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Article

Adsorption and adhesion of blood proteins and fibroblasts on multi-wall carbon nanotubes

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This article concerns the investigation of blood protein adsorption on carbon paper and multi-wall carbon nanotubes (MWCNTs). Mouse fibroblast cell adhesion and growth on MWCNTs was also studied. The results showed that fibrinogen adsorption on carbon paper was much lower than that on MWCNTs, which means that platelets readily aggregate on the surface of MWCNTs. Mouse fibroblast cells implanted on MWCNTs tended to grow more prolifically than those implanted on carbon paper. The cell concentration observed on MWCNTs increased from 1.2×10^5/mL for a single day culture to 2×10^5/mL for a 7-day culture. No toxicity reaction was observed during the culturing period. These results indicated that MWCNTs possessed excellent tissue compatibility.

multi-wall carbon nanotubes, cell adhesion, protein adsorption, toxicity, tissue compatibility

Carbon nanotubes (CNTs) are new nanoscale carbon materials. They have exhibited excellent electrical, thermodynamic, and mechanical properties due to their unique structure. CNTs also possess such physical and chemical characteristics of biomedical materials as high intensity, low modulus, corrosion-wear resistance[1].

Recently, the investigation of CNTs in biomedical applications has primarily focused on preventing nonspecific protein adsorption and identifying particular proteins by surface modification, and promoting cell growth as a culture medium by utilizing their uniquely individual shapes and electrical properties[2]. It has been reported that single-wall carbon nanotubes (SWCNTs) prevent the growth of embryonic kidney cells and reduce the adhesion ability of kidney cells[3]. CNTs have no toxicity and maintain their intrinsic cytoimmunity function[4,5]. However, there have been relatively few studies of blood protein adsorption on CNTs in recent literatures with regard to MWCNTs. Additional investigation of cell adhesion, growth, blood protein adsorption, toxic effects on animals are needed.

The aim of this work is to understand MWCNTs' biocompatibility by investigating blood protein adsorption and cell adhesion onto MWCNTs.

1 Materials and methods

A purpose-built AACVD system was used to synthesize MWCNTs on carbon paper (B-2/030 Toray Carbon Paper Designation TGPH-030, E-TEK Co.). Before synthesis, a layer of Al was sputtered as a buffer layer and a layer of Fe as a catalyst. One inlet introduced aerosol through the carrier with the aerosol solution into the reaction chamber. One additional argon inlet enabled to dilution of the resulting aerosol mixture. Hydrogen was introduced into the reaction chamber from the hydrogen inlet. The toluene solution was placed inside of the sonication generator and thermostated during the synthe-

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sis at room temperature by cooling water. The experiments were performed as follows. The carbon paper substrate (0.5×0.5□0.8×0.8 cm^2) was located in a ceramic boat, placed inside the chamber. The furnace was heated to 870℃ within 15 mins, after a 20 mins expulsion of the air in the chamber by argon. As soon as the temperature reached 850℃, the aerosol droplets were produced by ultrosonication and transported by argon gas introduced from the inlet. At the same time, dilute argon was introduced into the chamber. In this experiment, the growth time was 30 mins. The experiments were performed at atmospheric pressure.

The contact angles of water on the samples were measured at room temperature using a CAM 200 Contact Angle Instrument (KSV Company, Finland). Three different areas on the surface were chosen.

Albumin and fibrinogen were purchased from Sigma Company (USA). All chemical reagents from Shanghai Chemical Reagent Limited Company were analytically pure. A 5 mL albumin solution containing a concentration of 20 mg/mL was obtained from a pre-blend solution of 1 mL albumin solution at 100 mg/mL and 4 mL phosphate buffered saline (PBS, pH 7.4, 10 mmol/L). A fibrinogen solution in which the concentration was 20 mg/mL was obtained from a pre-blend solution of 1 mL albumin solution at 100 mg/mL fibrinogen powder. 300 μL albumin or fibrinogen solutions were inoculated on the surface of three MWCNTs and three pieces of carbon paper. After 2 h incubation at 37℃, the protein solution was removed and the samples were carefully rinsed two times using a PBS solution prior to fixation. A Fourier transform infrared (FTIR) spectrometer (Nicolet 5700, Thermo Co. USA) was used to measure the absorbance intensity of the amide II of proteins adsorbed on MWCNTs and carbon paper. In this test, the scan step length was 4 cm^{-1}, the scan time was 32, and the scan range was 650□4000 cm^{-1}.

