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Material behaviour

Thermal and hydrolytic degradation of electrospun fish gelatin membranes

D.M. Correia [a,b], J. Padrão [c], L.R. Rodrigues [c], F. Dourado [c], S. Lanceros-Méndez [b], V. Sencadas [b,d,*]

[a] Centro/Departamento de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Centro/Departamento de Física da Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[c] Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal
[d] Escola Superior de Tecnologia, Instituto Politécnico do Cávado e do Ave, Campus do IPCA, 4750-810, Barcelos, Portugal

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ABSTRACT

The thermal and hydrolytic degradation of electrospun gelatin membranes cross-linked with glutaraldehyde in vapor phase has been studied. *In vitro* degradation of gelatin membranes was evaluated in phosphate buffer saline solution at 37 °C. After 15 days under these conditions, a weight loss of 68% was observed, attributed to solvation and depolymerization of the main polymeric chains. Thermal degradation kinetics of the gelatin raw material and as-spun electrospun membranes showed that the electrospinning processing conditions do not influence polymer degradation. However, for cross-linked samples a decrease in the activation energy was observed, associated with the effect of glutaraldehyde cross-linking reaction in the inter- and intra-molecular hydrogen bonds of the protein. It is also shown that the electrospinning process does not affect the formation of the helical structure of gelatin chains.

1. Introduction

Gelatin is a biodegradable, biocompatible, non-toxic and non-carcinogenic biopolymer [1]. It is typically obtained through partial denaturation of collagen and accounts for 30% of the total animal protein in all animals. Collagen comprises all 20 amino acids in its three α-chains, which are stabilized and interlaced by hydrogen bonds into a triple helix that rotates clockwise [2–4]. Collagen may be partially degraded using two distinct pre-treatments, the acid and the alkali, resulting in type-A and type-B gelatin, respectively [3,5]. Gelatin possesses some drawbacks regarding long term applications, such as drug delivery systems [6] or smart packaging [4,7], because the protein dissolves quickly in an aqueous environment. To overcome this limitation, cross-linking of gelatin is necessary.

Electrospinning allows the production of flexible and highly porous nanofiber structures by applying a high electric field to a droplet of polymer solution or melt [8,9]. Although gelatin has been successfully electrospun into fibers, the preparation of electrospun membranes raises some critical issues, such as the use of highly toxic solvents. Electrospun gelatin membranes can be obtained by the dissolution of the polymer in acetic acid/formic acid mixtures [10], 1,1,1,3,3,3-hexafluoro-2-propanol [11], 2,2,2-trifluoroethanol [12] and with acetic acid and ethyl acetate aqueous solutions [13].

Cross-linking of gelatin fiber membranes is a necessary step to increase its stability in aqueous environments. This can be achieved either using physical methods, such as

* Corresponding author. Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal.
E-mail addresses: vsencadas@fisica.uminho.pt, vsencadas@ipca.pt (V. Sencadas).

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dehydrothermal treatment and ultraviolet or gamma irradiation, or chemical methods which exploit a large number of chemical agents to modify gelatin functional side groups [14].

Gelatin chemical cross-linking can be achieved by glutaraldehyde (GA) [15,16], genipin [17,18] and EDC/N-hydroxysuccinimide (NHS) [19] in order to maintain morphological integrity of the as-spun membranes. GA is one of the most widely cross-linking agents used in polymeric materials from natural origin, such as gelatin [12] or chitosan [20]. It has been reported that cross-linking of gelatin microspheres with GA takes place more rapidly and is more efficient than with genipin. A maximum cross-linking degree of approximately 90% is achieved in less than 4 h of exposure to GA, whereas just around 60% of cross-linking was achieved after 72 h of exposure to genipin [21,22].

In vitro degradation of gelatin is usually performed in a PBS collagenase solution at 37 °C. Rosellini et al. [23] studied the effect of the presence of collagenase in the phosphate buffer saline (PBS) solution during *in vitro* degradation of gelatin scaffolds cross-linked with GA, and found that a mainly hydrolytic degradation process occurs due to solvation and depolymerization of the polymeric chains [24].

Studies on the thermal properties of gelatin showed that it exhibits three main thermal degradation steps, the first being attributed to water desorption, and the others associated with protein degradation [25,26]. Gelatin thermal degradation kinetics has an activation energy (E_{act}) ranging between 175 and 275 kJ.mol^{-1}, the values increasing with the protein's molecular weight [25,27].

