Strikingly Extended Morphology of Cells Grown on Carbon Nanotubes

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The morphology of cells cultured on carbon nanotube (CNTs) scaffolds was investigated using a confocal laser scanning microscope (CLSM) and a scanning electron microscope (SEM) and it was shown that the cells extended strikingly in all directions and numerous filopodia extended far from the cells.

Carbon nanotubes (CNTs) have attracted great interest since their discovery was reported by Iijima in 1991 because of their unique structure-dependent electrical and mechanical properties.[1] However, there have been very few studies on their biomedical application.[2,3] Some reports suggest the possible toxicity of CNTs.[4] In contrast, our recent studies employing in vitro and in vivo experiments showed their excellent properties as scaffolds for cell culture.[5,6] Cell morphology and proliferation have been investigated in in vitro studies, most of which were performed using nerve cells.[7] CNTs support nerve cell functions and growth, and our previous studies showed that the cells on CNTs grew well.[5] Thus, further investigation of interactions between CNTs and cells was deemed necessary to evaluate their biocompatibility and develop biological applications. There are only a few reports about the morphology of osteoblasts on CNTs.[8] The present study reports observations on the relationship between cells and CNTs and quantitative analysis of the morphology of osteoblasts grown on CNTs examined via scanning electron microscopy and confocal laser scanning microscopy, respectively.

CNTs 5–20 nm in diameter and 20–40 μm in length synthesized by chemical vapor deposition (NanoLab, Inc. MA, U.S.A.) were treated with hydrochloric acid to remove the metal catalyst. The purity was about 98 wt %.[9] CNTs (200 μg) were dispersed in 100 mL of deionized water by sonication for 3 min. CNT scaffolds were made by vacuum filtration of the dispersed CNT slurry onto porous polycarbonate membranes (PC; 47 mm diameter and 0.8 μm pore size, ADVANTEC, Japan). After drying for 3 h at 60 °C, CNTs were fixed on membranes, and coated and noncoated membranes were placed in polystyrene dishes 60 mm in diameter and sterilized under UV light for 24 h. Then, 1.0×10^5 human osteoblast-like cells (SaOS2 cells) were seeded onto each scaffold (PC and CNTs) and cultured in Dulbecco's modified Eagle's medium (DMEM; SIGMA) with 10% fetal bovine serum (FBS; Biowest) and 1% penicillin/streptomycin under standard cell culture conditions (at 37 °C in a humidified 5% CO_2/95% air environment) for 7 days. After cell culture for 7 days, the samples were washed with PBS to remove nonadherent cells on the scaffolds and fixed with a solution of 2% glutaraldehyde, and post-fixed in 1% osmium tetroxide. Then, the samples were dehydrated in graded series of alcohol (50, 70, 80, 90, 95, and 100%) and isoamyl acetate following critical-point drying. The cell morphology was observed and profiled using a confocal laser scanning microscope (CLSM; VK-9500, KEYENCE, Japan). A scanning electron microscope (SEM; S4000, Hitachi, Japan) was used for investigation of the peripheral parts of SaOS2 cells grown on CNTs.

Figure 1 shows the CLSM images demonstrating the morphology of SaOS2 cells cultured for 7 days on PC and CNTs. Note the difference of the scale bars. Cells on PC were elongated in one direction (Figure 1a), whereas there was excellent proliferation with extension of cells in all directions on CNTs (Figure 1b). The cells on CNTs were about 10 times larger than those on PC. Figure 2 shows a comparison of the cross-section profiles of from the different cultures. The length and the height of the cell were 12.13 and 6.83 μm on PC, and 113.14 and 4.35 μm on CNTs, respectively. The contact angle for the cell and substrate was much smaller on CNTs (4.4°) than that on PC (28.3°). The cells on CNTs were wider and flatter than those on PC.

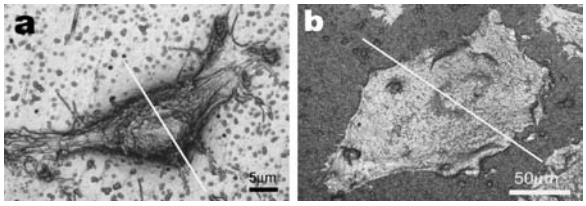

Figure 1. Cell morphology from culture on PC (a) and CNTs (b) observed by a confocal laser scanning microscope (CLSM). Note the difference of scale bars. Cells were fully developed on CNTs. The line show cross section referred to in Figure 2.