A 50 mL culture solution was obtained from fetal bovine serum thawed at 56℃. A 500 mL pancreatic enzyme solution was prepared by mixing 1.25 g of enzyme with a 250 mL of PBS solution and 250 mL of distilled water. The same density of mouse fibroblast cell suspension was grown on the surface of 8 MWCNTs and 8 pieces of carbon paper placed in a 24-well culture plate. 6 empty wells were used as a control group in this cell culture. The culture was placed in a 37℃ incubator with a humidified atmosphere containing 5% CO_2 in air. After 1 to 7 d in the incubator, the medium was removed and the cell monolayer washed several times with PBS, and fixed in methanol for scanning electron microscopy (SEM) observation and numeration.

2 Results and discussion

Table 1 gives contact angles of carbon paper and MWCNT. It is clear that MWCNT surface shows a stronger hydrophobic property than carbon paper.

The absorbance intensity of amide II at 1550 cm^{-1} in the FTIR spectrum exhibits a linear increase with the protein adsorbed on the surface of the materials[6,7]. It was used to indicate the total protein amount adsorbed on material surfaces according to the method described in the reference[7]. According to the intensity of amide II, it is possible to calculate the intensity ratio of albumin to fibrinogen ($R_{A/F}$).

Table 1 Contact angles of carbon paper and MWCNT

Materials	Carbon paper	MWCNT
Contact angle(°)	145.67 ± 3.60	160.08 ± 3.22

Figures 1 and 2 show the FTIR spectra of carbon paper and MWCNT with adsorbed fibrinogen on the surfaces. No apparent change is observed for the amide II FTIR intensity of albumin adsorbed on carbon paper and on MWCNT. The FTIR spectra of albumin are not given in this figure. The absorbance intensity of amide II for the two proteins and $R_{A/F}$ adsorbed on the two surfaces are summarized in Table 2. The amount of fibrinogen adsorbed on MWCNT is much more than the adsorbed on carbon paper.

Platelet coagulation and thrombosis are related to fibrinogen and albumin adsorption. The albumin adsorp-

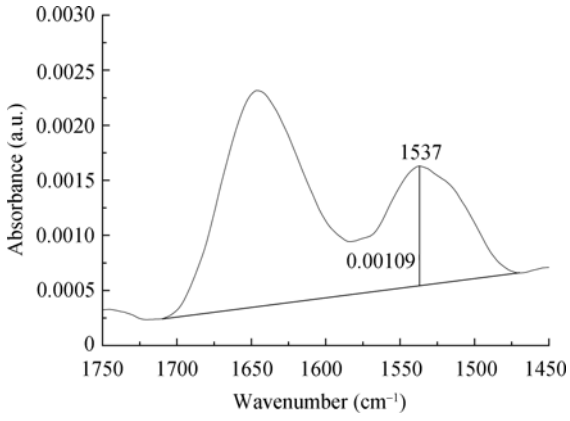

Figure 1 FTIR spectrum of fibrinogen adsorbed on carbon paper.

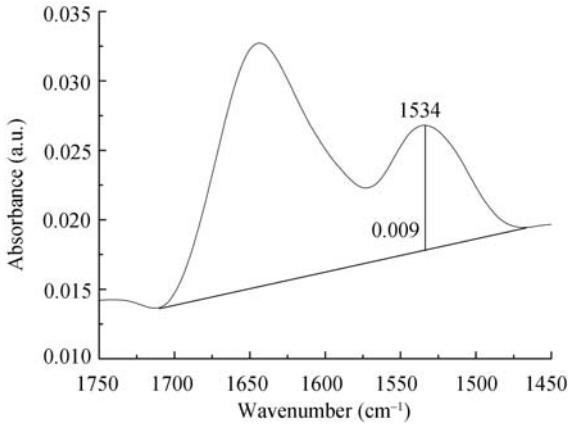

Figure 2 FTIR spectrum of fibrinogen adsorbed on MWCNT.

