Materials Science and Engineering C 58 (2016) 622–628

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Stimuli-responsive weak polyelectrolyte multilayer films: A thin film platform for self triggered multi-drug delivery

S. Anandhakumar [a,*], P. Gokul [a], A.M. Raichur [b]

[a] SRM Research Institute, SRM University, Kattankulathur, Chennai 603203, India
[b] Department of Materials Engineering, Indian Institute of Science, Bangalore 560012, India

ARTICLE INFO

Article history:
Received 14 May 2015
Received in revised form 12 August 2015
Accepted 22 August 2015
Available online 29 August 2015

Keywords:
Stimuli-responsive
Multilayer films
Dual drug delivery
Ciprofloxacin hydrochloride

ABSTRACT

Polyelectrolyte multilayer (PEM) thin film composed of weak polyelectrolytes was designed by layer-by-layer (LbL) assembly of poly(allylamine hydrochloride) (PAH) and poly(methacrylic acid) (PMA) for multi-drug delivery applications. Environmental stimuli such as pH and ionic strength showed significant influence in changing the film morphology from pore-free smooth structure to porous structure and favored triggered release of loaded molecules. The film was successfully loaded with bovine serum albumin (BSA) and ciprofloxacin hydrochloride (CH) by modulating the porous polymeric network of the film. Release studies showed that the amount of release could be easily controlled by changing the environmental conditions such as pH and ionic strength. Sustained release of loaded molecules was observed up to 8 h. The fabricated films were found to be biocompatible with epithelial cells during in-vitro cell culture studies. PEM film reported here not only has the potential to be used as self-responding thin film platform for transdermal drug delivery, but also has the potential for further development in antimicrobial or anti-inflammatory coatings on implants and drug-releasing coatings for stents.

1. Introduction

In recent years, PEM films prepared by alternate adsorption of oppositely charged polyelectrolytes on to a charged surface have received great attention in the biomedical field [1]. It is mainly due to their ability to be assembled in-situ on a wound bed, be pre-assembled and transferred from a flexible sheeting material to a wound bed or to be directly functionalized on implantable medical devices [2,3]. PEM films owe its popularity to the fact that it can be conformally coated over different surfaces, including biological and synthetic materials [4]. Various PEM film based drug delivery formulations have been developed to deliver therapeutics such as painkillers, anti-inflammatory drugs, antibiotics, proteins and growth factors [5,6]. Among various PEM films reported to date, PEM films made up of weak polyelectrolytes have attracted greater interest because of their distinct and reversible change of properties in response to external stimulus [7,8]. The use of weak polyelectrolytes is especially attractive for drug delivery applications because the risks of inducing toxicity are higher when strong polyelectrolytes are used. The intrinsic property of weak PEM is that the surface properties of the film such as thickness, roughness, composition and permeability could be easily manipulated by controlling the interactions between the polymers and the surface [9–12]. When there is a change in environmental stimulus such as pH, ionic strength and polarity, it induces charge imbalances in the film [8,13,14]. The films can even be destroyed at extreme conditions as the induced imbalance of charges overcompensates for the attractive polymer–polymer and/or polymer–surface interactions [2,15,16]. The softness and ability to swell and contract in aqueous media make these films compatible with biological systems, thus making them potential candidate for different biomedical applications such as cell adhesion, wound healing, drug delivery and antibacterial coatings [17,18].

Drug delivery with polymer films is an emerging field and needs new methodologies and tools to achieve its goals. For instance, there have been strong interests in the specific use of polymeric films for localized drug delivery. Various thin films have been reported for treating various diseases such as periodontal disease, glaucoma, and cancer by using the films directly as a means for drug encapsulation or as a polymer coating on metallic stents, and even as fully biodegradable stents [19–23]. One of the main challenges in fabricating thin film based drug delivery platform is to develop a system which is able to: 1) provide controlled release of loaded molecules, 2) preserve the biological activity of loaded molecules and 3) load more than one drug and/or protein in it. Many attempts have been devoted to achieve these requirements, it includes incorporation of nanoparticles in the film and use of diblock copolymers for the fabrication of polymer films to control and optimize the initial burst release [23,24]. Other factors, such as thickness, stiffness, chemistry, stability, permeability, composition, biofunctionality and dynamics, have also been explored and they can act as a key to modulate the drug release rates and profiles [25–27]. Though several attempts have been made, the success of formulating a single drug

* Corresponding author at: SRM Research Institute, SRM University, Kattankulathur, Chennai 603203, Tamil Nadu, India.
E-mail address: rsanandhakumar@gmail.com (S. Anandhakumar).

http://dx.doi.org/10.1016/j.msec.2015.08.039
0928-4931/

delivery system to fulfill all the requirements has only been minimal. Thus it is important to design multilayer films that can provide sophisticated control over the timing and the rate at which the drug is released. In addition, encapsulation and release of two or more drugs from a single system provide many advantages over conventional formulations, such as improved treatment and patient compliance, high drug concentration on site over extended periods and also reduces undesired side effects of the drug. Recently, codelivery of siRNA and cancer drug was reported by the group of Hammond [28] and codelivery of protein and CH by us [29]. In the latter work it was demonstrated that the encapsulated protein and CH were successfully released in a sustained manner when exposed to external triggers such as laser light and ultrasound [29].

