Single-walled carbon nanotubes displaying multivalent ligands for capturing pathogens†

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A single-walled carbon nanotube was exploited for its semi-flexible pseudo-one-dimensional nanostructure as a unique scaffold to display multivalent carbohydrate ligands, with a specific demonstration showing that galactosylated carbon nanotubes were effective in the capturing of pathogenic *Escherichia coli* in solution.

There has been considerable scientific interest in the understanding and mimicking of bacterial adhesin-specific interactions for various purposes, such as pathogen detection and the inhibition of bacterial infections *via* the chemotactic responses of the bacteria toward the corresponding ligands.[1,2] Both natural and synthetic multivalent inhibitors have been evaluated.[1] The latter includes the use of linear and branched polymers,[3] dendrimers,[4] proteins,[5] polymeric and other nanoparticles,[6,7] *etc.* to display multiple copies of sugar moieties.

Single-walled carbon nanotubes (SWNTs) represent a unique class of one-dimensional nanostructures, which offer many properties that are not available in traditional polymeric materials and nanoparticles.[8] Potential biological applications of carbon nanotubes have been discussed and explored.[9,10] Among those widely investigated have been the uses of the nanotubes in nanoscale biosensors.[10–12] Most of these applications require chemical modifications or functionalization of the nanotubes to impart aqueous solubility and/or to introduce biofunctionalities.[10] In fact, carbon nanotubes have been functionalized with a variety of bioactive groups,[9–16] and the functionalized carbon nanotubes have allowed the studies of their interactions with biological species.[17,18] These studies are not only important fundamentally but also critical to the development of practical biosensors.

Because of the versatile chemical modification and solubilization, the one-dimensional nanostructure of a SWNT, with the high surface area-to-weight ratio and some structural flexibility, may be exploited as a platform for a multivalent array of carbohydrates in solution under physiological conditions. Here we report that SWNTs can be solubilized *via* functionalization with derivatized galactoses and that the nanotube-bound galactoses could serve as polyvalent ligands and thus strongly interact with receptors on pathogenic *E. coli*, resulting in significant cell agglutination (Scheme 1).

† Electronic supplementary information (ESI) available: SEM images from the interactions between *E. coli* O157 : H7 and other water-soluble SWNTs (functionalized by α-D-mannose and bovine serum albumin, respectively). See http://www.rsc.org/suppdata/cc/b4/b415015e/

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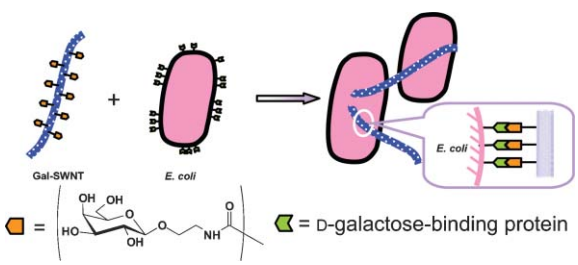
The galactose derivative 2′-aminoethyl-β-D-galactopyranoside was synthesized by following a procedure in the literature.[19] The functionalization of SWNTs was based on the carbodiimide-activated amidation of the galactose-tethered amino groups with the nanotube-bound carboxylic acids.[20] In a typical reaction, a purified SWNT sample (45 mg) was mixed with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, 112 mg, 60 mmol) in aqueous KH_2PO_4 buffer (45 mL, pH = 7.4). Upon activation *via* sonication for 2 h, 2′-aminoethyl-β-D-galactopyranoside (450 mg, 2 mmol) was added. After sonication for another 36 h, the reaction mixture was loaded into membrane tubing (cutoff molecular weight ~12 000) for dialysis against fresh deionized water for 3 days. The resulting suspension was centrifuged (1380 g) for 30 min, yielding a dark-colored homogeneous aqueous solution of the galactose-functionalized SWNTs (Gal-SWNTs).

The Gal-SWNT sample was characterized by a series of instrumental techniques including solution-phase NMR, scanning and transmission electron microscopy (SEM and TEM), Raman, and near-IR optical absorption. The NMR results suggested a significant effect on the galactoses from the nanotube attachment, consistent with the expected covalent functionalization. The SEM and TEM results showed that the nanotubes were exfoliated and well-dispersed (Fig. 1). The Raman and near-IR absorption spectra were also typical of solubilized SWNTs. For example, the S_{11} (1828 nm) and S_{22} (1035 nm) absorption bands associated with van Hove singularity pairs were preserved in the Gal-SWNTs (Fig. 1).[21] The nanotube content in the sample was estimated to be about 30% (wt/wt) in terms of the thermogravimetric analysis (TGA), in which the galactose functionalities were thermally defunctionalized and removed from the nanotube surface.[22] On the other hand, the Gal-SWNTs in aqueous buffer was tested for total sugar content in terms of classical spectrophotometry with an anthrone reagent.[23] The result thus obtained (65 wt% sugar, with the rest being primarily nanotubes) in reference to the



Scheme 1

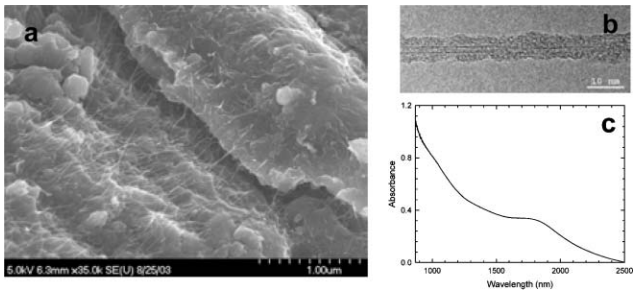


Fig. 1 (a) SEM image, (b) HR-TEM image (scale bar = 10 nm), and (c) near-IR absorption spectrum of Gal-SWNTs.

galactopyranoside before nanotube functionalization is in reasonable agreement with the TGA estimate of nanotube content.

