Improvement of Polycaprolactone Nanofibers Topographies: Testing the Influence in Osteoblastic Proliferation.

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ABSTRACT

A polymeric solution of polycaprolactone (PCL) was electrospun using collectors with different topographies, in order to obtain diverse porosities and textures in the nanofiber meshes. SEM micrographs demonstrated that it was possible to identify distinct areas of nanofiber deposition: random and aligned fibers, when a metallic wire net and aluminium tube were used as collectors. Direct contact studies indicate that osteoblastic-like cells (SaOs-2 cell line) maintained their normal phenotype morphology when seeded on the meshes. The cells seem to prefer structures where the fibers are not collapsed independently of the alignment of the nanofibers. The cells integrate with the surrounding fibers and migrate into the inner nanofibrous structure to form a three-dimensional cellular network. The topographies of nanofiber meshes developed in this study constitute promising scaffolds for tissue-engineered bone.

***Keywords*:** topography nanofiber meshes, electrospinning, biodegradable polymer, bone tissue regeneration

INTRODUCTION

Polymer nanofibers, an important class of nanomaterials, have attracted considerable interest in the last decade. Within the broad area of nanotechnology and nanostructured materials, a nanofiber generally refers to a fiber having a dimensions smaller than 100 nm. However, sub-micrometer fibers with diameters less than 1000 nanometers produced via ultra-fine fiber manufacturing techniques such as electrospinning may also be called nanofibers [1].

Traditional fiber reinforcements have dimensions of the micrometer range. The production of fibers at the nanometer scale allows obtaining properties that are significantly different from those of materials produced at the micrometer scale. Those properties include a much larger surface area to volume ratio [2] and improved mechanical properties, such as higher stiffness and particularly tensile strength [3]. Many nanostructured materials such as nanoparticles, nanotubes, and nanofibers have been fabricated aiming at taking the advantage of those unique properties of nanometer structures [1, 4]. One of the newest and most exciting possible applications of nanofibers is in the biomedical field, and particularly its use as scaffolds for tissue engineering [5-7]. Natural extracellular matrix (ECM) of various tissues is composed of randomly oriented collagen fibrils having diameters at the upper nanometer-scale. Indeed, the morphology and architecture of the electrospun nanofibrous structures is very similar to those of some natural ECMs [8].

Up to date, around one hundred different polymers have been successfully spun into ultrafine fibers using the electrospinning technique. The potential applications in cell biology and tissue engineering caused a large number of biodegradable polymers (including poly(caprolactone) [9], poly(lactic acid) (PLA) [10], poly(glycolic acid) (PGA) [11] and poly(lactide-co-glycolide) (PLGA) [12]) to be electrospun into nanofibers. In addition to these synthetic organic biodegradable polymers, natural biopolymers, such as silk fibroin [13], collagen [14] and chitosan [15], have also been successfully processed by electrospinning.

The main goal of the present work was to produce biodegradable nanofiber meshes with different topographies by electrospinning. It is aimed at obtaining diverse porosities and textures and to evaluate its biological relevance in *in vitro* studies with an established cell line of human primary osteogenic sarcoma (SaOs-2 cells).

MATERIALS & METHODS

A polymeric solution of 17% (w/v) PCL (MW = 80000), dissolved in an organic solvent mixture of chloroform/dimethylformamide (7:3 ratio) was electrospun. A voltage of 9.5 kV and a spinneret-to-ground collector distance of 200 mm was used in the process. Different collectors with diverse topographies were used, including a flat aluminium foil, a metallic wire net and an aluminium tube. PCL electrospun nanofiber meshes were imaged by scanning electronic microscopy (SEM) to characterize the morphology, diameter of fibers and porosity of the fiber meshes. Before cell seeding, electrospun PCL meshes (1 cm square) were sterilized by 70% ethanol incubation for 1h, followed by air-drying in a hood overnight followed by washing three times with sterile PBS.

The present study used an established cell line of human primary osteosarcoma (SaOs-2 cells), cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% of antibiotic-antimycotic mixture, and trypsinized before the following experiments. Cell seeding was performed with a concentrated droplet of $5x10^4$ cells/cm^2 of nanofiber mesh, which was previously placed in a 24-well plate. Cell-nanofiber meshes constructs were incubated in a humidified atmosphere at 37ºC, containing 5% CO_2, with medium changes every 3 to 4 days.

