

방사선 그래프트 중합에 의하여 제조된 폴리(아크릴 산)이 그래프트된 탄소나노튜브에 생체분자 고정화에 관한 연구

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A Study on the Immobilization of Biomolecules on Poly(acrylic acid)-grafted MWCNTs Prepared by Radiation-Induced Graft Polymerization

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초록: 본 연구에서는 방사선에 의한 그래프트 중합을 이용하여 생체분자가 고정화된 다중벽 탄소나노튜브(MWCNT)를 제조하였다. 생체분자의 고정화를 위하여 MWCNT에 아크릴 산을 그래프트 중합하였다. 열중량분석과 라만 분석을 통하여 MWCNT에 폴리(아크릴 산)이 효과적으로 그래프트 중합되었음을 확인하였다. 폴리(아크릴 산)이 그래프트된 MWCNT에 DNA와 단백질과 같은 생체분자들을 고정화하였다. X-선 광전자 분광법과 형광분석을 통하여 생체분자들이 성공적으로 MWCNT에 도입되었음이 확인되었다.

Abstract: In this research, biomolecule-immobilized multi-walled carbon nanotubes (MWCNTs) were prepared by using radiation-induced graft polymerization. For the immobilization of biomolecules, the surface of MWCNTs was functionalized by radiation-induced graft polymerization of acrylic acid. Based on the results of TGA and Raman spectroscopy it was found that acrylic acid was effectively graft-polymerized on the MWCNTs. Biomolecules such as DNA and proteins were immobilized onto the resultant poly(acrylic acid)-grafted MWCNTs. The results of the X-ray photoelectron spectroscopy and fluorescence microscopy confirmed that the biomolecules were successfully immobilized on the poly(acrylic acid)-grafted MWCNTs.

Keywords: biomolecule, multi-walled carbon nanotube, poly(acrylic acid), radiation-induced graft polymerization.

Introduction

Carbon nanotubes (CNTs) have been considered as one of the attractive functional nanomaterials because of their unique physiochemical properties and having a wide range of applications.¹ Recently, the functionalization of CNTs with biomolecules such as DNAs and proteins has attracted a considerable interest in the field of biological applications such as biosensors, genome analysis, biofuel cells and drug discovery.^{2,3} To immobilize biomolecules on the CNTs, the surface functionalization of the CNTs by various methods

including chemical, electrochemical, thermal and plasma oxidation, and graft polymerization have been developed to generate functional groups (e.g., COOH) on the CNTs.^{4,5}

The surface functionalization of the CNTs by radiation-induced graft polymerization is one of the attractive methods for the immobilization of biomolecules on the CNTs due to the several advantages as outlined in the following: 1) it does not require any harmful chemicals such as an initiator thereby making the process highly suitable for the immobilization of biomolecules and 2) it can control the degree of grafting by adjusting the irradiation conditions such as absorption dose, dose rate and monomer concentration and thus immobilizing biomolecules with various amounts.⁶⁻⁹

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However, the immobilization of biomolecules on the CNTs through radiation-induced graft polymerization has been rarely reported.

The immobilization of biomolecules on the CNTs has been performed by either a covalent or non-covalent attachment. The covalent binding-based methods have been preferred to the non-covalent-based ones because of their stability and durability of the attached biomolecules.¹⁰

In this study, we described an efficient method based on the radiation-induced graft polymerization technique to covalently immobilize biomolecules such as DNAs and proteins on the surface of multi-walled carbon nanotubes (MWCNTs). The biomolecule-immobilized MWCNTs were characterized by thermogravimetric analysis (TGA), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), and fluorescence microscopy.

Experimental

Control MWCNTs (purity: >95%, 10–15 nm overall diameter), prepared by chemical vapor deposition, were purchased from Ijin Nanotech and used without any further purification. Acrylic acid (AA), Mohr's salt ($(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (NHS) were purchased from Aldrich Company and used as received. *N,N*-Dimethylformamide (DMF) were supplied from TCI Company (Japan). Other chemicals were reagent grade and used without any further purification. All the oligonucleotides used in this study were purchased from Genotech Company (Korea). The oligonucleotide that has an amino group at its 3' position with the sequence 5'-CGACCACTTTGTCAAGCTCA-NH₂-3' was used as a probe-DNA (p-DNA) and the oligonucleotide that has been labeled with Cy5 at the 3' position with the sequence 5-TGAGCTTGACAAAGTGGTCG-Cy5-3' was used as a complementary-DNA (c-DNA). (+)-Biotinyl-3,6,9-trioxadecanediamine (biotin-amine) and fluorescein isothiocyanate-tagged streptavidin (SAv-FITC) were purchased from Pierce Company.

