Rational design of amphiphilic polymers to make carbon nanotubes water-dispersible, anti-biofouling, and functionalizable†

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We report rational design of amphiphilic polymers capable of making carbon nanotubes (CNTs) highly water dispersible and resistant to biofouling; such CNTs can be conjugated with bioactive molecules so as to be potential drug delivery vehicles.

Although carbon nanotubes (CNTs) have demonstrated potential as novel electronic or composite materials, their use in biomedical applications such as drug delivery vehicles[1] and scaffolds for tissue engineering[2] has drawn increasing attention in the past few years. Several recent reports have shown that CNTs are able to assist in the killing of cancer cells upon irradiation because of the near IR absorption property of CNTs, and that CNTs can also deliver therapeutic agents such as plasmid DNA, siRNA, or anticancer drugs to cells, in a specific manner.[3] When such *in vitro* data are translated to *in vivo* systems (CNTs as drug delivery vehicles), several special considerations arise.[4] First, CNTs must be well-dispersed in plasma for a reasonable period without aggregation which otherwise might cause severe embolization *in vivo* by blocking of capillary vessels. Second, nonspecific adsorption of plasma proteins onto CNTs surfaces should be prevented, as this would result in uptake of CNTs by macrophages of the reticuloendothelial system in the liver and spleen. Finally, it is desirable that suitable functional groups are present on the surfaces of CNTs, as such groups are used to immobilize bioactive ligands for targeting or therapeutic purposes. Whereas amphiphilic surfactants,[5] amphiphilic block copolymers,[6] pyrene-based derivatives,[7] and biomolecules such as peptides[8] and DNA moieties[9] serve to disperse or functionalize CNTs efficiently, none of these materials have met all three criteria required for drug delivery applications. Thus, we sought to develop a new polymer system that can provide robust water dispersibility, resistance to protein adsorption (anti-biofouling), and a functional group for modification. To ensure water dispersibility, the polymer should be amphiphilic so that the hydrophobic part can anchor to the CNTs surface while the hydrophilic portion is directed towards the aqueous environment. To this end, a poly(ethylene glycol) (PEG)-based copolymer may be suitable because PEG is known to block protein adsorption, is hydrophilic, and is biocompatible.[10] In addition, carboxylic acid seems to be a suitable functional group for immobilization of bioactive ligands because coupling reactions involving the carboxylic acid group are reasonably successful under aqueous conditions. With these considerations in mind, we synthesized two amphiphilic polymers as shown in Fig. 1. The polymers are composed of three functional parts: hydrophobic groups for anchoring onto the CNTs surface, PEG for blocking protein adsorption, and carboxylic acid to permit the introduction of bioactive molecules. Each polymer was synthesized from the corresponding monomers, with a molar feed ratio of 2 : 2 : 1, by radical polymerization (see ESI†). The dodecyl and benzyl groups in poly-**1** and poly-**2**, respectively, were selected as putative anchoring groups that would form multiple hydrophobic or van der Waals and π–π stacking interactions, in the case of poly-**1** and poly-**2**, respectively, with the CNTs surface. As expected, both polymers showed excellent solubility in water (up to ~50 wt%) as well as in organic solvents such as tetrahydrofuran (up to ~25 wt%), indicating their amphiphilic character.

The ability of each polymer to disperse single-walled CNTs in water was evaluated employing a probe sonicator as an emulsifier (Fig. 2). The amounts of CNTs dispersed in the aqueous polymer solution (10 mg ml^{-1} in distilled water) were quantified by measuring UV absorption.[3a] Poly-**1** and poly-**2** showed dispersion ability of 8 mg and 3 mg of CNTs ml^{-1}, respectively, suggesting stronger interactions between the dodecyl anchoring group of poly-**1** and the CNTs surface than was the case with the anchoring group of poly-**2**. The dispersion abilities of these polymers are better than those of previously reported polymers or surfactants, which were measured under similar conditions.[5–7] As comparison, we observed much lower dispersion ability from a well known amphiphilic polymer, Pluronic F-127, showing ~1.1 mg of CNTs ml^{-1}. The transmission electron microscopy image of poly-**1** coated CNTs showed well-separated individual nanotubes in solution (Fig. 2b). This dispersion pattern was further

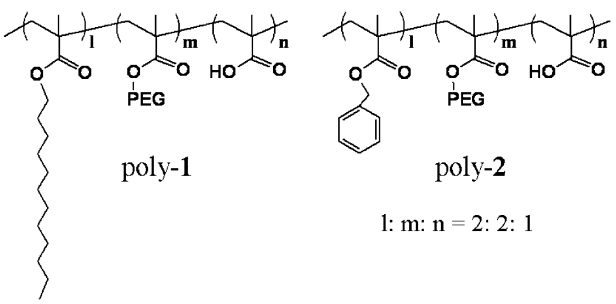

Fig. 1 Chemical structures of the amphiphilic polymers.

