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Plasma-induced polymerization as a tool for surface functionalization of polymer scaffolds for bone tissue engineering: An in vitro study

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ABSTRACT

A commonly applied strategy in the field of tissue engineering (TE) is the use of temporary three-dimensional scaffolds for supporting and guiding tissue formation in various in vitro strategies and in vivo regeneration approaches. The interactions of these scaffolds with highly sensitive bioentities such as living cells and tissues primarily occur through the material surface. Hence, surface chemistry and topological features have principal roles in coordinating biological events at the molecular, cellular and tissue levels on timescales ranging from seconds to weeks. However, tailoring the surface properties of scaffolds with a complex shape and architecture remains a challenge in materials science. Commonly applied wet chemical treatments often involve the use of toxic solvents whose oddments in the construct could be fatal in the subsequent application. Aiming to shorten the culture time in vitro (i.e. prior the implantation of the construct), in this work we propose a modification of previously described bone TE scaffolds made from a blend of starch with polycaprolactone (SPCL). The modification method involves surface grafting of sulfonic or phosphonic groups via plasma-induced polymerization of vinyl sulfonic and vinyl phosphonic acid, respectively. We demonstrate herein that the presence of these anionic functional groups can modulate cell adhesion mediated through the adsorbed proteins (from the culture medium). Under the conditions studied, both vitronectin adsorption and osteoblast proliferation and viability increased in the order SPCL ≪ sulfonic-grafted SPCL < phosphonic-grafted SPCL. The results revealed that plasma-induced polymerization is an excellent alternative route, when compared to the commonly used wet chemical treatments, for the surface functionalization of biodevices with complex shape and porosity.

1. Introduction

Tissue engineering (TE) emerged as an interdisciplinary field confronting the transplantation crisis caused by the shortage of donor tissues and organs. Since its inception, scaffolds composed of synthetic and natural polymers have been key elements of different TE approaches [1]. The use of an appropriate template to provide physical support and a local environment for cells and hence to enable and facilitate tissue development is an essential issue for a successful regeneration strategy. Nowadays, it is well accepted [2–4] that the ideal scaffold for bone TE must possess adequate porosity, resulting in an interconnected and permeable structure that allows the ingress of nutrients and cells. It is also believed that proper mechanical and physical properties, controlled biodegradability, biocompatibility and the ability to promote cellular interactions and tissue development are other main requirements for TE scaffolds [2,5–8]. Last but not least, cells and surrounding tissues interact with any external devise primarily through the surface and therefore properties such as surface chemistry and topography are also key determinants in material–bioentity interactions.

Starch-based polymers have been studied as valuable materials for several biomedical applications [4,9]. Their biocompatibility and non-cytotoxicity have been confirmed by both in vitro [4,10–12] and in vivo [13] assays. In this work, we have chosen fiber mesh scaffolds made from a blend of starch and ε-polycaprolactone (SPCL) which have been already proposed for bone tissue engineering [4,8,10,11]. Previous works have targeted their optimization in terms of degradability [7–9], porosity [4] and mechanical properties [9,14], but few studies have focused on their surface properties [15] and the possibility of improving them [12,16]. Herein, we propose plasma-induced polymerization as a way to render an appropriate surface for enhancing cell adhesion and speeding up cell proliferation, which will shorten the culture time

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in vitro, i.e. prior to implantation. We have previously demonstrated [17,18] that this method is a very effective way for grafting of vinyl polymers on regular two-dimensional (2-D) structures without modifying the bulk properties of the material. In this study, we report the effectiveness of this method for the functionalization of 3-D structures with a complex shape and with the negatively charged sulfonic and phosphonic groups, and the influence of these groups on osteoblast cell behavior in vitro.

Anionic scaffolds have been investigated because of their ability to facilitate morphogenetic processes for tissue engineering substitutes [19,20]. For example, the negative charge of glucosaminoglycans (GAGs) is associated with their bioactivity. GAGs interact with the positively charged amino groups of extracellular proteins and these interactions determine cell–matrix adhesion. Recent studies with sulfate-derived materials indicate enhanced adhesion and proliferation of osteoblast-like cells as a result of the presence of the sulfate groups [17,21]. On the other hand, the introduction of phosphate groups has been also proposed as an attractive modification strategy in targeting bone tissue engineering applications [18,22–24]. The rationale for the use of this functionality stems from mimicry of bone-promoting proteins and the mineral–bone matrix. Phosphate-rich proteins are known to initiate nucleation of mineralized bone and tooth matrix. It is also reported that many bone-promoting proteins naturally interact with acidic polymers [25].

