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Polyelectrolyte/silver nanocomposite multilayer films as multifunctional thin film platforms for remote activated protein and drug delivery

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ABSTRACT

We demonstrate a nanoparticle loading protocol to develop a transparent, multifunctional polyelectrolyte multilayer film for externally activated drug and protein delivery. The composite film was designed by alternate adsorption of poly(allylamine hydrochloride) (PAH) and dextran sulfate (DS) on a glass substrate followed by nanoparticle synthesis through a polyol reduction method. The films showed a uniform distribution of spherical silver nanoparticles with an average diameter of 50 ± 20 nm, which increased to 80 ± 20 nm when the $AgNO_3$ concentration was increased from 25 to 50 mM. The porous and supramolecular structure of the polyelectrolyte multilayer film was used to immobilize ciprofloxacin hydrochloride (CH) and bovine serum albumin (BSA) within the polymeric network of the film. When exposed to external triggers such as ultrasonication and laser light the loaded films were ruptured and released the loaded BSA and CH. The release of CH is faster than that of BSA due to a higher diffusion rate. Circular dichroism measurements confirmed that there was no significant change in the conformation of released BSA in comparison with native BSA. The fabricated films showed significant antibacterial activity against the bacterial pathogen *Staphylococcus aureus*. Applications envisioned for such drug-loaded films include drug and vaccine delivery through the transdermal route, antimicrobial or anti-inflammatory coatings on implants and drug-releasing coatings for stents.

1. Introduction

In recent years the use of polyelectrolyte multilayers (PEMs) fabricated by layer-by-layer (LbL) self-assembly of polycations and polyanions has emerged as a powerful and versatile strategy to engineer surface films for biofunctionalization and drug delivery [1–3]. Overcompensating adsorption, with more than equal charge, allows for charge reversal on the surface, which has two important consequences [1]: first, repulsion of equally charged molecules and thus self-regulation of the adsorption and restriction to a single layer; second, the ability of an oppositely charged molecule to be adsorbed in a second step on top of the first one. Although the driving forces for LbL assembly are primarily electrostatic interactions, they can also involve non-electrostatic interactions such as hydrogen bonding, host–guest interactions, hydrophobic interactions and charger transfer halogen interactions [3]. The physicochemical characteristics of the films, such as thickness, stiffness, chemistry, stability, permeability, composition, biofunctionality and dynamics, can be tunable to a large extent and they can act as a key to modulate the interaction with biomolecules and cells [4–7]. PEMs based on small organic molecules or inorganic compounds, macromolecules, drugs, biomacromolecules, e.g. proteins, DNA, lipids, polypeptides, polysaccharides or even colloids, and other functional components have been reported [3,8–10]. It is known that enzymes incorporated in films keep their catalytic activity and have a high tolerance to harsh conditions [11,12]. Adsorption and embedding of fibrinogen in multilayers made from polystyrene sulfonate (PSS) and poly(allylamine hydrochloride) (PAH) preserve the secondary structure of the protein [13]. The important feature of PEMs is that they can be conformally coated over different surfaces, including biological and synthetic materials. They can also potentially be prepared *in-situ* on a wound bed, be pre-assembled and transferred from a flexible sheeting material to a wound bed or be used to directly functionalize implantable medical devices [14,15].

One of the main challenges in biotechnology and nanomedicine is to develop a system which is able to provide the controlled release of bioactive compounds. Several reports have demonstrated that PEMs provide unique and attractive thin film platforms for the controlled release of both small molecular drugs and macromolecular therapeutics [2,8,10]. Various electrostatically assembled PEM films have been developed to release therapeutics such as painkillers, anti-inflammatory drugs, antibiotics, and growth factors [2,3]. Films containing functional components within the layered film architecture offer the ability to vary not only the amount of active agent but also to trigger the release and thus enable control "on demand" using external triggers [16]. In general

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drug release from PEMs can be activated either by changing the membrane permeability or directly disrupting the films using strategies based on (i) changes in environmental variables such as pH or ionic strength that disrupt ionic interactions in these assemblies, (ii) incorporation of polyelectrolytes with functionality that can be chemically or enzymatically cleaved, (iii) fabrication of films based on receptor–ligand interactions that can be disrupted in response to specific small molecules, or (iv) the application of light, magnetic, ultrasound, electrical potential, or other external stimulus [16]. In most of these cases, however, the films show rapid "burst" release of the encapsulated drug due to rapid and large changes in the film permeability induced by these methodologies. For instance, Caruso and co-workers reported that catalase was released from poly(L-lysine)/poly(L-glutamic acid) capsules within 30 min when the pH and ionic strength changed [17]. Thus it is necessary to design multilayer films that can provide sophisticated control over the timing and the rate at which the drug is released. This is highly attractive in view of the obvious advantages of controlled release, such as high efficiency and lower toxicity.

Among the different strategies mentioned above non-invasive release of bioactive molecules from the films requires a special focus, since it delivers bioactive molecules to a cell without any alterations to the surrounding medium. For example, cells are generally very sensitive to variations in pH, ionic strength, etc. [18]. In order to overcome this limitation in biomedical applications, non-invasive techniques such as laser irradiation and ultrasonication are used as triggers to induce remotely activated release of the loaded molecules. Thus it is desirable to obtain multilayer films possessing stimuli-responsive moieties for these triggers that would alleviate potential side-effects on biological tissues.

