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Pectin-chitosan membrane scaffold imparts controlled stem cell adhesion and proliferation

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ABSTRACT

Processing stable polysaccharide membranes with suitable mechanical properties has been challenging for applications in wound healing and tissue engineering. Here we expand the characterization of pectin/chitosan (PT/CS) membranes (without covalent crosslinking), which we recently reported. Membranes containing pectin (PT) excess were formed, and PT/CS ratio can be tuned to enhance the mechanical strength, and to modulate hydrophilicity and cytocompatibility. The surface wettability and swelling properties of the polyelectrolyte complexes (PECs) played an important role to promote the attachment of stem cells. These PECs membranes have ultimate tensile strength similar to that of human skin, which is on the order of ten times higher than similar previously reported polysaccharide materials. We show for the first time that these new PT/CS membranes may promote anchorage, adhesion and support human stem cell growth, making them candidate materials for tissue engineering purposes.

1. Introduction

Pectin (PT) is a polysaccharide with a complex structure, mostly composed of galacturonic acid units linked for α(1 → 4)-glycoside bonds, whereas chitosan (CS) is a linear polysaccharide, constituted of randomly distributed β(1 → 4)-D-glucosamine and *N*-acetyl-D-glucosamine units (Almeida et al., 2017; Facchi et al., 2017). PT and CS have anti-inflammatory, cytocompatibility, and biodegradability properties and they can be assembled to produce physical hydrogel membranes via acquisitions of polyelectrolyte complexes (PECs) (Maciel, Yoshida, & Franco, 2015; Nesic et al., 2014). PECs are mainly formed by mixing oppositely charged polyelectrolytes in solution, and they are stabilized by electrostatic and secondary forces (intra- and intermolecular associations) between polymer networks (Bhattarai, Gunn, & Zhang, 2010; Tentor et al., 2017).

One important advantage of PEC-based materials is that they can be produced without toxic crosslinking agents (glutaraldehyde, epichlorohydrin, genipin, and others), commonly used to create hydrogels (Bhattarai et al., 2010; Luo & Wang, 2014). Recently, our research group showed that PT/CS membranes could be obtained in high PT/CS weight ratios by forming PECs at a reduced pH (≈ pH = 1.0) (Martins, de Oliveira, Garcia, Kipper, & Martins, 2018). In this case, water stable PT/CS membranes were produced over a range of CS content, using PT with a high *O*-methoxylation degree (56%) (Martins et al., 2018). With respect to the biomedical and pharmaceutical applications, PT/CS or CS/PT-based membranes have received attention primarily for drug delivery purposes (Ofori-Kwakye & Fell, 2001; Tsai et al., 2014). In the present work, we intend to show for the first time that PT/CS membranes exhibit cytocompatibility that can be exploited for us as scaffolds for tissue engineering arena.

PT/CS polysaccharide-based membranes can play an important role in biomedical applications because they have similar structures to extracellular matrix (ECM) (Facchi et al., 2017). The ECM must mediate the cellular interactions with their environment (Facchi et al., 2017). The ECM provides mechanical support for cell anchorage, and signals that control cell proliferation, cell orientation, and maintenance of cell differentiation. The ECM thereby modulates so-called “cell fate decisions.” The essential ECM components are the fibrillar and adhesive proteins, glycosaminoglycans, and proteoglycans (Almodovar, Bacon, Gogolsky, Kisiday, & Kipper, 2010). Proteoglycans are chemical structures composed of glycosaminoglycans covalently bound to proteins.

Therefore, scaffolds used for tissue engineering purposes should

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similarly promote cell adhesion and provide mechanical and chemical cues that can guide cell shape, cell migration, and other important cellular activities. Therefore, we envision developing PT/CS membranes as wound dressings to promote healing and as scaffolds for tissue engineering. The PEC membranes will be created following a methodology previously reported by our research group (Martins et al., 2018). We report suitable PT/CS weight ratios for obtaining PEC membranes with high stability, tensile strength, cytocompatibility, and ability to support human adipose-derived stem cell culture.

2. Materials and methods

GENU® pectin (PT) type USP/100 from citrus peels with an *O*-methylation degree of 56% and molar weight of 190×10^3 g mol^{-1} (determined by GPC) was graciously donated by CP Kelco Co., Limeira-SP, Brazil. Chitosan with deacetylation degree equal to 85% and average molecular weight of 87×10^3 g mol^{-1} was purchased from Golden-Shell Biochemical (China) (Facchi et al., 2018).

2.1. Membrane preparation

The PT/CS-based membranes were produced following the same procedure recently reported by our research group (Martins et al., 2018). PT and CS-solutions (1.0 wt.%) were individually prepared in a 0.10 mol L^{-1} HCl aqueous solution at 60 °C (10 min). Immediately after preparation, CS-aliquots (5.0 or 10 mL) were added into the PT-solution, making 30 mL PT/CS-blends at 60 °C. The blending solutions were kept under magnetic stirring for 10 min (60 °C). Table 1 details the experimental conditions used to prepare the membranes. PT/CS PEC membranes are identified as M(X–Y); X and Y represent the PT-and CS solution volumes used to create the materials, respectively (Table 1). After blending (10 min), the PT/CS solutions (at 60 °C) were added to Petri dishes (PS, round-plate shape 85 × 10 mm). The solvent was evaporated in the oven at 35 °C for 24 h. Then, each PEC membrane was peeled off the Petri dish, soaked in deionized water (250 mL) at 25 °C, to achieve neutralization to at least pH 6.0 (Martins et al., 2018). Also, Table 1 shows the yield and the final PT/CS weight ratios of the membranes determined after neutralization step, following a previously reported experimental procedure (Supplementary information) (Martins et al., 2018).

