NANOTECHNOLOGIES

Proliferative Activity and Viability of Fibroblast and Glioblastoma Cell on Various Types of Carbon Nanotubes

I. I. Bobrinetskii, R. A. Morozov, A. S. Seleznev, R. Ya. Podchernyaeva*, and O. A. Lopatina*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 2, pp. 227-231, February, 2012
Original article submitted December 23, 2010

The effects of single-walled and multiwalled carbon nanotubes on proliferative activity and viability of human embryo fibroblasts and glioblastoma cells were studied. Low cytotoxic activity of single-walled carbon tubes was demonstrated. Possible mechanisms of nanotube effects on cell growth are discussed.

Key Words: *nanotubes; fibroblast; glioblastoma; proliferation; viability*

Carbon nanotubes can be used in biological tissue engineering as the scaffold material [7], because their structure is geometrically similar to that of collagen, the main connective tissue protein in animals [9]. Unique electron characteristics, high mechanical strength, flexibility, and specific surface of the nanotubes allow creation of biocompatible composite materials with new properties.

Creation of nanotube-based scaffold material for cell culturing is a promising trend [6]. Nanotube-based composite materials for bioengineering have been created, *e.g.* for bone and cartilage tissue regeneration [14,1] and fibroblast growth [13]. The scaffold material for tissue formation should create cell microenvironment supporting cell proliferation and differentiation (and ideally accelerating these processes in comparison with natural regeneration) [9]. The physiological functions of the forming tissues should be identical to those of normal tissues.

The nanotube cytotoxicity observed in some studies [12] remains a problem. Generally, cytotoxicity depends on various parameters of nanotubes, such as length and size distribution, presence of metal admixtures, dispersion and aggregation status, coating or functionalization, *etc.* [8]. Discovery of oxidative enzymatic cleavage of the tubes by human natural immune defense mechanisms became a breakthrough in the field of nanotube-biological object interactions [5].

We evaluated proliferative activity and viability of human cells cultures on substrates coated with carbon nanotubes.

MATERIALS AND METHODS

Single-walled carbon nanotubes (SWNT) and multiwalled carbon nanotubes (MWNT) from two Russian manufacturers were selected as the scaffold-forming materials. SWNT were obtained by arch evaporation of graphite at Institute of Chemical Physics Problems, Russian Academy of Sciences [4]. MWNT were obtained by catalytic pyrolysis of carbohydrates at GraNaT Company (Electrostal). SWNT formed bundles of solitary nanotubes with transverse size of 1-10 nm on the surface (Fig. 1, *a*), which corresponded to ~1-20 solitary SWNT 2.9±1.5 μ long. MWNT speci-

MIET National Research University; *D. I. Ivanovsky Institute of Virology, Ministry of Health and Social Development of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** vkn@nanotube.ru. I. I. Bobrinetskii

0007-4888/12/1532-0259

men contained nanotubes with several layers and was 4-9 nm in diameter (Fig. 1, *b*) and 4.9±0.5 μ long.

Slides (24×24 mm^2, 0.13-0.17 mm thick) were washed mechanically in 2-propanol and in an ultrasonic bath for 15 min at a frequency of 35 kHz (at 75 W power). The nanotubes (10 μg) were dissolved in 5 ml 2-propanol with ultrasonic treatment for 10 h. The nanotube solution was applied onto slides by multiple pooling from the solution until ~30% decrease in slide transparency (evaluated visually). The specimen was then dried in warm air flow and annealed at 500°C for 2 min. The quality of nanotube application was tested in some slides by atomic force microscopy (Fig. 2). The nanotubes formed a fine network of different porosity on the slide surface.

Normal human embryo fibroblast (HEF) cells and human glioblastoma (GL6) cells from Tissue Culture Collection of D. I. Ivanovsky Institute of Virology [3] were selected for culturing. Both cell strains were cultured on slides (experiment) coated and not coated (control) with nanotubes in Eagle's MEM with 10% FCS (PanEco) in 10-ml penicillin vials. Inoculation dose for cell culturing on slides was 10^5 cell/ml.

