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Assembled alginate/chitosan nanotubes for biological application

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Abstract

Biodegradable nanotubes were fabricated through the layer-by-layer (LbL) assembly technique of alternate adsorption of alginate (ALG) and chitosan (CHI) onto the inner pores of polycarbonate template with the subsequent removal of the template. The assembled materials present good film formation ability. The thickness of nanotubes wall can be controlled by changing the assembled layers. The assembled tubular structure was verified by the confocal laser scanning microscope (CLSM) using fluorescent-labeled ALG as well as the measurements of scan electron microscope (SEM) and transmission electron microscopy (TEM). Atomic force microscopy (AFM) images confirm the biodegradable feature of the assembled nanotubes as they are immersed in the pancreatin. Confocal microscopy images show that the assembled ALG/CHI nanotubes can be internalized into the cancer cell readily. The cell viability experiment proves the low cytotoxicity of ALG/CHI nanotubes. The final assembled nanotubes have presented good biodegradability and low cytotoxicity.

Keywords: Template synthesis; Nanotubes; Biodegradability; Layer-by-layer assembly; Chitosan; Alginate

1. Introduction

Nanotubes made from a variety of materials including carbon, ceramics, metals, and organic polymers have been summarized in the recent reviews due to their unique electronic, optical, and mechanical properties and their potential application in the field of nanodevices, catalysis, biology, and medicine as carriers [1–4]. They are often considered as biosensors and bioreactors or as templates for the immobilization or growth of biomolecules [5,6]. The recently developed layer-by-layer (LbL) assembly technique is a well-established to build composite multilayers [7–10]. This technique combined with template approach results in the successful fabrication of different types of polymer nanotubes, especially in an aqueous solution system [11–13]. It is well known that polyelectrolyte (PE) complexes of chitosan (CHI) with other polysaccharides are usually found in biotechnological and pharmaceutical application [14,15]. CHI is generally obtained by extensive deacetylation of chitin, a polysaccharide widely spread in nature. It is economical and nontoxic biomaterial. Recently the assembly of CHI/polyanion complexes leads to the novel structure of microspheres and microcapsules as carriers to encapsulate cells, proteins, or vaccines [16–22]. CHI complexes often exhibit good biodegradability. Therefore, synthesis and assembly of CHI together with alginate (ALG) as tubes are of great importance in developing novel biomaterial toward biological application. In the present work, we will demonstrate that the combination of CHI with ALG is able to form a tubular structure via LbL assembly through electrostatic forces and they are mechanically stable with the specific features of biodegradability. Such assembled tubular structure may have a potentional application in gene or drug delivery as carriers [23].

2. Materials and methods

2.1. Preparation of ALG/CHI Nanotubes

Commercially available microporous polycarbonate (PC) filters (purchased from Whatman Corp.) were used as the template membranes. These membranes have a nominal pore diameter of 400 nm and a membrane

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thickness of ca. 10 μm (quoted by supplier). Poly(ethylenimine) (PEI, $M_w = 5000–10{,}000$) was obtained from ICN Co., Ltd. ALG sodium, CHI (medium molecular weight), and fluorescein dichlorotriazine (DTAF) were purchased from Sigma. Pancreatin powder was obtained from Yakanglinuo bioengineering Co. (Jinan, China). All the chemicals have been used without further treatment. The water used in all experiment was prepared in a three-stage Millipore Milli-Q Plus 185 purification system and had a resistivity higher than 18.2 MΩ cm^{-1}. All experiments were performed at room temperature. Fig. 1 represents the chemical structures of CHI and ALG. CHI solution (1 mg ml^{-1} in 0.1 M NaCl, 0.02 M acetic acid) and ALG solution (1 mg ml^{-1} in 0.1 M NaCl) were used for the assembly of ALG/CHI multilayers in the inner pores of the template. The polysaccharide samples were dissolved under slight shaking at room temperature overnight. PEI solution of 1 mg ml^{-1} in water containing 0.5 M NaCl was used for deposition to produce the first PE layer with charges. All the solutions were filtered through a membrane filter of 8.0 μm pore size. Positively charged PEI was first absorbed on the inner pore of PC membranes by immersing the membranes into a solution of PEI for 30 min (gentle sonication for 1 min was applied at the beginning of adsorption to aid transportation of the PEI into the membrane pores). The PEI-modified membranes were then rinsed by immersing them into water for 10 min. This process was repeated twice. Next, negatively charged ALG and positively charged CHI were alternately adsorbed for 30 min in the pores of the membranes and then washed twice (each time 5 min) with water until the desired number of layers was obtained. The surface layers of the PC membranes were removed by polishing both faces of the membrane with 0.5 μm alumina powder. After polishing, the template with the inner pore composites was ultrasonicated in water in order to remove the residual alumina powder. The ALG/CHI nanotubes were obtained finally by dissolving the PC membranes template in dichloromethane. Then the product was purified by several dichloromethane washings and centrifugation cycles, followed by re-dispersing in ethanol. The assembled procedure has been illustrated in Fig. 1.

