Poly-L-lactic acid/hydroxyapatite electrospun nanocomposites induce chondrogenic differentiation on MSCs

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INTRODUCTION:
The use of mesenchymal stem cells (MSCs) for cartilage and bone tissue engineering has been widely investigated [1-2]. Scaffolds represent the pivotal structure of the engineered tissue and establish an environment for the synthesis of neo-extracellular matrix. Moreover, they can be associated to signals to modulate cell activity. Advances in stem cell biology have shown that differentiation of MSCs depends primarily on the environment in which they are placed [3]. In this study, we focused on the questions of whether MSC can differentiate when cultured upon a membrane of electrospun fibers of poly-L-lactic acic (PLLA) loaded with nanoparticles of hydroxyapatite (HAp) developed by the authors.

METHODS:
The PLLA/HAp membrane was prepared by electrospinning technique, starting from a dispersion of HAp nanopowder in a PLLA solution. The obtained suspension was electrospun (DC voltage 15kV, distance 15 cm) forming a non-woven cloth. Microstructure of the membranes was evaluated by Field Emission Scanning Electron Microscopy (FE-SEM). Prior to cell culture test, PLLA/HAp membranes were sterilized, punched out to disk 8 mm in diameter and placed into a 96-well plate. Then a standard static seeding was performed using human bone marrow MSCs (P2) at the density of 500 10^3 cells/cm^3 [4]. Cells were then cultured either in basal medium (DMEM) supplemented with 10% FBS, or Chondrogenic Differentiation Medium (Lonza Biologics). Media were changed every 2 days. Similar experiments have been performed on PLLA alone patches as a control for differentiation induction. 3 days after seeding cell attachment and engraftment was assessed by means of confocal microscopy staining cells on the scaffolds for F-Actin with Rhodamine Phalloidin and nuclei using TOTO as nuclear counterstain. 14 days after seeding the membranes were embedded in OCT, snap frozen, and cut. 7µm slides where immunostained with Antibodies against CD29, SOX-9 and Aggrecan. Nuclei were stained using TOTO. Slides were imaged under the confocal microscope. Slides were also stained with Toluidine blue and Safranin O and imaged under a light microscope.

RESULTS:
FE-SEM analysis revealed that the PLLA/HAp membrane obtained was composed of nanofiber of 7 µm ±1 of diameter with uniformly nano-dispersed hydroxyapatite aggregates (average diameter of 0.3µm) (fig. 1). 3 days after seeding, MSCs were well adhered on the PLLA/HAp fibers with a spindled shape. After 14 days of culture all MSCs were positive for the chondrogenic transcription factor SOX-9 in both basal and chondrogenic media groups. MSCs were either CD29 positive or negative (fig. 2). Aggrecan was present around the cells while it was not expressed by MSCs cultured on PLLA control membranes (fig. 3). Toluidine blue and Safranin O staining shown the presences of neo-extracellular-matrix produced around the cells.

DISCUSSION:
By using a combination of cells biomaterials, tissue engineering offers a technology that will both regenerate the matrix and fully restore normal function to either the bone or the articulating joint. In this study we demonstrated that PLLA/HAp membranes are able to induce differentiation of MSCs towards a chondrocyte-like phenotype that produces proteoglycan based matrix. The detected co-expression of CD29, a typical surface marker of MSCs and SOX-9, a transcription factor associated with chondrogenesis, suggests intermediate differentiation phases. These results could be obtained using a basal medium, reliably suggesting the idea of a differentiating influence exerted by the PLLA/HAp scaffold itself. Since HAp is known to have osteoinductive properties [5], the chondrogenic phenotype acquired by the MSCs induced by the PLLA/HAp may be either stable over time or an intermediate stage toward the enchondral bone formation process.

This membrane could be an amenable alternative scaffold for bone or cartilage tissue engineering using undifferentiated bone marrow MSCs. This functionalized scaffold would provide both a surrogate of the native ECM and the correct sequence of signals to allow an harmonic ongoing lineage-specific differentiation of pluripotent precursor cells

REFERENCES:
2. Risbud, M.V., and Sittinger, M. TRENDS in Biotech, 20, 8, 2002;