Poly(acrylic acid)-grafted MWCNTs were prepared according to our previous publication.⁹ Briefly, dried control MWCNTs were dispersed in 100 mL vials containing 10 vol% AA in DMF by sonicating for 30 min in an ultrasonic bath. Afterwards, the resulting mixtures were purged with dry nitrogen for 30 min, and then irradiated to 10 kGy by using γ -rays from a ⁶⁰Co source at a dose rate of 1 kGy/h. The irradiated mixtures were filtered through a 0.2 μm PTFE membrane. The resulting solids were redispersed in water by sonication and filtered through a 0.2 μm poly(ether sulfone)

membrane to remove any homopolymer. The poly(acrylic acid)-grafted MWCNT (MWCNT-*g*-PAA) was obtained after drying under vacuum for 24 h.

To immobilize the p-DNA onto the MWCNT-*g*-PAA, a solution containing 15 mM NHS, 45 mM EDC and 50 $\mu\text{g}/\text{mL}$ of the p-DNA was mixed with the MWCNT-*g*-PAA and allowed to react overnight.^{11,12} Afterward, the resulting mixed solution was washed thoroughly three times by redispersion and centrifugation in deionized water and used for a hybridization with the c-DNA. For this, the p-DNA-immobilized CNT solution was incubated with the c-DNA in a hybridization buffer solution containing 6 x saline/sodium phosphate/EDTA (0.9 M NaCl, 10 mM NaH₂PO₄ in H₂O, 1 mM EDTA, pH 7.4) and 20% (v/v) formamide. The hybridization was carried out at 36 °C for 6 h. After this time, the DNA-immobilized CNT was washed well with deionized water.

Immobilization of biotin on the MWCNT-*g*-PAA was carried out in a similar manner to that of the DNA immobilization.^{11,12} The MWCNT-*g*-PAA was immersed in a solution containing EDC/NHS and 10 mM biotin-amine overnight at room temperature. Afterwards, the resulting biotin-immobilized MWCNT was thoroughly washed by redispersion and centrifugation in deionized water. The prepared biotin-immobilized MWCNT solution was subsequently incubated with SAv-FITC (0.1 mg/mL) in a phosphate-buffered solution (PBS, pH 7.4) containing 0.1% (w/v) bovine serum albumin (BSA) and 0.02% (v/v) Tween 20 at room temperature. After 60 min, the SAv-FITC-bound MWCNT solution was washed with deionized water and dried.

Thermogravimetric analysis (TGA) was performed on a SDT Q-600 series thermal analysis system. This test was carried out under nitrogen atmosphere with a heating rate of 10 °C/min between 50 and 700 °C. Raman measurements were performed by using a LABRAM-HR Raman spectrometer (Jobin-Yvon) by utilizing an Ar-ion laser at an excitation wavelength of 514.5 nm. The surface chemical composition was measured by X-ray photoelectron spectrometer (XPS, MultiLab 2000, ThermoElectron Corporation, England) with MgK α X-ray source. The applied power was 14.5 keV and 20 mA, and the base pressure of the analysis chamber was less than 10⁻⁹ mbar. The fluorescence observation was conducted by using a fluorescence microscope (Olympus BX61).

Results and Discussion

The procedure for the immobilization of biomolecules on the MWCNT by radiation grafting process is depicted in Figure 1. The surface of the MWCNTs is first functionalized

by the radiation-induced graft polymerization of AA. Afterwards, the biomolecules, such as DNAs and proteins, are covalently immobilized on the MWCNT-*g*-PAA via the diimide-activated amidation in the presence of EDC and NHS.

To quantify the relative grafting degree (GD) of PAA onto the MWCNT, the TGA measurements were carried out and the results are shown in Figure 2(a). The weight loss of the

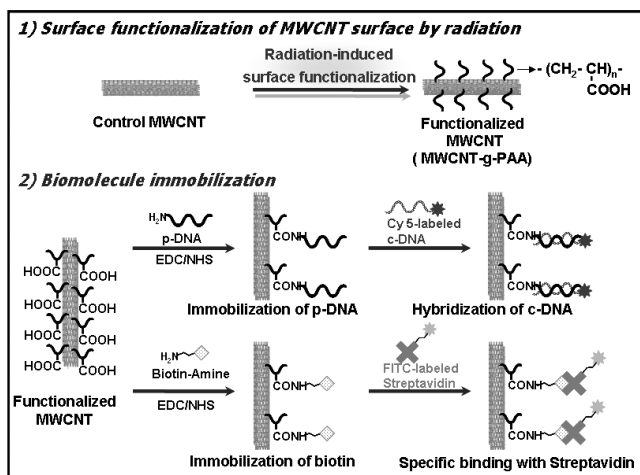


Figure 1. Schematic illustration for the immobilization of biomolecules on the MWCNTs by a radiation-induced graft polymerization method.

