ELECTROSPUN NANOFIBROUS STRUCTURES AS SCAFFOLDS FOR CONNECTIVE TISSUES REGENERATION

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Fibrous structures mimicking the natural extracellular matrix (ECM) morphology are considered promising scaffolds for Tissue Engineering (TE). Electrospinning has emerged as a very promising technology enabling to produce synthetic polymeric ultrafine fibers. These fibers in mesh, have diameters in the submicron range which results in a high surface area-to-volume ratio and high porosity. The meshes have a typically random distribution or, in some special cases, some preferential directions of alignment.

Aiming at obtain nanofiber meshes with different topographies (texture, alignment and porosity), a synthetic biodegradable polymer (polycaprolactone, PCL) was electrospun using specially-designed conductive static collectors. Scanning electron microscopy (SEM) analysis showed a random distribution of nanofibers, when a flattened collector was used. This is expected owing to the chaotic motion of polymeric jets of solution during the electrospinning process (Figure 1 A). However, when a metallic wire net was used as collector, it was possible to observe two distinct areas of nanofibers deposition (Figure 1 B). The fibers appeared parallely aligned and collapsed at the wire locations, where the electric field is more intense (Figure 1 C). In the spaces between the wires, the nanofibers deposition occurred more randomly, with diameters varying between 560 nm and 1,5 μm (Figure 1 D). Inversely, random collapsed fibers were present in protuberances (Figure 1 E) and aligned nanofibers deposition occurred in the spacing between protuberances (Figure 1 F) of a corrugated tube, presenting diameters between 200 nm and 1.2 μm. The former two nanofibrous structures presented distinctive properties in the same mesh, constituting the so called patterned nanofiber meshes.

The electrospun nanofibrous structures can be explored in diverse areas of tissue engineering, including the field of vascular regeneration. We considered an approach to controlled fabrication of biodegradable vascular substitutes by means of electrospinning technique. The procedure consisted of using a circular mandrel (4 mm diameter) as collector, rotating at 600 rpm. The electrospun fibrous scaffolds were manufactured with a length of approximately 15 cm and cut in pieces of 3 cm (Figure 1 G). In SEM micrographs was possible to observe a certain degree of nanofiber alignment induced by the rotating collector.

Despite the alterations in nanofiber mesh topography presented previously, we were also able to produce nanofibrous structures with control over the fiber composition. Thus, a polymeric solution of Polyvinylpirrolidone (PVP) and Tetraisopropanolato de Titânio ($Ti(O_iPr)_4$) was prepared and electrospun using a coaxial double capillary system as spinneret. By SEM analysis of the transversal section of PVP/Ti(OiPr)4 nanofiber mesh it was possible to observe that nanofibers were hollow with an external diameter ~1,2 μm and an internal diameter of 650 nm. These nanofibers can be used as a drug delivery system with fine tuned control over the release kinetics, depending on the polymer wall thickness.

Different cell types, such as fibroblasts (L929 cell line), osteoblasts (SaOs-2 cell line) and human bone marrow-derived stromal cells (hBMSCs), were seeded onto patterned nanofiber meshes. The aim was to study the relevance of the patterned nanofiber meshes for tissue engineering applications. SEM micrographs from the *in vitro* studies indicated that the different cells preferred to adhere to randomly distributed nanofibers. Fibroblasts and

hBMSCs also adhered and proliferate parallel to the aligned nanofibers of the patterned nanofiber meshes (Figure 2). hBMSCs were also induced to differentiate into the osteogenic pathway. Results supported that patterned nanofiber meshes are promising scaffolds for bone tissue engineering applications using hBMSCs as cell source.

Novel electrospun nanofiber meshes coated with biomimetic calcium phosphate (BCP) were also developed (Figure 3 B), mimicking the extracellular microenvironment found in the bone structure. The influence of the BCP on the viability, adhesion and proliferation of human osteoblast-like cells was assessed. It was shown that nanofiber meshes coated with a BCP not only support but also enhance the proliferation of osteoblasts for longer culture periods (Figure 3 D). The results suggested a high potential use of this Ca-P coated PCL nanofiber mats as components of polymeric scaffolds suitable for bone regeneration approaches.

