European Polymer Journal 88 (2017) 161–170

Contents lists available at ScienceDirect

European Polymer Journal

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

Electrospun multilayer chitosan scaffolds as potential wound dressings for skin lesions

Rafael Bergamo Trinca [a], Cecília Buzatto Westin [a], José Alberto Fracassi da Silva [b], Ângela Maria Moraes [a,*]

[a] Department of Engineering of Materials and of Bioprocesses – School of Chemical Engineering, University of Campinas (UNICAMP), Av. Albert Einstein 500, 13083-852 Campinas, Brazil
[b] Department of Analytical Chemistry, Institute of Chemistry, University of Campinas, R. Josué de Castro 126, 13083-970 Campinas, Brazil

ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form 22 December 2016
Accepted 20 January 2017
Available online 21 January 2017

Keywords:
Electrospinning
Scaffolds
Wound dressing
Chitosan
Polycaprolactone

ABSTRACT

In this work, double layer scaffolds were produced through the use of the electrospinning technique. One layer acts as mechanical support, being composed of polycaprolactone or a polycaprolactone/cellulose acetate blend (PCL and PCL/CA, respectively), while the other layer performs the role of primary wound dressing, being constituted by a chitosan/poly (ethylene oxide) blend (CHI/PEO). The scaffolds have the typical electrospun structure of interconnected open macropores due to the uniform distribution of randomly oriented fibers. The diameters of the PCL and PCL/CA fibers range from 1 to 4 μm while those of CHI/PEO fibers are smaller than 200 nm. The PCL or PCL/CA layers are responsible for the mechanical properties of the scaffolds: tensile strength of 1.4–1.8 MPa, Young's modulus of 10–15 GPa and elongation at break higher than 430%. The use of 10 wt% of CA in PCL/CA blends increases both tensile strength and Young's modulus, without loss of maximum elongation. There is a significant difference in surface wettability of PCL or PCL/CA and CHI/PEO, as verified by water contact angle assays, being the CHI layer more hydrophilic (contact angle around 35°) than the PCL-based layers (contact angle around 110°) The double layer scaffolds PCL + CHI/PEO/PEO and PCL/CA + CHI/PEO/PEO presents a water vapor permeation rate of around 730 g m^{-2} day^{-1} and a PBS solution uptake capability of up to 369%, being this characteristics attributed to the high volumetric porosity of the scaffolds (around 80%). The scaffolds properties meet the necessary requirements for application as dressings for skin lesions, showing also low cytotoxicity to L929 fibroblasts and promoting adequate cell proliferation.

1. Introduction

The skin stands out among the human tissues not only for its important biological functions, but also for its extension [1]. Traditional treatments for skin lesions such as burns, lacerations and ulcers consist in using protective wound dressings or more invasive approaches such as tissue transplants (removed from a donor area of the patient or from allogeneic sources). These treatments may be inefficient or unfeasible depending on several parameters, from the mere mechanical protection offered by traditional dressings to the limitations of skin transplants (due to affected area, tissue rejection, risk of

* Corresponding author.
E-mail address: ammoraes@feq.unicamp.br (Â.M Moraes).

http://dx.doi.org/10.1016/j.eurpolymj.2017.01.021
0014-3057/

contamination and necrosis, and others) [2,3]. For these reasons, the development of biomaterials useful in skin regeneration is of great scientific and clinical interest [2–7]. In skin wounds treatments, such biomaterials are commonly used in the form of dressings or scaffolds. In both cases, the presence of a interconnected pore structure is desirable, as it allows the transport of gases and nutrients [7].

In addition to mechanical protection, dressings have the classic function of barrier against bacterial infections and loss of fluids and proteins [8]. Modern dressings, however, must also provide an appropriate cell regeneration environment (transport of gases and liquids, moisture and temperature maintenance), as well as being desirable the use of materials able to stimulate cell migration, proliferation and reorganization of histological architecture [9]. The design of dressings and the area of tissue engineering comprise expertise from different fields of knowledge, combining the principles of materials engineering and life sciences in the development of artificial devices for regenerative medicine. Material properties must be tailored to meet the requirements determined by the tissue in which the device will be implanted.

