3D porous and protein decorated scaffolds for tissue engineering obtained by supercritical fluid technology

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INTRODUCTION

Regenerative medicine pursues the reconstruction of damaged tissues by the controlled growth of cells. A promising strategy in this field is the combination of cell/molecular biology and materials engineering in order to design 3D biomaterials which promote the cell colonization and proliferation [1]. In this context, the preparation of 3D scaffolds with appropriate properties is a critical issue in tissue engineering.

In this work we make use of supercritical fluid technology (SCF) as a new route to prepare porous matrices with novel physico-chemical and biological properties. In particular, we investigate the processing of the polylactic-acid (PLA) polymer to generate a 3D scaffold platform decorated with bacterial inclusion bodies. These submicron protein particles have been revealed in previous works as biocompatible, adhesive and mechanically stable protein materials that can be used to favor cell colonization and proliferation when used to decorate flat surfaces [2].

MATERIALS AND METHODS

Materials. In this study, a $P_{L,DL}LA$ polymer was purchased from Evonik Röhm GmbH (Darmstadt, Germany) in pellet form. CO_2 (purity 99.995%) was supplied by Carburos Metálicos S.A. (Barcelona, Spain).

Scaffold production by supercritical fluid technology. A desired mass of polymer was weighed and pre-heated in an oven, at 150ºC during 15 minutes in order to eliminate the crystalline part. The polymer was then placed into a special mould with a diameter of 13 mm and compressed with 3 tones to form a disk. This mould had two detachable parts to allow removal of scaffold after fabrication. The disks were then put into a high pressure vessel using a special stainless steel basket (Figure 1).

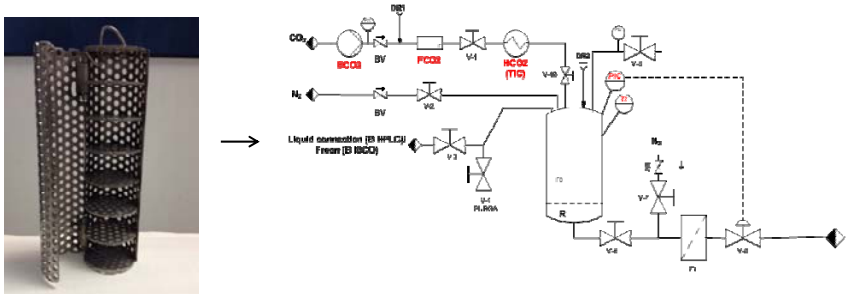

Figure 1: SS basket and high pressure plant used for scaffolds fabrication

The vessel R was heated at the working temperature (T). A high pressure syringe pump was used to introduce compressed CO_2 into the vessel to reach the working pressure (P). The polymer/compressed fluid mixture was maintained at pressure P during the soak time and, afterwards, depressurized to ambient pressure at a constant rate. Porous scaffolds were obtained with a layer of non-porous skin.

Scaffold decoration with Inclusion bodies. Bacterial inclusion bodies (IBs) formed by the Aequorea victoria green fluorescence protein (GFP) as biologically inert nanobiomaterials were supplied by the Institut de Biotecnologia i Biomedicina (IBB), Barcelona, Spain. A filtration procedure was implemented to decorate the porous scaffolds with Inclusion Bodies. Specifically, suspensions of IB's in 5 ml of PBS were filtrated through the scaffold three times.

Culture of human mesenchymal stem cells. hMSC´s were added on PLA scaffolds in 24-well plates and cultivated for 34 days. Cell viability was evaluated using alamarBlue assay which is working as a cell health indicator that uses the reducing power of living cells.

RESULTS

We have found that using the appropriate processing of PLA polymers with SCF methods it's been possible to obtain result matrices with improved properties concerning porosity, interconnectivity and mechanical properties. In addition, the decoration of these novel 3D porous scaffolds with bacterial inclusion bodies (IB) and the study of its impact into the cell proliferation [3], have lead to conclude that the presence of IBs in the scaffold does not result toxic and contrarily it significatively enhances cell proliferation on the substrate, as it can be seen in Figure 2.

This result shows how the SCF methods combined with the use of IBs represents a promising approach in the fabrication of hybrid matrices for multifactorial control of cell proliferation in tissue engineering.

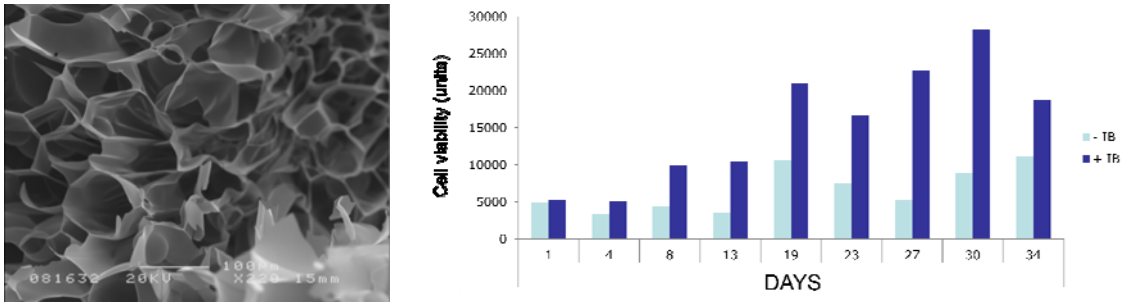

Figure 2: SEM image of SCF processed PLA scaffold (left) and experiments on hMSC cell viability with the comparison between scaffolds with and without IBs functionalization (right)

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