The cell activity at the surface of PCL nanofiber meshes was evaluated after 3, 7 and 14 days of culture. To observe viable cells attached to the PCL nanofiber meshes with different topographies, a vital nuclear cell staining was performed with methylene blue. Preferential cell adhesion at the surface of the scaffolds was evaluated by analysing cell-nanofiber mesh constructs in SEM. For that, the samples were washed in PBS, fixed in 2.5% glutaraldehyde, subjected to a dehydration process by immersion in a series of ethanol solutions with increasing concentrations, and air-dried overnight in a hood. The samples were sputter coated with gold and analyzed with a Leica Cambridge S360 scanning electron microscope.

RESULTS & DISCUSSION

Polycaprolactone (PCL) is a synthetic polymer extensively studied in biomedical applications due its low toxicity, biocompatibility and biodegradability. During the electrospinning of PCL solutions using collectors with diverse topographies, it was possible to observe differences in the fiber deposition. These observations were further confirmed by the SEM micrographs shown in Figure 1. When an aluminium foil was used as collector, a random distribution of nanofibers was observed (Figure 1 A). This is the most typical behaviour and is caused by the complex travel of the jet of polymeric solution during the electrospinning process [2]. When a metallic wire net was used as collector, it was possible to observe distinct areas with correspondent topographies. The fibers appear aligned and welded in the vicinity of the wires probably because of the local inhomogeneous intensity of the electric field (Figure 1 B). In the space between wires the nanofibers deposited randomly, with diameters varying between 560 nm and 1.5 μm (Figure 1 C). Conversely, when the aluminium tube was used as collector, random fibers were obtained in the protuberances (Figure 1 D) and aligned nanofibers correspond to the spacing between protuberances (Figure 1 E). The range of diameters of the fibers varied between 200nm and 1.2 μm.

The sensitivity of the cell adhesion to the topographies generated was also evaluated. Typically the adhesion and proliferation of cells in PCL surfaces is difficult because of its characteristic hydrophobicity. The same behaviour was observed with the fiber meshes. However, after 7 days of culture, some osteoblastic-like cells adhered to the nanofiber meshes and maintained a normal phenotype. This observation suggests that the cells are viable when seeded in those structures (Figure 2). The results from cells seeded over nanofiber meshes with defined topographies (metallic wire net and aluminium tube as collectors) indicate that osteoblasts prefer attaching to structures where the fibers are isolated entities, not welded by local solvent excess.

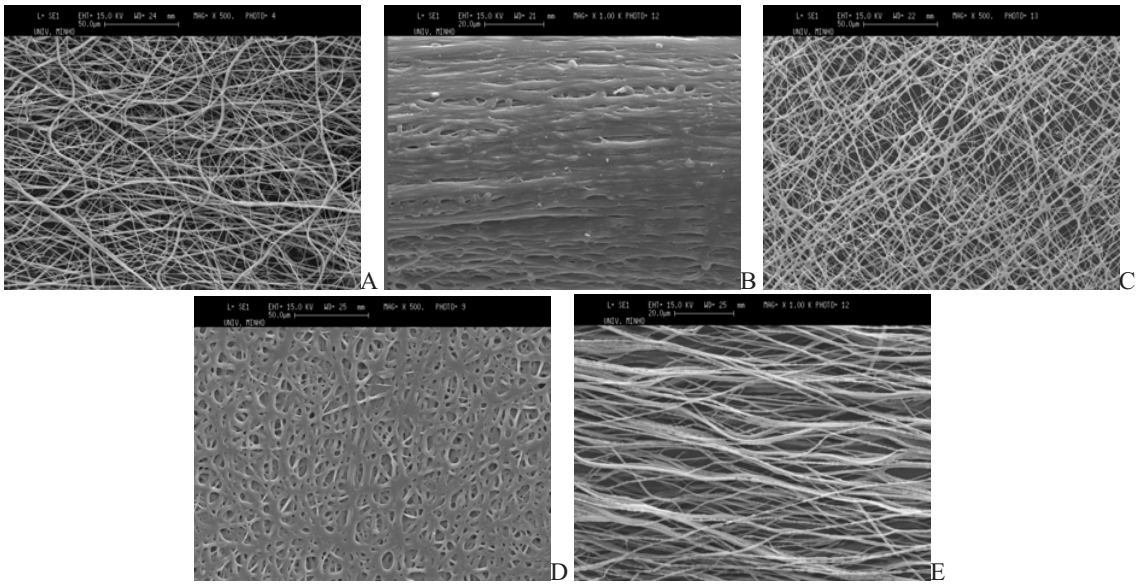

Figure 1 – SEM micrographs of PCL nanofibers deposited over grounded collectors with different topographies. (A) Nanofibers randomly deposited over a flattened aluminium foil; (B and C) nanofibers deposited over a metallic wire net, establishing areas of aligned nanofibers (B) and areas randomly distributed (C); random (D) and aligned (E) deposition was also observed when an aluminium tube was used as collector.

