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Corrugated round fibers to improve cell adhesion and proliferation in tissue engineering scaffolds

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ABSTRACT

Optimal cell interaction with biomaterial scaffolds is one of the important requirements for the development of successful in vitro tissue-engineered tissues. Fast, efficient and spatially uniform cell adhesion can improve the clinical potential of engineered tissue. Three-dimensional (3-D) solid free form fabrication is one widely used scaffold fabrication technique today. By means of deposition of polymer fibers, scaffolds with various porosity, 3-D architecture and mechanical properties can be prepared. These scaffolds consist mostly of solid round fibers. In this study, it was hypothesized that a corrugated fiber morphology enhances cell adhesion and proliferation and therefore leads to the development of successful in vitro tissue-engineered constructs. Corrugated round fibers were prepared and characterized by extruding poly(ethylene oxide terephthalate)-*co*-poly(butylene terephthalate) (300PEOT55PBT45) block co-polymer through specially designed silicon wafer inserts. Corrugated round fibers with 6 and 10 grooves on the fiber surface were compared with solid round fibers of various diameters. The culture of mouse pre-myoblast (C2C12) cells on all fibers was studied under static and dynamic conditions by means of scanning electron microscopy, cell staining and DNA quantification. After 7 days of culturing under static conditions, the DNA content on the corrugated round fibers was approximately twice as high as that on the solid round fibers. Moreover, under dynamic culture conditions, the cells on the corrugated round fibers seemed to experience lower mechanical forces and therefore adhered better than on the solid round fibers. The results of this study show that the surface architecture of fibers in a tissue engineering scaffold can be used as a tool to improve the performance of the scaffold in terms of cell adhesion and proliferation.

1. Introduction

In tissue engineering (TE), the general strategy is to seed cells within a scaffold, a structural device that defines the geometry of the replacement tissue and provides environmental cues that promote tissue regeneration [1]. To obtain a complex viable tissue, the TE scaffolds should have a high porosity/pore connectivity to ensure sufficient transport of oxygen and nutrients towards the cells and allow removal of metabolic waste products [2], since it is challenging to obtain a complex network of blood vessels and capillaries to perfuse the tissue for in vitro built tissues [3–6]. Moreover, micro and/or nano-topographical features are needed to aid the control of cell function, leading to engineering of better tissues [7,8].

Recent developments in biomaterials processing allowed the preparation of scaffolds with high porosity and transport of nutrients to the cells [9–17]. In particular, preparation of scaffolds by rapid prototyping techniques such as three-dimensional (3-D) plotting [18,19] and 3-D fiber deposition (3DF) [20] has been exploited extensively. For example, in 3DF, molten polymer is extruded through a needle to form a round fiber, which is deposited from an XYZ-axis motor-driven syringe on a stationary stage by applying pressure [21,22]. The scaffolds are built layer by layer, through material deposition on a stage with a defined structure, porosity and architecture, designed by means of CAD-CAM techniques. These scaffolds consist mostly of solid round fibers with a certain angle of curvature, depending on the fiber diameter, which

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may have a negative effect on cell adhesion. This work investigates whether a corrugated fiber morphology can enhance cell adhesion and proliferation, allowing the development of successful in vitro tissue-engineered constructs. To the best of the present authors' knowledge, this is the first study to investigate this systematically, aiming to develop methods for producing better scaffolds via rapid prototyping. Here, corrugated round fibers are prepared and characterized by extruding poly(ethylene oxide terephthalate)-*co*-poly(butylene terephthalate) (300PEOT55PBT45) block co-polymer through specially designed silicon wafer inserts. Corrugated round fibers with 6 and 10 grooves on the fiber surface are compared with solid round fibers with a variety of diameters. The culture of mouse pre-myoblast C2C12 cells on all fibers is studied under static and dynamic conditions by means of scanning electron microscopy (SEM), cell staining and DNA quantification.

2. Materials and methods

2.1. Polymer

For this study, poly(ethylene oxide terephthalate)-*co*-poly(butylene terephthalate) (PEOT/PBT) block co-polymer was used, kindly provided by Prof. Dr. C.A. van Blitterswijk (Tissue Regeneration group, University of Twente, The Netherlands). The chemical composition is represented by the notation aPEOTbPBTc, where "a" is the molecular weight of the starting poly(ethylene oxide) segments used in the polymerization process, while "b" and "c" refer to the weight ratio between PEOT and PBT blocks, respectively. For this study, 300PEOT55PBT45 co-polymer was used because of its high mechanical stability, low swelling, good cell adhesion and suitability for tissue formation [23].

