Acta Biomaterialia 8 (2012) 4380–4388

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

The influence of arrangement sequence on the glucose-responsive controlled release profiles of insulin-incorporated LbL films

Xingyu Chen [a], Jun Luo [a], Wei Wu [a], Hong Tan [a], Fujian Xu [b], Jianshu Li [a,*]

[a] College of Polymer Science and Engineering, Sichuan University, Yi Huan Road, South Section One, No. 24, Chengdu 610065, People's Republic of China
[b] College of Materials Science and Engineering, Beijing University of Chemical Technology, Beijing 10029, People's Republic of China

ARTICLE INFO

Article history:
Received 28 April 2012
Received in revised form 1 August 2012
Accepted 9 August 2012
Available online 16 August 2012

Keywords:
Drug delivery
LbL films
Star polymer
Controlled release
Insulin

ABSTRACT

Insulin, glucose oxidase and positively charged star polymers were incorporated into multilayer films by the layer-by-layer (LbL) assembly method. It is interesting to find that the arrangement sequence of the three components could significantly affect the glucose-responsive controlled release behaviors. The insulin release in vitro could be tuned to linear release and obtain desired "on–off" sensitivity in response to stepwise glucose challenge, just by rearranging the assembly sequence of LbL building blocks. Further, the controlled release of insulin in vivo, as well as the hypoglycemic effect, could be obviously prolonged from 17 days to 36 days by this simple strategy without changing the dosage of all the LbL components. In addition to provide a potential glucose-responsive delivery system for insulin, the strategy described in this paper could be valuable for various drug-incorporated LbL systems with three or more components.

1. Introduction

One of the most critical challenges in diabetes treatment is to design self-regulated insulin (INS) delivery systems, which could control INS release in response to the change in the blood glucose level (BGL), just like artificial pancreases. Various INS delivery systems with stimuli-sensitivity to different external stimuli, such as glucose [1–10], pH [11–18] and pH/temperature [19–22], which could control INS release in response to external environmental changes, were investigated. It is also important to maintain INS release levels within the narrow concentration window required to avoid hypoglycemia due to overdose or ineffective treatment from underdosing. Thus, it is required that a single dose could sustain INS levels within the desired therapeutic range for a long period in response to the change in BGL. For example, Gupta et al. [23] harnessed the inherent property of INS to aggregate into a supramolecular assembly, which generates a formulation that could release INS monomer for extended periods. Huynh et al. [21,22] fabricated a pH/temperature-sensitive hydrogel of pentablock copolymer, and found that a single injection of a complex mixture containing 10 mg ml^{-1} INS in 30 wt.% copolymer solution can achieve a therapeutic effect for more than 1 week in vivo.

The layer-by-layer (LbL) assembly method has been widely applied in drug delivery systems because of its simple yet highly versatile choice in component selection and flexibility of structural design, for which the mechanism and applications have been reviewed elsewhere recently [24–27]. For example, Shah et al. [28] demonstrated that a tunable delivery of two kinds of growth factors could be achieved from a series of designed LbL multilayer films. Yoshida et al. [12,15] and Sato et al. [29] fabricated INS-containing LbL films as potential oral delivery systems. The LbL films were assembled though alternate adsorption of positively charged INS and polyanions, which are satisfactorily stable even in the presence of the digestive enzyme at pH 1.4 and can release INS at weakly acidic or neutral pH. It is proved that the release mechanism is the loss of electrostatic forces of attraction between the INS and polyanions due to the charge reversal of INS from negative to positive. Recently, the present authors reported a glucose-sensitive multilayer film fabricated by the LbL assembly method with positively charged 21-arm poly[2-(dimethylamino)ethyl methacrylate] (star-PDMAEMA), and negatively charged INS and glucose oxidase (GOD). The multilayer film is sensitive to the environmental stimuli (pH and glucose), owing to the unique structure of star-PDMAEMA [30–32]. It could continuously release enough INS in vivo after being subcutaneously implanted in diabetic rats and reduce the BGL for 2 weeks [31]. However, a burst release at the beginning and a fluctuating release profile of INS could also be observed as well as the control effect on BGL.

