Materials Science and Engineering C 76 (2017) 365–373

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Characterization of membranes based on cellulose acetate butyrate/poly(caprolactone)triol/doxycycline and their potential for guided bone regeneration application

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ARTICLE INFO

Article history:
Received 22 July 2016
Received in revised form 20 December 2016
Accepted 12 March 2017
Available online 14 March 2017

Keywords:
Cellulose acetate butyrate
Poly(caprolactone)triol
Doxyciclyne
Guided bone regeneration

ABSTRACT

This paper discusses the feasibility of using membranes based on cellulose acetate butyrate/poly(caprolactone)triol loaded with doxycycline for guided bone regeneration. Those membranes were obtained by solvent casting varying the cellulose acetate butyrate: poly(caprolactone)triol:doxycycline (CAB:PCL-T:DOX) mass ratios and characterized by scanning electron microscopy, differential scanning calorimetry, dynamical mechanical analysis, swelling and weight loss, drug release, *in vitro* antimicrobial activity and *in vivo* inflammatory response. Neat CAB and CAB:PCL-T:DOX membranes exhibited inner porous structure, which has a pore-size reduced with increasing of the PCL-T ratio. DSC results demonstrated that the molecular dispersion of the DOX into the CAB:PCL-T membrane was conditioned by PCL-T amount. Elastic modulus reduced noticeably with increased of the PCL-T ratio in the membrane from 2 to 3, while the strain at failure showed an increase of *ca.* 10-fold on the same condition. The DOX release mechanism from the membranes was found to be Fickian or *quasi*-Fickian diffusion. Membranes assessed immediately after the preparation, and even as the membranes immersed in synthetic saliva during 7 days, demonstrated significant inhibition in the growth of *Staphylococcus aureus* and *Escherichia coli*. Subcutaneous implant test on rat *in vivo* showed that the CAB:PCL-T:DOX membrane (7:3:1) did not trigger chronic inflammatory responses. These results suggest the feasibility in applying the CAB:PCL-T:DOX membrane as a barrier for guided bone regeneration.

1. Introduction

Guided bone regeneration (GBR) is the most commonly technique applied for restoring the bone defects by using biosorbable or non-biosorbable membranes [1]. Those membranes act as a barrier capable to prevent the invasion of epithelial and connective tissues while guarding the space and the mechanical stability needed to allow the cells migration favoring the healing process [2,3]. Accordingly, membranes should also be biocompatible and capable to protect the wound from mechanical disruption and bacterial contamination [4,5].

The concepts of GBR have been used in clinical dental practice since the late 1980s [6]. Although different materials have been investigated for GBR procedures, membranes based on synthetic and natural polymers are the most widely used materials [7]. The enormous effort devoted to the development of GBR based on polymers are mainly due to the plethora of properties that can be manipulated through the chemistry and structural architecture of the polymer, blend of one or more polymers, and so forth. The above-mentioned properties include, but it is not limited to, degradation rate, mechanical properties, biocompatibility, and drug-encapsulating ability [3]. Currently, non-biosorbable (expanded polytetrafluoroethylene, *e.g.*, Gore-Tex®) and biosorbable (poly-DL-lactid/poly-L-lactid, *e.g.*, Guidor®; *co*-glycolid and poly-DL-lactid, *e.g.*, Result Adapt®; xenogenic collagen, *e.g.*, Bio-Gide®) polymeric membranes are commercially available [8].

Among these membranes, those based on biodegradable polymer have gained more attention due to the human body's capability to dissolve this material and slowly replace it by advancing bone tissue [9] and, hence, does not require a second surgical procedure to remove the membranes [10]. Despite the good bio-properties obtained for these polymers, some of them showed poor mechanical properties that may preclude the use as GBR [9]. Indeed, collapse of the polymeric

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http://dx.doi.org/10.1016/j.msec.2017.03.095
0928-4931/

membranes into the bone defect represents the main reason of failure for GBR [11]. Blending different polymers is a way to modulate the mechanical properties and conserve their bio-properties [12,13].

Actually, polymer blends have been extensively investigated for an application as GBR [3,14–18], especially those blends that combine natural polymers (*e.g.*, collagen, chitosan and cellulose) with synthetic polymer (*e.g.*, derived from aliphatic polyesters) in order to obtain biodegradable or even biosorbable membranes [19]. Undoubtedly, collagen-based blends are the most commonly investigated membrane for application as GBR [20,21]. However, collagen has poor mechanical strength and high solubility that may make it difficult to use as a GBR membrane [21–23].

Nevertheless, the use of membranes based on cellulose and its derivatives have gained space in tissue engineering field [5]. Recently, a biodegradable membrane obtained by blending cellulose acetate butyrate (CAB) and poly(caprolactone)triol (PCL-T) has been reported, but its feasibility for GBR applications have yet to be demonstrated [24]. Therefore, the development of membranes based on cellulose derivatives and polyesters blends capable to be applied, as GBR, is still a challenging issue.

