Cell Viability and Morphology on Carbon Nanotube Microstructures

NNIN Grad Program

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Abstract:

Carbon nanotubes (CNTs) are useful in a variety of applications including electronic, mechanical, energy, and biomedical due to their outstanding properties. The objectives of this study were to investigate the structural interaction between CNTs microstructures and cells as well as CNTs-biomolecule composites and cells. This study fills a gap in studies of cell behavior on the multi-scale geometries of CNTs forests. Vertically aligned CNTs were grown by chemical vapor deposition (CVD) and then, made in to three-dimensional (3D) shapes by capillary forming. After fabrication of the substrates, fibronectin was deposited on the CNTs microstructures and mouse fibroblast (3T3) cells were seeded and cultured for several days. Cells attached more readily to the CNTs-fibronectin composite than non-composited CNTs. Also, cell morphology was changed on CNTs micro-pillars compared to unprocessed substrates. In addition, cells were capable of growth on CNTs-fibronectin micropillars.

Introduction:

Carbon nanotubes (CNTs) are cylindrical macromolecules of carbon, and have unique mechanical, electrical, and thermal properties. Due to their outstanding properties, there are a variety of applications of CNTs such as electronics, mechanical, energy, and biomedical applications. In particular, CNTs could be used for studies of cell mechanotransduction, as scaffolds for tissue engineering, for regulation of stem cell differentiation, and for intracellular tracking and labeling. Recently, the fabrication of complex CNTs microstructures was achieved from vertically aligned CNTs and made into 3D shapes using liquid vapor condensation [1].

The objectives of this program were to investigate the structural interaction between CNTs micro-pillars structures and cells, CNTs-biomolecule composite and cells, because there are few studies of cell behavior on the multi-scale geometries of CNTs forests. This study focused on cell adhesion, morphology and viability on the substrate with CNTs and CNTs-fibronectin composite.

Experimental Procedure:

First, a catalyst film of 1/10 nm Fe/Al_2O_3 was deposited by e-beam evaporation on thermally-oxidized silicon wafers. The catalyst was patterned by photolithography using SPR 220 photoresist before catalyst deposition and lifted-off by ultrasonic agitation in acetone. Next, CNTs were grown by chemical vapor deposition (CVD) in a tube furnace with flows of 100/400/100 sccm $H_2/He/C_2H_4$ at 775°C.

After growth, the substrate was put to an aluminum mesh plate and reverse over a beaker, which was containing boiling acetone. The substrate was exposed to the acetone vapor and then the substrate was removed from the beaker to allow the acetone to completely evaporate. After densification of CNTs, the substrates were sterilized by UV exposure for 10 min. Next, the substrates were soaked to fibronectin solution (25 μg/mL) for 24 hours. Then, mouse fibroblast (3T3) cells (5,000 cells/cm^2) were seeded to the substrates and cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% bovine serum, 1% L-glutamine, 100 U/mL penicillin/streptomycin at 37°C with 5% CO_2 for 24 hours or three days. After cultured, cells were washed with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde in PBS for 30 min and then, washed with PBS again.

For scanning electron microscope (SEM) observation, the cells were dehydrate in ethanol solutions of 10% to 100% in 10% increments for 30 min, and twice of hexamethyldisilazane (HMDS) for five min. After drying, the substrates were sputtered with a thin film of gold (4-5 nm) and imaged by SEM.

Results:

After one day culture on the CNTs-fibronectin microstructures, various cell morphologies were observed including densely packed, aligned along CNTs, bridged between two pillars, widely extended and round, and so on.

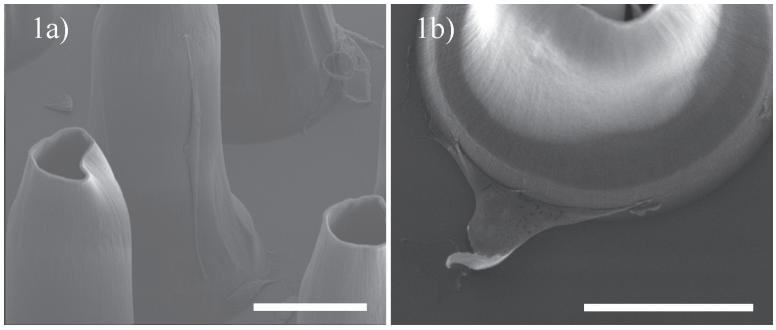

Figure 1: (a) Aligned cell to CNTs direction on CNTs microstructure (scale bar = 50 μm). (b) Perpendicular attached cell on CNTs microstructure (scale bar = 50 μm).

