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Interfacing Carbon Nanotubes with Living Cells

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Nanotechnology has infiltrated the field of cell biology in the form of quantum dots, nanofibers, and carbon nanotubes (CNTs), with applications ranging from imaging to tissue engineering.[1−4] Because of their scale and unique physical properties, these nanomaterials offer great opportunities for studying physiology at the level of single cells.[5] CNTs have attracted considerable attention due to their remarkable structural, electrical, and mechanical properties.[6] CNTs have been used to fabricate nanoscale sensors for detection of proteins[7] or carbohydrates.[8] Their unique near-infrared fluorescent properties might be exploited for biological sensing[9−11] and cancer therapy.[12] Several groups have shown that various cell types can engulf CNTs, suggesting their potential as delivery vehicles for biologically active cargo.[10,13−15] These applications, however, have relied upon nonspecific interaction between CNTs and cell surfaces, which precludes targeting to a particular cell type within a mixed population, or to a specific organelle within a cell. Moreover, the inherent cytotoxicity of CNTs has imposed severe limitations on their use in biological systems.[16−18] New strategies for controlling the interaction between CNTs and cells, and for curbing their toxicity, will be required in order to realize their full potential.

In this work, we coated CNTs with a biomimetic polymer designed to mimic cell surface mucin glycoproteins. The functionalized CNTs were then bound to cell surfaces via specific carbohydrate receptors. Whereas unmodified CNTs induced cell death, the functionalized CNTs were found to be nontoxic. This approach for interfacing CNTs with cells should accelerate their use in biological systems.

We were interested in tailoring the interface between CNTs and cells so as to more accurately reflect physiological interactions at the cell periphery. Glycans are major determinants of molecular recognition on the cell surface. They participate in diverse processes, such as pathogen binding, cell trafficking, endocytosis, and modulation of cell signaling.[19] Glycan structures vary as a function of cell type and physiological state,[20] and discrete epitopes are associated with specific organelles.[21] Thus, CNTs that are functionalized to engage in glycan−receptor interactions are ideal substrates for more refined applications in cell biology.

We previously demonstrated that CNTs can be coated with glycopolymers that mimic cell surface mucin glycoproteins.[22] As shown in Figure 1a, the polymers comprise a poly(methyl vinyl ketone) [poly(MVK)] backbone decorated with α-*N*-acetylgalactosamine (α-GalNAc) residues. These sugar residues are reminiscent of the O-linked glycans that decorate mucin glycoproteins. The C_{18} lipid tail provided a hydrophobic anchor for CNT surface assembly. The coated CNTs were stable in aqueous solution for several months without desorption of the polymer coating. We term CNTs coated with C_{18}-terminated α-GalNAc-conjugated polymers "C_{18}-α-MM-CNTs", where "MM" denotes "mucin mimic".

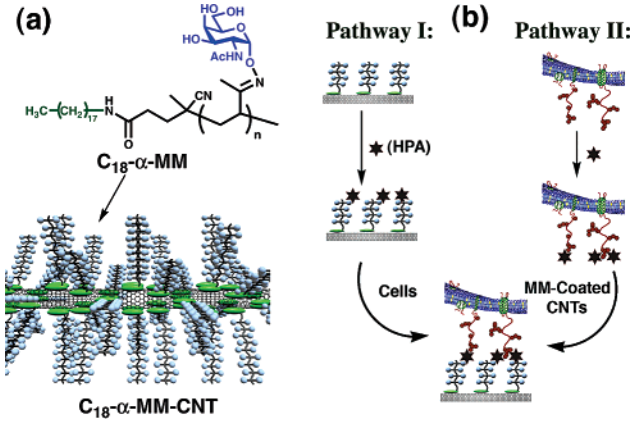

Figure 1. (a) Structure of C_{18}-terminated, α-GalNAc-conjugated mucin mimic (C_{18}-α-MM). The mucin mimic polymers assemble on CNT surface in aqueous media through hydrophobic interaction between the C_{18} lipid tails and CNT surface. The resulting coated CNTs (C_{18}-α-MM-CNTs) were soluble in water. (b) Schematic of interfacing CNTs on cell surfaces via carbohydrate−receptor binding. In pathway I, C_{18}-α-MM-coated CNTs were first bound to HPA, a hexavalent α-GalNAc binding lectin. The complex was then bound to cell surface glycoconjugates using available HPA binding sites presented on CNTs. In pathway II, HPA was first bound to cell surface glycoconjugates. The available HPA binding sites on cell surface were then bound to α-GalNAc residues on C_{18}-α-MM-coated CNTs.

