	Experimental conditions	ρ, kg/m^3	H_v, MPa
Nanocomposites based on MWCNTs	1th series	~ 1250	200±20
	2th series	~ 1300	180±10 (UV LED) 190±20 (Red LED) 220±10 (UV LED) ~ 200 (Hg lamp)
Nanocomposites based on SWCNTs	3th series	1250±60	320±60
Nanocomposites based on MWCNTs		1220±80	270±60
Albumin hydrate	Measurement	1100	~ 70
Iron	Kikoin (1976)	~ 1150	~ 250
Aluminum		~ 2800	~ 200
PMMA		~ 2700	~ 200

Vickers hardness H_v was measured using the pieces of the above described samples. They were ground and polished up to the surface roughness better than 2 classes. Hardness measurements were made using a microhardmeter PMT-3 by pushing the test material in the diamond pyramid (indent) with an angle of 136° between opposite faces. H_v values were calculated via division of the load by the surface area of the resultant indent.

The experimental values of hardness for the nanocomposites and construction materials selected for comparison, are shown in **Table 1**. Confidence intervals were equal to $\alpha = 0, 9$. As the table shows, the values of H_v for the bulk nanocomposites of SWCNT made by the laser method were ~ 300 MPa; these values are 4 times higher than those for albumin hydrate and comparable with the hardness of PMMA, iron and aluminum. While the density of all investigated composites is approximately the same, hardness of bulk nanocomposites fabricated by the laser method is noticeably higher than that of the unstable samples composites (~ 200 MPa) obtained by other techniques.

4. Discussion

Natural intercellular medium that consists of proteins, such as laminin, fibronectin and collagen, conditions cell growth. Synthetic conducting polymers such as PEDOT and polyaniline can be used to create electrically active substrates, but they are less suitable for fixing cells in comparison with 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 on such composite films in natural protein medium and the SWCNT provides the skeleton for the better cell adhesion. But in case of films the cell can form only a monolayer as shown in our experiments. For the 3D tissue organization we have developed the method for nanotube/albumin bulk nanocomposite formation by laser irradiation.

Experimental data point to the advantages of laser manufacturing of bulk nanocomposite materials. According to our observations, nanocomposites obtained by laser nanoforming can maintain stability over a period of five years. It should be noted that for the solution a denaturation temperature of pure bovine serum albumin is of 60-70 °C, while bulk nanocomposites obtained using laser techniques are much more stable even at higher temperatures up to 200 °C.

The mechanical hardness of these BNCs overtopped the hardness of the composites obtained by removing the liquid components from the aqueous dispersion of albumin with carbon nanotubes by prolonged heating, ultrasound exposure and liquid irradiation of LEDs emitting within different spectral regions, as well as a mercury lamp. Their hardness is as high as that of the known construction materials together with a low density which is close to the density of water.

The high strength and durability of BCNs obtained by laser irradiation can be caused by the bulk nanotube frame created inside them by the electric field of the directed laser radiation. According to Kamanina et al. (2011), CNTs can be oriented (perpendicular to the substrate surface) by the electric field of a $C0_2$ laser with N = 30 W at an electric field intensity of light E_1 = 10-20 kV/m. Under the power density of the diode lasers used in the present study (10-20 W/cm^2) E_1 = 5-10 kV/m. In these conditions the CNT orientation enabling the formation of BCNs nanoframe seems

probable. The high strength properties of bulk nanocomposites can also be explained by the formation of a nanotube scaffold inside them induced by the electric field of the laser radiation.

Moreover, the effect of electric field on the CNT orientation can be amplified at the nanotube tip depending on the size of CNTs and the nature of the structuring of their ensemble. At the tip of the nanotubes the light electric field strength is by (ma+b) times higher than the average intensity of the light electric field E_1, where the aspect ratio a = l/d, while l and d are the length and diameter of CNTs, respectively. For isolated CNTs m ≈ 0.7 and b ≈ 30 and tend to decrease when approaching nanotubes (Kim et al., 2009). For the SWCNTs (d≤ 1 nm, l ~ 1 μm) and MWCNTs (d = 20-100 nm, l ~ 1 μm) nanotubes used in the present study (Golikov et al., 2006; Shofner et al., 2006; Tkachev et at., 2007) a ≈ 1000 and 10-50, respectively.

5. Conclusions

Thus, we have developed an adhesive structure based on carbon nanotubes and BSA composites. According to the AFM results, this structure enables a good contact with cultured cells due to spatial nanotube organization in the albumin matrix. It provides the porous environment for better cell adhesion and biocompatibility, as well as nanotube fixation ensured by protein.

