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Biomimetic polyurethane – Based fibrous scaffolds

Chiara Tonda-Turo [a], Monica Boffito [a], Claudio Cassino [b], Piergiorgio Gentile [c], Gianluca Ciardelli [a,*]

[a] Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy
[b] Department of Science and Technology, University of Piemonte Orientale, Alessandria, Italy
[c] School of Mechanical and Systems Engineering, Newcastle University, Newcastle upon Tyne, UK

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ABSTRACT

The design of biomimetic scaffolds that reproduce the biochemical, mechanical and structural functions of the ECM is a fundamental requirement to develop functional substrate for tissue engineering. To this aim, materials which combine the easy processability and mechanical properties of synthetic polymers with the biochemical cues provided by natural materials, develop positive interaction with cells, enabling tissue regrowth in vivo. In this work, a polyester urethane (NS-BC2000) was synthesised using poly (ε-caprolactone) diol (PCL) as macrodiol, 1,4-diisocyanatobutane (BDI) as diisocyanate and N-Boc serinol as chain extender. After Boc cleavage (S-BC2000) to expose free amino groups, a S-BC2000/PEO (80/20 wt./wt.) solution was successfully electrospun fabricating aligned nanofibres with a fibre size of 2.8 ± 0.6 μm.

The aligned nanofibres were then functionalised with IKVAV peptide that was covalently bound exploiting the presence of free amino groups on S-BC2000 chains.

1. Introduction

The design of new synthetic biomaterials is a promising tool to overcome the current limitations in tissue engineering scaffold development. Specifically, scaffolds able to mimic natural extracellular matrix (ECM) with regard to its composition (biochemical mimicking), morphology (morphological mimicking) and mechanical properties (mechanical mimicking) can properly interact with cells enhancing adhesion, proliferation and differentiation processes [1–3]. Among other materials, synthetic polymers provide versatile bulk and mechanical properties and easy processability through different techniques [4,5]. However, they do not mimic ECM composition and surface modification techniques are required to introduce the desired bioactivity into biomaterials [6].

Polyurethanes have been recently applied in many TE applications thanks to their versatile polymer structure [7,8]. Furthermore, functional groups can be easily incorporated into the polymeric chain, reducing the need of additional functionalisation procedures on the polymer scaffold with consequent, possible modification of morphology and bulk properties [9].

In this work, a polyester urethane (PUR) having an amino acid derived diol (N-Boc serinol) as chain extender was synthesised as previously described [7]. After Boc cleavage, the polyurethanes were used to produce aligned fibres to mimic the aligned morphologies of soft tissue such as nerve and muscle [10].

Finally, a laminin-derived peptide (Ile-Lys-Val-Ala-Val;IKVAV) was covalently bound to the free amino groups, in order to introduce haptotactic cues on the scaffold and mimic the ECM composition [11,12]. The scaffold developed within this work combines the biomimetic requirements in terms of structure and composition, highlighting the unique properties of polyurethane-based materials that allowed easy surface functionalization without further process.

2. Materials and methods

2.1. Materials

Poly (ethylene oxide) (PEO) (M_n=100 kDa), dibutyltin dilaurate (DBTDL), N-Boc serinol, 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS) and all solvents (analytical grade) were purchased from Sigma Aldrich-Italy. Poly (ε-caprolactone) diol (PCL) (M_n=2000 Da) and 1,4-diisocyanatobutane (BDI) were purchased from Across Organics and Allorachem, respectively. Ile-Lys-Val-Ala-Val (IKVAV) and FITC-IKVAV peptides were purchased from Biomatik.

Anhydrous 1,2-dichloroethane (DCE) was prepared by leaving 1,2-dichloroethane over molecular sieves (4 Å, CarloErba Reagenti)

* Corresponding author.
E-mail address: gianluca.ciardelli@polito.it (G. Ciardelli).

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in a nitrogen atmosphere for at least 8 h prior the synthesis.

2.2. Scaffold fabrication

The polyurethane (NS-BC2000) was synthesised following a two steps procedure in an inert atmosphere using DCE as solvent, PCL as macrodiol, BDI as diisocyanate and N-Boc serinol as chain extender, as previously described [7].

In order to remove Boc groups from NS-BC2000, the polymer was first solubilised (40% w/v) in DCE in a nitrogen atmosphere at room temperature and 2,2,2-trifluoroethanoic acid (TFA, SigmaAldrich, Italy) was added (5% v/v with respect to DCE). After 1 h, rotary evaporation was performed to remove both TFA and DCE. Then, the de-protected polymer (S-BC2000) was again solubilised in DCE (10% w/v) for a second rotor-evaporation in order to completely remove all TFA traces. Subsequently, the polymer was solubilised in DCE (10% w/v), precipitated in diethyl ether (DEE) (5:1 with respect to DCE volume) and then dried under vacuum at 40 °C overnight.

NS-BC2000 and S-BC2000 were used for fibres fabrication using a Linari Engineering device. The electrospun fibres were prepared from NS-BC2000/PEO (80:20) and S-BC2000/PEO (80:20) polymer solution in chloroform (20% w/v). PEO was added to PUR solutions to stabilise the polymer jet and enhance fibres formation [13]. The solution and the electrospinning parameters were optimised and homogenous and aligned fibres were obtained using a static 21 G needle, a tip to collector distance of 15 cm, a flow of 0.5 ml/h, an electric potential of 20 kV and a rotating drum (diameter of 80 mm and length of 120 mm) with an angular speed of 2000 rpm.