In the present study, electrospun gelatin membranes were prepared by electrospinning and cross-linked with GA in vapor phase in order to improve the fiber membranes stability in a moisture environment. Hydrolytic degradation was evaluated in PBS solution at 37 °C. Moreover, thermal degradation before and after GA crosslinking was studied. The main goal is to evaluate the potential of fish gelatin for biomedical applications.

2. Experimental

Electrospun *membranes preparation*: Fish gelatin was purchased from Sigma–Aldrich and dissolved in a blend of N,N-dimethylformamide (DMF, from *Merck*) and formic acid (FA, from Sigma–Aldrich) (40/60 vol/vol) to achieve a gelatin concentration of 30 wt% of the final solution. Fish gelatin was dissolved by stirring at 50 °C [28]. The polymer solution was then placed in a commercial plastic syringe (10 mL) fitted with a steel needle with 500 μm inner diameter. Electrospinning was conducted at 1.5 kV.cm^{-1} with a high voltage power supply from *Glassman* (model PS/FC30P04). A syringe pump (from *Syringepump*) was used to feed the solutions into the needle tip at a rate of 0.2 mL.h^{-1}, and the electrospun fibers were collected in a grounded collector.

Cross-linking: Samples were placed in a vapor chamber and then collected at different times, between 2 and 48 h, with 20 mL of glutaraldehyde (GA, 50% water, Panreac), which was vaporized at room temperature, placed at the bottom of the chamber.

Hydrolytic degradation: In vitro degradation of cross-linked gelatin electrospun membranes was carried out in PBS solution. The membrane samples were cut into squares of 25 × 25 mm^2 (triplicate samples were used for statistical purposes), immersed in 15 mL of PBS (pH 7.4; 0.8 g NaCl; 0.2 g KCl; 1.44 g $Na_2HPO_4.2H_2O$ and 0.2 g KH_2PO_4 dissolved in 1 L of distilled water) and incubated in an air circulation oven (*HERAEUS Vacuotherm*) at 37 °C for 15 days. The pH of the PBS solution was measured periodically and PBS was renewed every 72 h. After specific periods of time, a membrane was removed from the PBS, washed with ultrapure water and dried in a vacuum oven (*JP SELECTA Vacuotherm*) at room temperature until constant mass was reached. Samples were weighed before and after degradation in an electronic quartz microbalance (M5P from Sartorius) with a resolution ≤ 0.001 mg. The extent of hydrolytic degradation (W_L) was calculated by:

$$W_L = \left(1 - \frac{m_s}{m_0}\right) \quad (1)$$

where, m_s is the sample mass after an incubation period and m_0 is the initial sample mass.

Characterization: Electrospun fibers were coated with a thin gold layer using a sputter coater (Polaron, model SC502) and their morphology was analyzed using a scanning electron microscope (SEM, Quanta 650, from FEI) with an accelerating voltage of 15 kV. Nanofiber average diameter and the distribution were calculated over approximately 40 fibers using SEM images (5000× magnification) and Image J software. Differential scanning calorimetry measurements (DSC) were performed in a *Perkin–Elmer* Pyris-1 apparatus at a heating rate of 10 °C.min^{-1}. Samples for the DSC studies were cut into small pieces from the middle region of the electrospun membranes and placed in 40 μL aluminum pans. All experiments were performed under a nitrogen purge. The thermal degradation kinetics of the samples was characterized by thermogravimetric analysis (TGA) in a *Perkin–Elmer* Pyris-1 TGA apparatus at heating rate scans from 10 °C.min-1 up to 40 °C.min^{-1} performed under a nitrogen atmosphere.

3. Results and discussion

The as-spun membranes (Fig. 1) show randomly oriented fibers with an average diameter of 240 ± 58 nm, with smooth surface and without bead defects (Fig. 1a). Since gelatin is a water soluble material, as-spun fiber membranes will partially or totally dissolve when in contact with an aqueous medium or high moisture environments [12]. To broaden the applications of these gelatin fibers to procedures requiring contact with water or biological medium gelatin fibers were exposed to a saturated atmosphere of GA in order to promote chemical cross-linking of the material. It was found that this chemical treatment does not influence the fiber average diameter for an exposure time up to 48 h [28]. Furthermore, the cross-linked fibers showed the same appearance as the as-spun ones (Fig. 1b). Similar behavior has been reported for other polymers such as chitosan [29].

Chemical cross-linking of gelatin with GA involves the reaction of ε-amino groups of Lys (lysine) or Arg (arginine)