Release of Bioactive Agent from Liposomes Immobilized on Electrospun Nanofibers Targeting Tissue Engineering Applications

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Introduction: The ability to manipulate and control the surface properties is of crucial importance in the designing of scaffolds for Tissue Engineering (TE) and Regenerative Medicine. Electrospun nanofibers (NFM), due to their morphology and fibrous structure have received much attention as potential biomedical devices, TE scaffolds and drug delivery carriers. Liposomes, a nanoparticle release system made by physiological material (phospholipids), hold tremendous promise as release systems. Liposomes may be combined with scaffolds to maintain a sustained and local delivery of the loaded drugs. The main objective of the present study is to evaluate the efficacy of dexamethasone (Dex) loaded liposomes immobilized on the surface of polycaprolactone (PCL) electrospun NFM as release system, for the induction of the osteogenic differentiation of mesenchymal stem cells (MSCs).

Methods: The PCL nanofiber meshes (NFM) surfaces were activated using the UV-Ozone irradiation technique. Aminolysis was performed to insert amine groups onto the NFM surfaces. Afterwards, SH groups were inserted at the surface of the NFMs through the reaction of the aminated surfaces with 2-iminothiolane. Ellman's reagent method was used to quantify the SH groups onto the NFM surfaces. BODIPY fluorescent dye was bound to the SH groups to study the spatial distribution at the surface of the PCL NFMs. Dex-loaded liposomes were produced by the lipid film method and immobilized at the surface of the SH-PCL NFM. The release study of Dex was performed using HPLC and UV detection at 247 nm. The effect of Dex-loaded liposomes immobilized at the surface of PCL NFMs was assessed by the study of the viability, proliferation, protein synthesis and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs) seeded at the NFMs.

Results: We report a chemical modification of PCL NFMs in order to immobilize Dex-loaded liposomes onto their surfaces. A maximum SH concentration, 5.52 ± 0.53 nmol/cm^{2}or 6.49 ± 0.58 nmol/mg, is achieved at 4 min of UV-Ozone irradiation and 1h aminolysis. It is visible the effect of the UV-Ozone irradiation at the surface of NFM with the appearance of surface cracks. However, the morphology of nanofibers present in the bulk of the electrospun meshes seemed unaffected by this physical treatment. Fluorescence microscopy using SH-reactive probes, BODIPY, showed that the binding sites on the NFM surfaces are well dispersed and cover uniformly the surface area of the PCL NFM (Figure 1). *In vitro* release studies demonstrated an initial burst release within 12h. Afterwards, the Dex continues to be released at a slower but steady rate until day 21. Biological activity showed that the Dex-loaded liposomes immobilized at the surface of PCL NFMs do not exhibited any cytotoxic effect, and promoting osteogenic differentiation of hBMSCs.

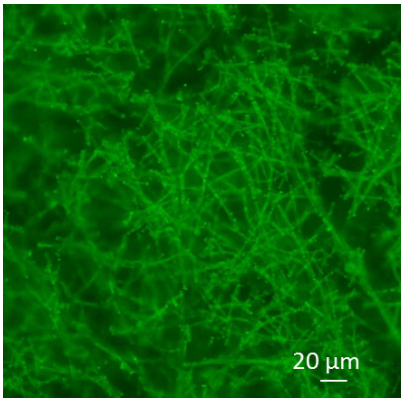

Figure 1 – Fluorescence micrographs of SH-functionalized PCL NFM reacted with BODIPY.

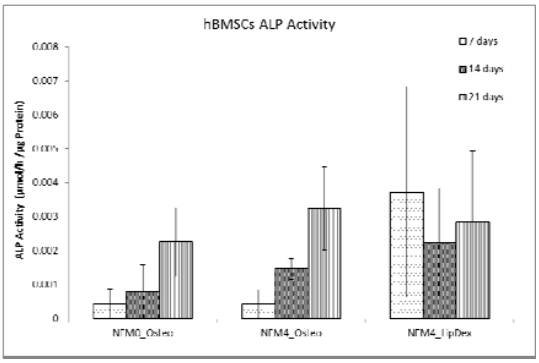

Figure 2 – hBMSCs ALP cultured on untreated (NFM0_Osteo) and treated (NFM4_Osteo and NFM4_LipDex) PCL NFMs.

Conclusions: We validate the concept of using liposomes immobilized at the surface of nanostructured system to be used as device for the local and sustained release of growth/differentiation factors relevant for regenerative medicine strategies.

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