Dressings and three-dimensional supports (scaffolds) constituted by natural polysaccharides such as chitosan, alginate and xanthan gum, or the polyelectrolyte complex (PEC) formed by the combination of these ionic polymers, are biocompatible and may present antibacterial properties and stimulate cell migration and proliferation [10–15]. Due to these properties, the application of these materials in skin tissue engineering has been extensively explored. However, the mechanical properties of these polysaccharide-based scaffolds and dressings are inferior to those presented by human skin.

Human skin possesses tensile strength in the range of 2–16 MPa, Young's modulus in the range from 6 to 40 GPa, and elongation at break in the range of 70–77% [16], thus the development of skin implants or dressings presents several challenges with respect to mechanical properties, because the porous matrices based on chitosan, alginate or even the PEC formed between these polymers possesses lower mechanical properties, showing some limitations for application in the skin tissue engineering [13–15]. Bellini and coworkers studied chitosan/xanthan PEC membranes. While tensile strength for porous and dense membranes ranged from 0.4 to 25 MPa, the elongation at break was lower than 2% [4]. Similar results have been found by Bueno [13] and Rodrigues [14] for chitosan/alginate PEC membranes, which reached up to 8% of elongation at break for non porous membranes. Therefore, the development of dermal implants based on these materials presents a variety of challenges, particularly when the implant region is near or over a joint.

Many attempts related to the production of blends of these biomolecules with synthetic polymers are reported in the literature, but the hydrophilic nature of the polysaccharides makes difficult the process of blending them with polymers that have more adequate mechanical properties, such as polycaprolactone (PCL) [5,6,17,18]. This particular synthetic polymer has been extensively used in many strategies for the development of materials for biomedical applications [5,19,20].

Different processing techniques can be applied in order to produce membranes and scaffolds, such as solvent casting, thermal or solvent-induced phase inversion and electrospinning. The electrospinning technique has been successfully applied in the production of porous membranes in the tissue engineering [21–23]. Electrospun membranes and scaffolds have a wide range of biomedical applications such as drug delivery, wound dressings and vascular grafts [24–26]. Different classes of natural and synthetic materials have been used to produce nanofibers [27–30]. However, electrospinning of natural polymers such as chitosan and alginate is quite harder than processing synthetic polymers. Chitosan, with a molecular structure comprising of β-(1-4) linked D-glucosamine and N-acetil-D-glucosamine units, presents very low solubility in most solvents, and the use of diluted or concentrated acid solutions seems to be the most reliable option to properly dissolve it (the protonation of chitosan amine groups greatly contributes to its solubility) [11]. While many strategies have been successfully adopted to obtain chitosan-based electrospun scaffolds [31–34], the mechanical limitations of such scaffolds and membranes are similar to those of traditional dense films.

The combination of PCL and chitosan in electrospun scaffolds has been reported in the literature. Typically, blends of the polymers are electrospun from strong acids or fluorinated solvents solutions [35–37]. In their work, Yang [37] and coworkers produced electrospun scaffolds from PCL and chitosan blends in hexafluoropropanol, with different proportions of chitosan in the final material, ranging from 3% to 23%. The mechanical properties of such scaffolds strongly depend on the composition, showing values of Young's modulus (9.8–12.9 MPa) and elongation at break (24–77%) suitable for skin dressings. Recently, Hassanin [36] and coworkers reported the development of multilayered scaffolds based on PCL and chitosan for drug delivery applications. Such scaffolds architecture comprises an inner layer of PCL and outer layers of PCL/chitosan blends, obtained by electrospinning. While possessing desirable rates of drug release, no mechanical properties or other parameters were investigated. Also, the use of trifluoracetic acid as solvent for electrospinning is very hazardous and strongly affects the properties of hydrolysable polymers.

In this work, multilayer scaffolds were produced through the use of the electrospinning technique, aiming at the manufacture of scaffolds with suitable mechanical properties for the treatment of skin applications while maintaining relevant properties of chitosan. In a simplified manner, the membranes were comprised of two layers: the first one composed of PCL or a PCL/cellulose acetate blend, which acts as mechanical support, and the second layer performs the role of primary wound dressing (this constituted by a chitosan/poly(ethylene oxide) blend) designed to be in direct contact with the lesion bed. The poly(ethylene oxide) play the role of both a spinning enabler/enhancer and of a hydrophilicity improver. The scaffolds were characterized by means of mechanical properties, water and moisture uptake, stability in phosphate-buffered saline, moisture permeation, water contact angle, macroscopic and microscopic morphology and regarding effects of direct and indirect contact to L929 cells.