2.2. Characterization

2.2.1. X-ray photoelectron spectroscopy (XPS)

Surface chemistry of the membranes was obtained using a Phi Electronics 5800 Spectrometer (Chanhassen, MN). Spectra were obtained with a monochromatic Al Kα X-ray source ($h\nu$ = 1486.6 eV), a hemispherical analyzer, and a multichannel detector. High-resolution spectra were obtained using a 23.5 eV analyzer pass energy with 0.1 eV steps and an X-ray spot of 800 µm. All spectra were obtained with a photoelectron take-off angle of 45°. A low-energy electron gun was used for charge neutralization. Spectra curve fitting was done using Origin version 8.5. Curve fitting of all spectra used a Shirley background. Gaussian peaks were fit according to expected functional groups. The height of each peak was fit first while keeping each peaks' position, full-width half max (fwhm) and percent Gaussian fixed. Then the fwhm, percent Gaussian, and finally position was fit while minimizing the chi-squared value (Romero et al., 2015).

Table 1
Experimental conditions used to yield the PT/CS PEC membranes and results of yielding.

Samples	PT (mL:mg)	CS (mL:mg)	Blend (mL)	PT/CS ratio[a]	Yield (%)
M(25-5)	25:250	5:50	30	6 ± 3	85 ± 4
M(20-10)	20:200	10:100	30	2.5 ± 0.8	93 ± 2

[a] Real PT/CS weight ratio determined after neutralizing step of the PEC membranes (Martins et al., 2018).

2.2.2. Scanning electron microscopic (SEM) and atomic force microscopy (AFM)

The surface morphology of the dry membranes was investigated through SEM and AFM. For SEM, the samples were sputter-coated with palladium-gold alloy (Polaron SC 7620 Sputter Coater, Quorum Technologies, Newhaven, UK) at a thickness of 10 nm (10–15 mA, under a vacuum of 130 mTorr). The SEM (JSM-6500F, field emission scanning electron microscope, JEOL, Japan) was operated at an accelerating voltage of 5 kV and three to six locations on each sample were imaged. AFM was conducted using a Bioscope Resolve AFM (Bruker) using ScanAsyst Air probes. AFM images were obtained using tapping mode from a scanning image probe processor version 4.2.2.0 software. AFM was performed at room temperature in air at a rate of one-line scan per second.

2.2.3. Mechanical analysis

Before mechanical analysis, the thickness of PEC membranes (50 × 25 mm) was determined using a digital micro-durometer (ZAAS model). The average result of the thickness was obtained utilizing five measurements on the total area of each sample (50 × 25 mm). The tensile properties were determined using the texture analyzer, Stable MicroSystems (model TATX2i, Surrey-England), according to the ASTM-D882-02 method. The samples were cut (50 × 25 mm), which were previously conditioned at 53 ± 2% relative humidity (RH), Mg $(NO_3)_2$ solution, at 25 ± 2 °C for 48 h. The crosshead speed was set at 0.83 mm s^{-1} (load cell of 50 kg), and the initial distance between the grips was 30 mm. The parameters, with five replicates, which were determined included the tensile strength (MPa) and elongation at break (%). The results were statistically analyzed using ANOVA with Tukey's post-hoc test for multiple comparisons (Graph Prism 6.0, GraphPad Software Inc., La Jolla, CA, USA) at a 5% significance level.

2.2.4. Water contact angle measurements

The water contact angle (WCA) measurements were assessed on the neutralized PEC surfaces (neutralized up to pH 6.0). Static contact angles were assessed by the sessile drop method with a contact angle goniometer (Krüss DSA 10, Hamburg, Germany), equipped with video capture (Almodovar et al., 2010). The automatic dosing feature of the DSA 10 dispenses a water drop on the PEC membrane surfaces, and the needle is manually withdrawn. Images were captured every 1.0 min after contact of a droplet with the surface by a camera leveled with the surface. The contact angle was monitored with time until the drop of water was entirely adsorbed by the PEC surface. Contact-angle measurements were analyzed by the circle fitting profile available with the DSA 10 imaging software. Three separate measurements were made on each sample at different locations (Almodovar et al., 2010).

2.3. Cytocompatibility assays

2.3.1. Cell culture

Human adipose-derived stem cells (ADSC cells) were used in this study. ADSC cells were cultured at 37 °C in a 5% CO_2 atmosphere in 175 cm^2 polystyrene vented tissue-culture flasks. The cell growth medium consisted of Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells of passage three were used. Before seeding the cells, dry membrane samples (diameter of 8.0 mm) were obtained using a handled puch. Then, these membranes were fixed on teflon supports (diameter of 8.0 mm) using carbon tapes and incubated into PBS for 30 min under UV exposure. After, the cells were seeded onto the samples at a concentration of 5000 cells/well in 48-well plates and they were cultured at 37 °C in a 5% CO_2.