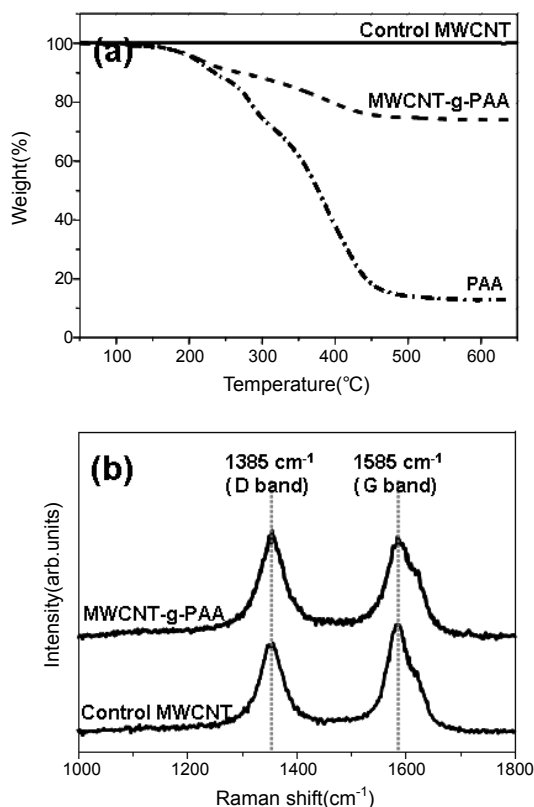


Figure 2. TGA curves (a); Raman spectra (b) of the control MWCNT and MWCNT-*g*-PAA.

control MWCNT was less than 1.5 wt% at 700 °C under nitrogen atmosphere. The weight loss of MWCNT-*g*-PAA was found in the temperature range of 150 to 500 °C, corresponding to the decomposition of the grafted PAA. The relative grafting degree of PAA on the MWCNT surface is 21 wt%. Moreover, to verify the covalent bonding between PAA and MWCNT, the control MWCNT and MWCNT-*g*-PAA were characterized by Raman spectroscopy.¹³ As shown in Figure 2(b), the G band due to the stretching vibrations of the sp²-hybridized carbon and the disordered D band attributed to defective carbon atoms, clearly appeared at around 1585 and 1385 cm⁻¹, respectively. The relative degree of defects present in the control MWCNT and MWCNT-*g*-PAA calculated by the intensity ratio of the D band to the G band were 0.90 and 1.02, respectively, indicating that, after graft polymerization, the defects of MWCNT increased due to the covalent bonding between PAA and MWCNT. Therefore, these results revealed that the PAA was successfully grafted on the MWCNT.

To investigate the changes in the surface chemical composition after the immobilization of biomolecules on the MWCNT-*g*-PAA, the XPS was employed and the results are shown in Figure 3.^{14,15} As shown in Figure 3(a), the

