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Protein Nanotube Arrays Immobilized on Solid Substrates: Molecular Trap in Aqueous Medium

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We describe template-assisted synthesis of protein nanotube arrays immobilized on solid substrates (e.g., glass plate) using a nanoporous polycarbonate membrane and the arrays' molecule-trapping capabilities in aqueous medium. The uniform array of human serum albumin-based nanotubes bearing an avidin surface interior captured efficiently fluorescein-labeled biotin in water.

Syntheses of smart nanotubes composed of biomolecules such as lipid amphiphiles, DNA, peptides, and proteins have been of particular recent interest.[1–14] Especially, protein nanotubes have attracted considerable attention because of their potential applications.[4–14] An efficient approach to create structure-defined hollow cylinders is template synthesis using a nanoporous polycarbonate (PC) membrane. We recently demonstrated a well-organized procedure to fabricate protein nanotubes comprising layer-by-layer (LbL) assembly of poly-L-arginine (PLA) and human serum albumin (HSA) [$(PLA/HSA)_3$ nanotubes].[9] The most abundant plasma protein in the bloodstream, HSA, serves as a transporter of endogenous and exogenous compounds.[15–17] Therefore, the cylindrical walls of the $(PLA/HSA)_3$ nanotubes in water capture several ligands for HSA, such as cyanine dye, zinc–protoporphyrin IX (ZnPP), and fatty acids.[9] This LbL deposition technique is extended to other proteins, and thereby various nanotubes can be produced.[9–12] Another advantage of the template-assisted synthesis is implantation of the core structure onto the solid substrate. That configuration confers high density to the homogeneous nanotube array on the flat surface if the nanotube-embedded PC membrane is pasted onto the substrate and the PC support is subsequently dissolved. Such a unique molecular architecture can be a novel molecular trap device that can strongly influence bioseparation chemistry. Herein, we describe the synthesis and structure of protein nanotube arrays immobilized vertically on solid substrates and highlight their molecule-trapping capabilities in aqueous medium.

First, we prepared seven-layered $(PLA/HSA)_2PLA/PLG/Avi$ nanotubes (PLG: poly-L-glutamic acid) into a track-etched PC membrane (pore-diameter: 400 nm, diameter: 25 mm).[9] To promote the biospecific interaction, avidin (Avi, MW: 68000) was introduced as the last layer of the wall. The Avi binds four biotins with the highest affinity of any known protein ($K > 10^{15}\,M^{-1}$).[18] Because Avi is a basic glycoprotein (isoelectric point: 10.0–10.5) with positive net charges at pH 7.0, an anionic PLG was sandwiched as the sixth layer. The obtained hybrid PC membrane was then glued onto a cover glass using epoxy resin, which was precoated onto the glass top before 10 min to avoid insertion into the channels. After drying at 4 °C for 12 h, the glass plate with the PC membrane was immersed into DMF solution to dissolve the PC framework. The DMF solution was changed several times and the glass plate was plunged into liquid N_2 with subsequent freeze-drying in vacuo. After lyophilization, white thin film remained on the planar surface. FE-SEM images of the white area revealed the formation of a uniform nanotube array in which the individual cylinders were fixed vertically onto the substrate (Figures 1A and 1B). The nanotube outer diameter was 407 ± 7 nm with wall thickness of 57 ± 5 nm. The maximum length of the tube (ca. 9 µm) corresponded to the PC template pore depth (Figure S1[19]). It is noteworthy that (i) the standing nanotube shape was identical to that of free tubes extracted directly from the membrane[9] and that (ii) the nanotube density (7.9×10^7 tubes/cm^2) showed good agreement with the porosity number of the PC template (7.9×10^7 pores/cm^2).

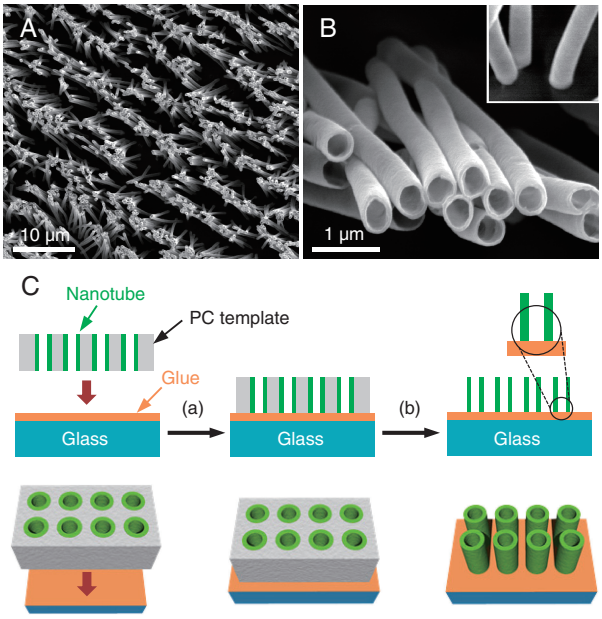

Figure 1. (A, B) SEM images of $(PLA/HSA)_2PLA/PLG/Avi$ nanotube array immobilized on glass plate. (C) Schematic illustrations of immobilization process of protein nanotubes on the glass surface; (a) pasting and drying, (b) PC template dissolution and freeze drying.