Although it is obvious that an increase in the number of drug-containing layers or the loading amount of drug on each layer could obtain a longer release time, there are very few reports about how to prolong the drug release time from LbL films at the same drug dose, except for several strategies based on chemical modifi-

* Corresponding author. Tel.: +86 28 85466755; fax: +86 28 85405402.
E-mail address: jianshu_li@scu.edu.cn (J. Li).

1742-7061/$ - see front matter © 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2012.08.014

cation [33–36]. For example, Zhang et al. [33] demonstrated that it is feasible to tune the erosion rate of LbL film and extend the release time of plasmid DNA from 2 days to 2 weeks using hydrolytically degradable polymers with different side-chain structures. Shukla et al. [36] and Macdonald et al. [36] found that modulating the degradability of synthetic polymers used during LbL films assembly could strongly influence the film degradation properties as well as the incorporation and release profiles of drugs. However, the above strategies involve the multistep synthesis of carefully designed polymers, thus a more facile method of prolonging the drug release time from LbL films at the same drug dose needs to be developed.

This work presents a simple strategy that the drug controlled release time from LbL films could be significantly prolonged simply by optimizing the arrangement sequence. As shown in Scheme 1, INS and GOD are incorporated into multilayer films, along with positively charged star polymers (star-PDMAEMA), in three different ways. Considering that the isoelectric points of INS and GOD are 5.4 and 4.2, respectively, the pH condition of adsorption solutions in the process of fabricating LbL films was adjusted to make them negatively charged, which could reasonably be absorbed on the layer of positively charged star polymers by electrostatic attraction. More details about the LbL films, e.g., the relative loading of building blocks and charge balance in the films, have been discussed in previous work [31]. In this system, the release of INS may result in a partial decomposition of the LbL film, since INS itself forms film layers. However, in addition to triggering the sensitivity of the LbL films by converting glucose into gluconic acid and changing the pH in the microenvironment, the GOD layer can also play a role in keeping the integrity of the film. As a result, a suitable arrangement sequence of INS/GOD/star-polymer within the LbL film could coordinately control the film decomposition and INS release profile. The mechanism was thoroughly investigated, and the hypoglycemic effect of the system was also studied in streptozotocin (STZ)-induced diabetic rats.

2. Materials and methods

2.1. Materials

INS was purchased from Xuzhou Wanbang Biological Pharmaceutical Enterprise (Jiangsu, China). Streptozotocin, heparin sodium, pentobarbital sodium, glucose, GOD and fluorescein isothiocyanate (FITC) were purchased from Baoxin Biotechnology Co. Ltd. (Chengdu, China). Poly(methyl methacrylate) tablets (PMMA, ϕ 20 mm) were used as film substrates. The "RCA clean" protocol (sonicated in a 1:1 mixture of water and 2-propanol for 15 min, followed by heating at 70 °C for 10 min in a 5:1:1 mixture of water, H_2O_2 (30% in water), and a 29% (v/v) ammonia solution) was applied to clean the PMMA tablets and hydrophilize the surface. Star-PDMAEMA (21-arm, MW 149,500) was synthesized following previous reports [30,31,37–39]. Quartz crystal microbalance (QCM) electrodes (titanium/gold crystals, resonant frequency 5 MHz) were from Stanford Research System. (USA). A home-built QCM, constructed using a Model QCM 100 analog controller (Stanford Research System Products, USA) and an Agilent 53131A universal counter, was employed for the microgravimetric experiments. Ultrapure water produced from a Millipore system with resistivity 18.2 MΩ·cm was used throughout.