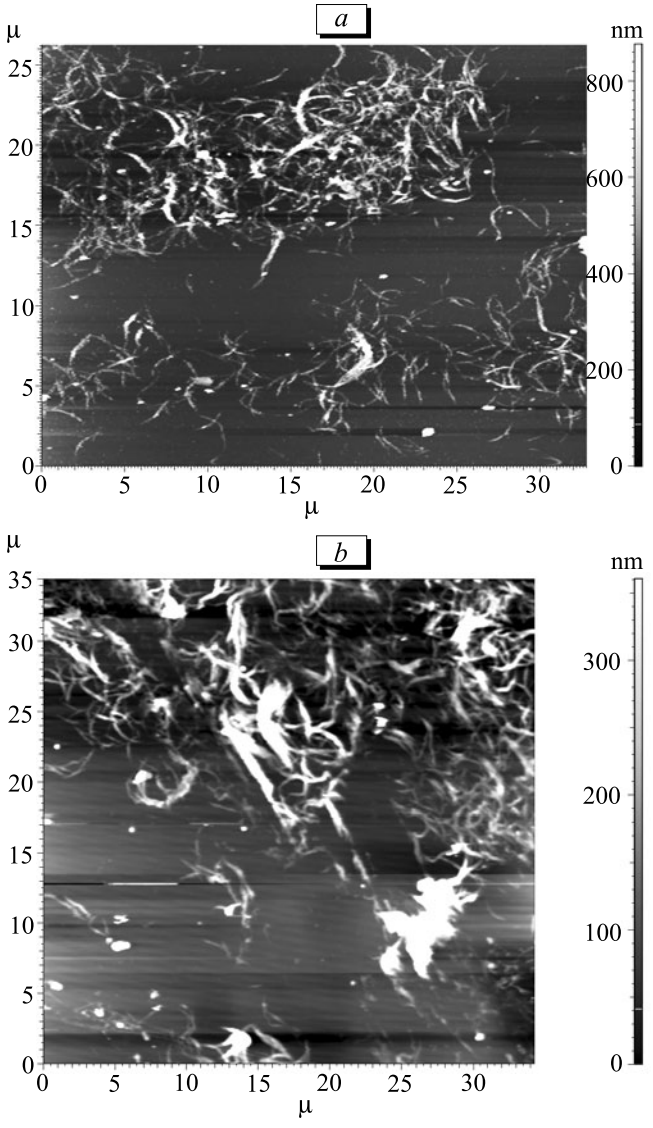

Fig. 1. Nanotubes on the slide surface, atomic force microscopy. *a*) SWNT; *b*) MWNT.

Three variants of prepared sublayers were studied: control slides, slides coated with SWNT, and slides coated with MWNT. Slides with nanotubes were placed into penicillin flasks and sterilized in an autoclave at 120°C and 1 atm. for 20 min, after which the cells were added. The cells (HEF and GL6) were seeded onto slides (control) and slides with nanotubes (experiment) and cultured in a thermostat at 37°C for 72 h. The specimens were then removed from flasks, stained with azur-eosin after Romanowskii for 10-15 min on air, washed in tap water, dried, and embedded in Canadian balm. Stained preparations were examined under a light microscope (10×40) in order to evaluate the cell morphology. Cell viability and proliferation index (PI) were evaluated. PI was calculated by the standard method as the ratio of grown to seeded cell numbers. The percentage of viable cells was evaluated by counting dead cells after trypan blue staining.

RESULTS

The control human diploid cell culture after 72-h growth consisted of fibroblast-like cells with oval nuclei. The nucleoli were large, 1-4 per nucleus, the cytoplasm was slightly reticular (Fig. 2, *a*). The morphology of cells on slides coated with SWNT was the same, but the monolayer was denser (Fig. 2, *b*). On slides coated with MWNT, the cell monolayer was dense, cell morphology was normal, but there were some black incorporations of different size and shape (Fig. 2, *c*).

