자기 조립된 펩타이드 나노튜브의 고정화

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Immobilization of Self-assembled Peptide Nanotubes onto a Gold Substrate

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Introduction

Recently, there has been an immerse concern in the fabrication of nanocomponents such as nanotubes, nanowires, and nanocrystals due to their potential to serve as building blocks for emerging nanometer-sized devices[1]. Organic tubular assemblies are of interest because of their plentiful possible applications, many of which are evident from a consideration of biological systems[2]. A decade ago, peptide nanotubes were synthesized from self-assembly of cyclic peptide being flat, ring-shaped peptide subunits made up of alternation D- and L-amino acid residues. Peptide self-assembly is directed by the formation of an extensible network of intersubunit hydrogen bonds[3]. Those peptide nanotubes have many advantages such as biocompatible, easy-to-made, and inexpensive compared to the general tubular structures. In this work, fabricated peptide nanotubes were modified with thiol-functionalized to immobilize the peptide nanotubes onto the gold substrate. This step will be anticipated to the further application on the fields of biosensors. Nanotube structures were characterized by Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM).

Experimental

Chemicals. All solutions were prepared in doubly distilled deionized water. Cyclic peptide (cyclo[(Gln-D-Leu)$_4$]) was obtained from jpt (Germany). Trifluoroacetic acid (TFA) was purchased from Fluka and N-hydroxysuccinimide (NHS), 11-Mercaptoundecanoic acid (MUA), and 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDAC) were obtained Sigma-Aldrich. All chemicals were used as received unless stated otherwise. Gold substrates prepared by e-beam evaporation of 30nm of gold onto the surface of cover glasses were obtained from Inostek (Korea). They were cleaned by dipping into a piranha solution with a 3:1 (v/v) mixture of sulfuric acid and hydrogen peroxide for 5min to remove organic residues on the surface. Those plates were rinsed with ethanol and deionized water (DI water).

Self-assembly of peptide nanotubes. Cyclic peptides were dissolved at a concentration of 1mg/mL in neat TFA. Typically 0.5mL of such a solution was floated in an open Eppendorf tube which was then placed in a 50mL conical which was partially filled with water. The conical tube was sealed and left undisturbed at room temperature for 2~3 days. Examination of the Eppendorf tube after this time revealed a milky white suspension forming at the surface of the TFA solution. Examination of the suspension under optical microscopy (OPTIPHOT2-POL, NIKON) and scanning electron microscopy (HITACHI S-4300) revealed that the suspension was composed of millions of tiny needle like microcrystals. The components of the suspension were also investigated by Fourier-transform infrared spectroscopy (FT/IR-620, Nicolet).

Immobilization of peptide nanotubes. The peptide nanotubes (200$\mu\ell$) were immersed in an aqueous solution 75 mM EDAC (200$\mu\ell$) and 15 mM *N*-hydroxy succinimide (200$\mu\ell$) for 30min. This solution was mixed with 400$\mu\ell$ of 15 mM MUA and then added to the above reaction mixture in tris buffer (pH 8) for 24h. The gold plates were placed in a flat-bottomed cylindrical reactor with a Teflon slab with four supporting legs. Fabricated thiol-functionalized peptide nanotubes in tris buffer (pH 8) were charged and reacted for 24h at room temperature. The peptide nanotubes were immobilized onto the gold substrates.

Results and Discussion

It was found that cyclic peptide assembled under appropriate conditions to form needle-shaped microcrystals. Figure 1 is an image of optical microscopy showed exclusive formation of needle-shaped crystalline objects. Formation of needle-shaped crystals is an expected consequence of the

faster rate of self-assembly or crystal growth along the tube axis where the extensive network of cooperative backbone-backbone intersubunit hydrogen-bonding stacking interactions takes place.

Figure 2 is a result of Fourier-transform Infrared spectroscopy (FT-IR) used to characterize and assigned the structural features of nanotube crystals. Nanotube crystal arrays formed by the self-assembly of the peptide subunits display FT-IR spectra in the amide Ⅰ and amide Ⅱ regions that are characteristic of extensively hydrogen-bonded β-sheet-like networks. Moreover, the N-H stretching frequency occurring 3280 cm^{-1} also correspond to a tightly hydrogen bonded ring-stacked network. FT-IR spectroscopy is a sensitive technique which can provide valuable structural information about the self-assembled nanotube structures and the mode of hydrogen-bonding interactions between the stacked peptide subunits. The observed amide Ⅰ bands at 1629 cm^{-1} and amide Ⅱ bands at 1546 cm^{-1} are characteristic of β-sheet-like structures. The position of N-H stretching frequency was used to gain valuable information regarding the geometry of the peptide subunits in the nanotube ensembles. Although N-H stretch is a highly localized mode, its frequency, normally seen between 3310 and 3270 cm^{-1}, depends on the strength of the N-H···O=C hydrogen bond and has been shown to be a sensitive reflection of structure and its variations.

