Carbohydrate Polymers 140 (2016) 129–135

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Layer-by-layer polysaccharide-coated liposomes for sustained delivery of epidermal growth factor

Gabriel A.T. Kaminski [a,b], Maria Rita Sierakowski [a], Roberto Pontarolo [b], Larissa Antoniacomi dos Santos [a], Rilton Alves de Freitas [a,*]

[a] BioPol, Chemistry Department, Universidade Federal do Paraná, R. Coronel F. H. dos Santos, Curitiba 210–81531-980, PR, Brazil
[b] CEB, Pharmacy Department, Universidade Federal do Paraná, Av. Prof. Lothário Meissner, Curitiba 3400–80210-170, PR, Brazil

ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form 31 October 2015
Accepted 7 December 2015
Available online 17 December 2015

Keywords:
DODAB
Xanthan
Galactomannan
EGF
Layer-by-layer
Liposomes

ABSTRACT

A three-dimensional layer-by-layer (LbL) structure composed by xanthan and galactomannan biopolymers over dioctadecyldimethylammonium bromide (DODAB) liposome template was proposed and characterized for protein drug delivery. The polymers and the surfactant interaction were sufficiently strong to create a LbL structure up to 8 layers, evaluated using quartz crystal microbalance (QCM) and zeta potential analysis. The polymer–liposome binding enthalpy was determined by isothermal titration calorimetry (ITC). The bilayer of biopolymer-coated liposomes with diameters of 165 (±15) nm, measured by dynamic light scattering (DLS), and ζ-potential of −4 (±13) mV. These bilayer-coated nanoparticles increased up to 5 times the sustained release of epidermal growth factor (EGF) at a first order rate of 0.005 min^{-1}. This system could be useful for improving the release profile of low-stability drugs like EGF.

1. Introduction

Therapies based on growth factors (GF) have promising potential in biomedical technology. In cases of chronic wounds proactive treatment is needed for healing and GF might provide the necessary stimuli to induce wound closure (Behm, Babilas, Landthaler, & Schreml, 2011). Of the various growth factors, epidermal growth factor (EGF) has been first and most successfully applied for treating wounds. It is a polypeptide composed of 53 amino acids that enhance epidermal and mesenchymal regeneration, cell motility, and proliferation (Choi et al., 2012).

EGF could be employed to accelerate re-epithelialization, reducing risk of infection and shortening hospitalization. However, due to short half-life, rapid dilution in the body, and the fact that EGF receptors are overexpressed in most squamous carcinomas, brain gliomas and breast cancers (Gedda, Olsson, Pontén, & Carlsson, 1996; Chen & Mooney, 2003), supply of exogenous EGF must be applied in a sustained and localized fashion to be effective and safe. The encapsulation of EGF in liposomes might be an alternative to maximize their stability and to avoid enzymatic degradation (Değim et al., 2011).

Liposomes have already been studied as delivery systems for GF (Değim et al., 2011). Nevertheless, liposomes have the limitations of spilling their contents over time and aggregating (Taylor, Davidson, Bruce, & Weiss, 2005). In order to prevent these events, the liposomes can be coated with polymers, modulating the drug delivery to achieve the desired release kinetics.

A very efficient technique to form polymeric coatings on two-dimensional and three-dimensional systems, such as liposomes, is Layer-by-layer (LbL), introduced by Decher (1997). This technique consists in the alternating depositions of polycations and polyanions, generating a multilayer coating supported mainly, but not exclusively, by the electrostatic interactions or of hydrogen bonds (Wang et al., 1997), covalent bonds (Sun et al., 1998), hydrophobic interactions (Lojou & Bianco, 2004) and van der Waals forces (Sato & Sano, 2005).

In order to coat the dioctadecyldimethylammonium bromide (DODAB) cationic liposomes, using the LbL technique, two biopolymers were chosen. Xanthan (XAN) an anionic biopolymer produced by *Xanthomonas campestris* and composed of a (1 → 4)-β-D-glucan cellulose backbone substituted with an acid trisaccharide in the side chain (Jansson, Kennark, & Lindberg, 1975), and galactomannan (GMC), a neutral biopolymer from *Ceratonia siliqua* seeds that is composed of a (1 → 4)-β-D-mannan backbone with (1 → 6)-α-D-galactose substitutions (Dea & Morrison, 1975). These polymers interact positively and synergistically, as previously described and

* Corresponding author. Tel.: +55 4133613260; fax: +55 4133613260.
E-mail address: rilton@ufpr.br (R.A.d. Freitas).

http://dx.doi.org/10.1016/j.carbpol.2015.12.014
0144-8617/

measured by rheology, differential scanning calorimetry and light scattering (Bresolin, Milas, Rinaudo, & Ganter, 1998; Khouryieh, Herald, Aramouni, & Alavi, 2007).

In this manuscript, we evaluate a new approach for LbL-three-dimensional systems structured with XAN and GMC, for sustained release of EGF. We employed DODAB, a cationic lipid with a quaternary ammonium salt as its polar head and two 18-carbon saturated chains, to form positively superficial charged liposomes potentiating the biopolymer LbL coating. The liposomes, including those with coatings, were characterized, and the EGF release rates were determined in vitro.

2. Material and methods

2.1. Polymer preparation

2.1.1. Polymer purification

XAN gum (Sigma-Aldrich) was purified by dialysis through a cellulose membrane (Sigma-Aldrich), first against 0.1 mol L^{-1} acetic acid for 3 days to ensure that all the molecules would be in the molecular conformation, and then against ultrapure water for 2 days to remove the acetic acid. GMC, from locust bean gum from *C. siliqua* seeds (Sigma-Aldrich), was purified by dispersion in ultrapure water at 40 °C overnight and centrifugation at 10,000 × *g* for 20 min at 40 °C in a 4K15 C (Sigma, Osterode am Harz, Germany) centrifuge to precipitate insoluble impurities. Ethyl alcohol (99%) was added to the supernatant to achieve a 70% alcohol concentration, and this suspension was centrifuged at 10,000 × *g* for 20 min at 5 °C to precipitate the purified XAN and GMC. The polymers were washed with 99% ethyl alcohol, centrifuged as described above and dried at 40 °C.