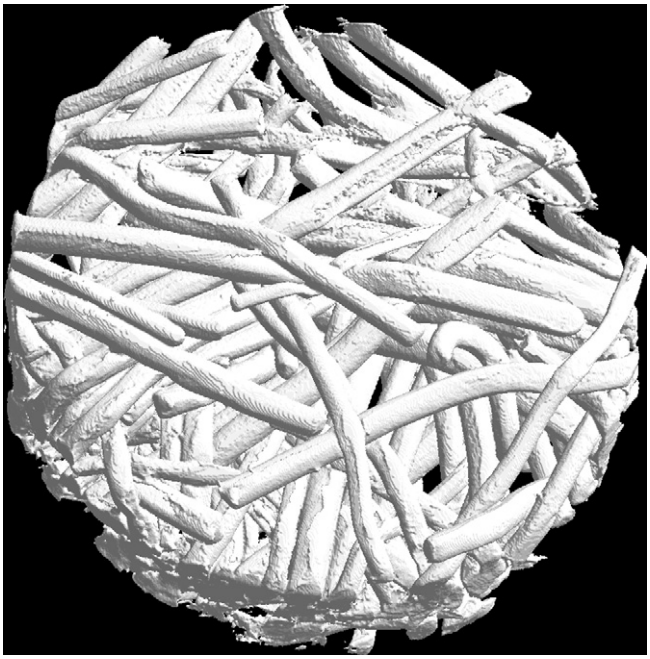

Fig. 1. Micro-computed tomography image from SPCL fiber mesh scaffold.

2. Materials and methods

2.1. Materials

In this work, we used a commercially available blend (Mater-Bi ZI01U, Novamont, Italy) of thermoplastic starch and poly(ε-polycaprolactone) (SPCL, 30/70 wt.%) [26,27]. The material was supplied in a granular form and processed by melt spun into fibers. Vinyl phosphonic acid (VPA) and vinyl sulfonic acid (VSA) were purchased from Sigma–Aldrich and used without further purification.

2.2. SPCL mesh production and modification

Fibers of SPCL were produced by melt spinning using a modular co-rotating twin screw extruder (Leistritz AG-LSM 36/25D, Germany) at a screw speed of 3 rpm and with a temperature profile in the barrel (from the feed zone to the die zone) of between 60 and 130 °C. The average output rate was 0.3 kg h^{-1}. Upon extrusion through the die, the filament was spun in two consecutive steps to a final draw ratio of approximately 1:100. The cooling of the filament was performed in air (average temperature of 17 °C). Melt-spun fibers presented a diameter in the 105–345 μm range, with a mean fiber diameter of 213 ± 50 μm. The fibers were cut into 0.5 cm lengths and used in the production of fiber mesh scaffolds by a custom-designed mould. Fiber bundles were randomly displaced into the mould cavities and subjected to thermal treatment at 60 °C for 30 min before predefined compression levels along the *Z*-axis were applied to ensure the bonding between neighboring fibers using a final compression ratio of 22%. Upon demoulding, scaffolds with dimensions 2.2 ± 0.2 mm thickness and 6 mm diameter were obtained. Their porosity was measured by micro-computed tomography and the obtained averaged value was 64.4 ± 4.4% (Fig. 1).

SPCL meshes were further modified by plasma-induced polymerization. Scaffolds were placed in a radio frequency (13.56 MHz) plasma reactor (Plasma Prep5, Gala Instrument, Germany) and exposed to O_2 plasma at 30 W of power for 15 min. During the treatment the pressure inside the reactor was maintained below 20 Pa by adjusting the gas flow. The activated meshes with free radicals formed on the surface were subsequently immersed in a degassed solution of VPA (100 mM in 2-propanol) or VSA (10 vol.% aqueous solution) at a ratio of 2 ml per scaffold. The reaction was carried out at room temperature for 2 h under stirring. The scaffolds were washed thoroughly with the solvent used for the reaction in order to remove any unreacted monomer and finally the modified samples were dried at room temperature.

2.3. Surface chemical composition

Surface elemental analysis of untreated and modified samples was performed by X-ray photoelectron spectroscopy (XPS). The spectra were obtained using an ESCALAB 200A instrument from VG Scientific (UK) with PISCES software for data acquisition and analysis. The spectrophotometer was calibrated with reference to Ag 3d5/2 (368.27 eV). Monochromatic Al Kα radiation ($h\nu$ = 1486.60 eV) operating at 15 kV (300 W) was used and the measurements were performed at a take-off angle of 90° relative to the sample's surface in constant analyzer energy mode (CAE). Survey spectra were acquire using a pass energy of 50 eV over a binding energy range of 0–1100 eV, and were used to calculate the elemental composition of the surfaces. High-resolution spectra for different regions were obtained using a pass energy of 20 eV. The peaks were fitted using the least-squares peak analysis software XPSPEAK version 4.1 and the Gaussian/Lorenzian sum function. Background counts were subtracted using a linear baseline and the sample charging was corrected assigning a binding energy of 285.0 eV to the saturated hydrocarbons C1*s* peak.

2.4. Surface topography

The topography of the samples was characterized by optical profiler analysis using a Wyko-NT 1100 interferometric profiler (Veeco) operating in vertical scanning interferometry mode. The images were processed and analyzed with the analytical software package WycoVision®32.

2.5. Protein adsorption

The effect of the surface treatments on protein adsorption was analyzed by fluorescent immunolabeling. Two adhesion proteins