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Biofunctionalization of electrospun poly(caprolactone) fibers with Maillard reaction products for wound dressing applications

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ABSTRACT

Bacterial colonization of open skin wounds can interfere with the healing process, contributing to an increase in the severity of the wound. To overcome such drawback, wound dressings with an improved bactericidal activity have been developed or are currently under development. Herein, poly(caprolactone) (PCL) nanofibrous membranes functionalized with biosynthesized Maillard reaction products (MRPs) were produced using an electrospinning apparatus and their properties (chemical, morphological, mechanical and biological) analyzed in order to evaluate their suitability for being used as wound dressings. The functionalization of PCL nanofibers with MRPs allowed the production of membranes with the mechanical, wettability and porosity features required for wound exudate absorption as well as nutrients and gas exchange. Furthermore, MRPs-modified PCL membranes were also able to inhibit *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth, without inducing any cytotoxic effect to human fibroblasts. These findings support the potential use of the produced membranes in the healing process.

1. Introduction

An open skin wound may occur as a consequence of superficial cuts, or can be caused by burns, punctures and diseases, as well as surgical procedures [1, 2]. Nowadays, skin infection remains as main cause of a delayed healing [3–5]. From a clinical management perspective, it is crucial to protect the wound against the external threats, which are responsible for skin contamination and subsequent infection.

In this way, wound dressings with antimicrobial properties have been developed in order to avoid wound microbial colonization, while supporting fibroblast migration and differentiation [6]. In order to accomplish a fully-functional antimicrobial dressing, three key points must be considered: (1) production method; (2) the materials used to produce the dressing that may have intrinsic antibacterial activity and/or (3) selection of an appropriate antimicrobial agent to be incorporated within the dressing [7, 8].

Up to now, wound dressings displaying antimicrobial activity have been produced using different techniques (e.g. self-assembly, phase separation and electrospinning) [9]. Among those, electrospinning, due to its simplicity, versatility, and low cost, is the most used, since it allows the production of nanofibrous meshes, capable of mimicking the fibrillar components of skin's extracellular matrix (ECM), displaying a high surface-to-volume ratio and high porosity. These features are essential for cell adhesion and proliferation [10–12].

The properties exhibited by the electrospun membranes are dependent on the materials used in their production. Synthetic polymers have been extensively used since they show excellent mechanical properties, thermal stability and have an adjustable degradation profile [9, 13]. Among the synthetic polymers, Poly(caprolactone) (PCL) is a semi-crystalline polymer well known for its good biocompatibility, mechanical properties, spinnability, non-immunogenicity and slow biodegradability, that has been used in the production of wound dressings [14–16]. However, its hydrophobic character and lack of cell recognition motifs on its surface can impair protein adsorption and subsequently, cell attachment as well as cell-dressing interactions, events that are essential for a successful healing be attained [17]. Thus, to improve PCL biological properties different strategies have been employed including surface functionalization (e.g. chemical grafting and plasma treatment techniques) or bioactive molecules (e.g. natural compounds, growth factors, vitamins) incorporation [18, 19]. For example, Ghasemi-Mobarakeh et al., improved the hydrophilicity of PCL nanofibrous membranes through alkaline hydrolysis and subsequently covalent attachment of Matrigel [20]. In the same way, Ma at el., modified the surface of PCL nanofibers properties by performing an air

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plasma treatment to introduce −COOH groups, followed by covalent grafting of gelatin molecules [21].

Furthermore, PCL membranes have been functionalized with antimicrobial agents such as antibiotics, nanoparticles and natural products, to confer PCL membranes bactericidal activity [16, 22, 23]. For example, Augustine et al. incorporated silver nanoparticles, a promising antimicrobial agent, into PCL membranes to be used for wound dressing applications [24].

Herein, PCL membranes were functionalized with biosynthesized Maillard reaction products (MRPs), glucose-arginine (GA) and fructose-arginine (FA), to enhance their biological properties and endows them with antimicrobial activity. Several authors proved that MRPs possess antioxidant, anti-inflammatory and antimicrobial activity, properties that are essential for improving the healing process [25–31]. The obtained results revealed the suitability of MRPs-modified PCL membranes to be applied as wound dressings. These membranes were able to promote cell adhesion and proliferation, as well as inhibit bacterial growth.

2. Materials and methods

2.1. Materials

D-Glucose anhydrous, D-fructose and 3,3,3 trifluoroethanol (TFE) was supplied by Acros Organics (Jersey City, NJ, USA). Fetal bovine serum (FBS) free from any antibiotic was purchased from Biochrom AG (Berlin, Germany). Normal human dermal fibroblasts (NHDF) cells were acquired from PromoCell (Labclinics, S.A., Barcelona, Spain). 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2 (4-sulfophenyl)-2H-tetrazolium (MTS) was bought from Promega (Madison, WI, USA). L-arginine, dulbecco's modified eagle's medium (DMEM-F12), ethylenediaminetetraacetic acid (EDTA), gentamicin, glutaraldehyde, LB Broth, phosphate-buffered saline solution (PBS), sodium hydroxide (NaOH), PCL (80,000 Da) and trypsin were purchased from Sigma-Aldrich (Sintra, Portugal). Quant-iT Pico Green dsDNA assay kit was obtained from ThermoFisher Scientific (Waltham, MA, USA). *Staphylococcus aureus* clinical isolate (*S. aureus*) ATCC 25923 and *Pseudomonas aeruginosa (P.aeruginosa)* obtained from a human sample were used as models of prokaryotic organisms to evaluate the bactericidal activity exhibited by the produced membranes. Propidium iodine buffer was gotten from Invitrogen (Carlsbad, Califórnia, EUA) and Calcein AM was supplied by Calbiochem (Merck Millipore, Oeiras, Portugal).

