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Biocompatible Carbon Nanotubes Generated by Functionalization with Glycodendrimers**

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The structural, mechanical, electrical, and optical properties of single-walled carbon nanotubes (SWNTs) have stimulated considerable interest in their biological applications[1-3]. SWNTs have been employed for biosensing[4], imaging[5], intracellular delivery[6], and cancer cell targeting[7,8]. However, expanded use of SWNTs in living systems will require strategies to diminish their cytotoxicity[9-12]. Thus, surface modifications that mitigate the toxicity of SWNTs while simultaneously enabling specific biological recognition are highly sought after[8,13-17].

A promising avenue we have recently explored is to coat SWNTs with synthetic glycopolymers that mimic glycoproteins found on cell surfaces[16,17]. We demonstrated

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that lipid-terminated poly(methylvinylketone)-based glycopolymers can coat carbon nanotube (CNT) surfaces and promote their binding to cells via receptor-ligand interactions[16,17]. Importantly, the modified CNTs were nontoxic to cultured cells. These findings were tempered, however, by the irregular surface and nonuniform thickness of the CNT coating, reflecting the high polydispersities (>1.7) of the polymers employed[18]. Such surface heterogeneity might undermine the use of glycopolymer-coated CNTs as sensors of protein binding.

Here we report the use of glycodendrimers as homogeneous bioactive coatings for CNTs. In addition to various biomedical applications[19-21], dendrimers have been used to functionalize CNTs with photoactive groups[22], to improve their solubility[23], and to introduce sites for metal detection[24]. Their branched architectures and high peripheral functional group density have prompted several groups to explore glycodendrimers as mimics of cell surface glycans[25-27]. Inspired by these examples as well as recent breakthroughs in dendrimer synthesis using click chemistry[28-30], we developed a new class of bifunctional glycodendrimers based on 2,2-bis(hydroxymethyl) propionic acid (bis-MPA), a biocompatible building block. As depicted in Figure 1 (compound **1**), the dendrimers comprised peripheral carbohydrate units and a pyrene tail capable of binding SWNT surfaces via π - π interaction. Their geometry is reminiscent of the multi-antennary N-linked glycans that populate eukaryotic cell surfaces.

The synthesis employed the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction also previously used by Sharpless and Hawker to prepare diverse dendritic structures[30,31]. In our work, the CuAAC reaction allowed for chemoselective ligation of azide-functionalized pyrene and glycan moieties to the alkyne-functionalized focal point and chain ends of a dendritic scaffold, respectively (Figure 1). The synthetic glycans, each with an azidoethyl aglycone[32], remained unprotected during glycodendrimer assembly. Applying this methodology, we prepared a panel of [G-2] (**2a-c**) and [G-3] (**1a-c**) glycodendrimers in near quantitative yield (see reference 33 for nomenclature key). Analysis of the dendrimers by NMR and MALDI-TOF mass spectrometry confirmed that the structures were homogeneous (Supporting Information).

The glycodendrimers were adsorbed onto SWNTs by ultrasonication in aqueous solution (see Supporting Information for experimental details) resulting in complete solubilization (Figure 2a I-VI). The suspensions of glycodendrimer-functionalized SWNTs were stable for several months in water, while the unfunctionalized SWNTs precipitated within one hour (Fig 2a VII). The [G-2] glycodendrimer-coated SWNTs were found to precipitate slowly after several months, most likely the result of interactions among coated nanotubes enabled by a relatively sparse hydrophilic coating. Accordingly, the precipitates were readily redissolved by ultrasonication. Control glycodendrimers lacking the pyrene moiety had no solubilizing effect on SWNTs (data not shown). Scanning electron microscopy (SEM) (Figure 2b) and transmission electron microscopy (TEM) (Figure 2c) analysis revealed small bundles and individual SWNTs coated entirely with a thin uniform layer of glycodendrimers. These images contrast markedly with the thick heterogeneous coatings observed with CNT-bound glycopolymers[16].

Specific binding of SWNT-bound glycodendrimers to receptors is critical for sensing and targeting applications. We probed this capability using a panel of fluorescein isothiocyanate (FITC)-conjugated lectins: *Canavalia ensiformis* agglutinin (Con A), *Arachis hypogaea* agglutinin (PNA) and *Psophocarpus tetragonolobus* agglutinin (PTA), which recognize α -mannose, lactose and β -galactose, respectively. SWNTs coated with different glycodendrimers were incubated with FITC-conjugated lectins then dialyzed to remove unbound protein and analyzed by fluorescence spectroscopy. Significant fluorescence was

observed for ConA-treated [G-3] Man-SWNTs, while only background fluorescence was observed for PTA- or PNA-treated [G-3] Man-SWNTs (Figure 3a). Similarly, [G-3] Gal-SWNTs bound to FITC-conjugated PTA, but not to PNA or Con A. Since the non-reducing terminal monosaccharide in lactose is β -galactose, [G-3] Lac-SWNTs were recognized by both PNA and PTA, but not Con A. Similar results were obtained from parallel studies using SWNTs coated with [G-2] glycodendrimers (data not shown).