To interface these functionalized CNTs with cells, we took advantage of the *Helix pomatia* agglutinin (HPA), a hexavalent lectin that is specific for α-GalNAc residues and is capable of cross-linking cells and glycoproteins.[23] We reasoned that the complex of HPA with C_{18}-α-MM-coated CNTs would possess sufficient available HPA binding sites for further complex formation with cell surface glycoproteins (Figure 1a, pathway I). Alternatively, HPA bound to cell surface glycans would present binding sites for α-GalNAc residues on C_{18}-α-MM-coated CNTs (Figure 1b, pathway II). In either scenario, binding of HPA to α-GalNAc residues would permit specific interaction of the cells and CNTs.

To evaluate pathway I (Figure 1b), we required a means for detection of HPA binding to the C_{18}-α-MM-coated CNTs. This was provided by the commercial reagent fluorescein isothiocyanate-conjugated HPA (HPA-FITC). We complexed C_{18}-α-MM-coated CNTs with HPA-FITC, and the protein-modified CNTs were then incubated with Chinese hamster ovary (CHO) cells. The labeling observed by fluorescence microscopy (Figure 2a) and flow cytometry analysis (Figure 2b) suggested the formation of α-GalNAc−HPA complexes at both the CNT and cell surfaces. As a control, we performed the same experiment using CNTs coated with a similar polymer bearing β-linked GalNAc residues (C_{18}-β-MM),[22] which do not bind HPA. In this case, no fluorescent labeling of the cells was observed (not shown).

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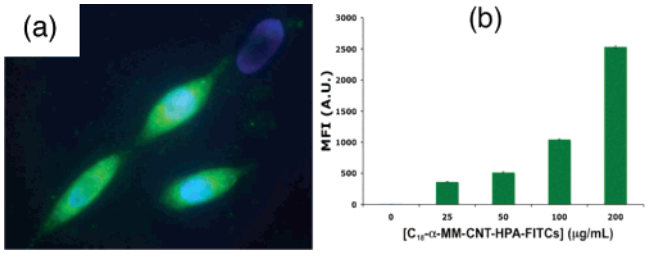

Figure 2. Binding of C_{18}-α-MM-coated CNTs to Chinese hamster ovary (CHO) cells via HPA cross-links. (a) Fluorescence micrograph of CHO cells labeled with FITC−HPA-conjugated C_{18}-α-MM-coated CNTs (see Supporting Information for experimental details). (b) Flow cytometry analysis of the cells in (a) treated with various doses of modified CNTs. MFI = mean fluorescence intensity. Error bars represent the standard deviation for three replicates.

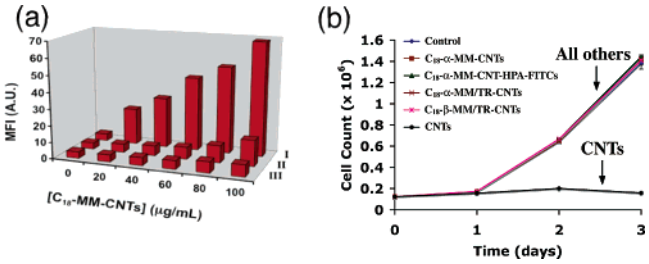

Figure 3. (a) Flow cytometry analysis of cells in pathway II of Figure 1b. Row I shows the specific binding of C_{18}-β-MM/TR−CNTs to CHO cells coated with HPA. Row II shows no binding of C_{18}-α-MM/TR−CNTs to cells in the absence of HPA. Row III shows that C_{18}-β-MM/TR−CNTs do not bind CHO cells coated with HPA receptors. These data are representative of results observed in triplicate experiments. (b) Effects of glycopolymer-coated and unmodified CNTs on the growth of CHO cells. Similar results were obtained for Jurkat cells. Error bars represent the standard deviation for three replicates.

