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Research Article

Magnetic Carbon Nanotubes for Protein Separation

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Magnetic separation is a promising strategy in protein separation. Owing to their unique one-dimensional structures and desired magnetic properties, stacked-cup carbon nanotubes (CSCNTs) with magnetic nanoparticles trapped in their tips can serve as train-like systems for protein separation. In this study, we functionalized the magnetic CSCNTs with high density of carboxyl groups by radical addition and then anchored 3-aminophenylboronic acid (APBA) through amidation reaction to achieve oriented conjunction of antibodies (IgG). It was also demonstrated that the obtained magnetic CSCNTs-APBA-IgG conjugates could readily react with target antigens through specific antigen-antibody reaction and be used as new magnetic systems for protein separation.

1. Introduction

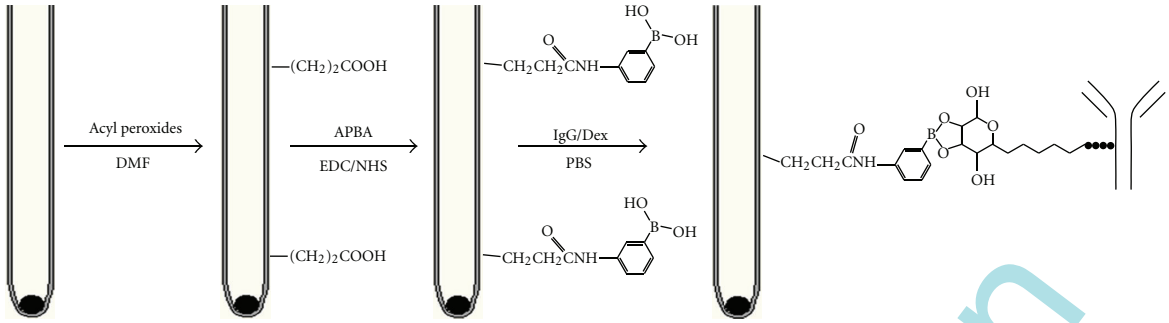
Effective separation of biomolecules, in particular target proteins, is important in biological and therapeutic applications [1, 2]. Different from the classic chromatographic method [3, 4], magnetic separation offers a cheap, fast, and gentle strategy to separate target species from crude samples with suspended solids [2, 5]. Biological targets can be captured on magnetic particles and then readily separated from the sample by applying an external magnetic field [6–8]. Many promising magnetic systems have been demonstrated and shed light on efficient separation of biomolecules or cells in many important bioprocesses [9, 10]. In these systems, spherical magnetic nanoparticles were usually used, and it was necessary to functionalize their surfaces to provide necessary stability in colloidal suspension and affinity for target species. However, functionalization of their surfaces with high density of functional groups or probe molecules is still a great challenge due to the structural limitation of spherical particles.

Carbon nanotubes [11] have one-dimensional structures with high surface area [12, 13]. Their surfaces can be readily modified with various functional groups and biomolecules [14, 15], offering a wide range of opportunities and application potentials in biology and medicine [16–19]. Notably, carbon nanotubes, especially the stacked-cup carbon nanotubes (CSCNTs) prepared by the floating catalytic method, usually have magnetic Fe nanoparticles trapped in their tips [20]. We hypothesized that this unique structure could be used as a train-like magnetic system for protein separation.

To test this idea, magnetic CSCNTs were chemically derivatized with abundant carboxyl groups by radical addition, followed by amidation reaction with 3-aminophenylboronic acid (APBA) [21, 22] to achieve oriented conjunction of antibodies through sugar-specific affinity interactions (Scheme 1). We also demonstrated that the oriented antibody-conjugated hybrid could serve as a new magnetic system for protein separation through specific antigen-antibody reaction.

1.1. Experimental Details.

1.1.1. Functionalization with Carboxyl Groups. The succinic acid acyl peroxides were prepared according to previous study. The magnetic CSCNTs were selected and functionalized with carboxyl groups by radical addition [23]. In brief, CSCNTs samples were dispersed in water by intense sonication. Magnetic CSCNTs were separated by magnetic decantation using a permanent magnet. The magnetic CSCNTs (50 mg) were then dispersed in 50 mL N,N-Dimethylformamide by intense sonication and reacted with the succinic acid acyl peroxides (1.5 g) at 90°C with interval adding of the peroxides. After reaction, the suspension was



Scheme 1: Functionalization of magnetic CSCNTs with APBA through radical addition and amidation reaction to achieve oriented conjunction of antibodies.