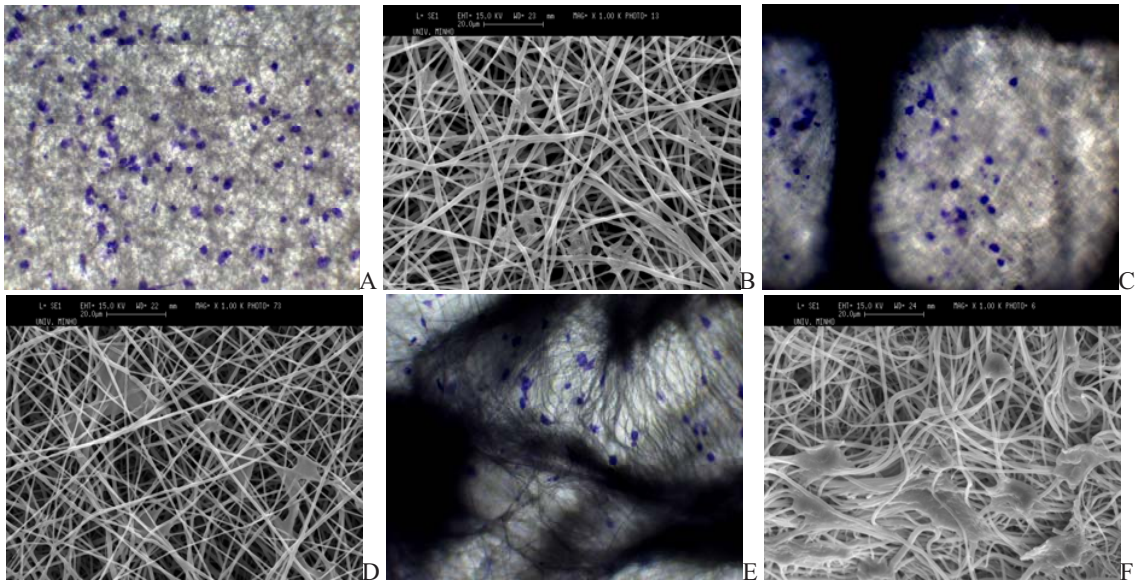

Figure 2 – Direct contact test with SaOs-2 cells growing over PCL nanofiber meshes with different topographies (flattened aluminium foil (A and B), a metallic wire net with (C and D) and a aluminium tube (E and F)) during 7 days. Methylene blue staining was performed in order to identify viable cells adherent to nanofiber meshes (A, C and E). SEM analysis was also performed in order to observed cell adhesion to nanofibers (B, D and F).

This preference is observed in random (Figure 2 C and D) or aligned fiber meshes (Figure 2 E and F). Aligned nanofibers cause the cells to assume a spindle-shape morphology following the nanofiber main axis (Figure 2 F). In the randomly oriented meshes do not follow (Figure 2 D). These results are in agreement with recent works of Xu *et al.*, Yang *et al.* and Lee *et al.*, where human coronary artery smooth muscle cells, neural stem cells and human ligament fibroblasts exhibited a classical contact guidance by aligning parallel to nanofibers of poly(L-lactid-*co*-ε-caprolactone) [P(LLA-CL)], polyurethane (PU) and poly(L-lactic acid) (PLLA), respectively [16-18].

SEM micrographs show that the cells stretch, integrate with the surrounding fibers and bridge between nanofibers to form a three-dimensional cellular network (Figure 2 F). Furthermore, some cells are found in inner regions of the nanofiber structure, indicating that these have sufficient porosity and interconnectivity to enable some cell migration, and permeation for oxygen and metabolites exchange.

Nanofiber structures provide a high surface area for cell attachment. Therefore, it is very important to be loaded with the adequate cell density. We believe that cell density used in this study could be increased to obtain a higher proliferating population covering totally the surface and the inner regions of the nanofiber mesh.

CONCLUSIONS

We developed collector-dependent PCL nanofiber meshes, with various topographies. It was possible to identify distinct areas of nanofiber deposition on the collectors. Direct contact test results demonstrated that the osteoblast-like cells maintain a normal phenotype morphology and proliferate, reacting locally to the aligned patterns. The developed nanofiber meshes can be considered promising for tissue engineering applications.

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