Biocompatible Nanotubes as potential Nanocarriers for hydrophobic model food and drug bioactives

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Abstract

Biocompatible nanotubes (NTs) have a great potential application especially in food and drug delivery. Nanotubes were produced using Layer-by-Layer (LbL) deposition of negatively charged bovine serum albumin (BSA) and positively charged , poly-D-lysine (PDL), into a nano-porous template. By deposition of three bilayers, robust nanotubes were produced. Curcumin was used as a hydrophobic bioactive model to evaluate the loading capacity of hydrophobic compounds. The NTs were prepared from interaction of BSA-curcumin as a negatively charged biopolymer layer. The SEM images show that the $(PDL/BSA\text{-}Cur)_2$ NTs had big cavities but the aqueous solution couldn't pass through it, which provides evidence of the hydrophobic nature of the cavities of the NTs.

Keywords: Biocompatible Nanotubes, LbL decomposition, Nutraceuticals, Curcumin, BSA.

1. Introduction

Nanotubes can be made from different materials such as carbon, ceramics, metals and organic polymers. Nanotubes have been very interesting in the recent decades due to their unique electronic, optical and mechanical properties. They have applications as nanodevices, catalysts, biological markers and diagnostic tools, and especially as carriers in medicine [1-6]. In recent years, interest in organic nanotubes has rapidly increased. They have been extensively investigated by researchers based on their biocompatibility. Amongst these biopolymers, proteins offer the most promise especially for biological and biomedical applications because of their functionality, safety and biocompatibility [7] [23]. Hou et al. [4] produced nanotubes from glucose oxidase using a porous Al_2O_3 membrane as a template for the first time. Nanotubes have several advantages over other nanoparticles like nanospheres: 1) the exterior and interior layers of nanotube may have different properties; 2) nanotubes have two open-ended terminals located on both ends of the nanotube, these ends are useful for uptake and delivery of substrates and controlled release; 3) nanotubes have high stability and they can retain their tubular shapes *in vivo* for a long time [8]. Geng *et al.* [9] reported that polymeric cylinders of 8 µm lengths could remain stable about 10 times longer than spherical particles of the same composition after intravenous injection in rat bloodstream.

Nanotubes are produced by two methods, the first one is the self-assembly method which has been successfully used to prepare nanotubes from different proteins [10-15], but has several limitations; only a few molecules could be used in self-assembly because of their unique chemistry and functionality. The morphology and dimensions of the self-assembled nanotubes are defined by their biomolecular characteristics. The self-assembly method has low efficiency and requires extremely pure protein, high concentration and precise conditions for self-assembly. The second method is template assisted layer-by-layer deposition which is more easily performed and a highly versatile alternative to prepare nanotubes from polyelectrolytes as well as proteins. This method can be performed to produce nanotubes from proteins either by the chemical crosslinking of proteins [7]; [16-17] or by the electrostatic interaction between protein layers [8]; [18-21]. The template assisted LbL method by the electrostatic interaction is more biocompatible because nanotubes can be produced by one or more biopolymers without using any chemical crosslinking agent. The LbL deposition method has been used for a long time to produce thin films on the surface of the flat substrates. This method is conducted by deposition of negatively and positively charged polymers respectively on the surface of a template. If the template configuration is flat, deposition results in a thin film on its surface but if the template has the nanoporous configuration, deposition causes the formation of nanotubes into the nanopores of the template. Different templates have been used for nanotube preparation, two common types of which are anodic aluminum oxide (AAO) [7]; [17]; [22-23]; and track-etched polycarbonate (PC) membrane [8]; [19]; [21]; [24-28].

Bovine serum albumin (BSA) is a very efficient carrier in blood and milk. The BSA has a wide range of physiological functions like binding, transportation and distribution of bioactive compounds [29]. Bovine serum albumin contains two tryptophan residues; Trp 134 which is

located on the surface of the molecule and Trp 213 which is in a hydrophobic pocket in the native form of BSA [29].

The hydrophobic molecules, which are practically insoluble in aqueous solutions, are the best model bioactive compounds to evaluate different micro and nano-carriers. Curcumin, a natural polyphenol, is the main yellow pigment of the turmeric and is used as spice and food coloring agent and as a medicine for some disease such as inflammation, skin wounds, and tumors [30], as an antioxidant [31] and as a cancer chemo-preventive agent [31-32]. In recent years, researchers introduced several carriers to improve activity and stability of curcumin molecules, such as liposomes [33-35], micelles [36-38], Colloidal micelles or vesicles [39], various nanoparticles [40-43], peptide hydrogels [44], hollow microcapsules [45]; multilayer vesicles, tubes, porous structures and organo gels [46], and magnetic nanoparticles [47].

