Journal of Colloid and Interface Science 525 (2018) 21–30

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

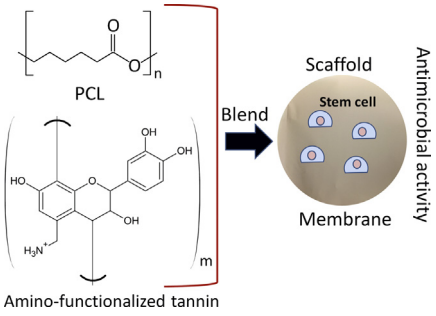
Regular Article

Novel poly(ε-caprolactone)/amino-functionalized tannin electrospun membranes as scaffolds for tissue engineering

Alessandro F. Martins [a,b,c,*], Suelen P. Facchi [b], Paulo C.F. da Câmara [c], Samira E.A. Camargo [c], Carlos H.R. Camargo [c], Ketul C. Popat [c], Matt J. Kipper [c]

[a] Postgraduate Program in Materials Science & Engineering (PPGCEM), Federal University of Technology (UTFPR-LD), 86036-370 Londrina, PR, Brazil
[b] Postgraduate Program in Environmental Engineering (PPGEA), Federal University of Technology (UTFPR-AP), 86812-460 Apucarana, PR, Brazil
[c] Department of Chemical and Biological Engineering, Colorado State University, 1370 Campus Delivery, Fort Collins, CO, United States

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 February 2018
Revised 13 April 2018
Accepted 15 April 2018
Available online 16 April 2018

Keywords:
Electrospinning
Tannin derivative
Medical application
Cell culture
Membranes

ABSTRACT

Poly(ε-caprolactone) (PCL) is a hydrophobic and cytocompatible aliphatic polyester that has been used to produce PCL-based nanofibrous for both wound healing and tissue repair. However, the high hydrophobicity and low water adsorptive have been challenges for developing PCL-based materials for use in tissue engineering field. Here, we report a new polymer (a hydrophilic amino-functionalized tannin (TN)) that is associated with PCL for developing PCL-TN blends at different PCL:TN weight ratios (100:0, 95:5, 85:15 and 78:22). PCL:TN ratio may be tuned to modulate hydrophilicity and cytocompatibility of the nanofibers. The neutralization step and surface wettability played an important role in the attachment of human adipose-derived stem cells (ADSC cells) on PCL-TN membranes. Also, fluorescence images confirmed great proliferation of ADSC cells on the PCL-TN electrospun surfaces. Yet, neutralized PCL-TN nanofibers promoted bactericidal activity against *Pseudomonas aeruginosa*. These membranes have potential to be used as scaffolds for tissue engineering purposes.

1. Introduction

Nanomaterials have received attention because nano-scale materials reveal exceptional properties not found in macro-scale materials [1,2]. In this scenario, nanofibers have attracted great interest, primarily due to their large surface area to volume ratio, and increased surface functionality compared to other forms of materials [1,2]. Nanofiber membranes can be used as devices for

* Corresponding author at: Postgraduate Program in Materials Science & Engineering (PPGCEM), Federal University of Technology (UTFPR-LD), 86036-370 Londrina, PR, Brazil.
E-mail address: afmartins@utfpr.edu.br (A.F. Martins).

https://doi.org/10.1016/j.jcis.2018.04.060
0021-9797/

drug delivery [3], scaffolds for wound healing [4], filters [2], prostheses [5], sensors [6], and membranes for food packing [7] and wastewater treatment [2,8]. Fibers are created from conventional textile methods, but electrospinning approach can be used to provide nanofiber membranes with diameters between 100 and 5000 nm [9].

Poly(ε-caprolactone) (PCL) is one of the most important synthetic polymers used for developing nanofiber scaffolds, for repairing soft and hard tissues [10]. PCL receives great attention in tissue engineering arena because it has promoted cytocompatibility, biodegradability, and mechanical resistance for PCL and PCL-based materials [11,12]. On the other hand, PCL comprises a semicrystalline biopolymer with high hydrophobicity and low water absorptivity [13]. These shortcomings should be overcome, whereas they reduce attachment of cells on PCL-based scaffolds. Therefore, PCL has been associated with polysaccharides (chitosan [14], starch [15], alginate [16], and others) and proteins (gelatin [17], fibroin [4], collagen [2], albumin [13] and others) to obtain blends. Polysaccharides and proteins enhance the hydrophilicity, biocompatibility, and biodegradability of PCL-based scaffolds. These natural biopolymers contain cell-recognition sites that improve cell seeding and adhesion [1]. Nanofiber scaffolds may mimic the biological environment, stimulating cellular responses [18].

Tannins are water-soluble polyphenols widely found in plants [19,20]. Plant parts containing tannins include wood, bark, fruit, fruit pods, leaves, roots and plant galls. They are mainly extracted from *Acacia* sp. that presents high contents of polyphenolic tannins, sugars and hydrocolloid gums [19]. Tannins are natural biopolymers that can protect plants against occurrence of pathogens. Tannins are classified as hydrolyzable and condensed. Hydrolyzable tannins are often molecules with a D-glucose as a central core. The hydroxyl sites of this carbohydrate are partially or entirely esterified with gallic acid molecules. Hydrolyzable tannins usually comprise low contents in plants. Condensed tannins are more abundant and they include oligomers and polymers of flavonoid units (favan-3-ol units) [19–21].