Table 2 Absorbance intensity of amide II of proteins and $R_{A/F}$ adsorbed on the surface of materials

Proteins	Materials	
	Carbon paper	MWCNT
Albumin	0.0052	0.0052
Fibrinogen	0.00098	0.0089
$R_{A/F}$	5.31	0.58

tion inhibits platelet adhesion and thrombosis. Fibrinogen adsorption also results in platelet coagulation and thrombosis. Therefore, $R_{A/F}$ indicates the antithrombogenicity of materials. In our protein adsorption, the $R_{A/F}$ value of carbon paper is 9 times higher than that of MWCNT, which indicates the antithrombogenicity of carbon paper. Protein easily adsorbed on hydrophobic surfaces[8]. Rough surfaces are related to platelet coagulation. MWCNT grown on carbon paper possesses higher roughness and hydrophobicity than that grown on carbon paper, which may be the primary reason for its fibrinogen increase.

Figures 3–6 show SEM images of fibroblast cellular morphology on carbon paper and MWCNT at different

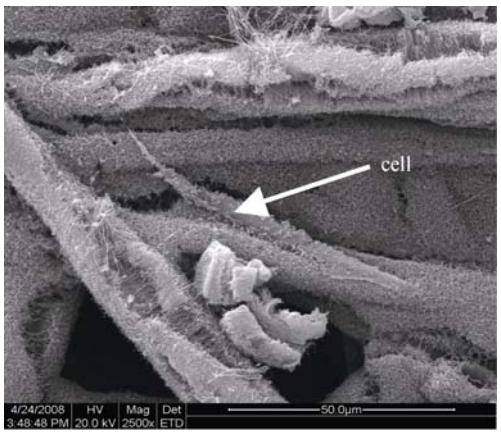

Figure 3 SEM image of a mouse fibroblast on MWCNT.

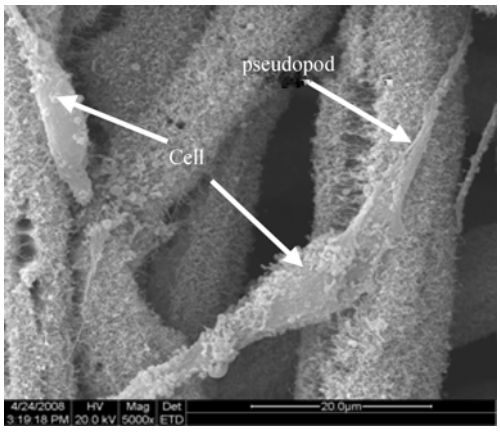

Figure 4 SEM image of a mouse fibroblast on MWCNT.

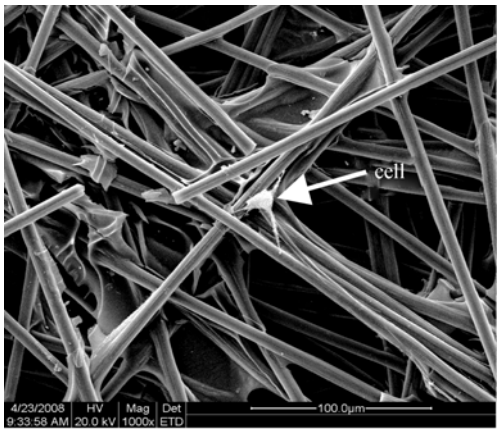

Figure 5 SEM image of a mouse fibroblast on carbon paper.

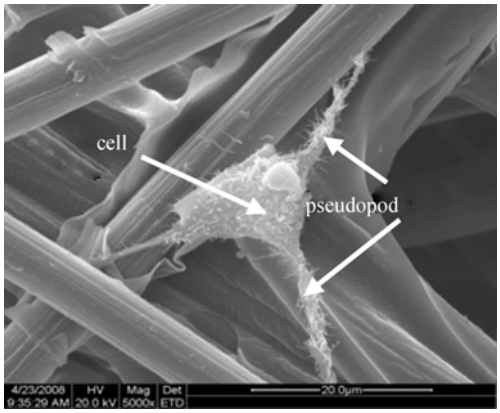

Figure 6 SEM image of a mouse fibroblast on carbon paper.

levels of magnification. The fibroblasts exhibit spindle-shape or polygon morphology with round nuclei on both MWCNT and carbon paper. Compared with carbon paper, MWCNT has better conditions for cellular stretching and pseudopod spreading, which may be related to its rough surface.