We performed closer inspection of the interface between the tube terminal and glass plane (Figure 1B inset). To elucidate the connection part in detail, the tubes were dissolved in an acidic condition (pH 2.0), and the remaining landscape was observed using SEM. The smooth surface had many circular traces of 400-nm diameter (Figure S2[19]), which indicates clearly that the glue did not invade into the central channels of the tubules when the nanotube-embedded PC membrane is pasted onto the cover glass (Figure 1C). Consequently, we conclude that the 9-µm hollow

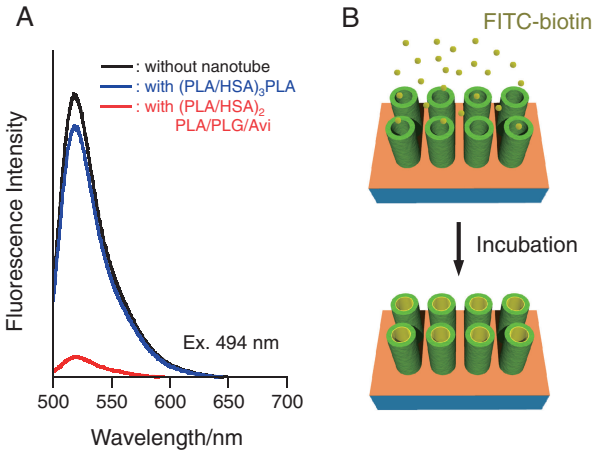

Figure 2. (A) Fluorescence spectra of PB solution (pH 7.0, 10 mM) of FITC-biotin (0.2 μM) after incubation with protein nanotube arrays immobilized on glass plate. (B) Schematic illustration of FITC-biotin capture in $(PLA/HSA)_2PLA/PLG/Avi$ nanotubes.

cylinders are immobilized by the tight connection between the tube edge and glass plate. Results have shown that this straightforward method is applicable for other combinations of protein nanotubes and solid substrates (glass, silicon wafer, graphite, etc.) (Figure S3[19]).

Next, we examined the molecule-capturing capability of the $(PLA/HSA)_2PLA/PLG/Avi$ nanotube array in water. As a ligand, 5-(6′-biotinamidohexanoylamino)pentylthioureidylfluorescein (FITC-biotin) was exploited. The nanotube array on the cover glass (1.27 cm^2, 7.9×10^7 tubes/cm^2) was immersed into the phosphate buffer (PB) solution (pH 7.0, 10 mM) of FITC-biotin (0.2 μM) and incubated for 3 h at room temperature. The fluorescence intensity of the aqueous phase became significantly lower (7%) than that of the FITC-biotin solution without the tube (Figure 2A). Immersing a control $(PLA/HSA)_3PLA$ nanotube array on the glass plate did not decrease the fluorescence, which indicates that (i) the Avi layer is necessary to capture FITC-biotin and that (ii) a nonspecific adhesion of the ligand to the tube is excluded. We reasoned that FITC-biotin enters the one-dimensional pore space of the tube rapidly and is then bound to the internal wall of the Avi layer (Figure 2B). In fact, the $(PLA/HSA)_2PLA/PLG/Avi$ nanotube array after incubation in the FITC-biotin solution fluoresced strongly on the glass surface (Figure S4[19]). The ratio of the FITC-biotin/Avi was estimated to be ca. 4.0 (mol/mol). The individual proteins making up the tubes were not eliminated from the assemblage during the experiment, as revealed by SDS-PAGE of the aqueous sample solution. It can be concluded that the ligand-binding ability of the $(PLA/HSA)_2PLA/PLG/Avi$ nanotube array on the glass plate is fundamentally identical to that of the corresponding free tubes dispersed in water.[9] In contrast, after immersing the same-sized cover glass (1.27 cm^2) coated with thin PLA/HSA/PLA/PLG/Avi film to the FITC-biotin solution, 83% fluorescence intensity remained, which is consistent with the fact that the total surface area of the inner pore walls of the nanotube array (5.71 cm^2) is 4.5-fold greater than the glass plate area (1.27 cm^2).

We demonstrated earlier that a $(PLA/HSA)_3$ nanotube can trap ZnPP efficiently in aqueous medium.[9] The ZnPP molecules can diffuse into the multilayered walls of the tubes, where HSA components capture them. The estimated ratio of the ZnPP/HSA was ca. 0.7 (mol/mol). The ZnPP-capturing capability of the $(PLA/HSA)_3$ nanotube array on the glass plate was much lower than that of the free tubes in water. The 72% fluorescence intensity of the ZnPP solution remained after immersing the $(PLA/HSA)_3$ nanotube array on the glass (1.27 cm^2, 7.8×10^7 tubes/cm^2) (Figure S5[19]). The nanotube-embedded PC membrane (Figure 1C middle sample) also showed the same result. These observations imply that the HSA inner surface wall trapped ZnPP, whereas the other two HSA layers in the wall did not bind the ligand. A possible explanation is that ZnPP cannot diffuse into the hydrated wall along the cylinder axis because of high mass transfer resistance when the solid surface blocks one terminal.

In conclusion, results show that template-assisted synthesis of protein nanotube arrays immobilized on solid substrates using nanoporous PC membrane. All protein cylinders fabricated in the template are implanted onto the planar surface. The terminal edges of the nanotubes are adhered tightly with epoxy resin at the bases. FITC-biotin can enter into the one-dimensional pore space of the tube, where the internal Avi wall captures it. These results suggest that simply depositing a specific protein at the last layer of the cylindrical wall can enable us to create a useful molecular trap system on a solid substrate for use as a separation device in biomedical applications, for instance virus removal. The flexibility of function program in the protein nanotube array might also develop a new biochip for use as an enzymatic chemical reactor or sensor.

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