Figures:

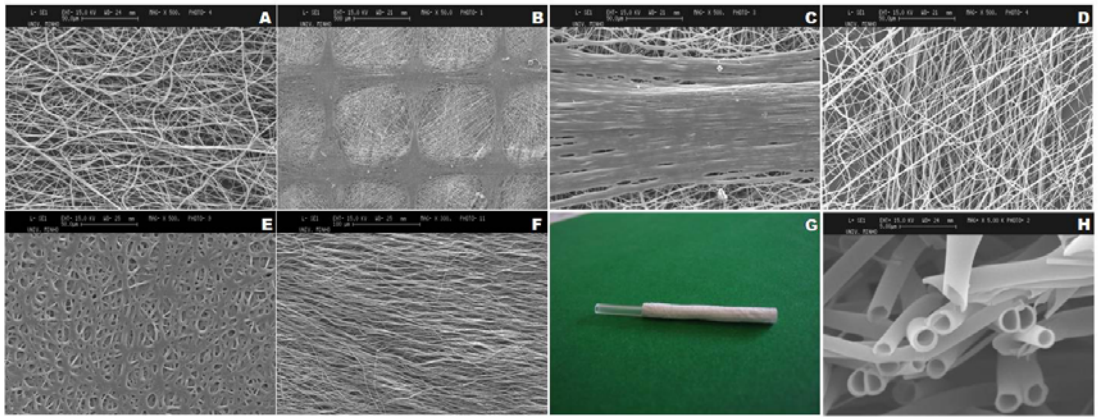

Figure 1 - SEM micrographs of random (**A**) and pattern PCL nanofiber meshs (**B-F**) composed by areas of randomly aligned nanofibers (**D** and **E**) and regions of parallely aligned fibers (**C** and **F**). Photograph of a electrospun vascular nanofibrous scaffold with 3 cm and an internal diameter around 4 mm (**G**). SEM micrographs of a transversal section of hollow PVP/$Ti(OiPr)_4$ nanofibers in a mesh (**H**).

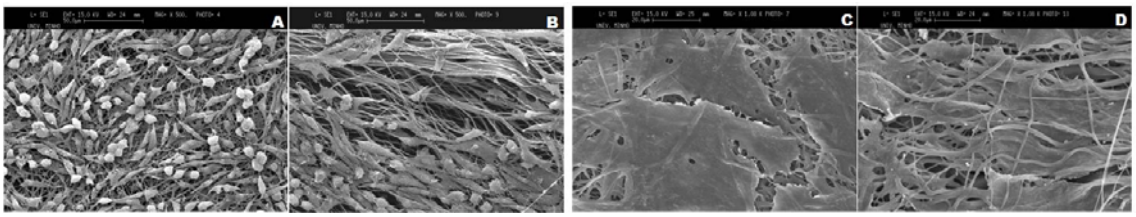

Figure 2 - Direct contact tests were performed fibroblasts (**A** and **B**) and human bone marrow-derived stromal cells (**C** and **D**). Phenotypic alterations of cells, reacting to areas of randomly alignment(**A** and **C**) and parallely aligned fibers (**B** and **D**) of pattern meshes.

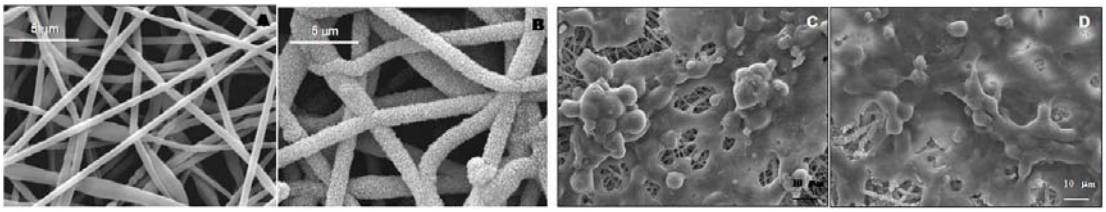

Figure 3 - PCL nanofibre mesh (**A**) with a biomimetic calcium phosphate (BCP) layer (**B**). Direct cell contact studies with osteoblast-like cells on PCL (**C**) and PCL-BCP nanofiber meshes (**D**).