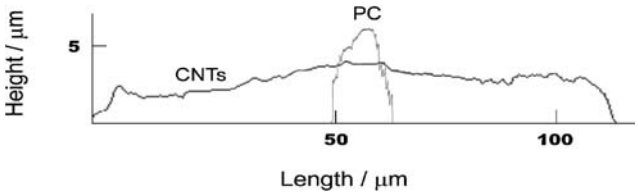

Figure 2. Comparison of cross-section profiles of cells shown in Figure 1.

Figure 3 shows the SEM images of the peripheral part of a cell on CNTs. Numerous filopodia 10–20 μm in length are extended and twisted on the CNT net with high density (Figure 3a). In the enlarged photograph (Figure 3b), the diameter of filopodia was comparable with CNTs and the apex of a filopodium is attached to the surface of CNTs.

In the present study, the difference of cell morphology on substrates with or without CNTs was investigated by SEM in vitro. Recently, toxicity of CNTs was reported;[4] however, our previous studies showed the number of cells on CNTs was larger

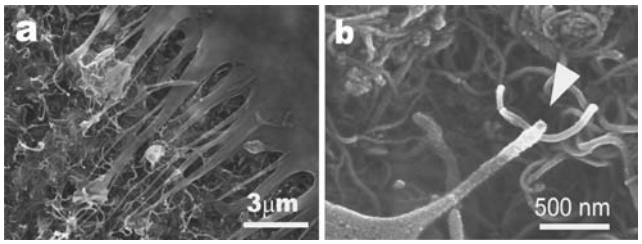

Figure 3. High-magnification SEM images of the peripheral part of SaOS2 cell grown on CNTs. a: Numerous fine filopodia extend toward CNT scaffolds. b: The apex of a filopodium attached on CNTs (arrowhead).

than the number of disseminated cells after 3 days, and cells on CNTs were more proliferated than those on PC.[5] The results of this study revealed that cells could adhere on CNTs and proliferate excellently.

The morphology of the cells with CNTs was markedly different from that of those without CNTs. Most of cells on CNTs were flat and spread in all directions. To evaluate the difference of cell morphology, quantitative analysis of cross-section profiles of cells was carried out using CLSM. The results showed that the length of cells on CNTs was as long as about 10 times that on PC, and the contact angle of cells on CNTs was less than one-sixth of that on PC. The cells on CNTs were clearly flatter. Additionally, the SEM images of cell periphery on CNTs revealed that numerous filopodia extended from cells toward the inside reticular CNTs. After treatment with trypsin to detach cells from CNTs, a few cells floated and showed a rounded-up shape. However, because of the mechanical binding of the filopodia extending from the cell bodies and twisted into CNT nets, most of the cells could not be detached from CNT scaffolds. The prominently flattered shape of cells, very small contact angle and growth of numerous filopodia suggested that CNTs had high affinity for adherence to human-derived osteoblastic cells. In contrast, few cells attached and grew on the graphite scaffolds. Although both CNTs and graphite consist of carbon, cell responses to them such as adhesion are quite different. Graphite is used as a material for heart valve prosthetics because it is nonthrombogenic. The difference of morphology between the fiber structure in CNTs and sheet structure in graphite would be one factor affecting their properties. The topology of the substrate affects the different aspects of cell behavior such as adhesion, proliferation, and morphology. Cell activity is influenced by microstructure and nanostructure. CNT scaffolds have a nanostructure, whereas PC membranes have a microstructure. The nanostructure, with its large surface area and high surface energy, may affect the morphology of cells on CNTs. In addition to surface topography, surface chemistry also plays a role in cell adhesion, proliferation, and morphology.[10] Various proteins such as extracellular matrix proteins and integrins may be related to cell adhesion. For example, the extracellular matrix of bone consists of collagenous proteins. In vitro, adsorption of proteins such as fibronection and vitronectin in serum onto materials may influence cellular responses. The CNTs surface was reported to be coated with various adsorbed molecules.[11] One possible explanation might be that the difference cellular response to CNTs and PC were caused by the difference of adsorption of protein. The excellent cell attachment and growth with numerous filopodia suggest that CNTs could be potential materials for various biomedical uses.

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