2.2. Fabrication of corrugated and solid round fibers

Corrugated and solid round fibers were fabricated using a Bioplotter device (Envisiontec, Gladbeck, Germany), which is an XYZ plotter for the preparation of scaffolds [18,20,22,24]. A few modifications were adapted before this device was used to extrude highly visco-elastic fibers with round and corrugated structures. Fig. 1 illustrates the fiber extrusion process, wherein polymer granules are loaded in a stainless steel syringe and heated at a temperature of 180–200 °C using a cartridge heating unit mounted on the device. After polymer melting, a pressure of 3 bar was applied to the syringe using nitrogen gas. Nitrogen was used rather than air in order to avoid co-polymer oxidation. The nozzles used to extrude solid and corrugated round fibers were made of silicon wafers by means of an etching technique and used in the form of inserts [25,26]. These inserts were placed in a special insert holder connected to the syringe containing the molten polymer. The polymer fibers were extruded without an air gap into an ethanol bath kept at ~−6 °C, to immediately cool down and solidify the fibers. In this way, the fibers retained their shape after extrusion through the inserts. Fibers were also extruded with 0.5 and 2 cm air gaps to test for possible shrinkage and/or deformation due to delayed solidification.

2.2.1. Gas plasma treatment

To improve cell attachment, the fibers produced were treated with an argon gas plasma, as previously reported [27]. The fibers were placed on a glass plate, which was placed inside the chamber of a radiofrequency glow discharge device (PlasmaFAB-508, Electrotech, Glenside USA). A vacuum of 0.01 mbar was first applied, and then the chamber was flushed four times with argon gas (purity ⩾ 99.999%, Hoekloos, Schiedam, The Netherlands). The fibers were treated with argon plasma (0.1–0.2 mbar) for 10 min.

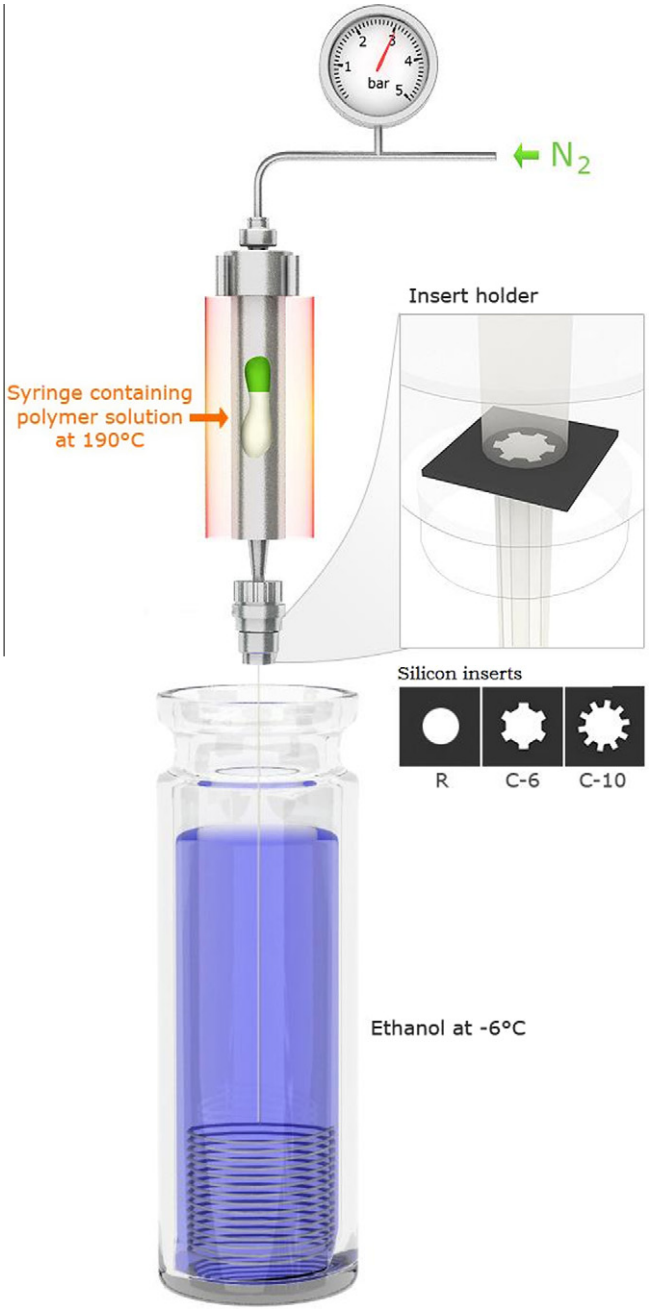

Fig. 1. Schematic representation of the fiber extrusion set-up; blow-up shows silicon insert holder and various silicon inserts.

After cooling, the fibers were turned around and again treated for 10 min as explained above.

2.3. Fiber characterization

2.3.1. Fiber shrinkage

After fabrication and complete solidification of corrugated and solid round fibers, the fiber diameters were measured with a digital screw caliper. Subsequently, fiber shrinkage was calculated with regard to the diameter of the silicon insert through which the fiber was extruded.

2.3.2. SEM

SEM images of dry fibers were obtained using a JEOL 5600LV (Tokyo, Japan) scanning electron microscope at an accelerating

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