The control GL6 cell culture was polymorphic consisting of epitheliocyte-like polygonal and spindle cells with fine cords of the cytoplasm. The cells were heterogeneous, of different size (Fig. 2, *d*). The monolayer structure on the slides coated with SWNT was the same: chaotically located cells of different shape (Fig. 2, *e*). On slides with MWNT, the monolayer was dense, multiwalled, with modified (often large) cells. Numerous large black incorporations were seen (Fig. 2, *f*).

In addition to cell morphology, proliferative activity and viability of HEF and GL6 cells cultured on slides with SWNT and MWNT nanotubes was studied (Fig. 3).

PI for cells cultured on SWNT and MWNT was about 2-fold lower than in the control. The viability of HEF cells was ~60% for both nanotube types and somewhat higher in the control (77%; Fig. 3, *b*). PI of GL6 cells was ~2-fold reduced in comparison with the

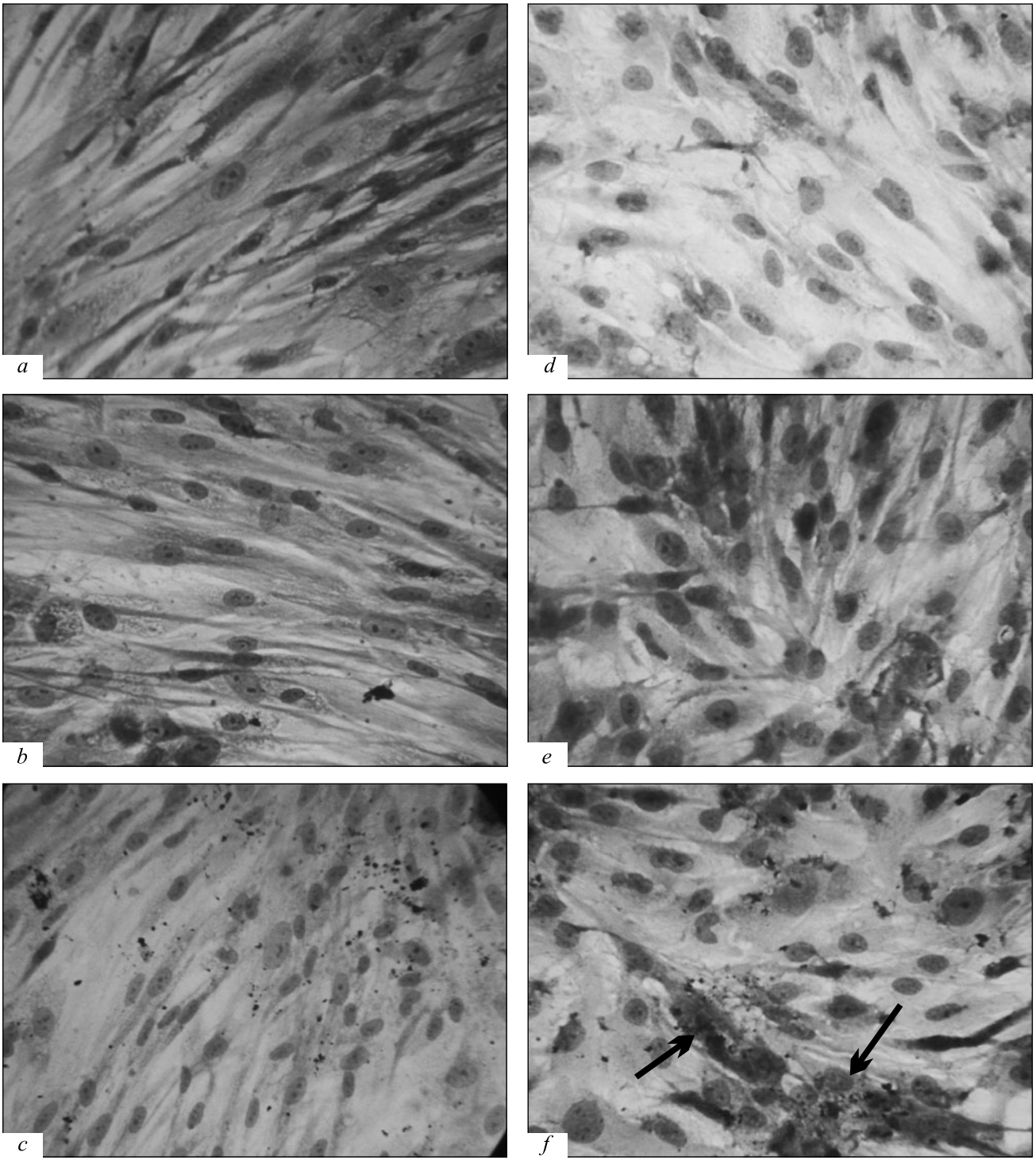

Fig. 2. Cells HEF (*a*, *b*, *c*) and GL6 (*d*, *e*, *f*): control (*a*, *d*) grown on SWNT (*b*, *e*) and MWNT (*c*, *f*). Arrows show accumulations of nanotubes, ×200.