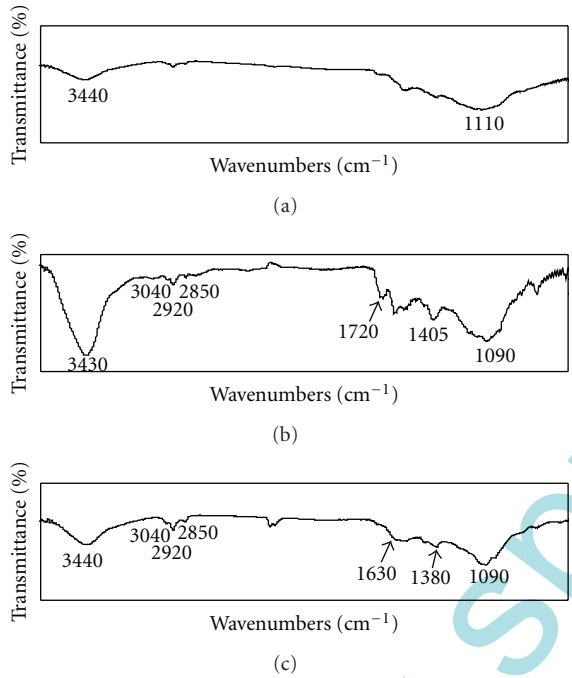


Figure 1: FTIR spectra of (a) magnetic CSCNTs, (b) f-CSCNTs, and (c) f-CSCNTs-APBA.

cooled and poured into a large amount of tetrahydrofuran with sonication and filtration to remove residual peroxides. The magnetic carboxyl groups-functionalized CSCNTs (f-CSCNTs) were then repeatedly washed with ethanol and vacuum dried overnight at 70°C.

1.1.2. Functionalization with APBA. The purified magnetic f-CSCNTs (20 mg) were dispersed in water to a concentration of 1 mg/mL. N-hydroxysuccinimide (24 mg) was then added to the suspension. With fast stirring, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (36 mg) was added quickly, and the mixture was continuously stirred for 1 h at room temperature. APBA (25 mg) was then added, and the solution was stirred overnight at room temperatures. The magnetic APBA-functionalized CSCNTs (f-CSCNTs-APBA) were then repeatedly washed with ultrapure water and vacuum dried overnight at 70°C.

1.1.3. Functionalization with Antibodies. The magnetic f-CSCNTs-APBA (2 mg/mL, 0.5 mL) were treated with goat anti-human IgG (2 mg/mL, 0.5 mL) and reacted at 4°C for 12 h. Then, the magnetic f-CSCNTs-APBA-IgG conjugates were collected by magnetic decantation and repeated washed with phosphate buffered saline (PBS, 0.1 M, PH 7.4). The black precipitate was further incubated with dextran (2.5 mg/mL, 100 μL) for 12 h at 4°C to prevent nonspecific adsorption. Finally, it was washed with PBS buffer and purified by magnetic decantation by using a permanent magnet.

