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Tailored electrospun nanofibrous polycaprolactone/gelatin scaffolds into an acid hydrolytic solvent system

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A B S T R A C T

Blended nanofibrous scaffolds based on polycaprolactone (PCL) and gelatin (Ge) were successfully prepared. A formic/acetic acid (1:1) mixture was used to dissolve PCL/Ge blends from 100/0 to 20/80 wt% in steps of 10 wt %. The hydrolysis of the PCL diluted in the formic/acetic acid mixture was considered as a method for tailoring the surface morphology and physicochemical features of the nanofibrous PCL/Ge scaffolds as a function of the dissolution time. The fibre diameter remained in the nanoscale range for all the studied scaffolds, which is crucial to mimic the extra-cellular matrix size. The reduction of the intrinsic viscosity, molar mass and hydrodynamic radius found for the PCL molecules as a function of the dissolution time, consequently diminished the entanglement capability of the polymeric chains. Subsequently, the fibre diameter decreased as dissolution time increased for all the studied compositions. While the crystallinity of the scaffolds with high PCL content increased as a function of the dissolution time, the scaffolds with high percentage of Ge showed the lowest crystallinity degree, which was ascribed to the hindering effect of the Ge that diffused among the PCL segments. The wettability increased as a function of the Ge content due to the high hydrophilic behaviour of these molecules. Water affinity also increased as a function of the dissolution time, due to the more hydroxyl groups available in the PCL segments to interact with water molecules. As a whole, the physicochemical assessment of the electrospun scaffolds revealed an effective tailoring procedure to obtain functionalised PCL/Ge scaffolds with specific properties as a function of the dissolution time before electrospinning.

1. Introduction

Tissue engineering involves the combination of engineering, materials and cells to improve or replace biological tissues. One of the fundamental approaches of this field is the fabrication of biocompatible scaffolds that provide the optimum conditions for cell adhesion and proliferation, along with tailored durability and performance. The development of synthetic nanoscaled fibrous scaffolds that structurally mimic the extra-cellular matrix (ECM) in size and porosity has brought new possibilities in the field of tissue regeneration [1].

The processing techniques to obtain artificial tissues are in continuous development [2]. Electrospinning stands out as one of the most promising techniques for the preparation of polymeric nanofibrous devices [3–5]. This method offers non-woven nanofibrous scaffolds with large area-to-surface ratio and high porosity that have fulfilled novel biomedical requirements [6,7].

A variety of natural and synthetic polymers have been used for nanofibrous scaffold fabrication, including polycaprolactone, poly (lactic acid), poly(glycolic acid) and their copolymers [8–11]. Among them, polycaprolactone (PCL) is a semicrystalline linear aliphatic polyester, widely applied in biomedicine due to its good mechanical properties, biocompatibility and slow biodegradability, being suitable for applications which require certain structural durability [12–17]. However, the lack of hydrophilic functional groups in the chemical structure of the PCL macromolecules have prevented this material from an extended application as an individual component. Several strategies such as coating, grafting or blending with other components have been suggested in order to improve the cell affinity of the scaffolds [18]. Actually, several hydrophilic biopolymers including collagen, gelatin, fibrinogen or elastin have been considered to improve the scaffold hydrophilicity and biocompatibility [19]. Gelatin (Ge) is a natural biopolymer of excellent biocompatibility, biodegradability and low cost in comparison to collagen. It contains Arginyl-Glycyl-Aspartic amino acid sequence, which offers biochemical signals to promote cell adhesion, migration, proliferation and differentiation [20]. Thus, the combination of PCL and Ge into a nanofibrous scaffold would retain the mechanical properties of PCL with an improved cell affinity brought by the Ge. Actually, PCL/Ge nanofibrous scaffolds have been proposed as a

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versatile substrate for cell seeding of different tissues, including skin [21–26], muscle [27], cardiovascular [28,29], nerve [30–32], bone [33–36] and cartilage [37,38].

The suitability of the electrospinning process is known to depend on the synergistic effect of the solution and processing conditions [39–41]. Traditionally, highly toxic halogenated solvents such as chloroform, hexafluoroisopropanol, trifluoroethylene, dimethylformamide, methylene chloride or dichloroethane have been required for the effective electrospinning of PCL and Ge [40,42,43]. In the last years, some alternative solvents such as acetone [44], acetic acid [45], acetic acid/ethyl acetate [46], tetrahydrofuran/methanol [47], tetrahydrofuran [40] or formic/acetic acid mixture [48–50] have been proposed for the electrospinning of the PCL. These solvents allow minimizing the risk to health during manipulation [51] and reduce the toxicity of the scaffolds due to retained residual solvent [52]. For the electrospinning of Ge, low-toxic alternative solvents have also been considered, including acetic acid [53,54] or ethyl-acetate/acetic acid in water [55]. Specifically, the formic/acetic mixture has been considered as a suitable candidate for the electrospinning of PCL/Ge scaffolds [56–59].

Some studies in the bibliography correlate the influence of several features of the scaffolds on their subsequent performance such as the composition, the fibre diameter, the scaffold porosity, the molar mass, the glass transition temperature or the crystallinity degree, among others [60–65]. The hydrolytic degradation of the PCL molecules in the formic/acetic mixture results in the reduction of the polymer molar mass, and thus, some of the physicochemical properties of the electrospun scaffolds could be expected to be altered as a function of the dissolution time [49]. Indeed, the physicochemical behaviour of PCL/Ge scaffolds has not been correlated to the dissolution time of the polymer in the formic/acetic acid solution before electrospinning, and therefore represents an interesting line of research for the tailoring of biomedical scaffolds.