control (PI=1.0) for SWNT and was 0.3 for MWNT. PI correlated with reduction of GL6 cell viability: viability was higher (~72%) for SWNT than for MWNT (~64%), but lower than in the control (82%).

The decrease in cell viability after culturing on sublayers with nanotubes of both types was obvious. The viability evaluated as the proportion of viability on slides with nanotubes/viability on control slides was higher for GL6 cells cultured on SWNT; the viability of these cells on MWNT was somewhat lower than that of HEF cells. This correlated with the lower PI for GL6 cells cultured on MWNT, which indicated

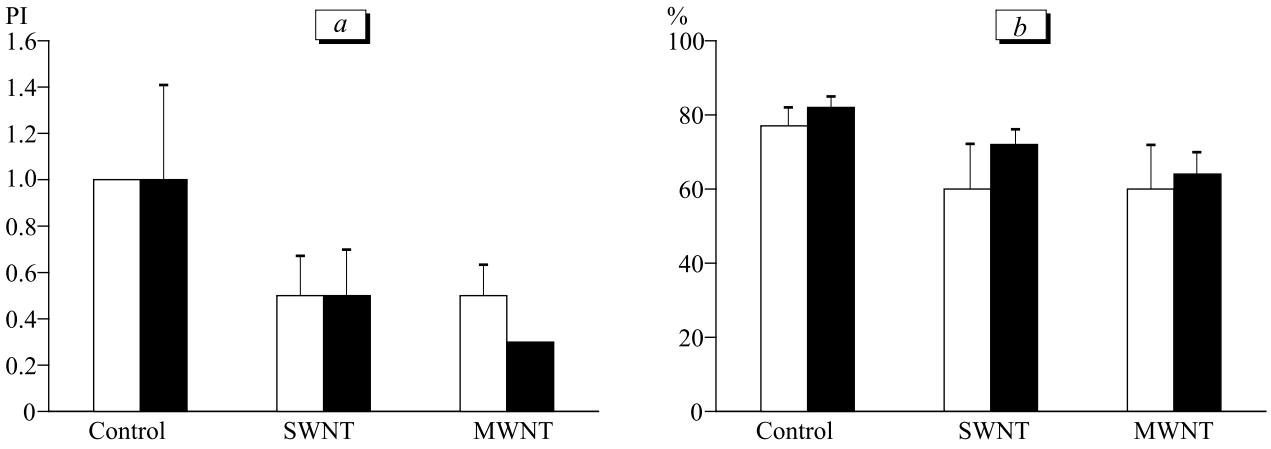

Fig. 3. Proliferative activity (*a*) and viability (*b*) of HEF (light bars) and GL6 cells (dark bars) on different nanotubes. The mean PI and viability percentage for three substrates and two tube types are presented.

generally higher cytotoxic effect of MWNT towards glioblastoma cells, while PI decrease for fibroblasts did not depend on the nanotube type.

