Acta Biomaterialia 51 (2017) 161–174

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Solution blow spinning fibres: New immunologically inert substrates for the analysis of cell adhesion and motility

Rafaella T. Paschoalin [a,b], Bruna Traldi [a], Gülcan Aydin [c], Juliano E. Oliveira [d], Stephan Rütten [e], Luiz H.C. Mattoso [a,b,*], Martin Zenke [c], Antonio Sechi [c,*]

[a] National Nanotechnology Laboratory for Agribusiness (LNNA), Embrapa Instrumentation, 13560-970 São Carlos, SP, Brazil
[b] Department of Chemistry, Federal University of São Carlos (UFSCar), 13565-905 São Carlos, SP, Brazil
[c] Institute of Biomedical Engineering, Dept. of Cell Biology, Uniklinik RWTH Aachen, Pauwelsstrasse, 30, D-52074 Aachen, Germany
[d] Department of Engineering, Materials Engineering, Federal University of Lavras (UFLA), 37200-000 Lavras, MG, Brazil
[e] Electron Microscopy Facility, Uniklinik RWTH Aachen, Pauwelsstrasse, 30, D-52074 Aachen, Germany

ARTICLE INFO

Article history:
Received 13 July 2016
Received in revised form 16 December 2016
Accepted 5 January 2017
Available online 7 January 2017

Keywords:
Solution blow spinning
Dendritic cells
Cell motility
Cytoskeleton

ABSTRACT

The control of cell behaviour through material geometry is appealing as it avoids the requirement for complex chemical surface modifications. Significant advances in new technologies have been made to the development of polymeric biomaterials with controlled geometry and physico-chemical properties. Solution blow spinning technique has the advantage of ease of use allowing the production of nano or microfibres and the direct fibre deposition on any surface *in situ*. Yet, in spite of these advantages, very little is known about the influence of such fibres on biological functions such as immune response and cell migration. In this work, we engineered polymeric fibres composed of either pure poly(lactic acid) (PLA) or blends of PLA and polyethylene glycol (PEG) by solution blow spinning and determined their impact on dendritic cells, highly specialised cells essential for immunity and tolerance. We also determined the influence of fibres on cell adhesion and motility. Cells readily interacted with fibres resulting in an intimate contact characterised by accumulation of actin filaments and focal adhesion components at sites of cell-fibre interactions. Moreover, cells were guided along the fibres and actin and focal adhesion components showed a highly dynamic behaviour at cell-fibre interface. Remarkably, fibres did not elicit any substantial increase of activation markers and inflammatory cytokines in dendritic cells, which remained in their immature (inactive) state. Taken together, these findings will be useful for developing new biomaterials for applications in tissue engineering and regenerative medicine.

1. Introduction

The development of novel biomaterials and their use in biomedicine has received much attention in recent years [1,2]. Nanofibres have a wide medical application, ranging from scaffolds for tissue regeneration to drug delivery systems. Based on the type of application, significant advances in new technologies have been made to the development of polymeric biomaterials with controlled geometry and physico-chemical properties. Since the 1990s there has been an increasing interest in methods for polymeric fibres production, such as electrospinning [3]. Recently, nanometric fibres from a variety of polymers, including polyvinyl chloride (PVC), nylon-6, poly(lactic acid) (PLA) and poly(D, L-lactic acid) (PDLLA) have been produced by another method known as solution blow spinning (SBS) [4–11]. This innovative technique is conceptually similar to electrospinning but does not require a high voltage and, therefore, it is much safer and simpler. Additionally, it allows fast fibre production and versatility in solvent choice [4]. In contrast to other methods, SBS techniques enable the deposition of fibres directly onto any type of surface, including living systems as in the case of surgical scaffolds, sealants or tissue adhesives [4,9,11]. Nanofibres scaffolds fabricated by SBS have been shown to support osteogenic differentiation of human bone marrow stromal cells [10] and prevent both bacterial attachment and thrombosis [11].

Despite the promise of such techniques, their ultimate functional success to new medical therapies depends on how cells respond to the nanostructured biomaterials obtained. One main

* Corresponding authors at: National Nanotechnology Laboratory for Agribusiness (LNNA), Embrapa Instrumentation, 13560-970 São Carlos, SP, Brazil (L.H.C. Mattoso). Institute of Biomedical Engineering, Dept. of Cell Biology, Uniklinik RWTH Aachen, Pauwelsstrasse, 30, D-52074 Aachen, Germany (A. Sechi).
E-mail addresses: luiz.mattoso@embrapa.br (L.H.C. Mattoso), antonio.sechi@rwth-aachen.de (A. Sechi).

http://dx.doi.org/10.1016/j.actbio.2017.01.020
1742-7061/

strategy in tissue engineering involves the generation of hybrid structures containing biomaterials and cells. Biomaterials provide the initial support structure for cells to organise and assemble themselves into a functioning tissue. Hence, biomaterials must be highly adaptable polymers that can be easily engineered to achieve topographic and physico-chemical properties to be able to mimic native microenvironments required for the regulation of cell function and fate [12]. In addition, the biomaterial must be biocompatible enough not to elicit host immune response [13–17]. Many polymers have been used in the attempt to produce such microenvironments including, poly-L-lactic acid (PLLA), polyglycolic acid (PGA) and poly-lactic-co-glycolic acid (PLGA). Although it was found that these materials could be easily used to fabricate various architectures with controlled degradation characteristics, they also caused unwanted effects including activation of immune cells thus potentially leading to their rejection [15,18–22]. Hence, to avoid host reactions and improve implant safety, it is essential to understand how cells of various origin including immune cells interact with biomaterials and how these interactions may prove pivotal to evaluate the material biocompatibility.

