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Nanoporous multilayer films for controlled antigen protein release

Miso Yang, Daheui Choi, Moonhyun Choi, Jinkee Hong *

School of Chemical Engineering and Material Science, Chung-Ang University, 47 Heukseok-ro, Dongjak-gu, Seoul 156-756, Republic of Korea

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ABSTRACT

We have studied the preparation and characterization of nanoporous thin films fabricated from a layer by layer assembly of branched poly(ethylene imine), gold nanoparticles (gold NPs) and hyaluronic acid. Gold NPs embedded in the multilayer thin film structure were easily dissolved using an aqueous cyanide solution, generating the nanoporous film. This allows for the direct and precise comparison of the porosity of the polymer based multilayer thin films in both the presence and the absence of gold NPs. Additionally, the layer by layer (LbL) assembly method has advantages in that it increases the possibilities for mass production of multilayer films, and allows the precise control of film structures prepared on large substrates. Therefore, this method offers a new route to allow the introduction of nanoporosity into multilayer thin films, allowing the development of more complex functional multicomponent nanoporous structures for protein delivery. Furthermore, these novel materials could be used in biomedical applications.

Introduction

The functional surface engineering of nanomaterials is essential to improve the properties of materials used in the biomedical field, including drug delivery systems [1–3]. In particular, thin films that control the release of biologically active molecules, such as proteins [4,5], therapeutics [6–8], and DNA [9] from their outer surfaces for local 'smart' delivery, could improve the development of nano-coatings for biomedical devices and tools. A number of challenges, which had the goal of producing the time controlled release of biomolecules from thin films, have been published [7,10,11]. Despite the fact that significant progress in achieving this goal has been made, many of the published processes still have limited number of modes of molecule delivery and present challenging demands. In particular, for certain clinical events, therapeutics must be released within a specific time period; in practice this has been difficult to achieve.

Layer by layer (LbL) assembly has been widely employed as a versatile method for fabricating multilayer nano-sized films that have a controlled structure and composition [10,12–15]. It is based on the sequential adsorption of molecules and nano-objects that have complementary interactions such as electrostatic interactions [16–18] or hydrogen bonding [15,19,20]. Recent advances in surface drug delivery materials prepared by LbL assembly include [21–23] growth factor [11,24,25], siRNA [26,27], and protein delivery [28–30], all of which were incorporated into nanoporous multilayer films. However, considerable attention has been brought to the idea of directly incorporating therapeutics into thin films during the fabrication process. One useful approach would be to enable the incorporation of proteins as a building block of the films themselves. That is, the proteins act as a deliverable cargo and are incorporated in the LbL films.

Herein, we report the preparation of a protein delivery thin film that would enable the delivery of ovalbumin (Ova), which is a 45 kDa globular protein used as a model vaccine antigen [31]. We have examined a novel and simple method to develop nanoporous thin films by introducing a nano-sized space via gentle dissolution of the nanoparticles from composite multilayer films containing gold nanoparticles (gold NPs) and polymers. We found that the most important factor in preparing a well ordered nanoporous structure in the films was to achieve an even distribution of gold NPs in the multilayer structures. For this reason, we have taken full advantage of the LbL assembly method. We used a tetralayer structure that comprised positively charged gold NPs, branched poly(ethylene imine) (BPEI), and negatively charged hyaluronic acid (HA). The tetralayered (BPEI/HA/gold NPs/HA)$_n$ (n = number of tetralayers) structures were used to generate the empty nanopore structures, which were obtained upon the cyanide induced chemical dissolution of the gold NPs embedded in the (BPEI/HA/gold NPs/HA)$_n$ multilayer films. We have employed these

* Corresponding author.
E-mail address: jkhong@cau.ac.kr (J. Hong).

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nanoporous films as carriers of ova, thus demonstrating the integration of a globular protein within a polyelectrolyte multilayer. We have demonstrated that the resulting films released protein (ova) into the surrounding medium under physiological conditions by the diffusion of encapsulated ova out of the nanoporous film (Fig. 1).

Experimental

Materials

The positively charged gold nanoparticles (gold NPs) were synthesized following the previously reported method of D. I. Gittins and F. Caruso [32]. Chemicals for synthesis of the gold NPs were purchased from Sigma–Aldrich, except 4 (Dimethylamino) pyridine (DMAP), which was purchased from Aldrich chemistry. Branched Poly(ethylene imine) (BPEI) with a molecular weight of ~25,000 was obtained from Sigma–Aldrich. Sodium hyaluronate (HA) with a molecular weight of 10,000 was obtained from Lifecore Biomedical. Potassium cyanide (KCN) was purchased from Sigma–Aldrich and used without further purification. Ovalbumin (ova) was purchased from Bio Basic Inc. Minimum essential medium alpha (MEM α) was obtained from Gibco® Life Technologies.