To evaluate pathway II (Figure 1b), we required a method for direct detection of C_{18}-α-MM-coated CNTs that is independent of HPA. Thus, we synthesized a C_{18}-α-MM polymer in which ~3% of the GalNAc residues were substituted with the fluorescent dye Texas Red (C_{18}-α-MM/TR, Figure S1). CHO cells were incubated with unmodified HPA to introduce α-GalNAc receptors onto the cell surface. The cells were then treated with various concentrations of C_{18}-α-MM/TR-coated CNTs and analyzed by flow cytometry. As shown in Figure 3a, dose-dependent labeling was observed (row I), and the labeling was dependent upon precomplexation of the cells with HPA (row II). The control CNTs modified with C_{18}-β-MM/TR showed no significant cell surface binding in the presence of HPA (Figure 3a, row III). At the highest doses of C_{18}-α-MM/TR-coated CNTs (>80 μg/mL), some nonspecific fluorescent labeling of cells was observed. This may result from nonspecific binding to the cell surface or from internalization during the 1-h incubation. However, at lower concentrations of C_{18}-α-MM/TR-coated CNTs, cell labeling was highly specific.

Recent reports that unfunctionalized CNTs show potent cytotoxicity toward alveolar macrophages,[16] HacaT cells,[17] and HEK293 cells[18] have stimulated concern regarding their potential utility in biological systems. We were therefore interested in evaluating the cytotoxicity of our glycopolymer-coated CNTs. CHO cells were cultured with C_{18}-α-MM-, C_{18}-α-MM/TR-, C_{18}-β-MM/TR-, or HPA-FITC-conjugated, C_{18}-α-MM-coated CNTs (each at 100 μg/mL) for 3 days. In control experiments, the cells were cultured with unmodified CNTs or with media alone. Viable cells were counted each day (Figure 3b). Cells cultured with the glycopolymer-coated CNTs were indistinguishable from cells grown in the absence of CNTs. By contrast, cells cultured with unmodified CNTs were unable to expand during the course of the experiment. Presumably, the unmodified CNTs either inhibited cell growth or induced cell death at a rate comparable to the proliferation rate. Similar results were obtained with Jurkat cells (not shown). Thus, the glycopolymer coating renders the CNTs nontoxic while simultaneously providing a means for specific cell surface binding.[24]

In summary, we have demonstrated a strategy for interfacing biocompatible CNTs with cell surfaces by virtue of carbohydrate−receptor interactions. The synthetic methods used to produce the glycopolymers permit the facile introduction of myriad alternative ligands, in addition to sugars, that could encode interactions with numerous receptor types. This strategy may offer new opportunities for probing biological processes. Experimental and theoretical studies have indicated that the environment surrounding the nanotube can influence CNT properties. For example, charge transfer to the CNT from attached chemical species can alter the electrical conductance, and mechanical properties are altered by changes in moments and local bonding structure.[6] Therefore, variations in a cell's local environment might be monitored by changes in the electrical, mechanical, or optical properties of CNTs.