In Brazil, condensed tannins are extracted from *Acacia decurrens*, popularly called as "Black wattle" [19]. These tannins have commercial interest because they are used to prepare an amino-functionalized tannin (TN), using formaldehyde and ammonium chloride, following a Mannich-type reaction (acid catalysis) [20]. TN has received great attention for use in environmental field because it is extensively employed as a coagulation-flocculation agent for wastewater and effluent treatments. Recently, our research group showed that TN and TN/alginate-based polyelectrolyte complexes (PECs) had antimicrobial and antioxidant properties [19]. The presence of substituted amine groups (pKa ≈ 6.0) imparts cationic feature to the TN [19,20].

Electrospinning of PCL and PCL-chitosan blends have been investigated in formic acid:acetone [22], dimethyl formamide:methylene chloride [23], chloroform:methanol:acetic acid [11] and acetic acid:formic acid (AA:FA) [24] mixtures. Van der Schueren et al. [25] showed that PCL nanofibers could easily be obtained combining both AA acid FA solvents. PCL membranes are prepared in several AA:FA volume ratios, ranging from 75:25 to 10:90 ratio, respectively. Van der Schueren et al. [24] also created PCL-chitosan nanofibers in AA:FA mixtures, using PCL:chitosan blend ratios ranging from 86:14 to 72:28 at different PCL contents (6.0–14 wt%). Therefore, the AA:FA set may be a suitable system for producing PCL-TN electrospun membranes because it offers production of nano-scale fibers, and it comprises low toxicity than conventional chloroform solvent, commonly used for electrospinning of PCL solutions [24–26]. Besides, TN comprises a cationic polymer like chitosan [27], forming uniform blends with PCL at the AA:FA solvent.

We envision developing PLC-TN membranes to produce scaffolds for tissue engineering. PCL-TN membranes are produced in an AA:FA mixture (at 70:30 ratio). We report a new polymer (amino-functionalized tannin) for forming these membranes using PCL-TN blends at different PCL:TN weight ratios. The washing step of the membranes played an important role in the cell culture results. The PCL-TN membrane produced in a 78:22 PCL:TN ratio supports the attachment, adhesion, and proliferation of human adipose-derived stem cells (ADSC cells). A PBS neutralized membrane also exhibits antimicrobial activity against *Pseudomonas aeruginosa* (*P. aeruginosa*). We report a novel polymer (tannin derivative) with great potential for combining to PCL membranes, allowing development of scaffolds with healing and antimicrobial properties.

2. Materials and methods

The amino-functionalized tannin (TN), commercially labeled as tanfloc-SG was graciously donated by Tanac SA Company (Montenegro-RS, Brazil) [19]. The TN has been synthesized from natural condensed tannins extracted from the black wattle. According to the Graham et al. [20], TN comprises a moderate-to-high molecular weight (600,000 g mol^{-1}), including 1000–2000 repeating units with a narrow distribution. Poly(ε-caprolactone) (80,000 g mol^{-1}) with water impurity below 0.5 wt%, formic acid (>88 vol%), and membranes for dialysis (7.0 kDa) were purchased from Sigma Aldrich (USA). Acetic acid (>98 vol%) was acquired from Acros Organics (USA).

2.1. Tanfloc purification

The dialysis process is used to purify the as-obtained TN. A TN-solution (50 mL at 10 wt%) is prepared in ultrapure water, filtered (twice) and dialyzed in ultrapure water for 3 days (performing the change of water twice a day). After dialysis, the TN solution was removed from the dialysis bags, filtered, frozen in liquid nitrogen, and lyophilized for 72 h.

2.2. Electrospinning of PCL-TN blends

Electrospun membranes are created following experimental conditions proposed by der Schueren et al. [24,25] with some adaptations. PCL-TN blends (3.0 mL) provided in an AA:FA mixture (70:30) were prepared (1.0 h) at different PCL:TN weight ratios, using a vortex apparatus to dissolve the polymers (Table 1). The TN content (wt%) used to create PCL-TN blends is determined according to the whole PCL concentration (wt%) in the final blend composition (Table 1). PCL-TN blends were pumped (1.0 mL h^{-1}) in a Kent Scientific Genie Plus syringe pump (Torrington, Connecticut). The blending solutions were electrospun from a high voltage DC power supply (Gama High Voltage Research Ormond Beach) at 15 kV (20 ± 2 °C and 22% relative humidity). The tip to collector distance was set at 12.5 cm. Nanofibers were neutralized in PBS buffer (pH 7.4) for 24 h, changing PBS five times (the first four exchanges were performed in each 3 h, and the last one was carried out after 12 h). Also, another method for neutralizing the membranes was followed. They were neutralized in a 14 vol%/vol % NH_4OH solution [14] for 15 min, and washed five times in ultrapure water (10 min each cycle of washing). Before cell culture assays, all PCL and PCL-TN membranes were added to PBS buffer and sterilized (30 min) by ultraviolet irradiation.

2.3. Characterization

The morphology of the TN (before and after dialysis) and PCL-TN membranes was investigated through SEM analyses. The samples were sputter-coated with palladium-gold alloy (Polaron SC