Another major challenge in GBR procedures is related to the bacterial colonization, which has a negative effect on the healing process with reduced bone regeneration potential [25–27]. Nowadays, tetracycline derivatives including tetracycline, minocycline and doxycycline are the most commonly used antibiotics for topical treatment of periodontitis and peri-implantitis [28–30]. Among them, doxycycline (DOX) has showed the most promising results. DOX has bacteriostatic activity against both Gram-positive and Gram-negative bacteria, including the periodontal pathogens *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* [31], and also "non-oral" pathogenic bacteria as *Escherichia coli* and *Staphylococcus aureus*. It is noteworthy that the presence of "non-oral" bacteria may play a significant role in the progression of periodontitis [32].

Furthermore, DOX facilitates the bone regeneration through initiating the demineralization of its external surface, resulting in the release of osteogenic factors that trigger bone induction [33]. Compare to tetracycline, DOX shows also greater oral adsorption, longer half-life and enhanced lipid solubility [34].

The objective of our study was to prepared membranes based on natural biodegradable polymer CAB and synthetic biosorbable polymer PCL-T containing DOX incorporated by using a solvent casting protocol, and investigated the membrane physical-chemical properties and also their potential for GBR application. According to the best of our knowledge, this is the first study that evaluates the potential application of CAB:PCL-T membranes as GBR barrier.

2. Materials and methods

2.1. Materials

Cellulose acetate butyrate (13.5% acetyl, 37% butyl, M_n of 70.000 g mol^{-1}) and poly(caprolactone)triol (M_n of 900 g mol^{-1}) were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, USA). Doxycycline hydrochloride was obtained from Henrifarma (São Paulo, Brazil) and used as received. Acetone (99 + % purity) was purchased from Fmaia (São Paulo, Brazil) and used as received. Müeler-Hinton was obtained from Becton, Dickinson and Company (Franklin Lakes, USA) and Tryptic Soy Broth was purchased from Himedia Laboratories (Mumbai, India). *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were obtained from Newprov (Paraná, Brazil).

2.2. Preparation of membranes

Membranes were prepared by dissolving different mass ratios of CAB:PCL-T:DOX (8:2:0.5, 8:2:1, 7:3:0.5, and 7:3:1 m/m/m) in 20 mL of acetone and stirred until complete dissolution. Then, the solutions were poured onto Teflon®-coated plates and dried under dry air flux. Subsequently, the blend was removed from the plate and stored in a vacuum desiccator before use.

2.3. Membrane morphology

Scanning electron microscopy (SEM) was performed using a JEOL (JSM-6390LV) microscope (Peabody, MA) operating at an accelerating voltage of 5 kV. The membranes were mounted on aluminum stubs using adhesive carbon tape and then coated with a thin layer of gold using a sputter coater (D2 Diode Sputtering System). The pore diameters in the membrane cross-section were measured using ImageJ software for Windows, which is available in the public domain (http://rsb.info.nih.gov/ij/).

2.4. Thermal analysis

Differential scanning calorimetry (DSC) was performed using a differential scanning calorimeter (DSC 50, Shimadzu) by heating the samples from 25 to 200 °C at a heating rate of 10 °C min^{-1} under an N_2 atmosphere (50 cm^3 min^{-1}). Samples having weight around 6 mg were placed on an aluminum pan. Standard Indium (156.6 °C) and Zinc (419.5 °C) were used for calibration.

2.5. Fourier transform infrared spectroscopy

Fourier infrared transform (FTIR) spectra were recorded in a transmission mode on a Bomem MB-100 series spectrometer in the range of 4000 to 400 cm^{-1} using potassium bromide (KBr) pellets. The pellets were subjected to 64 scans at a resolution of 4 cm^{-1}.

2.6. Mechanical properties

Membranes having 60 mm in length, 10 mm in width, and thickness range from 0.075 to 0.09 mm were used to determine the elastic modulus (E, MPa), ultimate strength (σ_f, MPa) and strain at failure (ε_f, %) from the stress-strain curves. Mechanical assays were performed on EMIC DL 2000 equipment using a two grips (one stationary and another movable) operating at a crosshead speed of 10 mm min^{-1} and equipped with a 100 N capacity load cell. The tests were conducted at 25 °C. The initial grip separation was set at 40 mm. Data were expressed as the mean values of at least six measurements.

2.7. Swelling and weight loss

Briefly, membranes were weighed (W_o) and then introduced into small flasks filled with phosphate buffer pH 7.0. The flasks were maintained at 37 ± 0.5 °C in a Dubnoff bath (Quimis SA, Brazil) under constant shaking for a period of 7 days. After this period, each membrane was withdrawn from the flask and wiped with absorbent paper to remove the excess water from the membrane surface. Subsequently, membranes were weighed (W_{wet}) and oven-dried at 50 °C until constant weight (W_{dry}). Swelling and weight loss values were determined following the Eq. (1) and Eq. (2), respectively [35]:

$$\text{Swelling}\ (\%) = \left[\left(\frac{W_{wet} - W_{dry}}{W_{dry}}\right)\right] \times 100 \quad (1)$$

$$\text{Weight loss}\ (\%) = \left[\left(\frac{W_0 - W_{dry}}{W_0}\right)\right] \times 100 \quad (2)$$

2.8. Drug release tests

The drug release tests were performed using horizontal diffusion cells and a diffusion area of 6.8 cm^2. The donor compartment containing