The laser nanoforming of bulk nanocomposites from aqueous albumin dispersion of carbon nanotubes differs from other known methods of preparing such composites because it allows producing stable materials with high mechanical properties. Orientation of carbon nanotubes by the electric field of the directed laser radiation make conditions for the formation of the nanotube scaffold inside nanocomposites, which can induce the more effective self-organization of the cellular material as compared with CNT planar structures in which the rise, development and branching of the living nerve, bone and stem cells were observed (Mattson et al., 2000; Correa-Duarte et al., 2004; Kam et al., 2009).

Distinctive features of the laser method of manufacturing nanocomposite materials also comprise the non-intrusive and disinfecting ability of high-power optical radiation.

It is interesting to note that the laser technique used to irradiate aqueous dispersions of several types of cellulose and synthanol with the CNTs in the above described experiments did not bring about bulk nanocomposites. The same negative results were also obtained when using an alcohol-albumin dispersion with CNTs.

Biocompatibility of the bulk nanocomposites obtained is mainly determined by the properties of high soluble globular protein albumin perfoming the transport function in many living organisms (Peters, 1996). Complex formation (functionalization) of CNT and albumin can significantly widen the scope of biomedical applications of the bulk nanocomposites studied as well as can improve the toxicological properties of nanotubes (Huang et al., 2002; Zhao et al., 2010).

The results of the studies on stability, density, hardness and internal structure of bulk nanocomposites produced by the laser technique point to the possibility of their use as filling materials for general-purpose surgical implants.

The investigations of stability, density, hardness, internal structure and cell adhesive capacity of nanocomposites produced from a nanotube and albumin suggest the possibility of their use as surgical implants filling materials of 2D and 3D tissue structure.

Acknowledgements

We are very grateful to Dr. I. Suetina (D. I. Ivanovsky Institute of Virology) for the cell growth experiments and R.A. Morozov (MIET) for the discussion on atomic –force microscopy.
This work was provided by the Ministry of Education and Science of the Russian Federation (Agreement 14.132.21.1789) and the Foundation of the President of the Russian Federation for state support of young Russian scientists (Grants SP-2477.2012.4, MD-170.2014.8).

References

Ageeva, S. A., Bobrinetskii, I. I., Nevolin, V. K., Podgaetskii, V. M., Selishchev, S. V., Simunin, M. M., Konov, V. I., Savranskii, V. V. (2009). Nanotube-based three-dimensional albumin composite obtained using continuous laser radiation. Semiconductors, 43(13), 1714-1718. http://dx.doi.org/10.1134/S1063782609130211

Ageeva, S. A., Eliseenko, V. I., Gersimenko, A. Yu., Ichkitidxe, L. P., Podgaetskii, V. M., Selishchev, S. V. (2010). Possible medical application of laser Nanoengineering. Biomedical engineering, 44(6), 233-236.

Bagratashvili, V. N., Sobol, E. N., Schechter, A. B. (2006). Laser engineering cartilage, M. Francis, London, UK, 488.

Belova, L. M., Potekhina, Y. P. (2003). Study of conformational changes of the albumin molecule in various conditions by wedge dehydration. Nizhny Novgorod meditsinkii jurnal, 3-4, 89-90.

Correa-Duarte, M. A., Wagner, N., Rojas-Chapana, J., Morsczeck, C., Thie Giersig, M. M. (2004). Fabrication and biocompatibility of carbon nanotube-based 3D networks as scaffolds for cell seeding and growth. Nano letters, 4(11), 2233-2236. http://dx.doi.org/10.1021/nl048574f

Dvir, T., Timko, B. P., Kohane, D. S., Langer, R. (2010). Nanotechnological strategies for engineering complex tissues. Nature nanotechnology, 6(1), 13-22. http://dx.doi.org/10.1038/nnano.2010.246

Elisseeff, J. H., Lee, A., Kleinmann, H. K., Yamada, Y. (2002). Biological response of chondrocytes to hydrogels. Annals of the New York academy of sciences, 961(1), 118-122. http://dx.doi.org/10.1111/j.1749-6632.2002.tb03062.x

Fu, K., Huang, W., Lin, Y., Zhang, D., Hanks, T. W., Rao, A. M., Sun, Y.-P. (2002). Functionalization of carbon nanotubes with bovine serum albumin in homogeneous aqueous solution. Journal of nanoscience and Nanotechnology, 5, 457-461. http://dx.doi.org/10.1166/jnn.2002.135

