Selective immobilization of PRP-derived growth factors at the surface of nanofibrous substrates for Tissue Engineering and Regenerative Medicine

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Statement of Purpose: Biological fluids are described to have a high concentration of different proteins, including growth factors (GFs), that play a pivotal role in most tissue healing processes[1,2]. The immobilization of biomolecules at the surface of different biomedical devices has attracted enormous interest, in order to enhance their biological functionality at the cellular level. This work aims to develop a biofunctional nanofibrous substrate capable of selectively immobilize GFs of interest, taking advantage of the specific interactions between a certain antibody and its antigen[3]. This biofunctionalized system will allow the specific and efficient capture of GFs from biological fluids, namely Platelet Rich Plasma (PRP). This strategy involves single or multiple antibodies/GFs immobilization (i.e. TGF-β1, FGFb and VEGF) distributed in a random or patterned fashion.**Methods:** A 17% (w/v) polycaprolactone (PCL) solution was prepared with a solvent mixture of Chloroform and DMF (7: 3 ratio) and electrospun by applying a differential potential of 13.6 kV and a flow rate of 1ml/h. The nanofiber meshes (NFMs) were exposed to UV-Ozone irradiation during 4 minutes (each side) and immersed in a 1 M HMD solution during 1h at 37ºC. The NFMs were then incubated with the primary antibody at different concentration (4ºC, overnight). Each NFM was washed with PBS and a blockage of 3% BSA was performed for 30 minutes at RT. A fluorescent secondary antibody (Alexa Fluor® 488 and 595 at 1:200 in PBS) was incubated for 1h at RT and the amount of unbound antibody was measured. The GFs from the PRP and the recombinant proteins were incubated for 1h at RT, after the blocking step. The bioactivity of the captured GFs was evaluated by seeding two different cell lines: HPMEC-ST1.6R for immobilized VEGF and Saos-2 for immobilized FGFb. The VEGF and FGFb antibodies were mixed in the same solution at the concentrations optimized before in order to develop a random immobilization system. To develop a patterned substrate, a compartmental watertight device was developed capable of physically divide a single functionalized electrospun NFM into two distinct areas, without allowing the mixture of the different antibodies solutions. **Results:** In order to determine the maximum immobilization capacity of the TGF-β1, VEGF and FGFb antibodies, a wide range of concentrations (0 µg/ml – 20 µg/ml) were tested. As observed in Figure 1, each antibody is present at a defined density over the functionalized NFMs and this data perfectly correlates with the antibodies size. The results presented in Figure 2 validate the immobilization efficiency of recombinant proteins (almost 100% of the total amount), as well as of GFs derived from PRP (immobilization efficiency range from 50% to 85%, depending on the GF).

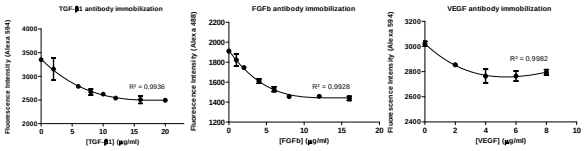

Figure 1 - Maximum immobilization capacity of a single antibody at the surface of electrospun nanofibers.

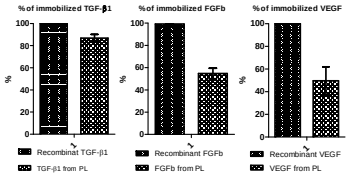

Figure 2 - Quantification of captured TGF-β1, FGFb and VEGF recombinant proteins, and GFs derived from PRP samples.

The bioactivity of the captured GFs was confirmed by culturing defined cell types on those substrates. Cell viability and proliferation, total protein synthesis and lineage-specific markers data demonstrate the beneficial outcomes of the captured GFs. Two multiple antibodies immobilization designs were also tested: one with a random distribution of GFs and another with a pattern of distinct antibodies in the same nanofibrous substrate. In Figure 3 presents the spatial distribution of the multiple antibodies, demonstrating the efficacy of the designs.

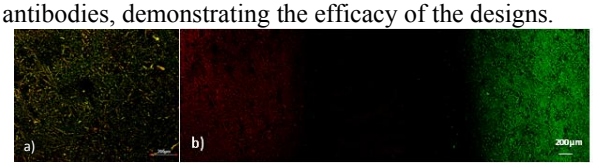

Figure 3 – Random a) and Patterned b) antibodies distribution

Conclusions: We herein demonstrate that immobilized antibodies (single or multiple) in different patterns and designs allows an efficient capture of the corresponding proteins. This technology enables selecting specific GFs from a pool present in a biological fluid. These results, suggest that this platform has a transversal potential use in TERM approaches, since it is possible to immobilize different GFs depending on the target application. Ultimately, using both biological fluids and cells from an autologous source, it will be possible to implement very effective and personalized therapies. **References:** 1. Barrientos, S., et al. Wound Repair Regen. 2008:16: 585–601 2. Anitua, E., et al. Biomed. Mater. Res. A. 2011: 97:536 3. Dixit, C. K., et al. 2011 **Acknowledgements:** I would like to thank Maxbone and Osteography projects and also QREN (Project(s) "[RL1 - ABMR - NORTE-01-0124-FEDER-000016" cofinanced by North Portugal Regional Operational Programme (ON.2 – O Novo Norte), under the NSRF, through the (ERDF)). for financing this research work.