Figure 7 shows the growth curves for the fibroblasts

growing on carbon paper, MWCNT, and the control group. For the control group, the cell number is the highest in the initial incubation. Cellular growth, however, starts to decline after 4 d of incubation. Compared with the control group, cell numbers on carbon paper and MWCNT continuously increase with incubation time ranging from 1 to 7 d. MWCNT has much better conditions for proliferation than carbon paper. This result shows that mouse fibroblasts on both carbon paper and MWCNT exhibit normal adhesion, proliferation, and growth trends. There appeared to be no evidence of extensive cell death on the surfaces of both materials. The three-dimensional configuration of carbon paper and MWCNT provides larger space, which allows cells to proliferate and metabolize.

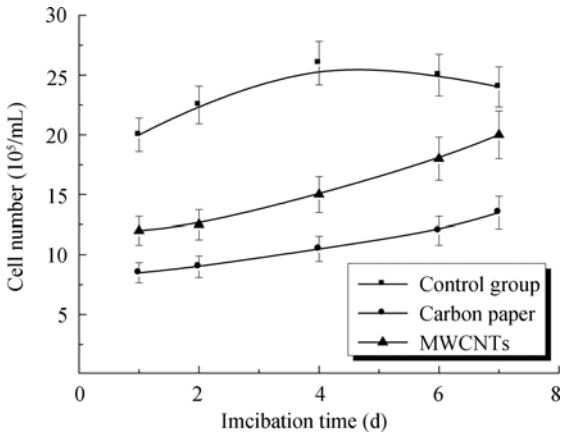

Figure 7 Growth curves for the fibroblasts growing on carbon paper, MWCNT, and the control group.

3 Conclusion

In addition to adsorbing more fibrinogen, MWCNTs possessed the same good tissue compatibility as carbon paper. Due to have a larger surface area and a three-dimensional configuration, MWCNTs provided a larger space for cell adhesion, proliferation, and growth than carbon paper. MWCNTs have exhibited good physical, chemical, mechanical properties, and biocompatibility. Therefore, it is conceivable that MWCNTs may be an effective biomedical material which might have clinical applications in the future.

1 Gu X T, Meng J, Kong H, et al. Protein adsorption and PEG modification on the surface of single-walled carbon nanotubes non-woven film. Chin J Biomed Eng, 2005, 24: 764—765

2 Carrero-Sanchez J C, Elías A L, Mancilla R, et al. Biocompatibility and toxicological studies of carbon nanotubes doped with nitrogen. Nano Lett, 2006, 8: 1609—1616

3 Cui D, Tian F, Ozkan C S, et al. Effect of single wall carbon nanotubes on human HEK293 cells. Toxicol Lett, 2005, 155: 73—74

4 Pulskamp K, Diabate S, Krug H F. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. Toxicol Lett, 2007, 168: 58—60

5 Dumortier H, Lacotte S, Pastorin G, et al. Functionalized carbon nanotubes are non-cytotoxic and preserve the functionality of primary immune cells. Nano Lett, 2006, 7: 1522—1528

6 Zeng H, Chittur K K, Lacefield W R. Analysis of bovine serum albumin adsorption on calcium phosphate and titanium surfaces. Biomaterials, 1999, 20: 377—384

7 Fink D J, Hutson T B, Chittur K K, et al. Quantitative surface studies of protein adsorption by infrared spectroscopy II. Quantification of adsorbed and bulk proteins. Anal Biochem, 1987, 165: 147—154

8 Ying P, Yu Y, Jin G, et al. Competitive protein adsorption studied with atomic force microscopy and imaging ellipsometry. Colloids Surf B Biointerfaces, 2003, 32: 1—10