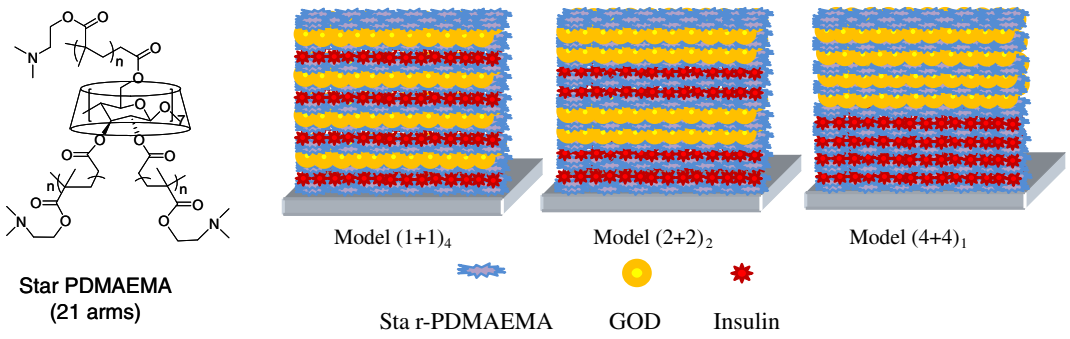
2.2. Insulin loading and release in vitro

2.2.1. Insulin loading

LbL films were fabricated on PMMA tablets with three different arrangement sequences, as shown in Scheme 1: (1) Model $(1+1)_4$, $(\text{star-PDMAEMA/INS/star-PDMAEMA/GOD})_4$+star-PDMAEMA; (2) Model $(2+2)_2$, $[(\text{star-PDMAEMA/INS})_2+(\text{star-PDMAEMA/GOD})_2]_{2+}$-star-PDMAEMA; (3) Model $(4+4)_1$, $(\text{star-PDMAEMA/INS})_4$+$(\text{star-PDMAEMA/GOD})_4$+star-PDMAEMA. In Model $(x+y)_n$, x means a double layer of star-PDMAEMA/INS, y means a double layer of star-PDMAEMA/GOD, and subscript n means the repeat units. The aqueous solutions of star-PDMAEMA (1 mg ml^{-1}, with 0.1 M NaCl, pH 6.0) and GOD (2 mg ml^{-1}, pH 6.0) were prepared, and 100 mg INS was dissolved in 100 ml of HCl (pH 3.0). Then the pH of the INS solution was adjusted to 6.3 ± 0.1 using 0.1 M NaOH. The fabricating process of Model $(4+4)_1$ film was detailed as follows: 200 μl star-PDMAEMA solution was initially dropped and extended on the cleaned PMMA tablet for 10 min and rinsed by 1 ml Milli-Q water, followed by drying with a nitrogen stream. Then 200 μl INS solution was dropped and extended on the substrate for 10 min and followed with the same rinsing and drying steps as described above. Four-bilayer film was obtained by sequential adsorption in star-PDMAEMA and INS solutions. The following four bilayers were fabricated in the same way by alternating adsorptions of star-PDMAEMA and GOD. Finally, a covering layer of star-PDMAEMA was deposited on the top of the film. Similar processes were carried out to prepare Model $(1+1)_4$ and Model $(2+2)_2$ films with different LbL arrangement sequences.

2.2.2. QCM measurements

The assembly steps of LbL multilayer films were monitored by a home-built QCM device with a frequency counter (described in Section 2.1). The films prepared for QCM analysis were manufactured by dropping and extending 200 μl solution onto a certain area of the QCM electrode. After each solution was dropped and extended on the cleaned QCM electrode for 10 min, it was rinsed by 1 ml Milli-Q water, and then the surface was dried with nitrogen stream before performing data collection.



Scheme 1. LbL films with different arrangement sequences.

ID	Title	Pages
354	The influence of arrangement sequence on the glucose-responsive controlled release profiles of insulin-incorporated LbL films	9