Golikov, F. V., Pozharov, A. S., Obraztsova, E. D., Arutyunyan, N. R., Terekhov, S. V., Chernov, A. I., Konov, V. I., Iskhakov, L. D., Lobach, A. S. (2006). Synthesis and characterization of single-walled carbon nanotubes grown by chemical deposition of ethanol vapor. Advances in science and technology, 48(1), 31-36. http://dx.doi.org/10.4028/www.scientific.net/AST.48.31

Harrison, B. S. Atala, A. (2007). Carbon nanotube applications for tissue engineering. Biomaterials, 28(2), 344-353. http://dx.doi.org/10.1016/j.biomaterials.2006.07.044

Kam, N. W. S., Jan, E., Kotov, N. A. (2009). Electrical stimulation of neural stem cells mediated by humanized carbon nanotube composite made with extracellular matrix protein. Nano letters, 9(1), 273-278. http://dx.doi.org/10.1021/nl802859a

Kamanina, N. V., Vasilyev, P. Y., Studenov V.I. (2011). Features of nanostructured coatings obtained by laser deposition of carbon nanotubes. Technical physics letters, 37(2), 106-108. http://dx.doi.org/10.1134/S1063785011020088

Kikoin, I. K., Ed. (1976). Tables of physical quantities, Atomizdat, Russia, Moscow, 1008.

Kim, D., Bouree, J.-E., Kim, S. Y. (2009). Calculation of the field enhancement for a nanotube array and its emission properties. Journal of applied physics, 105(8), 084315-5. http://dx.doi.org/10.1063/1.3091282

Langer, R., Vacanti J. P. (1993). Tissue engineering. Science, 260 (5110), 920-926. http://dx.doi.org/10.1126/science.8493529

Liu, X., Ma, P. X. (2004). Polymeric scaffolds for bone tissue engineering. Annals of biomedical engineering, 32(3), 477-486. http://dx.doi.org/10.1023/B:ABME.0000017544.36001.8e

Ma, P. X. (2008). Biomimetic materials for tissue engineering. Advanced Drug Delivery Reviews, 60 (2), 184-198. http://dx.doi.org/10.1016/j.addr.2007.08.041

Mattson, M. P., Haddon, R. C., Rao, A. M. (2000). Molecular functionalization of carbon nanotubes and use as substrates for neuronal growth. Journal of molecular neuroscience, 14(3), 175-182. http://dx.doi.org/10.1385/JMN:14:3:175

Peters, T. (1996). All about albumin: biochemistry, genetics, and medical applications, Academic Press, San Diego, US, 432.

Podgaetskii, V. M., Savranskii, V. V., Simunin, M. M., Kononov, M. A. (2007). Laser-induced preparation of volume nanocompositions in aqueous albumin solutions. Quantum electronics, 37(9), 801-803. http://dx.doi.org/10.1070/QE2007v037n09ABEH013656

Rice, M. A., Dodson, B. T., Arthur, J. A., Anseth K. S., 2005. Cell-based therapies and tissue engineering. Otolaryngologic clinics of north America, 38(2), 199-214. http://dx.doi.org/10.1016/j.otc.2004.10.010

Shofner, M. L., Khabashesku, V. N., Barrera, E. V. (2006). Processing and mechanical properties of fluorinated single-wall carbon nanotube-polyethylene composites. Chemistry of materials, 18(4), 906-913. http://dx.doi.org/10.1021/cm051475y

Tkachev, A. G., Mishchenko, S. V., Konovalov, V. I. (2007). Catalytic synthesis of carbon nanotubes from gas-phase products of hydrocarbons pyrolysis. Russian nanotechnologies, 2, 100-108.

Whitesides, G. M., Grzybowski, B. (2002). Self-assembly at all scales. Science, 295(5564), 2418-2421. http://dx.doi.org/10.1126/science.1070821

Yuen, F. L. Y., Zak, G., Waldman, S. D., Docoslis, A. (2008). Morphology of fibroblasts grown on substrates formed by dielectrophoretically aligned carbon nanotubes. Cytotechnology, 56(1), 9-17. http://dx.doi.org/10.1007/s10616-007-9113-0

Zanello, L. P., Zhao, B., Hu, H., Haddon, R. C. (2006). Bone cell proliferation on carbon nanotubes. Nano letters, 6(3), 562-567. http://dx.doi.org/10.1021/nl051861e

Zhao, X., Liu, R., Chi, Z., Teng, Y., Qin, P. (2010). New insights into the behavior of bovine serum albumin adsorbed onto carbon nanotubes: comprehensive spectroscopic studies. Journal of physical chemistry B, 114(16), 5625-5631. http://dx.doi.org/10.1021/jp100903x