Engineering functional microparticles to fabricate instructive cell microenvironments

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Statement of Purpose: The development of instructive substrates with application in tissue regeneration has become the focus of intense research in the last years [1]. This work reports a new system that is simultaneously bioinstructive, due to the presence of immobilized growth factors (GFs) that control biological function, and bioresponsive as it promotes the assembly into microgels using cells as attachment points.
It is well established that platelets are an important source of autologous and complex mixture of GFs that can modulate cell proliferation and differentiation [2]. Herein we propose antibody-conjugated chitosan-based microparticles (μPs) as a method to select specific GFs from platelet lysates (PLs), which will then be used to modulate cell function. Furthermore, these instructive microgels can also be used as an injectable system for non-invasive tissue engineering applications.

Methods: Microparticles (μPs) were fabricated by spraying a chitosan solution (1.5% w/v) through an aerodynamically assisted jetting equipment (Nisco Encapsulation Units VAR J30) into a gelling bath of NaOH (1M). μPs were functionalized with anti-human Platelet Derived Growth Factor-BB (PDGF-BB) antibody through cardbodiimide (EDC) chemistry. Briefly, EDC was used to activate the carboxylic groups of the antibodies. The activated samples were then mixed with the μPs with gentle rotation for 3hours at 4ºC. PLs were obtained from different platelet collections performed at Instituto Português do Sangue (Porto, Portugal). Collected samples were subject to three repeated temperature cycles (− 196 °C; 37 °C). The selection of a specific GF, in this particular case PDGF-BB, was obtained by simply mixing the μPs with the PLs at 4ºC for 15min. μPs were then washed and ressuspended with human adipose stem cells (hASCs). Morphological analysis of the constructs was done by Scanning Electron Microscopy (SEM) and confocal microscopy. *In vitro* studies were performed for up to 7 days to analyze cell viability and proliferation.

Results: PDGF-BB activation results in signaling cascades that initiate proliferation, migration, and differentiation of many of cell types including hASCs. Cells present cell surface receptors that recognize and bind to this GF. Thus functionalized μPs with PDGF-BB will work as binding points for cells, leading to the formation of a 3D hydrogel-like structure while simultaneously promoting cell proliferation and differentiation within the construct (Fig. 1A). The capture of PDGF-BB from PLs was assessed by ELISA assay on the remaining lysates solution post-incubation with the μPs. Results clearly show a decrease in the amount of this GF in solution when compared to TFG and VEGF. This results support our hypothesis that the functionalization of the μPs was effective and that they are selective for PDGF-BB. The assembly of the microgels was followed for up to 24hours. Controls in absence of cells or by using non-modified μPs were used. Results show (Fig.1B) that after 90min, a 3D structure is already formed on its own. On the other hand, controls were not able to form a stable structure. Biological activity of the obtained constructs was assessed up to 7 days by alamar blue assay and DNA quantification. Results show an increase in cell proliferation and activity up to 7 days. SEM and confocal images revealed a homogeneous 3D construct with hASCs entrapped within the μPs. (Fig. 1C)

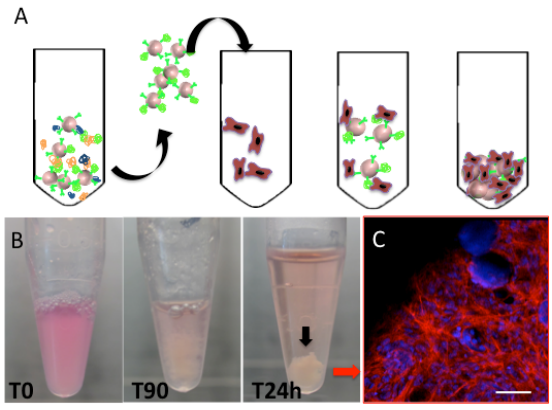

Figure 1. A) Schematic diagram of the proposed strategy. μPs modified with specific antibodies are used to recruit GFs of interest from PLs. Functional μPs are then mixed with cells. The construct is formed through cell-particle interaction. B) Images of the assembly process at different time points. C) Confocal Z-stack image of the construct after 3D in culture. Scale bar 100 μm

Conclusions: The present study addresses the hypothesis that the combination of μPs tailored with specific GFs and stem cells can generate bioactive microgels with tunable cell function through cell crosslinking. The obtained construct simultaneously provides support for stem cell proliferation, as well as localized and sustained presentation of factors to modulate cell function. Different formulations with a combination of GF can be used for a particular application based on the desired composition and intended cellular function. Furthermore, these functionalized μPs can also be used as tools for specific GF sorting from complex mixtures such as PLs.

References: 1. Kim, J.; Hayward, R. C., **2012,** *30* (8), 426-439. **2.** Santo, V. E.; Gomes, M. E.; Mano, J. F.; Reis, R. L., *J Tissue Eng Regen M* **2012**.