Strong interactions of nanotubes with cell membrane with subsequent penetration of nanotubes through the membrane and movement are assumed to be responsible for nanotube cytotoxicity [11]. The nanotube length is essential for this process: shorter nanotubes act as "nanoneedles", while longer nanotubes tend to form balls and cords, which prevented their penetration into the cell membrane [10]. Another important factor responsible for nanotube accumulation in the cells is the technique of nanotube application: it is essential whether they are fixed to the carrier or free (in solution). The toxic effect is more pronounced for solution [8]. This could be explained by greater force of nanotube interactions with the substrate, about tens and hundreds of nanonewtons [2]. The lesser is nanotube diameters, the greater is the strength of interaction with the substrate, and hence, loss of contact with the carrier is energetically unfavorably for the nanotube. Hence, a lesser toxic effect can be expected from SWNT on a carrier than from MWNT, which correlates with the data on lower viability and proliferative activity of GL6 cells on MWNT.

Hence, carbon nanotubes manufactured in Russia are not toxic and can be used for culturing of various cells. Morphological changes in cells cultured on SWNT and MWNT nanotubes were noted. Cell culturing on nanotubes of different kinds was associated with negligible reduction of cell proliferative activity and viability. A high toxic effect of MWNT produced the most pronounced toxic effect on GL6 cells.

The study was carried out within the framework of realization of Federal Target Programs "Scientific and Pedagogical Cadres of Innovation Russia" for 2009-2013 (state contract No. P979) and "Research and Development of the Priority Trends of Scientific and Technological Complex of Russia for 2007-2012" (state contract No. 16.512.11.2135).

REFERENCES

1. I. I. Bobrinetskii, S. A. Ageyeva, V. K. Nevolin, *et al.*, *Izvestiya VUZov. Elektronika*, No. 5, 33-40 (2008).
2. I. I. Bobrinetskii and V. K. Nevolin, *Mikrosistemnaya Tekhnika*, No. 4, 20-21 (2002).
3. *All-Union Cell Culture Collection. Catalogue* [in Russian], Moscow (1991), p. 118.
4. A. V. Krestinin, *Ros. Khim. Zh.*, No. 5, 21-27 (2004).
5. L. B. Allen, G. P. Kotchey, Y. Chen, *et al.*, *J. Am. Chem. Soc.*, **131**, No. 47, 17,194-17,205 (2009).
6. M. Correa-Duarte *et al.*, *Nano Lett.*, **11**, No. 4, 2233-2236 (2004).
7. B. S. Harrison and A. Atala, *Biomaterials*, **28**, No. 2, 344-353 (2007).
8. M. A. Hussain, M. A. Kabir, and A. K. Sood, *Cur. Sci.*, **96**, No. 5, 664-673 (2009).
9. P. X. Ma, *Adv. Drug. Deliv. Rev.*, **60**, No. 2, 184-198 (2008).
10. V. Raffaa, G. Ciofania, and S. Nitodasb, *Carbon Volume*, **46**, No. 12, 1600-1610 (2008).
11. C. M. Sayes, F. Liang, J. L. Hudson, *et al.*, *Toxicol. Lett.*, **161**, No. 2, 135-142 (2006).
12. S. K. Smart, A. I. Cassady, G. Q. Lu, and D. J. Martin, *Carbon*, **44**, No. 6, 1034-1047 (2006).
13. F. L. Yuen, G. Zak, S. D. Waldman, and A. Docoslis, *Cytotechnology*, **56**, No. 1, 9-17 (2008).
14. L. P. Zanello, B. Zhao, H. Hu, and R. C. Haddon, *Nano Lett.*, **6**, No. 3, 562-567 (2006).