Biomimetic S-BC2000 fibres were obtained after surface functionalisation of the electrospun membranes by IKVAV grafting. Briefly, 1 mL of an aqueous solution containing 1 mg/ml of IKVAV (or FITC-IKVAV) was mixed with 1 mL of distilled water containing EDC/NHS (4/1) (pH 5.5) for 1 h at 4 °C [14]. Then, water was added to the obtained solution, namely IKVAV_EDC/NHS, in order to have a final IKVAV concentration of 0.25 mg/mL and the pH was raised to 7.4 by adding drops of 1N NaOH. S-BC2000 nanofibrous membranes (2×2 cm^2 size) were immersed in 1 mL IKVAV_EDC/NHS solution for 20 h at 5 °C. Then, the solution containing unreacted IKVAV_EDC/NHS was withdrawn and the samples were washed three times using distilled water and dried under vacuum overnight at room temperature.

2.3. Scaffold characterization

NS-BC2000 and S-BC2000 samples were characterised through Size Exclusion Chromatography (SEC) (Agilent Technologies 1200 Series, USA) and Attenuated Total Reflectance Fourier Transform Infrared (ATR-FT-IR) Spectroscopy (Perkin Elmer Spectrum 100) according to previously published protocols [8].

^{1}H-NMR spectra of NS-BC2000 and S-BC2000 were recorded in anhydrous deuterated dimethyl sulfoxide (DMSO-d6) by means of an Avance III Bruker spectrometer equipped with a 11.75 T superconductor magnet (500 MHz ^{1}H Larmor frequency). The spectra were recorded by averaging 12 runs, with 10 s relaxation time. The ^{1}H-NMR signals were referenced to TMS at 0 ppm.

Scanning Electron Microscopy (SEM, LEO 1450VP) was used to evaluate the morphology of the produced fibres. Samples were sputtered using gold under vacuum for 100 seconds by Agar Auto Sputter Coater. Fibre diameters and pore size were measured on three different images using ImageJ software.

Surface composition was determined by XPS on Theta Probe (Thermo Scientific), which uses a micro-focused AlKa X-ray source (1486.6 eV), operated with a 400 μm spot size (100 W power). Survey spectra were collected at a pass energy of 200 eV, a step size of 1 eV and a dwell time of 50 ms, with the spectrometer operated in standard (not angle-resolved) lens mode. Three points were analysed on each sample surface as received. Charge neutralisation was used throughout the analysis. High resolution regional spectra were collected using a pass energy of 40 eV, a step size of 0.1 eV and a dwell time of 200 ms. High resolution spectra envelopes were obtained by curve fitting synthetic peak components using the CasaXPS software.

Finally, the amount of grafted peptide was estimated through the difference between the initial FITC-IKVAV solution concentration and the sum of the concentration of the solutions removed from the S-BC2000 membranes after grafting procedure and three rising steps. Solution concentrations were measured through a Perkin Elmer Lambda 25 UV/Vis Spectrometer in the spectral range from 550 to 400 nm.

3. Results

NS-BC2000 was successfully synthesised as demonstrated by ATR-FT-IR spectroscopy and SEC. NS-BC2000 average numeral molecular weight was in the range of 33,000–44,000 Da. The low polydispersity index (1.3–1.5) indicated a narrow distribution of the molecular weight. After the cleavage of the Boc groups, S-BC2000 numeral molecular weight obtained by SEC was in the range of 23,000–34,000 Da, showing a degradation percentage of 22–30% and a polydispersity index of 1.4–1.5. No significant increase in polydispersity index was observed for S-BC2000 showing that the Mw distribution is shifted to low molecular weight without further dispersions. S-BC2000 ATR-FT-IR spectrum confirmed the integrity of polymer chain bonds and the absence of TFA residues.

^{1}H-NMR spectroscopy was performed to verify Boc cleavage (Fig. 1).

A reduction in the area of the peak associated to the methyl protons of the Boc protecting group was observed and about 15% of the Boc protecting groups were removed, while all the other polymer signals in the ^{1}H-NMR spectrum remained constant indicating that Boc cleavage treatment did not affect polymer structure significantly.

NS-BC2000/PEO and S-BC2000/PEO electrospun fibres showed an aligned morphology with a fibre size of 2.2 ± 0.7 μm and 2.8 ± 0.6 μm for NS-BC2000/PEO and S-BC2000/PEO respectively (Fig. 2). The cleavage of N-BOC groups did not affect the properties

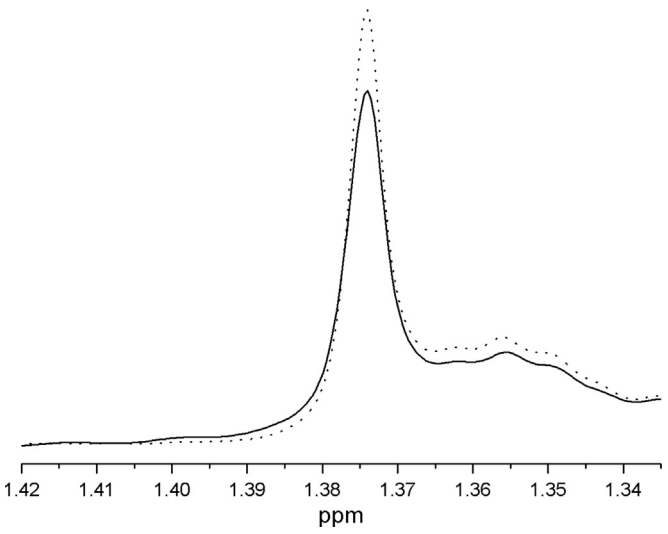

Fig. 1. ^{1}H-NMR spectra of NS-BC2000 (dotted line) and S-BC2000 (solid line); expansion of BOC protons signal region.