The ultrastructure of the peptide nanotubes on the surface of gold substrate was characterized by SEM as shown in Figure 3. This figure showed that the peptide nanotubes are immobilized on gold substrate by modifying their side-chains with the thiol-functionalized components. Currently, the immobilization of functional groups onto the surface of nanotube using biosensor are under consideration.

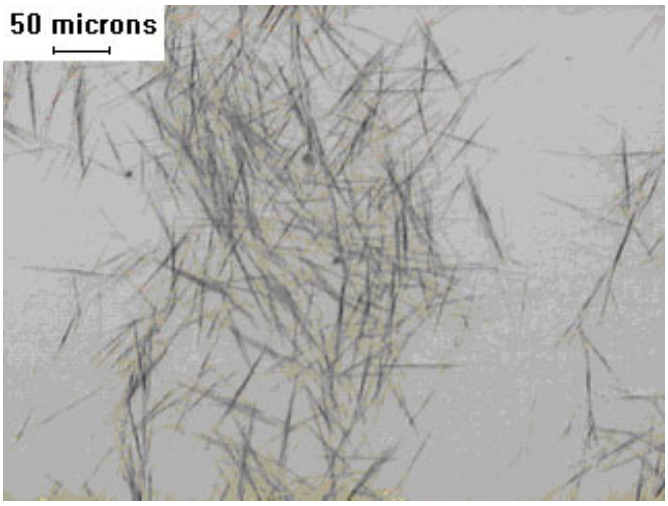

Figure 1. Microscope image of self-assembled peptide nanotubes.

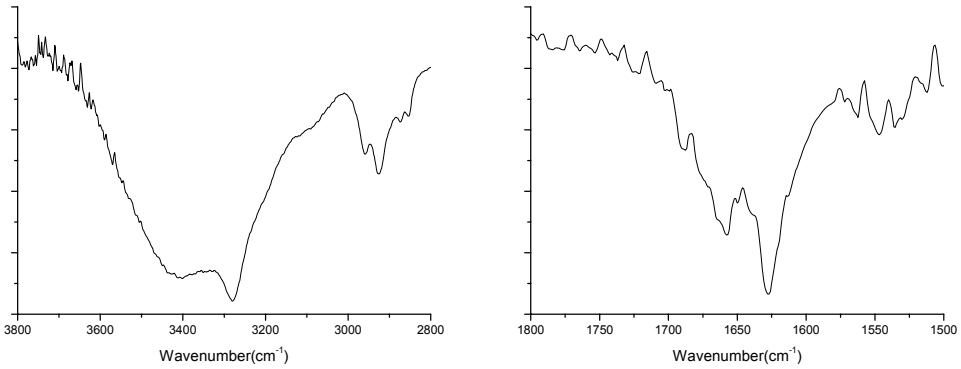

Figure 2. Fourier-transform infrared spectroscopy of N-H stretch and amide Ⅰ and amide Ⅱ regions of nanotube crystals.

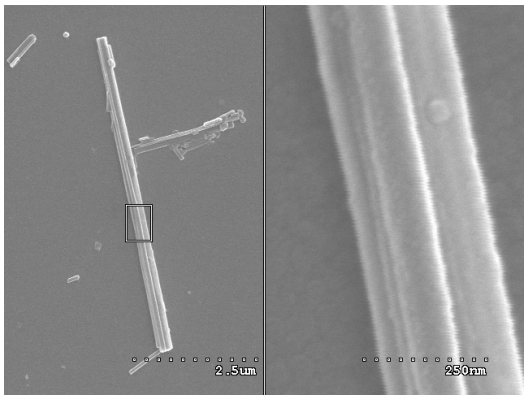

Figure 3. SEM image of immobilization of a peptide nanotube immobilized on a gold substrate.

Acknowledgements

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Reference

1. Ipsita A. Banerjee, Lingtao Yu, and Hiroshi Matsui, *Nano Lett.*, 3(3), 283-287(2003)
2. Dennis T. Bong, Thomas D. Clark, Juan R. Granja, and M. Reza Ghadiri, *Angew. Chem. Int. Ed.*, 40, 988-1011(2001)
3. Jeffrey D. Hartgerink, Juan R. Granja, Ronald A. Milligan, and M. Reza Ghadiri, *J. Am. Chem. Soc.*, 118, 43-50(1996)