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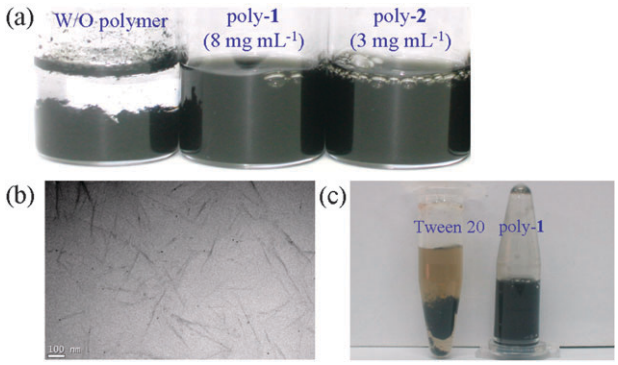

Fig. 2 (a) A photograph of single-walled CNTs dispersed in water by each of two polymers. (b) Transmission electron microscopy image of poly-**1** coated CNTs where the scale bar is 100 nm. (c) A photograph of Tween-20 and poly-**1** coated CNTs after incubation in 10% serum-containing medium for 7 days.

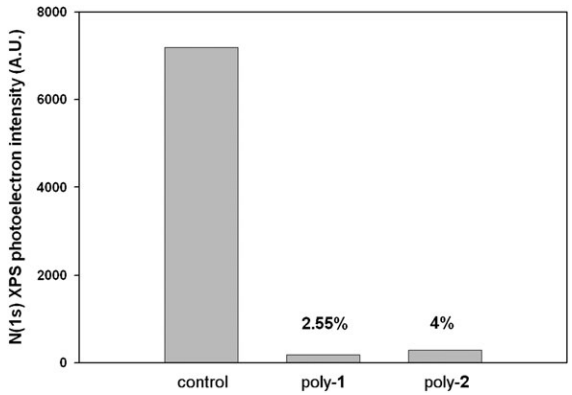

Fig. 3 High resolution N (1s) XPS intensity of the control, bare single-walled CNTs and each polymer-coated CNTs measured after incubation with BSA (0.1 mg ml^{-1} in distilled water) for 2 h. The amounts of protein adsorption on each surface (denoted as percentage) were calculated relative to that of the bare CNTs as 100% of BSA adsorption.

confirmed by an atomic force microscope (Fig. S1†). To further examine the presence of the polymer coating layers on CNTs we characterized the poly-**1** coated CNTs by using a Raman spectroscope. A slight upshift ($\sim$2.2 cm^{-1}) in the RBM (radial breathing mode) peaks as well as much increased peak intensity as compared to that of bare CNTs was evident for the firm polymer coating on the CNT surface (Fig. S2†).[11] Furthermore, it is noteworthy that the CNTs dispersed by poly-**1** were quite stable on incubation in serum-containing medium (10%, v/v, fetal bovine serum) even for 4 weeks at ambient temperature, showing no precipitation (Fig. 2c). In contrast, CNTs dispersed by the polymer surfactant Tween-20, which is widely used to disperse CNTs in aqueous medium, resulted in extensive precipitation after only 3 days of incubation under the same conditions. The Pluronic F-127 coated CNTs showed similar stability in the serum to the case of Tween-20 (Fig. S3†). Unlike Tween-20 which has only a single dodecyl group for interactions with the CNTs surface, the robustness of the coating layer afforded by the use of poly-**1** may be attributed to the multivalent anchoring of multiple dodecyl groups. In addition, the presence of multiple PEG groups might play an important role in maintaining long-term water dispersibility because these groups are known to block the adsorption of proteins onto the surfaces of nanomaterials and thus prevent protein-mediated aggregation.[10]

To examine the anti-biofouling property of each polymer-coated CNTs, the extent of nonspecific protein adsorption was measured using bovine serum albumin (BSA) as a model plasma protein. After a drop of BSA solution (0.1 mg ml^{-1} in PBS buffer, pH 7.4) was loaded on each polymer-coated CNTs film[12] for 2 h, the CNTs were washed, air-dried, and characterized by X-ray photoelectron spectroscopy (XPS). High resolution N (1s) XPS data revealed that the polymer-coated CNTs showed only $\sim$2.5% and 4% level of BSA adsorption for poly-**1** and poly-**2**, respectively, relative to that of control uncoated CNTs (Fig. 3). This result clearly demonstrates that the present polymers impart excellent anti-biofouling properties to CNTs.