The aim of this study was therefore to obtain tailored nanofibrous PCL/Ge scaffolds in terms of the blend composition and the dissolution time into a hydrolytic formic/acetic acid (1:1) solvent, and correlate them with the impact on the structure, morphology and performance of these scaffolds.

2. Materials and methods

2.1. Materials

Polycaprolactone (PCL) was supplied by Perstorp as 3 mm diameter pellets under the grade CAPA™ 6800 ($M_n = 85000\,\text{g·mol}^{-1}$ and T_m 58–60 °C). Gelatin (Ge) from porcine skin Type A, gel strength 300, was supplied by Sigma-Aldrich. Formic acid and acetic acid (≥99%) were used as solvents for electrospinning. Tetrahydrofuran (≥99.8%) was used for SEC sample preparation and analysis. All solvents were supplied by Sigma-Aldrich and were used without further purification.

2.2. Polymer solution and electrospinning

Nine blended compositions of PCL/Ge were prepared, ranging from 100/0 to 20/80 by weight proportion, by steps of 10 wt%. The solutions for electrospinning were prepared in a 1:1 formic/acetic acid mixture, with a total solid concentration of 15 wt% and were electrospun after being subjected to 30 °C and magnetic stirring for 24, 48, 72, 96 and 120 h. The dissolution time ranged between the minimum time to reach complete dissolution (24 h) and the time in which the viscosity of the solution still ensured stable electrospinning (120 h).

Nanofibrous scaffolds were obtained by means of a Bioinicia FLUIDNATEK® LE-10 electrospinning equipment, which contents a high voltage source, a programmable syringe pump, a HSW NORM-JECT 20 mL Luer Lock syringe, Teflon® tubing, a gauge 21 metallic needle and a grounded flat collector. The tip-to-collector distance was maintained constant at 15 cm. The feeding rate and voltage varied as dissolution time increased between 1 and 0.2 mL·h^{-1} and 25 to 19 kV, respectively. The working time was adjusted for each case as a function of the feeding rate in order to obtain nanofibrous structures with comparable surface density. The temperature and relative humidity (RH) were kept constant along electrospinning at 22 °C and 35% RH, respectively. The nanofibrous scaffolds were collected on waxed paper, dried and stored for further analyses.

2.3. Scaffold characterization

2.3.1. Field-emission scanning electron microscopy (FE-SEM)

The surface topology of the specimens was analysed by means of a Zeiss Ultra 55 field emission scanning electron microscope (FE-SEM). The samples were cut into small pieces and dried at 50 °C in a vacuum oven for 24 h and then kept in a desiccator during 48 h. Afterwards, the specimens were mounted on metal studs and sputter-coated with a platinum layer during 10 s using a Leica EM MED020 sputter coater. FE-SEM images were taken at 22 °C with a 2 kV voltage. The fibre diameters were measured from the scanning electronic microscope images (10000×) at random locations ($n = 100$) with the aid of the Image J® software.

2.3.2. Thermogravimetric analysis (TGA)

The thermo-oxidative decomposition profiles were obtained by means of a Mettler-Toledo TGA 851 thermogravimetric analyser. The samples, with a mass of about 4 mg were introduced into TGA Mettler-Toledo perforated alumina crucibles, with capacity of 70 µL. The samples were analysed in the temperature range of 25–800 °C with a heating rate of 10 °C·min^{-1}, under atmosphere of oxygen at a flow rate of 50 mL·min^{-1}. The experiments were performed in triplicates to ensure reproducibility.

2.3.3. Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) was carried out by means of a Malvern Instruments OMNISEC RESOLVE chromatograph. It combined an integrated pump, a degasser, an autosampler and a column oven, along with a Malvern Instruments OMNISEC REVEAL multi-detector –Ultraviolet (UV), Refractive Index (RI), Low and Right Angle Light Scattering (LALS and RALS) and Viscosity (VISC)–. A monodisperse polystyrene standard with dn/dc value of 0.185 was used for universal calibration. Two columns from Malvern Instruments (T2000 and T4000) were used (300 × 8 mm). Tetrahydrofuran (THF) was used as mobile phase at a flow rate of 1 mL·min^{-1} and a column temperature of 35 °C. The samples were dissolved in THF with concentrations of around 2.0 mg·ml^{-1} and filtered through 0.45 µm PTFE filters. Two specimens per sample were analysed and the obtained data were assessed in triplicates with the aid of the OMNISEC V10™ software, and the averages were taken as representative values.

2.3.4. Differential scanning calorimetry (DSC)

The calorimetric data were obtained by means of a Mettler-Toledo DSC 820^e differential scanning calorimeter, previously calibrated following the procedure of In and Zn standards. The samples, with a mass of about 4 mg, were analysed between 0 and 80 °C with a heating/cooling/heating rate of 10 °C·min^{-1}. All the experiments were run under nitrogen atmosphere at 50 mL·min^{-1}. The specimens were characterised at least by triplicate and the averages of temperatures and enthalpies were taken as representative values.

The crystallinity degree (X_c) was evaluated from the melting enthalpy results, by means of the Eq. (1),

$$X_c(\%) = \frac{\Delta h_m}{w_{PCL}\cdot\Delta h_m^0}\cdot 100 \tag{1}$$

where Δh_m is the melting enthalpy of the PCL melting, w_{PCL} is the weight fraction of the PCL in the sample and $\Delta h_m{}^0$ is the melting enthalpy of a perfect crystal of PCL (148 J·g^{-1}) [66].

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