The *E. coli* O157 : H7 strain C7927 was kindly provided by Prof. Michael P. Doyle, University of Georgia. After growth, repeated washing, and separation *via* centrifuging, the *E. coli* cells were suspended in sterile saline solution (0.85% NaCl) to an optical density of 0.5 ($\sim 5 \times 10^8$ CFU per mL according to McFarland standard #2; CFU = colony forming units). An aliquot of the suspension (200 μL) was added to an aqueous solution of the Gal-SWNTs (0.32 mg mL^{-1}, 0.1 mL), and the mixture was rotated (40 rpm) at room temperature for 1 h. Upon centrifuging at 14 000 g (Eppendorf 5417R), the supernatant was discarded and the pellet was washed with phosphate buffered saline (PBS) *via* the same centrifuging–suspending procedure twice. The final pellet was suspended in a gluteraldehyde solution (1 mL 2.5% gluteraldehyde in 0.2 M sodium cacodylate–hydrochloric acid buffer, pH 7.5) for fixing at room temperature for 30 min. The suspension was collected on a polycarbonate filter (Whatman nucleopore 0.2 μm) and allowed to fix for another 30 min. The filter with the specimen was rinsed with the sodium cacodylate–hydrochloric acid buffer (shaking for 5 min) 3 times, followed by post-fixing with freshly prepared osmium oxide solution (1%, enough to cover the filter) for 1 h. Upon repeated rinsing with double-distilled water (shaking for 5 min each time), the specimen was dehydrated with graded ethanol and shaken: 50% for 30 min, 75%, 85%, and 95% each for 10 min, and 100% for 10 min twice. The specimen was subjected to critical point drying to remove the ethanol completely, and was then mounted onto an aluminium stub with double-sided carbon tape for platinum coating before the electron microscopy analysis. Shown in Fig. 2 are typical SEM images of the specimen. There are apparently strong interactions between Gal-SWNTs and the pathogenic *E. coli* cells, with multiple nanotubes binding to one cell and some nanotubes "bridging" adjacent cells to result in significant agglutination.

The observed binding in Fig. 2 is specific to the Gal-SWNTs. In the control experiments, SWNTs covalently functionalized with either α-D-mannose or bovine serum albumin (BSA) protein,[16] and thus similarly soluble in aqueous PBS buffer, were used to replace Gal-SWNTs under essentially the same experimental conditions, but no apparent binding was observed (see ESI†). This suggests that the nanotube-bound galactoses in Gal-SWNTs were responsible for the binding and cell agglutination shown in Fig. 2. This is consistent with the report that there are periplasmic galactose-binding proteins on the *E. coli* cell surface to couple with galactose ligands.[24]

Multivalent ligands carried on polymers, dendrimers, or proteins are known to be more potent than their monovalent counterparts in cell adhesion.[25] For the Gal-SWNTs in this study, the high aspect ratio and large surface area of the nanotubes enable the display of abundant sugar arrays, which are excellent polyvalent ligands toward the specific receptors on the cell surface. The semi-flexible nature of the nanotube scaffold may also facilitate the binding of multiple galactose ligands with the *E. coli* cell.

In summary, the results reported here suggest that a SWNT could serve as a unique carrier for multiple carbohydrate ligands and that the Gal-SWNTs are highly efficient in the capturing of pathogenic *E. coli* in physiological solutions. The same materials may be applied to other pathogens bearing galactose receptors. In addition, the aqueous soluble SWNTs displaying galactoses and other bioactive ligands may also be developed as potent inhibitors or effectors for specific cellular responses.

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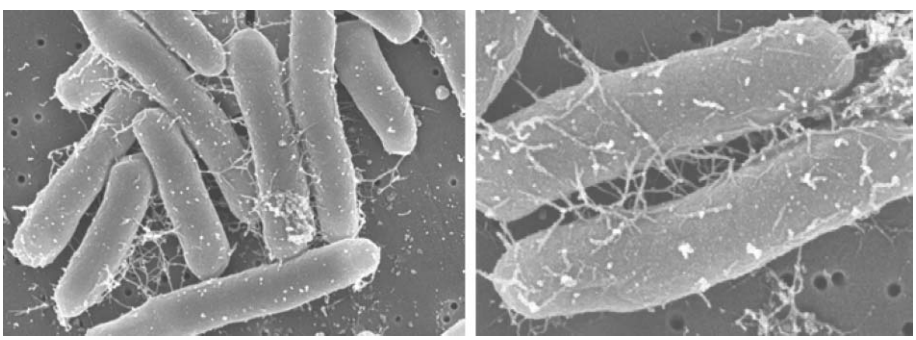

Fig. 2 SEM images for Gal-SWNTs capturing pathogenic *E. coli* cells.

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