In general gold/silver nanoparticles (NPs) or infrared dyes have been included in the PEM films in order to make them responsive to non-invasive triggers. Here we have used silver NPs for our studies, since silver is used to control bacterial growth in various applications, such as dental work, catheters, and burn wounds. Two methods have been reported to incorporate silver ions or particles into PEM films for antibacterial coating purposes, depending on the PEM film preparation process [19–22]. In the first method, a complex of a polyelectrolyte with silver ions or preformed silver NPs is prepared, followed by assembling with another polyelectrolyte of opposite charge. In the second method a PEM is assembled from weak polyelectrolytes and then, by pH manipulation, free ionized groups (ion binding sites) are created in the film, capitalizing on the dissociation equilibria of the weak electrolytes. These ionized groups are used to bind silver ions via ion exchange, and the silver ions can be reduced *in-situ* to produce silver NPs. However, the reduction of silver ions (Ag^+) to silver metal (Ag^0) is often achieved using hazardous chemicals, such as $NaBH_4$, dimethylformamide or hydrazine. Other safer biomolecules such as sugar, sodium alginate or alcohol can also be used for this but then surfactants are required to stabilize the NPs [23,24]. Among various alcohols diols like polyethylene glycol (PEG) can be used to prepare silver NPs, acting as a reducing as well as stabilizing agent [9]. Compared with other reducing agents PEG is a biologically benign material and is widely used as a prodrug in the pharmaceutical and biomedical industries. Here we report a new method to introduce silver ions into a PEM by a rapid ion exchange process, which can then be converted into silver NPs using PEG to create composite thin films. This method provides a convenient route to the fabrication of silver-containing PEMs with controllable silver loading. Further, the process is simple and economical, does not involve any toxic chemicals and all the polyelectrolytes used in this study are considered non-toxic.

With this background we aimed to add increased functionality to these silver-containing PEM thin films for multiple drug delivery and investigate their potential for remote activated delivery of bovine serum albumin (BSA) and ciprofloxacin hydrochloride (CH). We demonstrate that using our newly developed nanoparticle loading protocol, transparent silver-containing PEM films can be easily prepared. These films allow spatial control over the absolute and relative locations of proteins and pharmaceutical drugs within them (e.g. by depositing the protein as one layer and loading the drug within the layer porosity). Provided that film disassembly can be induced by laser light or ultrasonic exposure and this approach can be a new method of loading and releasing multiple drugs for remotely activated delivery. In addition, films having such a molecular structure provide the opportunity to design films that could provide sophisticated control over the timing and order with which the protein and drug are released (e.g. simultaneous release, sequential release, pulsatile release).

2. Experimental

2.1. Materials

Dextran sulfate (DS) (M_W 500 kDa), poly(allylamine hydrochloride) (PAH) (M_W 70 kDa), poly(ethylene glycol) (PEG) (M_W 6 kDa), BSA (M_W 66 kDa), fluorescently labeled BSA (FITC-BSA), rhodamine, phosphate buffered saline (PBS) and silver nitrate are commercially available and were purchased from Sigma–Aldrich. All chemicals were used without any further purification. CH was a gift from Dr. Reddy's Laboratories Ltd. (India). Water from a Milli-Q system with a resistivity greater than 18 MΩ cm was used for all experiments. All pH adjustments were done with 0.1 M HCl or 0.1 M NaOH.

2.2. Substrate preparation

Quartz slides (substrates) were initially cut into 8 mm × 8 mm pieces and washed ultrasonically with isopropanol–water mixture (75:25 vol.%) for 15 min followed by rinsing with deionized water. This cleaning step was repeated at least three times and then the substrates were blow-dried with N_2 gas.

2.3. PEM film preparation

PEMs of PAH and DS were assembled on cleaned quartz slides using a hand dipping procedure. Aqueous solutions of PAH and DS (1 mg ml^{-1}) were prepared in 0.2 M NaCl solution, adjusted to the desired pH using either 0.1 M HCl or 0.1 M NaOH, and used to prepare the PEMs. Multilayer assembly was performed in 5 ml plastic vials at pH 5. The multilayer construction was accomplished by successively dipping the quartz substrates in the PAH and DS solutions (alternating between PAH and DS), followed by 15 min adsorption and subsequent rinsing three times with pH adjusted water for 1 min. Deposition of a PAH layer and a DS layer completes one cycle, termed a single bilayer formation. This process of sequential polyelectrolyte adsorption and rinsing was repeated to obtain the desired number of polyelectrolyte bilayers. After assembly the PEM films were dried under vacuum for 1 h and stored under ambient conditions.

2.4. Nanoparticle synthesis in the PEMs

Synthesis of silver NPs within the PEMs was initiated by incubating the pre-assembled PAH/DS multilayers (6 bilayers) in an aqueous solution of silver nitrate (25 or 50 mM) at pH 6 for 1 h. As mentioned in earlier reports [25,26], Ag^+ ions in the solution diffuse into the PEMs and exchange with acid protons of SO_4^- groups present in DS. After ion exchange the films were washed to remove Ag^+ ions present in the bulk solution. Subsequent reduction of the