In this article, we demonstrate the feasibility of preparation of Poly-D-Lysine (PDL) and BSA NTs via electrostatic bonds in the interior of the Poly-Carbonate template pores. This article also reports on our efforts to evaluate the possibility of entrapment of a hydrophobic bioactive compound (curcumin) into the NTs. We also report the preparation of NTs resulting from the interaction of a protein (BSA) and a hydrophobic bioactive (curcumin) as a negatively charged layer.

2. Materials and Methods

2.1 Materials

Poly-D-Lysine hydrochloride (PDL, Mw ≥ 300 kDa), Bovine serum albumin (BSA, lyophilized powder, ≥98%, essentially fatty acid free, essentially globulin free), N, N-Dimethylformamide (DMF) (99.8%) were purchased from Sigma-Aldrich Co. Curcumin was kindly donated by ChromaDEX company. The water was deionized (18.2 MΩcm) using water purification systems. The track-etched polycarbonate (PC) membrane (Isopore membrane, 13 mm, pore diameter 400 nm) was purchased from Millipore Corporation.

2.2 Bovine serum albumin NTs fabrication

The protein nanotubes were prepared according to the previously reported technique with some modifications [8].

2.3 BSA-curcumin NTs fabrication

The BSA solution in PBS (2 $mgmL^{-1}$, pH 7.4, [NaCl]=0.138M) was mixed with curcumin dissolved in ethanol, with the molar ratio of curcumin to BSA of 1. The mixture was stirred for 30 min and the solution was used to produce the PDL/BSA-curcumin NTs using same method.

2.4 Loading capacity of NTs

The stock solution of curcumin in ethanol was diluted in BPS (pH 7.4, [NaCl]=0.138 M) to the final concentration of 0.01 $mg.mL^{-1}$. 3 mL of this solution was added to NTs released from one template (diameter 13 mm). It was found that the weight of $(PDL/BSA)_3$ NTs produced into each template pores was around 50 μg. The solution was stirred for 5 min to complete the hydration of NTs, then sonicated (for 25 s) and stirred for 1.5 h to enhance loading of curcumin molecules into the NTs cavities. After that the NTs were separated by centrifugation (5000×g, 15 min) and the supernatant was used for measurement of absorbance using UV/visible spectrophotometer (Infinite M200, Tecan Inc., USA). All mentioned steps were applied on the same concentration of curcumin without NTs, as the control sample. All experiments were conducted in triplicate. Averages are being reported as results.

2.5 Characterization of nanotubes by Scanning Electron Microscopy (SEM)

The morphology of NTs and template surface was characterized by field emission scanning electron microscopy (FE-SEM) under an acceleration voltage of 10 KV (S-4700, Hitachi, Japan).

3. Results and Discussion

The opposite electrostatic charges of PDL and BSA in a wide range of pH were the main driving force for the interaction of the molecules. By deposition of PDL and BSA onto the template pores and cleaning both surface sides of the template, biopolymers formed very thin tubular films into the pores of the membrane. Fig. 1 shows the SEM images of the surface of a template before and after deposition of three bilayers. For three bilayers deposition $(PDL/BSA)_3$, the nanotubes were strong and robust, they maintained their tubular structures after removing from template and drying (Fig. 2).

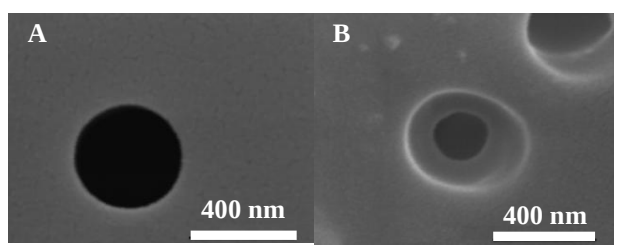

Fig. 1: The SEM images of surface of template without any treatment (A), and deposition of 3 BL after cleaning and wetting for 8 h (B).

3.1 Nanotubes dimension

The thickness of three bilayers of PDL/BSA was 61±6 nm; it is thicker than nanotubes produced from Poly-L-Arginine (PLA) (MW *ca.* 70 kDa) and human serum

albumin (HSA) reported by the method mentioned earlier [8].

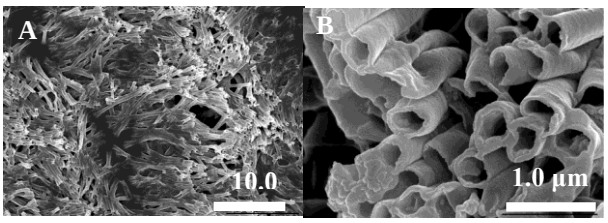

Fig. 2: The SEM images of NTs which produced by deposition of 3 BL (A-D).

