3D hydrogel fibers based system to design heterotypic bone vascularization approaches

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Statement of Purpose: Angiogenesis, a critical process in bone formation, development, remodeling and healing, relies on the growth of new blood vessels and on the organization of tridimensional vascular networks. This process, sustained by endothelial cells of microvasculature origin, is finely orchestrated by osteoblastic cells. Recently, we have proven that the SSEA-4$^+$ sub-population, selected amongst heterogeneous adipose-derived stem cells (hASCs), is capable of differentiate towards the endothelial and osteogenic lineages [1]. Even though 2D co-cultures provide powerful insights about heterotypic cellular crosstalk, 3D platforms that more closely translate their native microenvironment allowing mimicking their functionalities, have increased relevance. Naturally occurring polysaccharide *kappa*-carrageenan (κ-CA) hydrogels have been exploited as cell carries. Upon cooling and under appropriate salt conditions, k-Ca undergoes coil-helix conformational transitions forming hydrogels [2]. Herein, we propose an innovative 3D co-culture system based on κ-CA hydrogel microfibers loaded with endothelial derived SSEA-4$^+$hASCs incorporated within a κ-CA hydrogel matrix containing osteo-derived SSEA-4$^+$hASCs, as a bone tissue strategy. Therefore, the κ-CA fibers, carrying the endothelial cells, are expected to act as promoters of vascularization within a 3D system, loaded with osteogenic cells aimed at assisting bone regeneration.

Methods: The SSEA-4$^+$cells were selected from the stromal vascular fraction of human lipoaspirates and differentiated towards the endothelial and osteogenic lineages as previously described [1]. κ-CA fibers with different diameters were obtained using a method based on ionotropic gelation principles. A 1.5% (wt/v) κ-CA solution was loaded in a syringe and extruded into a coagulation bath (potassium chloride, KCl, 5% wt/v) through 18 to 27G needles. Physico-chemical characterization of the developed microfibers (swelling ratio, weight loss, scanning electron microscopy) was performed. Endothelial derived SSEA-4$^+$hASCs were incorporated within the κ-CA solution prior extrusion to obtained cells-loaded fibers. Cells survival and proliferation (Alamar Blue, MTS and DNA quantification), as well as organization within the fibers (Calcein, DAPI, H&E) were assessed. The stability of cells endothelial phenotype, before and after encapsulation, was analysed by flow cytometry (CD31, vWF) and using the Matrigel assay.

Results: κ-CA fibers with different diameters, within the microscale, were obtained. These were shown to be stable for prolonged culture periods up to 21 days, independently on the diameter. Water uptake profile confirmed the high water uptake and swelling capability of the fibers, characteristic of hydrogels. Moreover, the monitoring of the weight loss in culture media showed a controlled and progressive degradation rate along the time. In what concerns cell behavior, the maintenance of the viability of the cells when loaded into the κ-CA fibers confirmed the mild character of the processing (Figure 1A-B). After 21 days of culture, the viability of the endothelial derived SSEA-4$^+$hASCs retrieved from the fibers was kept around 80%. Additional the endothelial phenotype of those cells as well as their capacity to form tubular like-structures when seeded on Matrigel were confirmed.

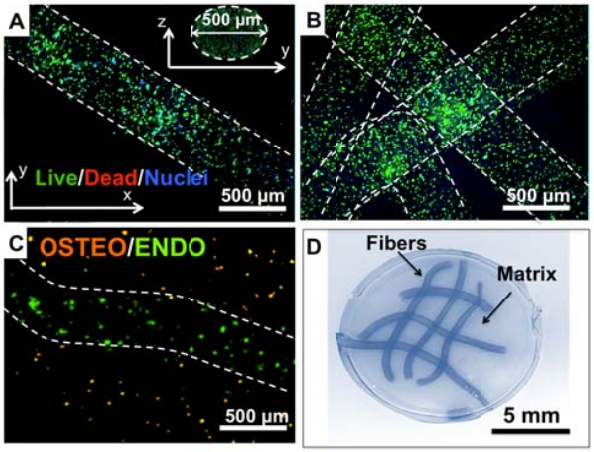

Figure 1. (A) Endothelial derived SSEA-4$^+$hASCs are viable (green) and evenly distributed (green/blue) within the κ-CA fibers microfibers. (B) Random stacking of microfibers containing endothelial derived SSEA-4$^+$hASCs. (C) 3D co-culture system based on osteo- (red) and endothelial derived SSEA-4$^+$hASCs (green), (D) macroscopic view of the 3D construct. κ-CA fibers were stained with methylene blue (blue).

The κ-CA processing versatility enabled to stack cell-loaded fibers into different designs and shapes (Figure 1B) and its incorporation within a second κ-CA hydrogel containing osteo-derived SSEA-4$^+$hASCs (Figure 1C-D).

Conclusions: We were able to fabricate κ-CA hydrogel microfibers with tunable features capable of retaining encapsulated endothelial derived SSEA-4$^+$hASCs cells viability and phenotype. Furthermore, these fibers could be integrated, in a random or organized fashion, within a κ-CA hydrogel containing osteo-derived SSEA-4$^+$hASCs. Together with the cellular components, derived from a pluripotent cell subpopulation within the adipose-derived stem cells, this data suggests the possibility of developing 3D κ-CA hydrogel-based co-culture platforms relevant for bone tissue engineering, starting from a single cell source.

References: 1. Mihaila *et al*, Tissue Engineering, 19, Oct 2012; 2. Popa *et al*, Biomacromolecules. 2011 14;12(11):3952-61