The utility of the carboxylic acid group as a functionalizable site in the polymer coating was examined. As an example, the carboxylic acid group in poly-**1** coated CNTs was converted to the *N*-hydroxysuccinimide (NHS) ester by treatment with the EDC/NHS reagents, followed by reaction with a biotin derivative containing an amine group (biotin-NH_2, see ESI†). After incubation of biotinylated CNTs in streptavidin (0.1 mg ml^{-1}), the amount of bound streptavidin was assessed by high resolution N (1s) XPS which revealed much higher streptavidin binding to biotinylated CNTs than to poly-**1** coated CNTs lacking biotin, indicating specific binding of streptavidin to biotinylated CNTs (Fig. 4). This result demonstrates that the carboxylic acid group of the poly-**1** coating can be suitably functionalized by a biomolecule in aqueous solution.

Recently, there has been increasing interest in the use of CNTs as novel drug delivery vehicles.[1,3] We thus examined the feasibility of poly-**1** coated CNTs as a drug delivery vehicle. An anticancer drug, doxorubicin (Dox), was chosen for this purpose, because the drug has aromatic rings that may interact with CNTs surfaces as well as a positively charged amine group that can interact with the carboxylic acid residue of the polymer.[3e,13] Poly-**1** coated CNTs (2 mg ml^{-1}) were incubated in an aqueous solution of Dox (2 mg ml^{-1}) for 12 h and the resulting Dox-loaded CNTs were then collected by membrane filtration. The drug loading efficiency was $\sim 72 \pm 2\%$ ($n = 3$), corresponding to a $\sim$42 wt% of Dox in the resulting complex. As indicated in recent reports,[3e,13] it appears that the high Dox loading was achieved by combinations of π–π stacking, hydrophobic or van der Waals, and electrostatic interactions between Dox and CNTs. The cytotoxicity of poly-**1** coated CNTs before and after Dox loading was measured by the

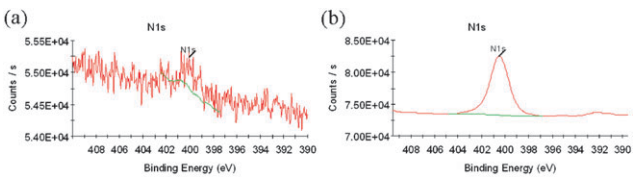

Fig. 4 High resolution N (1s) XPS intensity of poly-**1** coated CNTs (a) and the biotinylated CNTs (b) measured after incubation with streptavidin (0.1 mg ml^{-1}).

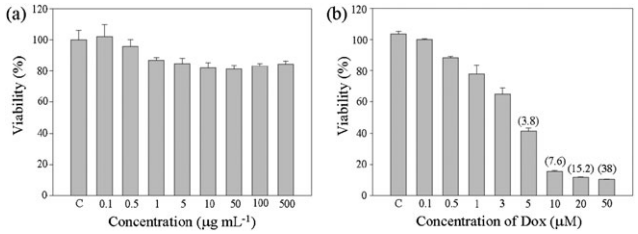

Fig. 5 Cell viability of B16F10 cells after 24 h incubation with increasing amounts of poly-**1** coated CNTs (a) and Dox-loaded CNTs (b) where the numbers in parentheses indicate the amount of poly-**1** coated CNTs contained in the complex (μg ml^{-1}).

XTT assay[14a] against B16F10 melanoma cells *in vitro* (Fig. 5). According to recent reports, CNTs tend to aggregate into ropes and bundles, decreasing aqueous solubility and thus increasing cytotoxicity.[14b] The poly-**1** coated CNTs exhibited significantly lower toxicity (Fig. 5a), even at relatively high CNTs concentration (500 μg ml^{-1}), compared to uncoated CNTs (data not shown), indicating increased water dispersibility and biocompatibility of CNTs after the polymer coating. As expected, however, Dox-loaded CNTs exhibited cytotoxicity comparable to that of Dox alone at a similar concentration (Fig. 5b), indicating that an active form of Dox was released from polymer-coated CNTs. Timecourse drug release tests revealed that Dox was released in a controlled manner with a half life of 38 h in PBS at pH 7.4 (Fig. S4†). This result indicates that poly-**1** coated CNTs may hold promise as a potential drug delivery vehicle.

In conclusion, we rationally designed and synthesized amphiphilic polymers capable of making CNTs highly water dispersible and resistant to biofouling, and such CNTs can form conjugates with bioactive molecules. It is noteworthy that the polymer formed robust coating layers on CNTs. Further, we demonstrated that drug loading of polymer-coated CNTs could be achieved, suggesting the potential use of CNTs as drug delivery vehicles. The design criteria employed herein can also be applied to the coating of other nanomaterials. The present polymer system could potentially be applied in CNT-based biomedical fields such as diagnostics and drug delivery.

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