Film construction

The films were fabricated onto silicon wafers and quartz glass. The substrates were treated with either O_2 plasma for 2 min or RCA treatment for 10 min prior to use. The substrate was subsequently dipped into pH adjusted aqueous solutions of BPEI (pH 9.5), HA (pH 9.5), gold NPs (pH 10.5), and HA (pH 9.5), each for 10 min, and then washed with pH adjusted water for 3 min. The concentrations of the BPEI and HA solutions were both 1 mg/mL. Gold NPs were diluted with DI water (1:50 vol%). The multilayer films were then heated in vacuum oven at 90 °C for 3 h.

Dissolution of the gold NPs

To obtain the nanoporous film, the gold NPs were removed from the multilayer films according the reaction equation shown below. The fabricated films were placed into an aqueous solution of KCN (5 mM) for 30 s.

$$4Au + 8KCN + O_2 + 2H_2O \rightarrow 4K[Au(CN)_2] + 4KOH$$

Characterization equipment

Energy filtering transmission electron microscopy (EF TEM) images of the gold NPs were obtained using a LIBER 120 (Carl Zeiss). The ζ potentials of the NPs were measured using a particle size analyzer (SZ 100, Horiba). Film thickness was determined by profilometer (Dektak 150, Veeco). The film and pore morphologies were characterized by field emission scanning electron microscopy (FE SEM) (SIGMA, Carl Zeiss). Film absorbance and transmittance were measured using an ultraviolet visible spectrometer (Evolution 300, Thermo Scientific). The refractive indices of the multilayer films on Si wafers were measured by ellipsometry (Gartner Scientific Corp., L2W15S830) with 632.8 nm He Ne laser light. The refractive index was allowed to vary in fitting the ellipsometric data by an iterative process. Atomic force microscope (AFM) measurements were carried out in tapping mode (Nanoscope IIIa, Digital Instruments).

Release characterization

Ova loaded films were dipped into alpha minimum essential media (αMEM) (15 mL) at 37 °C in an incubator. At a series of different time points, 0.3 mL of the αMEM solution containing the films was transferred to a conical tube and then filled with fresh solution of αMEM and made up to same volume.

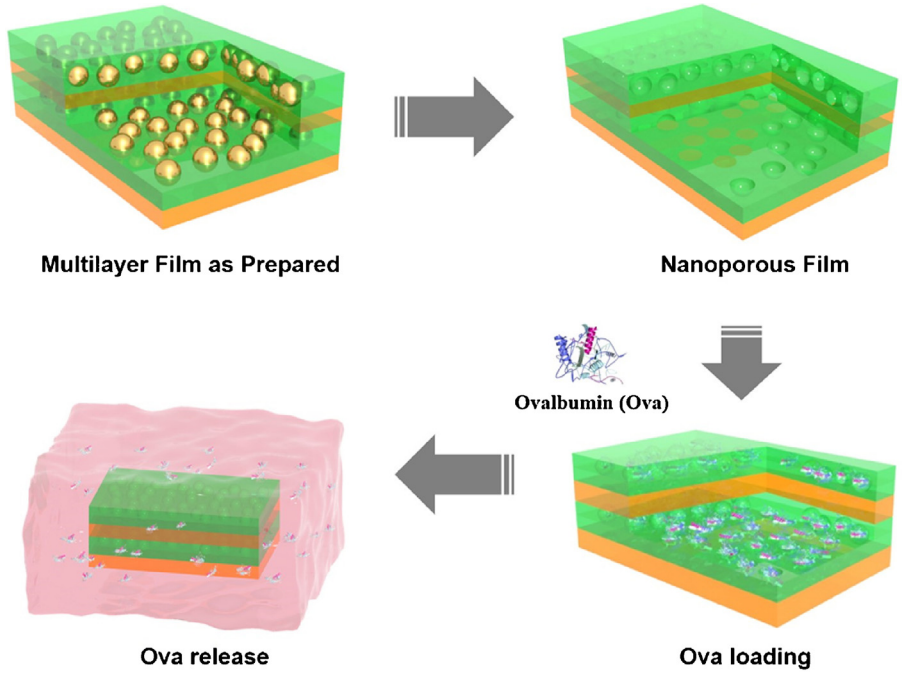

Fig. 1. General strategy for the preparation of the (BPEI/HA/gold NPs/HA)$_n$ (n = number of tetralayers) structure based nanoporous films and subsequent ova release under model physiological condition.