2.2. Coupling of DTAF to alginate

We added 8 mg DTAF dissolved in 10 ml of 1 mg ml^{-1} ALG solution (adjust pH > 11 with NaOH). The mixture was allowed to react overnight at room temperature with gentle stirring. DTAF-labeled ALG were separated from free DTAF by extensive dialysis against water in avoiding light environment. Then DTAF-labeled ALG solution was used to fabricate DTAF-ALG/CHI nanotubes for confocal laser scanning microscopy (CLSM) measurement.

2.3. Characterizations of the obtained nanotubes

Scan electron microscopy (SEM) and energy-dispersive X-ray (EDX) spectroscopy images were obtained by a Hitachi S-4300. Transmission electron microscopy (TEM) measurements were performed by Philips TECNAI-20. Fourier transform infrared (FT-IR) spectra were recorded on a TENSOR 27 instrument (BRUKER, Germany). The CaF_2 plate was used for FT-IR spectra measurement. CLSM images were taken with an Olympus FV500 confocal system. The atomic force microscopy (AFM) images were taken by means of a Digital Instrument Nanoscopy IIIa instrument in the tapping mode.

To prove the biodegradability of the obtained nanotubes, we left the dried sample in the pancreatin solution and stirred at room temperature overnight. The nanotubes suspension was dropped on a mica plate and silicon substrate, respectively, and washed with deionized water carefully and then dried in air for characterizations.

2.4. Cell culturing

Standard cell culture techniques were used for all cell experiments. The HeLa or MCF-7 cells were cultured in complete DMEM medium containing 10% fetal calf serum and 1% penicillin and streptomycin at 5% CO_2 with humidity. When the HeLa cells reached about 90% confluency, they were split into a 35 mm glass-bottom Petri dish using a standard trypsin-based technique. After the cells have been incubated in the logarithmic growth phase, ALG/CHI nanotubes were added for 24 h of co-culturing. Then the cells were washed twice with PBS to remove the dispersed nanotubes and dead cells in growth media and observed by CLSM. Then, after trypsin treatment, the cell suspension was obtained

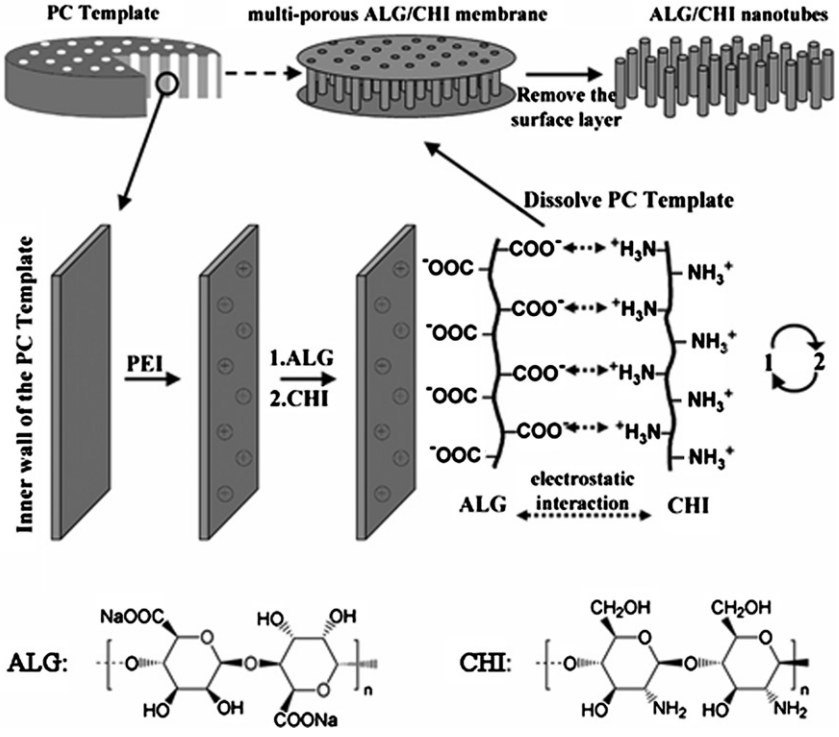

Fig. 1. A schematic illustration for the fabrication of ALG/CHI nanotubes and multi-porous ALG/CHI membrane.