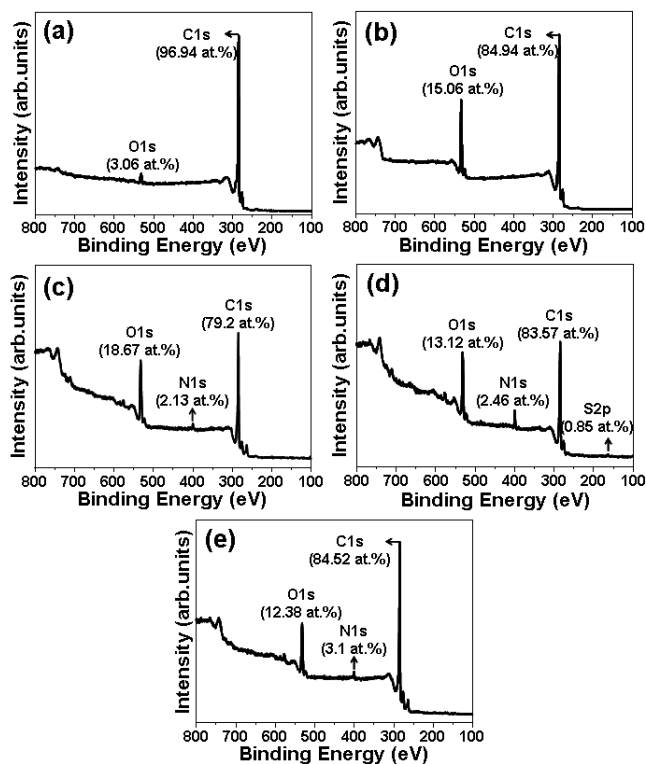


Figure 3. XPS spectra of the control MWCNT (a); MWCNT-*g*-PAA (b); *c*-DNA hybridized, *p*-DNA-immobilized DNA-immobilized MWCNT-*g*-PAA (c); biotinylated MWCNT-*g*-PAA (d); streptavidin-bound, biotinylated MWCNT (e).

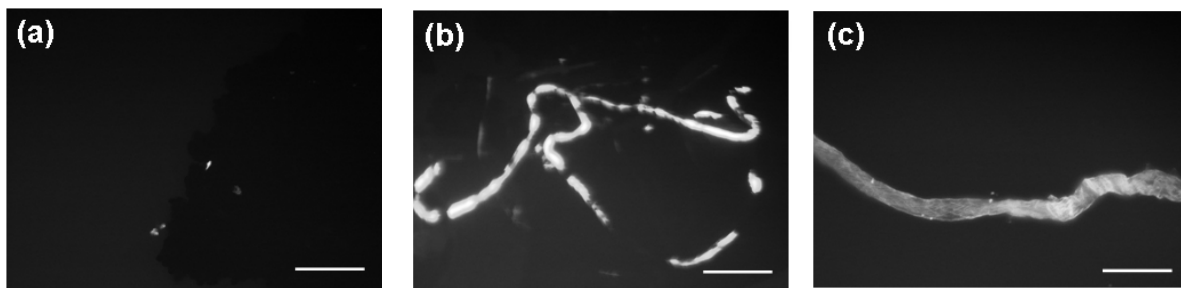


Figure 4. Fluorescence microscope images of the control MWCNT (a); the Cy5-labeled *c*-DNA hybridized to *p*-DNA-immobilized MWCNT-*g*-PAA (b); the SAV-FITC bound to biotin-immobilized MWCNT-*g*-PAA (c) (scale bar: 50 μm).

carbon (C) and oxygen (O) concentrations were 96.94 and 3.06 at.% in the XPS spectrum of the control MWCNT, respectively. For the MWCNT-*g*-PAA in Figure 3(b), the O concentration dramatically increased up to 15.06 at.% due to the grafted PAA. After the hybridization of *c*-DNA to *p*-DNA immobilized MWCNT-*g*-PAA, the nitrogen (N) corresponding to the constituent atoms of the DNA was newly detected and its concentration was 2.13 at.% in Figure 3(c), meaning that the DNA was definitely present on the MWCNT-*g*-PAA. In case of biotinylated MWCNT-*g*-PAA, the N and sulfur (S) corresponding to the constituent atoms of biotin were newly observed in Figure 3(d) in comparison with that of the MWCNT-*g*-PAA and their concentration was 2.46 and 0.85 at.%, respectively. After binding biotinylated MWCNT-*g*-PAA with streptavidin, the atomic concentration of the N, main constituent of the streptavidin, increased to 3.1 at.% while the S present in the biotin disappeared in Figure 3(e). Therefore, the streptavidin was successfully bound to the biotinylated MWCNT-*g*-PAA.

The DNA- and streptavidin-immobilized MWCNTs were observed with a fluorescent microscope and the results are shown in Figure 4. The control MWCNT did not show fluorescence property as shown in Figure 4(a). On the other hand, Cy5-labeled *c*-DNA-immobilized and FITC-labeled streptavidin-immobilized MWCNT-*g*-PAA samples exhibited a strong fluorescence as shown in Figure 4(b) and (c), respectively. Therefore, this result revealed that the DNA and streptavidin are successfully immobilized onto the MWCNT-*g*-PAA.

Conclusions

The immobilization of biomolecules on the MWCNT by radiation-induced graft polymerization was successfully demonstrated in this study. The results of the TGA and Raman spectroscopy revealed that the PAA was covalently grafted on the MWCNT by the simultaneous graft polymerization. The effective immobilization of biomolecules

such as DNAs and proteins on the MWCNT-*g*-PAA was confirmed by the XPS and fluorescence microscopy. The biomolecule-immobilized CNTs developed in this study can be utilized in a variety of biological applications.

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