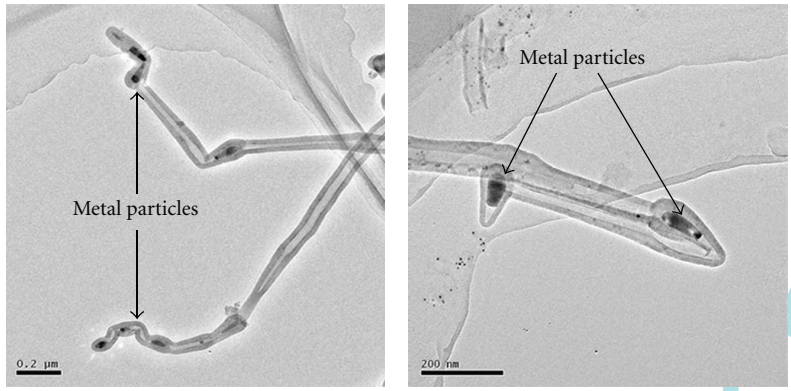
1.1.4. Specific Protein Separation by Antigen-Antibody Reaction. For the specific protein separation by antigen-antibody reaction, aliquots (200 μL) of the magnetic f-CSCNTs-APBA-IgG conjugates and the f-CSCNTs-IgG control prepared by direct amidation reaction were separately added into 1 mL PBS buffer (PH 7.4). Then, target antigens (FITC-labeled goat anti-human anti-IgG, 20 μL, 1 mg/mL) were added and reacted at room temperatures for 90 min with slow rotation. After reaction, the magnetic f-CSCNTs-APBA-IgG-antigen conjugates were magnetically separated, and the supernants were collected for fluorescent tests. The collected magnetic f-CSCNTs-APBA-IgG-antigen conjugates were purified by repeated washing with tri(hydroxymethyl) amino methane hydrochloride buffer and deionized water for later fluorescent tests.

2. Sample Characterization

The samples were characterized by fourier transform infrared spectroscopy (FTIR) (Nicolet Nexus FTIR), X-ray photoelectron spectroscopy (XPS) (PerkinElmer PHI-1600 spectrometer), atomic force microscopy (AFM) (CSPM 5000), and fluorescence spectrometer (PerkinElmer LS 55).

3. Results and Discussion

To provide abundant sites for covalent integration of APBA, the magnetic CSCNTs were chemically derivatized with carboxyl groups by free radical addition. FTIR was adopted to characterize the succinic acid acyl peroxides (see Supporting Information). FTIR spectrum of the obtained magnetic carboxyl groups-functionalized CSCNTs (f-CSCNTs)



(a) (b)

Figure 2: TEM images of (a) *f*-CSCNTs and (b) *f*-CSCNTs-APBA.

(a) (b) (c) (d)

Figure 3: Photos of magnetism test of magnetic CSCNTs (a and b) and magnetic *f*-CSCNTs-APBA (c and d) by using a magnet.

(Figure 1(b)) shows significant increase in the adsorption at 3430~3440, 1720, and 1090 cm^{-1}, corresponding to the carboxylic O–H, C=O, and C–O stretches, respectively. Characteristic C–H stretches at 2800~3050 cm^{-1} region from the anchored $–CH_2CH_2COOH$ can be also observed. After functionalization with APBA (Figure 1(c)), however, the carboxylic O–H stretches obviously reduce with the shift of the C=O stretches from 1720 to 1630 cm^{-1} [24]. In addition, characteristic B–O stretches at 1380 cm^{-1} can be also observed after reaction, suggesting the successful amidation reaction of the anchored carboxyl groups with the amine groups of APBA. This conclusion was also supported by XPS measurement (see Supporting Information).

Typical TEM image of magnetic CSCNTs shows clearly the Fe nanoparticles in the ends of CSCNTs. Different from conversional acid treatment method [25], introducing carboxyl groups on CSCNTs by the radical addition strategy did not destroy the trapped Fe nanoparticles (Figure 2(a)). This desired structure was successfully reserved after further amidation with APBA (Figure 2(b)).

The magnetic nature of the trapped Fe nanoparticles as well as the large length-diameter ratio of CSCNTs renders the obtained *f*-CSCNTs-APBA great potentials as high efficient magnetic systems for protein separation.

To test this idea, the magnetic response of the APBA-functionalized magnetic CSCNTs was preliminarily evaluated by their responses to the applied magnetic field. As shown in Figure 3, similar to the magnetic CSCNTs, the *f*-CSCNTs-APBA could be successfully attracted by a permanent magnet. Notably, the magnetic *f*-CSCNTs-APBA show excellent stability in water, and no sedimentation or aggregation can be observed for days. This impressive solubility renders it great biocompatibility for protein separation [17].

