Chondrogenic Inductive Nanofibrous Mesh Biofunctionalized with Human Fibronectin

M.R. Casanova[1,2,3], R.L. Reis[1,2,3], A. Martins[1,2], N.M. Neves[1,2,3]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark – Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco – Guimarães, Portugal; [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães 4805-017, Portugal; [3]The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal;

Statement of Purpose: Articular cartilage is a connective tissue with low self-regeneration potential due to its avascular nature and limited access to progenitor cells [1]. Furthermore, this tissue is characterized by a dense and specific extracellular matrix (ECM). Fibronectin is a key constituent of the pericellular ECM, assembled into a fibrillar matrix through a cell-mediated process. Specifically, fibronectin links cell surface integrin receptors with collagens and other ECM proteins [2]. Although fibronectin is essential in chondrocytes condensation during cartilage development, its role during chondrogenic differentiation of mesenchymal stem cells (MSCs) has not been demonstrated. In accordance, herein it is hypothesized that the availability of this glycoprotein at the surface of a biomaterial substrate would conduct the chondrogenic differentiation of MSCs. For that, polycaprolactone (PCL) electrospun nanofibrous mesh was biofunctionalized with human-derived Fibronectin (hFN) bond from platelet-rich plasma (PRP).

Methods: The surface of PCL nanofibrous meshes (NFM) was activated by UV-Ozone irradiation and functionalized by the insertion of amine groups, in order to achieve a covalent immobilization of an antibody, as described elsewhere [3]. Quantification of a fluorescent secondary antibody (Alexa Fluor® 488) was used as an indirect method to quantify immobilized fibronectin antibody (anti-FN). After anti-FN immobilization, the amount of fibronectin not bond by the anti-FN was quantified by ELISA. The chondrogenic potential of the different biofunctionalized NFMs was further assessed by culturing human bone marrow-derived MSCs (hBM-MSCs) during 28 days in basal medium. The controls comprise hBM-MSCs cultured on NFMs in basal medium (***Basal***), standard chondrogenic differentiation medium (***Chondro***) and soluble commercial fibronectin (***Soluble cFN***).

Results: The antibody against fibronectin was successfully immobilized at the surface of NFMs at the maximum concentration of 8 μg/mL. The binding efficiency of immobilized anti-FN was assessed by using commercial or human-origin fibronectin, being capable to bind them at the maximum concentration of 8 μg/mL.

The performance of hBM-MSCs cultured on the different biofunctional nanofibrous meshes was evaluated by viability and proliferation assays, and total protein and glycosaminoglycan (GAG) synthesis. Biological data confirms the bioactivity of the captured fibronectin, since the biofunctional nanofibrous meshes (cFN and hFN) are more effective when compared to the control conditions (Figure 1). The overexpression of chondrogenic transcripts confirms the genotype of hBM-MSCs cultured on the NFMs biofunctionalized with fibronectin. The typical spherical morphology of chondrocytes, as well as the Alcian Blue staining, and the immunolocalization of collagen type II confirmed the formation of a cartilaginous ECM.

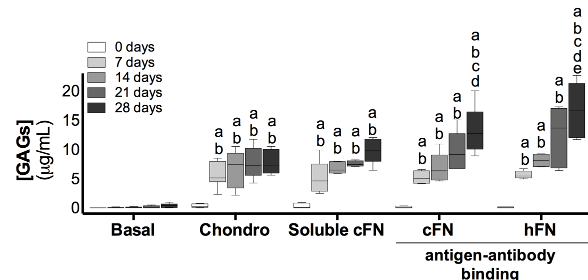

Figure 1. GAG synthesis by hBM-MSCs cultured on NFMs biofunctionalized with fibronectin from commercial (***cFN***) or human–origin (***hFN***) by antigen-antibody binding. Unfunctionalized NFMs cultured in basal medium (***Basal***), chondrogenic medium (***Chondro***) and soluble cFN (***Soluble cFN***) were used as controls.

Conclusions: The fibronectin biofunctionalized NFMs are able to successfully promote chondrogenesis of hBM-MSCs under basal conditions, being more effective than the standard chondrogenic differentiation condition. Therefore, the NFMs biofunctionalized with human fibronectin can enhance the efficacy of a cartilage tissue engineering strategy, operating as an active ECM-like support for stimulating hBM-MSC growth and chondrogenic differentiation.

References: [1]Correa, D. Lietman, *Semin Cell Dev Biol*. 2016; 62:67-77; [2]P. Singh, *J Cell Sci*. 2014; 127:4420-8; [3]Oliveira, C. Biomacromolecules, 2014; 15:2196-2205.

Acknowledgments: Authors acknowledge the financial support from FCT/MCTES and FSE/POCH/PD/169/2013, for a PhD grant (No. PD/BD/113797/2015), the IF grant (IF/00376/2014), and the projects SPARTAN, PTDC/CTM-BIO/4388/2014 and the FRONthera, NORTE-01-0145-FEDER-0000232.