To more accurately mirror the complexity of biological glycoconjugates, which typically display multiple glycan epitopes, we functionalized SWNTs with a mixture of [G-2] Man (**2a**) and [G-2] Lac (**2c**) at various ratios. After dialysis to remove unbound glycodendrimers, the coated SWNTs were incubated with a 1:1 (molar ratio) mixture of Texas Red-conjugated PNA and FITC-conjugated ConA. As shown in Figure 3b, the SWNT-associated Texas Red fluorescence decreased while the FITC fluorescence increased along with increasing percentage of [G-2] Man in the SWNT coating. Thus, multiple epitopes displayed on SWNTs can bind simultaneously to discrete proteins.

We hypothesized that after binding to the [G-2] Man-SWNTs, the tetravalent lectin Con A[34] would still possess open sites for further complexation with α -mannose residues on cell surface glycans. The carbohydrate-lectin interaction could thereby promote SWNT binding to cell surfaces. Toward this end, [G-2] Man-SWNTs were first treated with FITC-conjugated Con A (FITC-ConA) and then incubated with Chinese hamster ovary (CHO) cells. Fluorescence microscopy analysis revealed specific binding of modified SWNTs to the cell membrane (Figure 4). As a control, we performed the same experiment using SWNTs coated with the [G-2] Gal (**2c**) dendrimer. In this case, no fluorescent labeling of the cells was observed (data not shown). By contrast, [G-2] Gal-SWNTs and [G-2] Lac-SWNTs labeled the CHO cells robustly when PTA and PNA were used as crosslinkers, respectively (data not shown).

Finally, to evaluate their cytotoxicity we co-cultured [G-2] and [G-3] glycodendrimer-coated SWNTs (100 μ g/mL) with HEK293 cells for four days. In control experiments, the cells were incubated with unmodified SWNTs or with media alone. Viable cells (trypan blue assay) were counted each day. Cells cultured with glycodendrimer-coated SWNTs proliferated at the same rate as cells grown in the absence of SWNTs (Figure 5). By contrast, unmodified SWNTs greatly hampered the growth of HEK293 cells. Notably, the relatively thin coating produced by glycodendrimers appears to passivate SWNTs against cytotoxicity as effectively as much thicker glycopolymer coatings[17].

In conclusion, glycodendrimers can function as homogeneous bioactive coatings for SWNTs that also mitigate their cytotoxicity. The synthetic method used to construct the glycodendrimers can be readily adapted to ligands for other receptor interactions. Future applications include biosensors for carbohydrate-binding proteins and delivery agents that target specific cell surface receptors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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33. Glycodendrimer nomenclature: Man denotes α -D-mannopyranoside, Gal denotes β -D-galactopyranoside, Lac denotes 1,4-(β -D-galactopyranosyl)- β -D-glucopyranoside, [G-2] denotes generation 2 and [G-3] denotes generation 3. [G-2] Man, [G-2] Gal, [G-2] Lac, [G-3] Man, [G-3] Gal, and [G-3] Lac denote the corresponding glycodendrimers.
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Figure 1.

Synthesis of glycodendrimers (generation 3 [G-3] dendrimers are depicted; generation 2 [G-2] dendrimers were synthesized using a similar process). The pyrene tail was conjugated to the focal point of **4**[25] by click chemistry and the resulting dendrimer **5** was further coupled with pent-4-ynoic anhydride (**6**) to introduce additional alkyne groups to the periphery of the dendrimer. The resulting dendrimer **7** was then reacted with a 2-azidoethyl mono- or disaccharides **8a-c** using click chemistry to furnish the desired [G-3] glycodendrimers **1a-c**. A panel of [G-2] (**2a-c**) and [G-3] glycodendrimers with a variety of carbohydrate structures was synthesized.

Figure 2.

a) Photographs of vials containing SWNT suspensions. [G-2] Man-SWNTs (I), [G-2] Gal-SWNTs (II), [G-2] Lac-SWNTs (III), [G-3] Man-SWNTs (IV), [G-3] Gal-SWNTs (V) and [G-3] Lac-SWNTs (VI) formed stable suspensions in water for more than three months, while as-produced SWNTs (VII) precipitate from water within several hours. b) SEM and c) TEM images of [G-3] Man-SWNTs. The glycodendrimer coating can be visualized as the density surrounding the SWNT.

Figure 3.

a) Lectin binding to glycodendrimer-coated SWNTs. Con A and PNA exhibit specific binding to [G-3] Man-SWNTs and [G-3] Lac-SWNTs, respectively. PTA binds to both [G-3] Gal-SWNTs and [G-3] Lac-SWNTs. Error bars indicate the standard deviation for 3 replicate experiments. b) Modulation of lectin bindings to SWNTs co-functionalized with two different glycodendrimers. SWNTs coated with [G-2] Lac and [G-2] Man at various ratios were incubated with a 1:1 (molar ratio) mixture of Texas Red-conjugated PNA and FITC-conjugated Con A. The intensities of Texas Red and FITC emission paralleled the ratio of [G-2] Lac and [G-2] Man components in the SWNT coating, respectively. Error bars indicate the standard deviation for 3 replicate experiments.

Figure 4.

a) The multivalent lectin Con A can bind glycodendrimer-coated SWNTs to cells. b) Fluorescence micrograph of CHO cells labeled with complexes of [G-2] Man-SWNT and FITC-Con A.

Figure 5.

Effects of glycodendrimer-coated SWNTs on the proliferation of HEK293 cells. Live cells were quantified by the Trypan blue dye exclusion method. Control cells were grown in the absence of SWNTs. Error bars represent the standard deviation for 3 replicates.