3.2 Curcumin loading into Nanotube cavities

Figure 3 shows the visible spectrum of curcumin solution before and after mixing with ~50 µg $(PDL/BSA)_3$. The loading percent of curcumin into $(PDL/BSA)_3$ after 1.5 h was 44.9±3.9 %. The main reason for this high deviation in the loading ratio could be due to the variability in weight of NTs manufactured. This means that the entrapment efficiency of curcumin is 270±24 $\mu g.mg^{-1}$ of $(PDL/BSA)_3$ NTs. Manju and Sreenivasan [45], used hollow microcapsules which were produced from six bilayers of poly(ethylene imine) (PEI) and poly(sodium 4-styrene sulfonic acid) (PSS) to entrap curcumin. They reported that the entrapment efficiency of curcumin in microcapsules was 4.5 $\mu g.mg^{-1}$. However, the diameter of these microcapsules was bigger than our nanotubes, but the inherent affinity of BSA to interact with curcumin has been effective in improving entrapment efficiency. Qu and Komatsu (2010) produced PLA/HSA NTs in which the interior layer was formed by deposition of avidin from egg white. They found that 5-(6-biotinamidohexanoylamino) pentylthioureidyl-fluorescein (FITC-biotin) could incorporate into the NTs cavities if their particle size was smaller than cavity diameter [8]. It is concluded that the manipulation of the interior layer of NTs could be used to load special molecules into the NTs pores.

3.3 Preparation of NTs from BSA-curcumin interaction

In this case, the BSA solution is exposed to curcumin solution to interact, where the ratio of curcumin molecules to BSA molecule was 1.0. The zeta potential of the mixture of curcumin-BSA (-20.24±1.75 mV) was the same as BSA (-21.33±1.09 mV) solution in the same condition (2 $mg.mL^{-1}$, in PBS at pH 7.4). The deposition process was conducted with the same method, but after deposition of two bilayers, the template was completely clogged. To better understand the reason for this blockage, the template surfaces were cleaned with swab and saline solution (pH 12, [NaCl]=3 M), then water. Even after cleaning and drying for 1 h at high vacuum the flux was very low and the template was blocked in the first minute. After releasing the NTs from the template and drying them, SEM images were taken which showed clearly the NTs had big cavities (diameter bigger than 300 nm in dry state); the only reason for blocking of the template could be the higher hydrophobicity of cavities which could decrease passing of aqueous solution through the template. Fig. 4 shows the SEM images of $(PDL/BSA\text{-}Cur)_2$. The images showed that the walls of NTs produced from $(PDL/BSA\text{-}Curcumin)_2$ were not completely strong to endure all stresses during removing from template, washing and drying, since the NTs had been slightly deformed after these steps. It was similar to $(PDL/BSA)_2$. This method is very useful to improve the hydrophobicity of the NTs cavities which may improve loading of hydrophobic compounds into the NTs cavities.

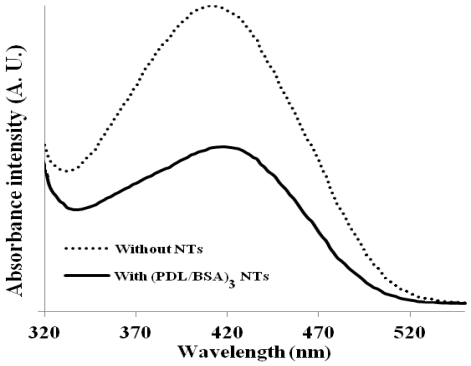

Fig. 3: The Visible spectrum of curcumin solution after mixing without and with NTs for 1.5 h

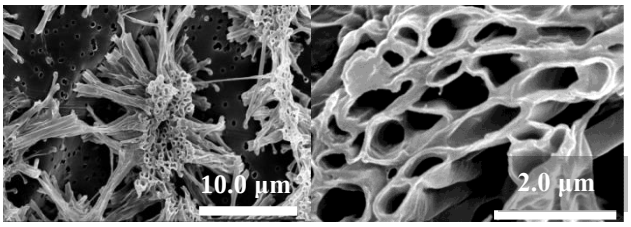

Fig. 4: The SEM images of $(PDL/BSA\text{-}Cur)_2$

4. Conclusion

The deposition of three bilayers led to strong and robust NTs which were completely tubular and stable. The average thickness of three bilayers of PDL/BSA was 61±6 nm. The main factor to entrap the hydrophobic bioactives into the NTs cavities was the hydrophobicity generated by the orientation of the hydrophobic groups towards the curcurmin in the interior layer of NTs. When the interior layer was fabricated from BSA, about 45% of curcumin molecules loaded into NTs. After deposition of two bilayers of PDL/BSA-Cur into the template pores the template was blocked. The SEM images showed that the $(PDL/BSA\text{-}Cur)_2$ NTs had relatively larger cavities allowing for the PDL solution to flow through it, until ultimately it clogged the NTs; the only reason for blocking the template could be the higher hydrophobicity of the NTs cavities which

prevented the passing of aqueous solution through the NTs cavities.

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