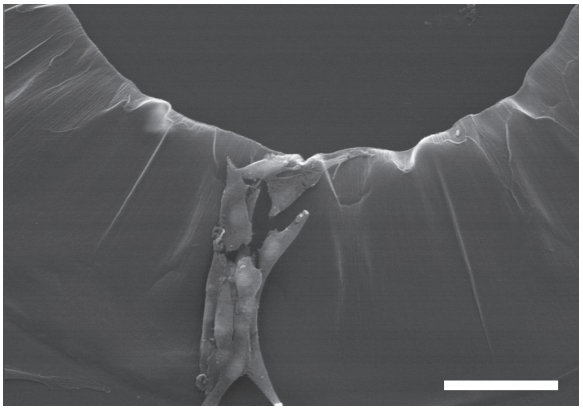

Figure 2: Multiplied cells on CNTs microstructure (scale bar = 50 μm).

As a result of more detailed observation by SEM, some of cells on the CNTs-fibronectin microstructures were aligned along CNTs direction, and some were perpendicularly attached (Figure 1). In addition, cells were capable of growth on CNTs-fibronectin microstructure and maintained their direction of alignment even after three days culture (Figure 2). In contrast, cells were not attached to CNTs microstructure without fibronectin coating. Finally, cell morphologies were sorted into five different types (parallel, perpendicular, bottom of substrate, bridged, attached to the top) and cell number were counted.

In the region with CNTs micropillars, which spacing and diameter were about 35 μm and 90 μm respectively, there were more than the twice number of cells on the fibronectin coated substrate than non-coated substrate. Moreover, about 70% cells were perpendicularly attached to CNTs pillars in fibronectin coated substrate (Figure 3). In contrast, most of cells were attached to the bottom of substrate in non-coated substrate.

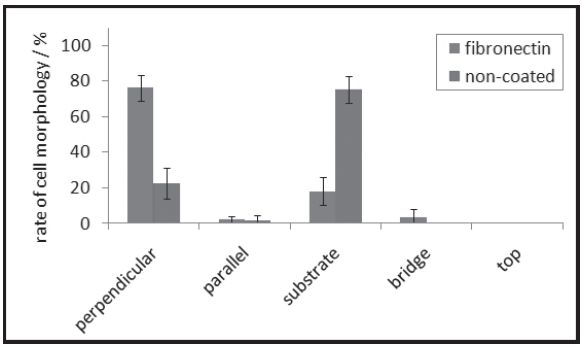

Figure 3: Evaluation of cell morphology in the region with CNTs microstructures (spacing: 35 μm, diameter: 90 μm).

In addition, in the region with twisted CNT micropillars with spacing and diameter of about 75 μm and 30 μm, respectively, the cell number was about same and more than 60% of cells attached to the bottom of substrate on both fibronectin coated substrate and non-coated substrate (Figure 4).

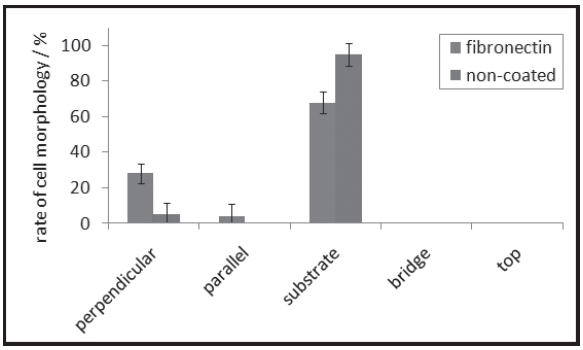

Figure 4: Evaluation of cell morphology in the region with CNTs microstructures (spacing: 75 μm, diameter: 30 μm).

Conclusions and Future Work:

Cells attached readily to CNTs-fibronectin microstructures and their morphology was altered on CNTs-fibronectin microstructures. Cells were capable of growth on CNTs-fibronectin microstructures. The morphology of cells depends on the spacing and size of CNTs-fibronectin microstructures. More detailed observations such as orientation of focal adhesion and distribution of cytoskeleton are needed to understand cell attachment on CNTs-fibronectin microstructures.

Acknowledgments:

I would like to thank Professor John Hart, Davor Copic, and Megan J. Roberts and the Mechanosynthesis group, and Professor Jianping Fu and Jennifer Mann for guiding me throughout this program. Also, I would like to thank Pilar Herrera-Fierro for taking SEM images and the site coordinators: Sandrine Martin, Brandon Lucas, and Trasa Burkhardt, and the staff at Lurie Nanofabrication Facility for their help. Finally, I would like to acknowledge the National Science Foundation, and the National Nanotechnology Infrastructure Network Research Experience for Graduates Program, and the National Institute for Materials Science.

References:

[1] M. De Volder, S. Tawfick, S.J. Park, D. Copic, Z. Zhao, W. Lu, A.J. Hart. Diverse 3D microarchitectures made by capillary forming of carbon nanotubes. Advanced Materials (in press), (2010).