Biodegradable nanofibrous multilayer composite scaffolds as 3D supports for the osteogenic differentiation of bone marrow mesenchymal cells under dynamic conditions

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Introduction:

Emergent new (nano) technologies offer the possibility to improve scaffold designs that may be critical to obtain highly functional tissue substitutes for bone applications.

This study aims to develop novel biodegradable composite scaffolds made of tricalcium phosphate (TCPs) and electrospun nanofibers of poly(ε-caprolactone) (PCL), in order to combine the osteoconductive properties of TCPs with PCL biocompatibility and mechanical properties. We hypothesized that these composite scaffolds would stimulate the proliferation and osteogenic differentiation of goat bone marrow cells (GBMCs).

Materials and Methods:

Composite Preparation: β-TCP was obtained from a solid state reaction between stoichiometric amounts of dicalcium phosphate anhydrate and calcium carbonate and further sinterization at 800ºC for 24h. TCPs powder was characterized by X-Ray diffraction (XRD) and by Fourier Transform Infrared (FTIR) techniques. A polymeric solution of 17% (w/v) PCL was processed by electrospinning technique. Composite scaffolds were developed using consecutive stacked layers of PCL and TCPs at a ratio of 0.5g TCP/130cm^2 PCL meshes. Scaffolds were characterized by FTIR, XRD and Scaning Electronic Microscopy (SEM). Scaffolds were cut into ϕ 5mm discs. PCL nanofiber meshes without TCPs were considered as controls of the experiment. Scaffolds were sterilized by two 30 minute-cycle of UV irradiation before cellular studies.

Cell Culturing Procedures: GBMCs were isolated from iliac crests of adult goats and cultured in basal medium: DMEM supplemented with 10% FBS and 1% antibiotic/antimicotic (A/B). Cells were cryopreserved, expanded and sub-cultured twice (P2) before seeded onto the TCPs/PCL composite at 5.0x10^4 cells/scaffold. After the seeding, cell-scaffold constructs were maintained in basal medium for 24h and then cultured in static or dynamic conditions (60rpm) in either basal or osteogenic medium containing α-MEM, 10% FBS, 1% A/B and osteogenic supplements, ascorbic acid (50 μg/ml), dexamethasone (10^{-8}M) and β-glycerophosphate (10 mM) for 7, 14 and 21 days. Cell viability and proliferation were assessed by MTS and DNA quantification assays, respectively, and cell morphology was assessed by SEM. Osteogenic differentiation was also measured not only by ALP activity and Alizarin Red staining but also by immunocytochemistry (ICC) and polymerase chain reaction (PCR) for Type I collagen, osteocalcin and bone sialoprotein.

Results and Discussion:

The calcium phosphate obtained through the chemical reaction presented a rhombohedrical structure, typical of β-TCP, and vibrational bands associated to the phosphate groups, as demonstrated by XRD and FTIR analysis, respectively.

SEM analysis demonstrated a dispersion of TCPs granules throughout the electrospun nanofiber structure in the produced composite scaffolds. A random distribution was observed not only with TCPs granules but also in nanofibers of the mesh-like stratified structure. Also, a perfect integration of TCPs was observed in the multi-layered nanofiber scaffolds.

GBMCs seeded onto TCPs/PCL scaffolds were able to adhere and proliferate in both static and dynamic conditions as well as in basal and osteogenic cultures (Figure 1).

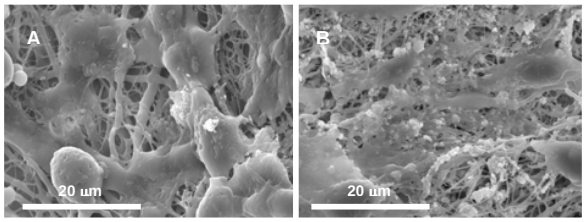

Figure 1- SEM micrographs of GBMCs seeded onto PCL/TCP scaffolds on basal (A) or osteogenic (B) conditions after 21 days in dynamic culture.

Nevertheless, this is particularly evident in a dynamic osteogenic environment. Indeed, the DNA and ALP results demonstrated higher cell activity at these conditions. The phenotype of differentiated cells was confirmed by Alizarin Red staining and ICC against osteogenic specific markers. Additionally, PCR analysis for osteocalcin and type I collagen is still on going.

Conclusions:

The development of multi-layered TCPs/PCL scaffolds was successfully achieved. The combination of PCL nanofibers with ceramic materials, in a multi-layered structure, is an innovative methodology with great potential that might allow the development of more adequate scaffolds for bone tissue engineering approaches.

The results obtained so far, suggest that β-TCP/PCL composites seem to be a suitable 3D support for GBMCs proliferation and differentiation towards the osteogenic phenotype. We believe that the presence of TCPs positively stimulates cell osteogenic process, especially under dynamic conditions.

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