Polyurethane Scaffolds for the Regeneration of the Infarcted Myocardium.

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Statement of Purpose: Cardiac tissue engineering/regenerative medicine (TERM) promises to revolutionize the treatment of patients undergoing a myocardial infarction and provide solutions to the serious problem of heart donor shortage[1]. In this context, a polymer scaffold can provide a temporary structural and mechanical support to the repairing infarcted tissue. Such substrates should meet strict requirements concerning material physico-chemical properties, scaffold geometry and mechanical behavior. In this work, an elastomeric degradable polyurethane (PU) was synthesized and processed via Thermally Induced Phase Separation (TIPS) to produce 3D porous scaffolds. The scaffolds were functionalized with fibronectin by plasma treatment. Finally, cell culture tests with neonatal rat cardiac cells, were performed on functionalized structures.

Methods: The PU used in this work was synthesized from poly-ε-caprolactone diol (Mn=2000Da), 1,4-butanediisocyanate and lysine ethyl ester, as previously described[2]. The PU was characterized in terms of physico-chemical (Size Exclusion Chromatography – SEC-, Attenuated Total Reflectance -ATR-IR-), thermal (Differential Scanning Calorimetry -DSC-), mechanical (Tensile Tests) and properties. 3D porous scaffolds were fabricated by TIPS. Typically, the PU was dissolved in dimethyl sulfoxide (DMSO) and heated. The solution was poured in a mold and cooled. Then, the frozen scaffolds underwent solvent extraction in EtOH/H_2O (70:30) followed by freeze-drying. The surface functionalization was performed by plasma treatment with acrylic acid, followed by the activation of carboxylic groups by 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (Sigma Aldrich) and N-hydroxysuccinimide (Sigma Aldrich), and the coupling with fibronectin (YO Proteins). The scaffolds were characterized by Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), DSC and Tensile Test. The functionalization steps were followed by ATR-IR, Contact Angle and X-ray photoelectron spectroscopy (XPS). Image J software analysis was used to evaluate porosity, pore size and distribution. Primary cultures of cardiac cells were prepared from neonatal (1- to 2-day-old) Sprague-Dawley rats as previously described[3]. Cardiac cells were plated onto the scaffolds and maintained in a 5% CO_2 atmosphere at 37°C. Alamar tests were conducted in order to estimate cell vitality. Cardiomyocyte beating activity was recorded using time lapse microscopy.

Results: The PU (Mn=40.000Da) was successfully synthesized as confirmed by ATR-IR. Stress-strain tests showed the elastomeric behavior of the PU (Young Modulus of about 9 MPa and strain at break of about 700%), that made it a promising candidate for the fabrication of porous substrates in the regeneration of cardiac tissues. Porous scaffolds were obtained by TIPS. It was observed that, by changing fabrication parameters, such as solution concentration and temperature, scaffolds with different morphologies were obtained. When the quenching was performed under application of a thermal cooling gradient, pore formation in a preferred direction was obtained, as observed by SEM micrographs (Figure 1). The porosity and pore size were respectively in the range of 85-90% and 100-150μm. TIPS allowed the fabrication of scaffolds with a good resistance to suture and suitable mechanical properties for cardiac TERM application (Young modulus of about 1/1.2 MPa in dry state, and about 0.3/0.5 MPa in wet conditions; strain at break values were higher than the typical deformation of the heart during each cardiac cycle). Contact angle was about 133° for the non functionalized scaffold, 84° for the scaffold functionalized with acrylic acid and 102° after fibronectin coupling. ATR and XPS analysis showed slight differences between functionalized e non functionalized scaffolds.

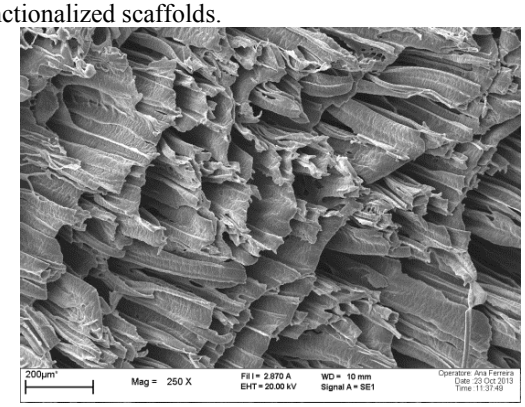

Figure 1. SEM micrograph of a representative PU scaffold.

Neonatal rat cardiomyocytes cultured directly on polyurethane scaffolds showed high vitality and colonized the scaffold readily. The synchronized beating of cardiomyocytes was observed after 7 days of cell culture.

Conclusions: Porous scaffolds showing anisotropic properties were successfully produced by TIPS. Surface functionalization with fibronectin was obtained. Scaffolds turned out promising for cardiac TERM application in terms of mechanical properties and biological response.

References:

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