This work was centred on the fabrication and characterisation of polymeric fibres composed of pure PLA or various PLA/PEG blends by solution blow spinning technique. Cells easily interacted with SBS fibres resulting in an intimate contact characterised by accumulation of actin filaments and focal adhesion components at sites of cell-fibre interactions. Moreover, cells movement was guided along SBS fibres and actin and focal adhesion components showed a highly dynamic behaviour at cell-fibre interfaces. Finally, SBS fibres did not elicit any substantial increase of activation markers and inflammatory cytokines in dendritic cells, which remained in their immature (inactive) state. Our findings suggest that SBS fibres could serve as novel inert biomaterials for tissue engineering applications.

2. Materials and methods

2.1. Chemicals

Poly(lactic acid) (PLA, mol. wt., 76,000) and polyethylene glycol (PEG, mol. wt., 8000) were purchased from Biomater, Ltda (São Carlos, Brazil) and Sigma-Aldrich (Brazil), respectively. Chloroform and acetone were purchased from Sigma-Aldrich and were not subjected to purification processes.

2.2. Preparation of polymer solutions

Five samples were prepared composed of pure PLA or PLA/PEG blends at PLA:PEG ratios of 99:1, 95:5, 90:10 or 80:20 (w/w). The overall polymer concentration was maintained constant at 12% for all blends. Polymer solutions were prepared by solubilizing weighed portions of the two chemicals in chloroform:acetone (3:1 v/v) with stirring at room temperature for 3 h. The polymer solutions were transferred into 25 mL glass syringes (Becton Dickinson, USA) and placed in a syringe pump (SBS apparatus).

2.3. Solution blow spinning (SBS) apparatus

The SBS apparatus was composed of a polymer injection pump (NE-1010-US One Channel 100 lb.), a collector consisting of a spinning cylinder positioned horizontally, a source of compressed air and a concentric nozzle system with an inner nozzle (0.5 mm in diameter) through which the polymer solution is injected at a rate of 7.2 μL/min and an outer nozzle through which compressed air is delivered at a constant pressure of 2.4 kPa. The inner nozzle was positioned so that it protruded 2 mm from the outer nozzle. SBS fibres were deposited directly on the collector spinning at 180 rpm that was positioned at a fixed working distance of 18 cm from the nozzle system. SBS fibre production was done at 21 °C and 35% humidity.

2.4. Rheological analysis

Viscosity of PLA and PLA/PEG blends was determined using the rotational and oscillatory Rheometer MCR 302 (Anton Paar, Austria). Measurements were done with a concentric cylinder geometry (DG26.7/Q1) in rotational mode at 25 °C using a shear rate between 1 and 100 s^{-1}.

2.5. PEG dissolution analysis

To determine the release of PEG from PLA/PEG SBS fibres, fibres were incubated with PBS (pH 7.2) or cell culture medium at 37 °C for up to 48 h in a shaking water bath (shaking speed: 150 rpm). Samples were collected at 2, 6, 12, 24 and 48 h, washed with distilled water and dried in a vacuum oven at room temperature for 24 h. Dried samples were analysed by infrared spectroscopy. For quantifying PEG dissolution, each spectrum baseline was corrected by subtracting a background absorbance calculated from the absorbance value between 2800 and 3100 cm^{-1}. Afterwards, each spectrum was normalised as already described [23] and then the intensity ratio blends/PLA for the 2800 cm^{-1} peak was determined.

To determine the loss of PEG from SBS fibres, we used 80:20 fibres due to their high content of PEG that allows more precise measurements. Dried SBS fibre mats (2.5 × 2.5 cm) were weighed, placed in a vial filled with 15 mL phosphate-buffered saline (PBS) (pH 7.0; Invitrogen Life Technologies) or cell culture medium supplemented with 10% FCS and then incubated with constant shaking (150 rpm) at 37 °C for 4, 8 or 12 h. At these time intervals, triplicate specimens were collected, rinsed thoroughly with distilled water and dried under vacuum to constant weight at room temperature. The percentage of elution was denoted as weight loss (%) = $(W_0 - W_t)/W_0 \times 100$, where W_0 was the dry weight before incubation and W_t was the dry weight at time t. The percentage of PEG elution was corrected by taking into account PLA mass loss.

2.6. Infrared spectroscopy

Infrared spectra were collected on dried samples using the attenuated total reflectance (ATR) method with a spectrophotometer (Vertex 70, Bruker, Germany). Spectra were collected for all samples at a resolution of 2 cm^{-1} in the range between 3300 and 600 cm^{-1} for a total of 128 scans.

2.7. Thermal properties

The thermal transition of pure PLA and PLA/PEG blends was measured under nitrogen at a flow rate of 20 mL/min using a differential scanning calorimeter (DSC; TA Instruments calorimetric analyser, model Q100, New Castle, USA). DSC measurements were done using 5–6 mg for each sample, which were sealed in an aluminium pan and heated from 0 to 180 °C at a rate of 10 °C/min.

Glass transition temperature (T_g), cold crystallization temperature (T_{cc}), melting temperature (T_m), and degree of crystallinity (χ_c) were determined from the first heating cycle to observe the effect of SBS processing. T_g was taken at the mid-point of heat capacity changes, T_m at the peak value of the endotherms and the enthalpy was calculated from area under the curve. All parameters were calculated by the software used to drive the DSC calorimeter. The degree of crystallinity (χ_c) of PLA and PLA/PEG