For an exemplary separation experiment, the *f*-CSCNTs-APBA were reacted with goat anti-human IgG (antibodies) through sugar-specific affinity interactions and then separated by magnetic decantation. To evaluate the interactions between IgG and *f*-CSCNTs-APBA, the IgG, *f*-CSCNTs, *f*-CSCNTs-APBA, and separated sample after reaction with IgG were characterized by AFM. As shown in Figure 4, the *f*-CSCNTs and *f*-CSCNTs-APBA show clear surfaces but with obvious increases of lateral dimensions in *f*-CSCNTs-APBA, probably due to the increased dilatation tip effect between *f*-CSCNTs-APBA and the probe tip. However, typical AFM image of *f*-CSCNTs-APBA after reaction with IgG shows interesting wormlike structures. The outer surfaces and the ends of CSCNTs are fully covered by antibodies. Different from common carbon nanotubes, CSCNTs have truncated conical graphene layers (cups) and abundant open edges on the outer surfaces and the ends. Therefore, abundant carboxyl groups can be introduced to not only the ends but also their surfaces, providing great opportunities for covalent integration. This result suggests that abundant IgG were

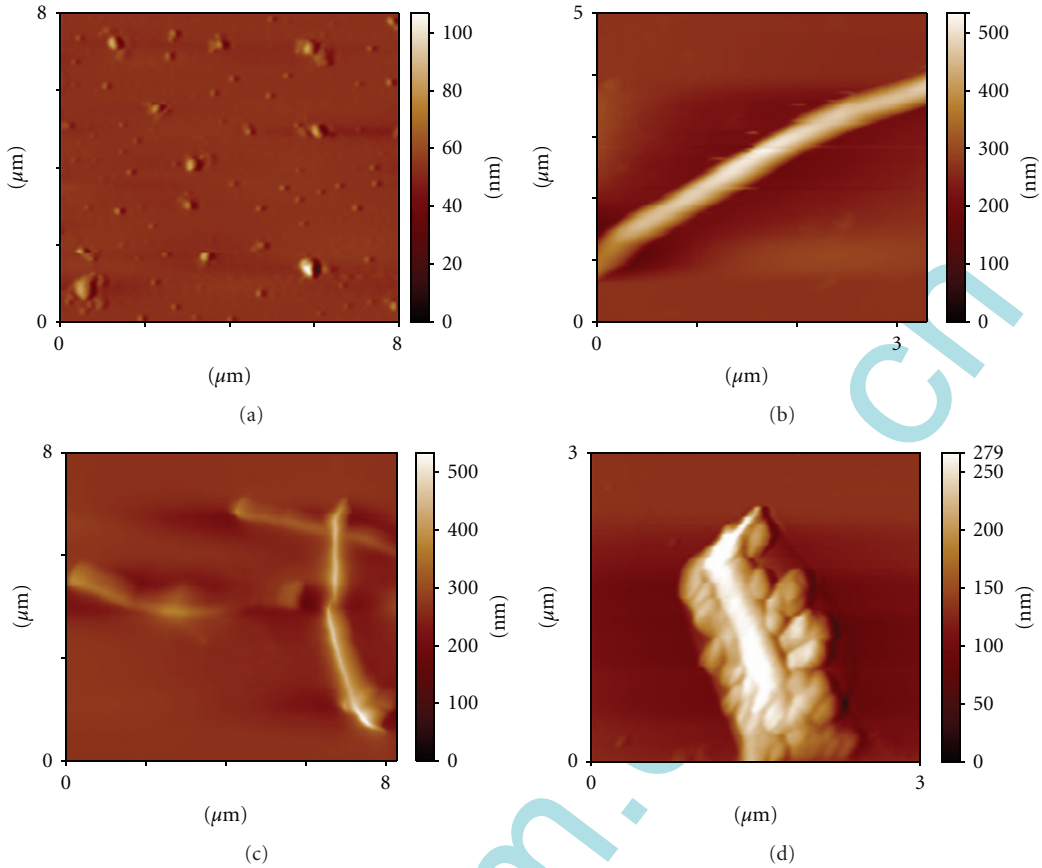


Figure 4: AFM images of (a) pure IgG, (b) f-CSCNTs, (c) f-CSCNTs-APBA, and (d) f-CSCNTs-APBA-IgG.