2.2. Methods

2.2.1. Preparation of the sugar-amino acid model Maillard reaction products (MRPs)

MRPs were synthesized following a slightly modified version of the protocol previously described by Wu et al. [31]. Equimolar (0.01 mol) amounts of glucose-arginine and fructose-arginine were separately dissolved in Milli-Q water. The pH of both solutions was adjusted to 10.7 with NaOH (1 M), to promote the first step (condensation) of the reaction. Then, the solutions were transferred to a flask and refluxed in an oil bath at 100 °C for 1 h. After that, the heated solutions were immediately cooled in an ice-water bath and subsequently freeze-dried for further use.

2.2.2. Attenuated total reflectance-Fourier transform infrared spectroscopy analysis of the MRPs

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) was used to study the final composition of the MRPs. The samples' spectra were obtained with an average of 128 scans, a spectral width ranging from 400 and 4000 cm^{-1}, and a spectral resolution of 4 cm^{-1}, using a Nicolet iS10 FTIR spectrophotometer (Thermo Scientific, Waltham, MA, USA). All the components used for the Maillard reaction (glucose, fructose and arginine) were also analyzed in pure state for further comparison purposes (see further details in the Supplementary information).

2.2.3. Production of electrospun MRPs-modified PCL nanofibrous membranes

Different nanofibrous membranes composed of PCL, PCL_GA and PCL_FA polymeric blends were produced using a conventional electrospinning apparatus. The system setup was comprised of a high voltage source (Spellman CZE1000R, 0–30 kV), an automatic precision syringe pump (KDS-100), a plastic syringe with a stainless-steel needle (21 Gauge) and an aluminum foil connected to a copper collector, at a working distance of 14 cm. To accomplish the production of the PCL_GA and PCL_FA membranes, a PCL solution (9% *w/v*) was initially prepared by dissolving PCL in TFE 80% (*v*/v). Then 25 mg/mL of GA or FA MRPs were added to the PCL solution and maintained under stirring for 15 to 20 min. After homogeneous solutions be obtained, they were electrospun at a constant flow rate of 2.5 mL/h and an applied voltage of 25 kV. Additionally, PCL membranes were also produced as described above, for comparison purposes.

2.2.4. Evaluation of the morphological, physical and mechanical properties of the produced electrospun membranes

2.2.4.1. Characterization of the surface morphology and composition of the produced membranes. Scanning electron microscopy (SEM) analysis of the produced membranes was used to characterize nanofibers' surface morphology and fibers' diameters distribution. Samples were initially mounted onto aluminum stubs using Araldite glue, and sputter-coated with gold using a Quorum Q150R ES sputter coater (Quorum Technologies Ltd., Laughton, East Sussex, UK). SEM images were acquired using a Hitachi S-3400 N Scanning Electron Microscope (Hitachi, Tokyo, Japan) at an accelerating voltage of 20 kV. The average diameter of electrospun nanofibers was measured using ImageJ (Scion Corp., Frederick, MD).

Furthermore, ATR-FTIR spectra were acquired to characterize the chemical composition of the produced membranes, following the protocol described above (Section 2.2.2).

2.2.4.2. Assessment of the mechanical properties of the membranes. The mechanical properties of PCL, PCL_GA and PCL_FA membranes were evaluated using a Shimadzu AG-X Tensile Testing Machine (Tokyo, Japan), at room temperature (RT), under wet and dry conditions, following the guidelines established by Standard Test Method for Tensile Properties of Polymer Matrix Composite Materials (ASTM standard D3039/D3039M) [32]. To perform this assay, samples ($n = 5$) with a width of 2 cm, gauge length of 6 cm and thickness ranging from 0.15 to 0.3 mm were used. The length between the clamps was set to 2 cm and the speed of testing was set to 15 mm/min. For the wet conditions, membranes were immersed in a PBS solution (pH = 5.5), for 24 h at 37 °C. Load-extension data were recorded and the stress–strain curve of the membranes was constructed by applying Eqs. (1) and (2), respectively:

$$Stress = \sigma = \frac{F}{A} \quad (1)$$

$$Strain = \varepsilon = \frac{\Delta l}{L} \quad (2)$$

where F is the applied force; A is the cross-sectional area; Δl is the change in length; and L is the length between the clamps.

2.2.4.3. Evaluation of the membranes' porosity. The total porosity of the membranes was determined using a fluid displacement method adapted from Miguel et al. [33]. In this assay, five specimens were weighed and then immersed in absolute EtOH for 1 h. Subsequently, the samples were reweighed and the porosity of the membranes was assessed by determining the amount of ethanol absorbed by the membranes, through Eq. (3):

$$Porosity\ (\%) = \frac{W_s - W_d}{D_{ethanol} \times V_{membrane}} \times 100 \quad (3)$$