Given the ability of multilayer films consisting weak polyelectrolytes to be altered from pore free film to porous film by changing the environmental stimuli such as pH and ionic strength, and to load various drug molecules and to regulate the release of drugs, we are interested in investigating these films for transdermal drug delivery. This method not only offers advantages of needle free drug delivery but also provides an easy option to limit first-pass drug metabolism. We believe that drug and/or protein-loaded multilayer films coated on the surface of the skin adhesive patch or wound dressing could provide several attractive features for transdermal drug delivery: i) multilayers preserve the loaded proteins/biomolecules in native form [30,31]; ii) high drug concentration in the film would provide a strong driving force for the drugs to penetrate into the skin; iii) more than one drug could be loaded in the film and the kinetics of release of individual drug could be easily regulated to optimize the therapeutic response [28,29]; iv) the change in wound pH when compared to normal skin could be successfully utilized to trigger the release of loaded molecules from the film; and v) the versatility of PEM coating on various substrates would allow this concept to be implemented in many different ways, including coatings of simple skin adhesive patches, woven fiber adhesive patches, micro-needle arrays, implants and drug-releasing coatings for stents.

In this study, we report a thin film platform for the encapsulation and release of protein and pharmaceutical drug for self triggered (environmental stimuli responsive) drug delivery. Here, protein is only a model molecule, it can be growth factors, antigens and proteins in actual applications. In this work, we extended our research on PEM films composed of weak polyelectrolytes for encapsulation and release of BSA and CH. Since the weak polyelectrolytes have special tendency to realign their polymeric configuration under the influence of pH and ionic strength, it is interesting to study on how these properties can be utilized to release the loaded molecules from the film. This work will not only expand the knowledge on the preparation of PEM films based on weak polyelectrolytes, but also would benefit the application of stimuli-responsive drug delivery.

2. Experiment

2.1. Materials

PAH ($M_w = 70$ kDa), PMA ($M_w = 483$ kDa) and BSA were all purchased from Sigma Aldrich (India) and used without any further purification. Ciprofloxacin hydrochloride (CH) was a gift by 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 were initially cut into 8 mm × 8 mm pieces and used for LbL assembly of PAH and PMA. Before assembly, quartz pieces were cleaned ultrasonically with isopropanol/water (75 mL/25 mL) mixture for 30 min followed by rinsing three times with water.

2.3. PEM film preparation

Polyelectrolyte solutions of 1 mg/mL were prepared in 0.2 M NaCl solutions. PEM film was assembled at pH 5 which maintained the polyelectrolytes at the fully charged state. Since the quartz substrates are negatively charged, the assembly was started with PAH adsorption. The adsorption step was 15 min, followed by washing three times with water to remove loosely bound molecules from the surface. The adsorption and washing processes were then repeated for PMA. Deposition of one PAH layer and one PMA layer is regarded as one cycle and is termed a single bilayer formation. This process of sequential adsorption and washing was repeated until a desired number of PAH/PMA bilayers were obtained. After assembly, the films were rinsed thoroughly with water, dried in a nitrogen stream and stored in a desiccator for further characterization.

2.4. Loading of BSA and CH in PEM films

The procedure for selective deposition of BSA and CH has been described in our previous study [29]. In brief, six bilayers of PAH/PMA PEM films were prepared as described above. Then these samples were dipped in BSA for 30 min at pH 4 and washed three times with pH adjusted water to remove BSA present in supernatant. Here BSA is a model protein, and can be deposited as a layer by multimode interactions such as electrostatic interactions, hydrogen bonding and hydrophobic interactions [18,32]. After BSA adsorption, two bilayers of PAH/PMA were added to maintain the surface charge negative, which favors the loading of CH by electrostatic interactions [29]. For CH loading, $(PAH/PMA)_8$ films were dipped in CH solution (5 mg/mL) for 30 min at pH 4, which allowed the drug to permeate and be loaded in the film. Rinsing was not performed after CH loading, which provided a means of incorporating excess unbound drug available for immediate diffusive release upon immersion in a physiological environment. It is important to note that the drug excess is advantageous since it could serve as a loading dose to reach therapeutic concentration. The sustained release obtained from loaded drug could be used for maintaining the drug concentration within therapeutic limit for extended periods. The final drug loaded samples were vacuum dried, stored at 4 °C and later used for in-vitro release experiments.

Protein/drug loading of the film was quantified as the absorbance difference prior to and after loading protein/drug in the film using a UV–Visible (UV–Vis) spectrophotometer (Nanodrop 2000c, Nanodrop Technologies, U.S.A.).

2.5. Atomic force microscopy (AFM) imaging

The surface morphology of PAH/PMA films was investigated by AFM (MFP-3D AFM, Asylum Research, U.S.A). Imaging was done in air by tapping mode using NCR-20 cantilevers having a resonance frequency of 285 kHz and force constant of 42 N/m (Nanoworld, Switzerland). At least 5 images were recorded from different areas of the sample to obtain consistent results.

2.6. Thickness measurements

The film thickness was determined using a SE850 spectroscopic ellipsometer (Sentech Instruments, Germany) over a spectral range of 300 to 800 nm at an incidence angle of 70°. At least 5 measurements were made at different spots and averaged.

2.7. In-vitro release experiments

To investigate the influence of pH on drug release, the drug loaded films were immersed into 4 mL of pH adjusted water (pH 1.2 or 7.4) in a tightly capped plastic vial maintained at 37 °C in an incubator shaker. The vial was kept sealed during the experiments to minimize the