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Supporting Information Available: Synthetic procedures and spectral data for C_{18}-α-MM/TR and C_{18}-β-MM/TR, and biological procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; Doose, S.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. *Science* **2005**, *307*, 538.
(2) Kasili, P. M.; Song, J. M.; Vo-Dinh, T. *J. Am. Chem. Soc.* **2004**, *126*, 2799.
(3) Wu, W. Z.; Huang, W. H.; Wang, W.; Wang, Z. L.; Cheng, J. K.; Xu, T.; Zhang, R. Y.; Chen, Y.; Liu, J. *J. Am. Chem. Soc.* **2005**, *127*, 8914.
(4) Mattson, M. P.; Haddon, R. C.; Rao, A. M. *J. Mol. Neurosci.* **2000**, *14*, 175.
(5) Andersson, H.; van den Berg, A. *Curr. Opin. Biotechnol.* **2004**, *15*, 44.
(6) Lin, Y.; Taylor, S.; Li, H. P.; Fernando, K. A. S.; Qu, L. W.; Wang, W.; Gu, L. R.; Zhou, B.; Sun, Y. P. *J. Mater. Chem.* **2004**, *14*, 527.
(7) Chen, R. J.; Bangsaruntip, S.; Drouvalakis, K. A.; Kam, N. W. S.; Shim, M.; Li, Y. M.; Kim, W.; Utz, P. J.; Dai, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4984.
(8) Wang, J.; Musameh, M.; Lin, Y. H. *J. Am. Chem. Soc.* **2003**, *125*, 2408.
(9) O'Connell, M. J.; Bachilo, S. M.; Huffman, C. B.; Moore, V. C.; Strano, M. S.; Haroz, E. H.; Rialon, K. L.; Boul, P. J.; Noon, W. H.; Kittrell, C.; Ma, J. P.; Hauge, R. H.; Weisman, R. B.; Smalley, R. E. *Science* **2002**, *297*, 593.
(10) Cherukuri, P.; Bachilo, S. M.; Litovsky, S. H.; Weisman, R. B. *J. Am. Chem. Soc.* **2004**, *126*, 15638.
(11) Barone, P. W.; Baik, S.; Heller, D. A.; Strano, M. S. *Nat. Mater.* **2005**, *4*, 86.
(12) Kam, N. W. S.; O'Connell, M.; Wisdom, J. A.; Dai, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11600.
(13) Kam, N. W. S.; Jessop, T. C.; Wender, P. A.; Dai, H. J. *J. Am. Chem. Soc.* **2004**, *126*, 6850.
(14) Pantarotto, D.; Singh, R.; McCarthy, D.; Erhardt, M.; Briand, J. P.; Prato, M.; Kostarelos, K.; Bianco, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5242.
(15) Kam, N. W. S.; Dai, H. J. *J. Am. Chem. Soc.* **2005**, *127*, 6021.
(16) Jia, G.; Wang, H. F.; Yan, L.; Wang, X.; Pei, R. J.; Yan, T.; Zhao, Y. L.; Guo, X. B. *Environ. Sci. Technol.* **2005**, *39*, 1378.
(17) Shvedova, A. A.; Castranova, V.; Kisin, E. R.; Schwegler-Berry, D.; Murray, A. R.; Gandelsman, V. Z.; Maynard, A.; Baron, P. *J. Toxicol. Environ. Health, Part A* **2003**, *66*, 1909.
(18) Cui, D. X.; Tian, F. R.; Ozkan, C. S.; Wang, M.; Gao, H. J. *Toxicol. Lett.* **2005**, *155*, 73.
(19) Collins, B. E.; Paulson, J. C. *Curr. Opin. Chem. Biol.* **2004**, *8*, 617.
(20) Hollingsworth, M. A.; Swanson, B. J. *Nat. Rev. Cancer* **2004**, *4*, 45.
(21) Helenius, A.; Aebi, M. *Science* **2001**, *291*, 2364.
(22) Chen, X.; Lee, G. S.; Zettl, A.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 6111.
(23) Lisgarten, J. N.; Chattopadhyay, T. K.; Pitts, J. E.; Palmer, R. A.; Reynolds, C. D.; Dao-Thi, M. H.; Van Driessche, E.; Beeckmans, S. *Acta Crystallogr. D Biol. Crystallogr.* **1999**, *55*, 1903.
(24) Sayes et al. recently reported that CNTs functionalized with phenyl sulfonic acid or phenyl carboxylic acid show reduced cytotoxicity. Sayes, C. M.; Liang, F.; Hudson, J. L.; Mendez, J.; Guo, W.; Beach, J. M.; Moore, V. C.; Doyle, C. D.; West, J. L.; Billups, W. E. *Toxicol. Lett.* **2006**, *161*, 135.

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