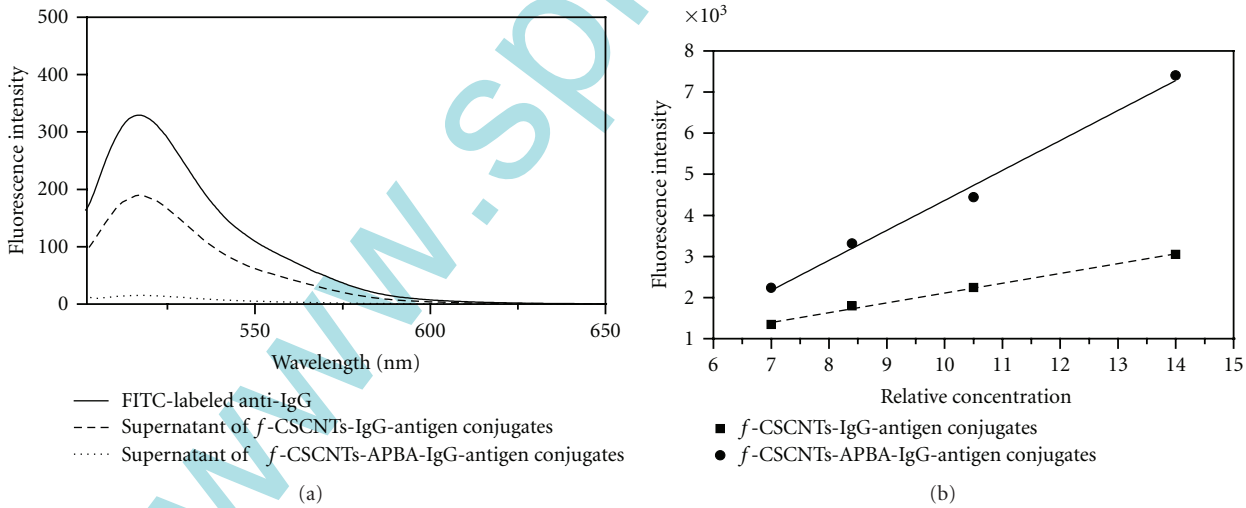


Figure 5: (a) Fluorescence spectra of the FITC-labeled goat anti-human anti-IgG in the supernatant before and after reaction with f-CSCNTs-APBA-IgG and f-CSCNTs-IgG; (b) fluorescent test of the separated conjugates. To avoid interferences, the obtained conjugates were purified by repeated washing and then diluted to different concentrations for fluorescence measurements.

successfully loaded on the surfaces of f-CSCNTs-APBA. This conclusion was also supported by XPS measurement (see Supporting Information).

As control, IgG was also directly coupled with f-CSCNTs through conventional amidation reaction. The loaded antibody amounts on the two samples were detected by quantification of the free IgG in the supernatant. And the amounts of antibody immobilized on f-CSCNTs and f-CSCNTs-APBA are 78 and 56 μg/mg, respectively. Although relatively fewer antibodies were immobilized on the f-CSCNTs-APBA, the obtained f-CSCNTs-APBA-IgG conjugates show dramatic increase in the capture efficiency of target antigens. As shown in Figure 5(a), almost no free fluorescent FITC-labeled goat anti-human anti-IgG (target antigens) can be detected in

the supernatant after reaction with f-CSCNTs-APBA-IgG. As comparison, only limited fluorescent antigens were separated by f-CSCNTs-IgG (Figure 5(b)), probably due to greater exposure of the antigen binding domain and reduced steric hindrance of the antigen binding site.

4. Conclusions

In summary, we successfully functionalized magnetic CSCNTs with high density of APBA through radical addition and subsequent amidation reaction. The obtained APBA-functionalized CSCNTs (f-CSCNTs-APBA) can react with antibody through sugar-specific affinity interactions to achieve oriented conjunction. We also demonstrated that the magnetic f-CSCNTs-APBA-IgG conjugates can readily catch target antigens through specific antigen-antibody reaction and serve as a high-efficient train-like system for protein separation. Because of its combination of specificity and high efficiency, this novel magnetic system may also find promising applications in separation of cells and other biomolecules.

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