PP517
Internalization of magnetic iron oxide nanoparticles for stem cell functionalization

AI Gonçalves, MT Rodrigues, RL Reis and ME Gomes
3B's Research Group, University of Minho, Guimarães, Portugal

Introduction: Cell monitoring and cell localization can be potentially achieved by the internalization of magnetic nanoparticles (MNPs) in the cells. This might allow for the investigation of migratory patterns through tracking studies, the targeting of particle-labelled cells to desired locations via the application of an external magnetic field and, finally, for activation stem cells to initiate desired cellular responses as inducing the differentiation process [1]. This study focus on determining the effect of magnetic stimulation in human adipose stem cells (hASCs) differentiation towards tendon cells. Firstly, the MNPs uptake by the cell and magnitude/frequency of the external magnetic field was verified. Afterwards, initial studies on understanding the endocytic mechanisms involved in the uptake of MNPs were performed. For this, cells were treated with pharmacological inhibitors before exposure to fluorescent labeled MNPs.
Materials and Methods: hASCs cells were seeded onto 24-well plates (50,000 cells/well) and incubated with red-fluorescent crosslinked magnetic dextran nanoparticles (nanomag®-CLD-redF, Micromod), at different concentrations, with and without magnetic stimulation provided by a magnefect nano device for up to 16 h. MNPs internalization was confirmed using fluorescence microscopy and prussian blue staining (PB) for iron detection. Cellular viability was also assessed through time to check potential cytotoxicity effects. Moreover, cells were incubated with 100×10^{-6} M Genistein, 80×10^{-6} M Dynasore and 10 μg/mL Chlorpromazine for 1 h at 37°C. After this, the medium was changed and 100 μg/mL of MNPs were added. Cells were visualized after 4 h for MNPs detection.
Results: MNPs were successfully internalized by hASCs. MNPs were detected inside cells by fluorescence microscopy as well as by PB staining (Fig.1). The detection of ferric iron is more intense for longer incubation periods under magnetic 2stimulation. Cell metabolic activity seems not to be affected by the increasing concentrations of MNPs and tends to increase with incubation time. MTS assay also show an increment in cell viability levels when the magnetic stimulus is applied.

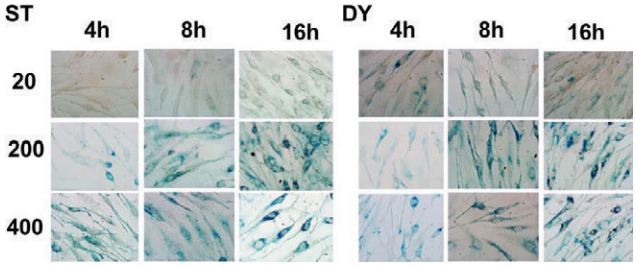

Figure 1. Prussian blue staining images of hASCs cultured with redF MNPs under static (ST) or magnetic stimulated (DY) conditions at different concentrations (20, 200 or 400 μg/mL) for 4 h, 8 h or 16 h of incubation. Blue dots represent the iron core of MNPs (60x magnification).

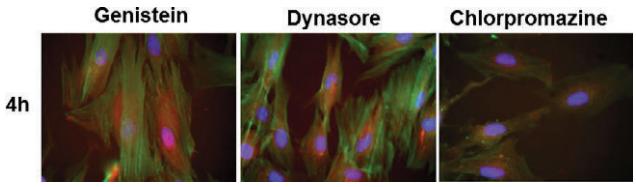

Figure 2. Fluorescent microscopy images of hASCs incubated with endocytic inhibitors and cultured with 100 μg/mL redF MNPs for 4 h (60x magnification).

It was observed that redF MNPs were internalized by hASCs after 4 h of incubation in all inhibitory conditions (Fig.2).

Discussion and Conclusions: Studies with redF MNPS didn't affect cell viability and functioning.

The treatment with genistein, dynasore and chlorpromazine did not influence the internalization process of the nanoparticles. RedF MNPs have the potential to be used in stem cell therapy as a promising tracking and/or functionalization tool.

Acknowledgments: ERDF through Atlantic Area Transnational Cooperation Programme Project MARMED (2011-1/164). NORTE-01-0124-FEDER-000020 under ON.2 SR&TD Integrated Program. 7th Framework Programme (FP7/2007-2013) under grant agreement no. REGPOT-CT2012-316331-POLARIS.
Disclosures: Authors have nothing to disclose.
Reference
1. Wimpenny *et al*. Stem Cell Research & Therapy. 3:13, 2012.

PP518
Bioactive nanofibrous materials for wound healing

H Hajiali, E Mele, I Liakos, IS. Bayer and A Athanassiou
Nanophysics Department, Italian Institute of Technology IIT, Genova, Italy

Introduction: Traditional scaffolding methodologies, such as solvent casting and particulate leaching, gas foaming and freeze drying, have limited ability to form scaffolds mimicking the native tissue structure. On the other hand, electrospining (ES), a technique based on the use of a high voltage to extrude ultra-fine fibers, has been advantageously used to fabricate nanofibrous substrates with controlled mechanical properties and porosity, ideal for tissue engineering applications [1, 2]. In addition, electro-spun fibers are excellent candidate for wound dressing and healing, due to their characteristics of efficient absorption of wound exudates, gas permeability, protection against bacterial infections, easy incorporation of bioactive molecules and drugs, good conformability, promoted cell attachment and proliferation. Here, we present the realization smart textile for wound dressing by using composite nanofibers based on polymers derived from natural sources and functionalized with active agents.
Materials and Methods: Biopolymers, including sodium alginate, cellulose and zein protein, were dissolved in the appropriate solvent and extruded in form of nanofibers by ES. Optimized amount of active agents, like essential oil of cinnamon, mint and lemon grass, were incorporated into the nanofibers. The realized fibrous scaffolds were characterized in terms of morphology by Atomic Force Microscopy (AFM) and Scanning Electron Microscope (SEM), mechanical and wetting properties, antimicrobial activity and biodegradability, and chemically by FT-IR and Raman spectroscopy.
Results: We focused our attention on biopolymers, because they offer remarkable advantages in terms of biocompatibility, biodegradability and environmental friendliness. We encapsulated natural substances, which possess antimicrobial properties or enhance cell growth, in mats

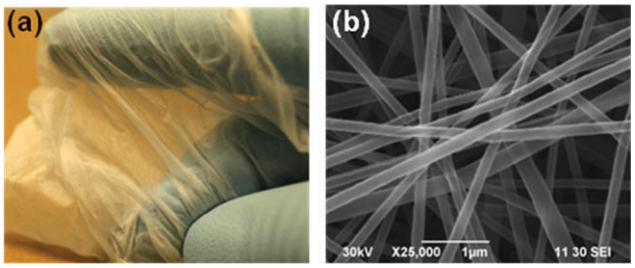

Figure 1. (a) Picture of the electro-spun textile; (b) SEM image of alginate nanofibers.