Enhanced Cellular Behaviour on Plasma-modified Electrospun Biodegradable Nanofiber Meshes

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Statement of Purpose: Biomaterials play a significant role in the modern strategies of regenerative medicine and tissue engineering. Recently, much attention has been devoted to the electrospinning technique as a processing method of biodegradable polymers. It is a versatile technique of producing polymeric ultrafine fibres with diameters ranging from few microns down to tens of nanometers, aiming of mimic the extracellular matrix (ECM) of almost all tissues of the body [1].

A critical aspect in the development of ideal biomaterials comprises the optimization of the surface properties in order to achieve an adequate cell response. In the present work, a plasma-based treatment was used as a method to modify the surface properties or introduce desired chemical groups onto the surface of a material, without comprising the bulk properties. The final goal was to define the optimum plasma treatment conditions to modify electrospun biodegradable nanofiber meshes.

Methods: Electrospun Polycaprolactone (PCL) nanofiber meshes were submitted to plasma-treatment under various conditions, such as atmosphere (Ar or O_2), electrical power (20 or 30 W) and exposure time (5 or 10 min). The resulting effects of plasma treatment on physicochemical characteristics (i.e. roughness, hydrophilicity and chemical composition) of the surface of nanofiber meshes were analyzed by: Scanning Electron Microscopy (SEM); Optical Profilometry; Contact Angle measurements; Attenuated Total Reflection-Fourier Transform InfraRed (ATR-FTIR) Spectrophotometry; and X-ray photoelectron spectroscopy (XPS).

Different cell types, namely fibroblasts (L929 cell line), chondrocytes (ATDC5 cell line) and osteoblasts (SaOs-2 cells), were seeded on plasma-treated and untreated PCL nanofiber meshes. Tissue culture polystyrene (TCPS) was used as a successful plasma-treated substrate for two-dimensional (2D) cell culture. The biological relevance of each plasma treatment was assessed analyzing the cell attachment and morphology by SEM, and the cell viability and proliferation by MTS assay.

Results/Discussion: SEM micrographs and roughness parameters showed the induction of topographical alterations in plasma-treated PCL nanofiber meshes, mainly an increase of roughness when compared to untreated meshes. The contact angle analysis revealed that the electrospun nanofiber meshes became more hydrophilic after plasma treatment, owing to a pronounced decrease in the contact angle, particularly in the case of O_2-treated nanofiber meshes.

ATR-FTIR and XPS results indicated the presence of oxygen-containing groups at the surface of plasma-treated nanofiber meshes, including hydroxyl (C-OH) and carbonyl (C=O) functionalities (Figure 1 A). Comparing high resolution signals of untreated with plasma-treated nanofiber meshes, only a slight decrease of hydroxyl (C-H) or hydrocarbon (C-C) groups was observed, eventually due to etching of the material during the plasma treatment. Additionally, the elemental composition of the O_2-treated electrospun nanofibers demonstrated that the Oxygen contents increased in accordance to the increment of power and treatment time.

SEM micrographs from direct contact tests revealed that the different cell types maintain a normal phenotype when growing onto plasma-treated PCL nanofiber meshes. In most of the plasma-treated samples, the three cell types adhered to the surface of the materials, interacting with the nanofibrous structure to form a cellular construct.

Regarding cell viability and proliferation, the PCL nanofiber meshes treated with Oxygen at 30W during 5 minutes (Figure 1 B) and Argon at 20W during 5 minutes presented higher cell viability compared to TCPS and untreated nanofiber meshes, independently of the cell type. It was also possible to verify that nanofiber meshes subjected to the treatment with Oxygen at 20W during 5 minutes presented the lowest viability and proliferation rate. In the remaining plasma treatment conditions, the different cell types showed a proliferation rate similar to the observed on untreated nanofiber meshes.

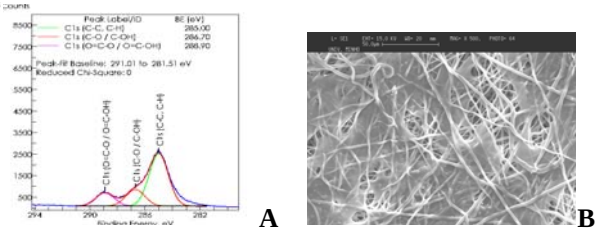

Figure 1 - C1s high resolution spectrum of a plasma-treated electrospun PCL nanofiber mesh (A); SaOs-2 cells growing on the same surface (B).

Conclusions: It was possible to define optimal plasma-treatment conditions in terms of cell behaviour. Plasma treatments are able to improve the proliferation of different cell types (fibroblastic, chondrogenic and osteogenic) when seeded at the surface of the meshes.

References: 1. Huang, Z.-M., *et al*. Comp. Sci. Tech. 2003; 63:2223-2253.

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