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197376, Санкт-Петербург, ул. Проф. Попова, 5

Тел.: (812) 346-44-87

Факс: (812) 346-27-58

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CARBON NANOTUBE COATINGS FOR LOCAL ELECTRICAL STIMULATION AND VISUALIZATION OF LIVING CELLS IN VITRO

A.S. Seleznev [1], ***I.I. Bobrinetskiy*** [1], ***A.V. Romashkin*** [1], ***R.A. Morozov*** [1], ***O.A. Lopatina*** [2], ***R.Y .Podchernyaeva*** [2], ***I.A. Suetina*** [2]

[1] ***MIET, Moscow***

[2] ***Ivanovskiy Institute of Virology***

One of the most exciting areas of biotechnology today is the integration of biological elements in traditional electronic elements to create physiological environment sensors, monitoring the body state and for artificial implants development. This includes the formation of artificial relations between single cell or cell networks and multi-electrode systems. Ideally, such a system should provide non-invasive connections between cells and electrodes, as the damage caused by the introduction of the electrode may affect the composition of intracellular fluid. [1] In this case the signal ratio problem arises: extracellular stimulation signals reaches the units of volts, whereas the potential taken from the extracellular electrodes in the tens of microvolts. To solve this problem, measuring systems are supplied with precise and expensive measuring equipment. An alternative solution to this problem may be found through the development of devices that generate large local electric fields.Carbon nanotubes (CNT), in particular, are one of promising nanotechnology products, which can be used in as s scaffold material for tissue engineering and drug delivery, as well as for development of conductive transparent ultra-light and biocompatible electrodes [2].

In this study we have developed a biocompatible current-conductive coating based on carbon nanotubes and bovine serum albumin (BSA) and have shown its efficiency in culturing cells in vitro. To make CNT substates, 2.5 mg of 99.5 mass % of single walled carbon nanotubes provided by A.V. Krestinin (Institute of Problems of Chemical Physics of RAS, Moscow, Russia) were placed into a BSA solution (10 mg of BSA in 5 mL of water) and ultrasonicated for 10 hours. Cover-slips, 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 ultrasonicated as well. On one of the surfaces of the cover-slip two gold contact pads, 30 nm thick, were formed by magnetron sputter deposition of gold. 25 μL of nanotube solution in albumin were applied onto this surface with a microdispenser 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 ℃ and scanned with AFM (Figure 1, f). To improve the film adhesion and conductance the structure was annealed on air at 150℃ for 2 minutes.

We use two different types of cells for two sets of experiments. Human embryonic fibroblast (HEF) were incubated in 37 ° C and 5% CO2 for 72 hours. After first 24-hour incubation with no voltage applied, a signal of 5 pulses was sent with 5 ms pulse duration, 5 ms spacing between pulses, 1 s interval between pulse groups. We applied different amplitudes from 10 mV to 5 V to the cells. In the experiment, for proliferation estimation we used samples of the same order of resistance from 3 to 10 MOhms. For microscopic analysis the cell were removed from the culture medium, fixed for 30 min in 2.5% glutaraldehyde and

washed in phosphate buffer 2-3 times for 2 min, and then dehydrogenate the exposure to the solution of 50%, 70% and 96% ethanol in for 2 min each and scanned in AFM (Figure 1, a-d). It is assumed that the transport mechanism accompanied by higher synthesis of proteins and their polymerization may increase proliferative activity at low voltages. At higher voltages the motility and spatial organization of HEF cell is observed. As a result, a novel technique of supplying the cells with electric field through a system of micro- and nanosized electrodes and a biocompatible composite have been developed.

Correlated optical and topographical studies of Neuro 2A cells on CNT surfaces were performed. Despite the slight decrease in cell proliferation, cell morphology remains unchanged and we did not observe any significant toxic effects. The possibility of identifying the formation of the electrochemical interface between the cells and carbon nanotubes by atomic force microscopy and Raman spectroscopy was shown. The results of this study can be used for further investigation of cells and conducting nanomaterial interaction for the development of neuro-replacement implants and bio-electronic interface devices as well as for bio-signals registration, transmission and recognition.

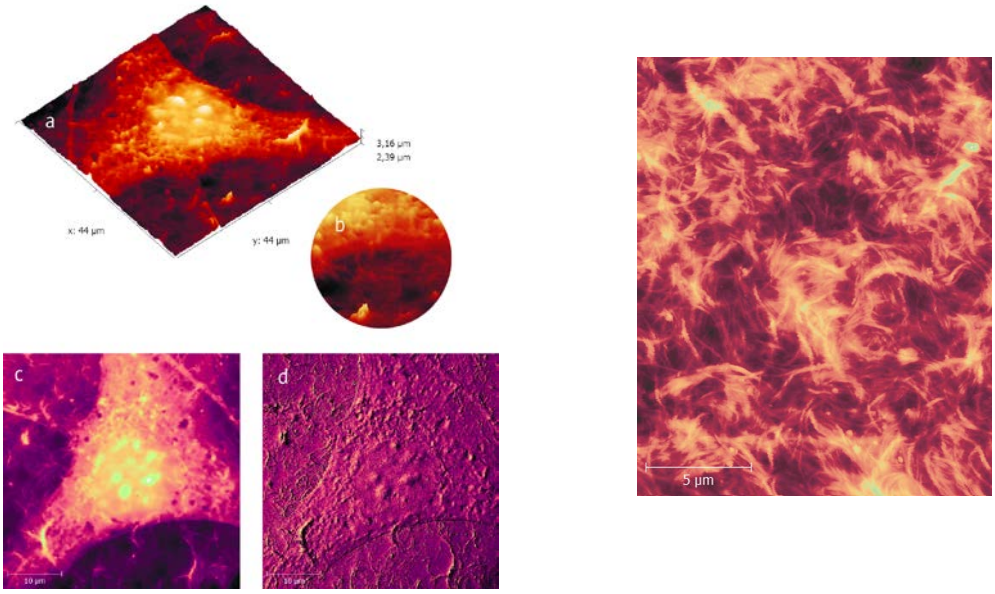

Fig. 1. 3D visualization of AFM data (a), close-up view (b), 2D AFM (c) and phase contrast image (d) of HEF cells interaction with nanotubes on a cover-slip modified with a SWNT/BSA film (bar 10 µm) and AFM image of CNT substrate (f).

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197376, С.-Петербург, ул. Проф. Попова, 5