2.1.2. Polymer characterization

The polymers were characterized at 0.5 mg mL^{-1} by high performance size exclusion chromatography (HPSEC) with 0.1 mol L^{-1} $NaNO_3$ and 200 ppm sodium azide at 0.4 mL min^{-1} as the mobile phase at 40 °C. The system was composed of a UV/vis detector, refractometer, light scattering detector at 7° and 90° and a differential viscometer detector (Viscotek, Westborough, MA, USA) with an OHpak SB-806 M HQ column (Shodex, New York, NY, USA).

The persistence length (L_p) was determined as previously described for other galactomannans by Salvalaggio, de Freitas, Franquetto, Koop, and Silveira (2015).

The zeta potential was determined for polymers dispersions at 0.5 mg mL^{-1} in ultrapure water using the electrophoretic mobility measured on a Zetasizer Nano-ZS (Malvern, Westborough, MA, USA) at 25 °C with 120 s of stabilization.

The protein quantification of purified GMC was determined by the Hartree method (Hartree, 1972).

Infrared spectroscopy (FTIR), using an attenuated total reflectance (ATR) mode was determined in a VERTEX 70 (BRUKER) with 4 cm^{-1} of resolution and 4000–600 cm^{-1} (Supplementary material S1).

2.2. Liposome preparation

Liposomes were prepared by a modified method (Alves et al., 2009). DODAB (Sigma-Aldrich, Switzerland) was dispersed in chloroform at 5 mmol L^{-1}, the solvent was removed by rotary evaporation at 40 °C, and the lipid was resuspended in a 5 μg mL^{-1} EGF (Caregen) solution at 35 °C, similar to Alves et al. (2009) and Değim et al. (2011). The suspension was then submitted to sonication at 25 °C in pulse mode for 5 min.

The EGF-liposome solution was diluted 1:10 in ultrapure water and centrifuged at 10,000 × *g* for 20 min at 25 °C. The supernatant was discarded, and the liposomes were resuspended in 0.5 mg mL^{-1} XAN solution, followed by centrifugation and washing with ultrapure water. The same procedure was performed with a 0.5 mg mL^{-1} GMC solution.

2.3. Liposome characterization

The hydrodynamic diameters of the coated liposomes were analyzed and compared with those of the plain liposomes by dynamic light scattering (DLS) on a NanoDLS (Brookhaven Instruments, Holtsville, NY, USA). The uncoated liposomes and polymer-coated liposomes were also characterized by their ζ-potential and AFM as described elsewhere.

The zeta potential of the liposomes was determined for 5 mmol L^{-1} dispersion of DODAB in ultrapure water using the electrophoretic mobility measured on a Zetasizer Nano-ZS (Malvern, Westborough, MA, USA) at 25 °C with 120 s of stabilization.

2.4. Polymer and DODAB/DODAB vesicle interaction

2.4.1. Isothermal titration calorimetry (ITC)

Experiments were performed in a VP-ITC (Microcal, Westborough, MA, USA) calorimeter with a normal cell (1.464 mL) at 25 °C. The DODAB vesicle dispersion was injected into ultrapure water or into a polymer solution at 0.5 mg mL^{-1}. Each titration consisted of a preliminary 2 μL injection followed by 29 subsequent 10 μL injections with 600 s intervals between each injection. The syringe tip acted as a blade-type stirrer to ensure proper mixing at 300 rpm. Data were collected and processed with Origin 7.0 software (OriginLab, Northampton, MA, USA).

2.4.2. Quartz crystal microbalance (QCM)

Analyses were performed in triplicate in a SRS QCM200 using the flow cell mode. The QCM (Gold/Cr 5 MHz, SRS, Sunnyvale, CA, USA) crystals were cleaned by immersion in 1:3 (v/v) H_2O_2:H_2SO_4 for 5 min and followed by rinsing with ultrapure water. To mimic the liposome coating process, the gold surface was modified with hexanethiol (Sigma-Aldrich) to form a hydrophobic surface and then coated with DODAB by immersion in a 5 mmol L^{-1} chloroform solution similar to Morita, Nukui, and Kuboi (2006). One milliliter of each polymer solution (0.5 mg mL^{-1}) was alternately injected at 0.1 mL min^{-1} with a syringe pump (KD100, KD Scientific, Holliston, MA, USA), and 1 mL of ultrapure water was injected between each solution.

2.4.3. Atomic force microscope (AFM)

Images of each layer on the QCM crystal were obtained on a PicoPlus Molecular Imaging microscope (Agilent, Santa Clara, CA, USA) in the intermittent contact mode in air at 25 °C with silicon cantilevers, an oscillating amplitude of 50 to 100 nm and a resonance frequency close to 300 kHz. The dynamic tapping mode was used with an oxide-sharpened micro-fabricated silicon μ-Masch cantilever with a 4.7 N m^{-1} nominal spring constant and tip curvature radius of less than 10 nm. The image processing and root mean square roughness (rms) determination were performed with Gwyddion software (Czech Metrology Institute, Brno-sever, Czech Republic).

2.4.4. Contact angle

The angles of the polymer substrates were determined with $OCA15^+$ (DataPhysics, Filderstadt, Germany) device equipped with SCA20 software by the sessile drop method at 25 °C with the delivery of 10 μL ultrapure water drops onto the QCM crystal-coated surface.