Synergistic Effect of Silicon and Calcium Ions on Osteogenic Differentiation of Human Adipose Stem Cells

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Statement of Purpose: Bone is a mineralized tissue that confers multiple mechanical and metabolic functions to the skeleton. Among those functions, bone work as storage of different elements, namely being a major sink and store of calcium [1]. Also there is another important trace element, which is silicon (Si), the most abundant trace element after iron and zinc, two other elements of physiological importance, although its function continues to be unclear. Nevertheless, it is known that Si has an influence on calcium phosphate deposition and on the differentiation of bone precursor cells [2, 3]. Also, in our previous work [4] we demonstrated that a wet-spun fiber mesh based on a blend of corn-starch with polycaprolactone (30/70 wt.%, SPCL) with silanol (Si-OH) groups sustained human adipose stem cells (hASC) proliferation and osteogenic differentiation when cultured under dynamic conditions. To address this, our aim is to study the synergistic effect of combining Si and Ca on osteogenic gene expression of hASC.

Methods: SPCL scaffolds were produced by wet-spinning technique. For that, we used three coagulation baths: methanol for SPCL (control), a calcium silicate solution for SPCL-CaSi and a silicate solution for SPCL-Si. Wet-spun fiber meshes were characterized by X-Ray elemental mapping, energy dispersive spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS). The mechanical characterization of the scaffolds was performed using dynamic mechanical analysis (DMA). Then, the scaffolds were sterilized and cultured with hASC for 4, 7, 14 and 18 days using α-MEM or osteogenic medium. Cell proliferation, morphology and adhesion were evaluated by the quantification of DNA and scanning electron microscopy (SEM). hASC differentiation was assessed by ALP quantification and quantifying the osteogenic gene expression by real time RT-PCR analysis. Ions release into the culture medium was measured by inductively coupled plasma (ICP).

Results: X-Ray elemental mapping of Si and Ca indicated a uniform distribution of those elements on the surface of the materials (Fig. 1a). EDS spectrum also confirmed the presence of both ions in the SPCL-CaSi scaffolds, although in the case of SPCL-Si, only Si was detected. Results showed that hASC readily proliferated and migrated into the interior of the wet-spun fibre mesh scaffolds, and calcium and phosphate on the cell surfaces were detected, within only 18 days of culture (Fig. 1b). The osteogenic differentiation seems to be influenced by the release of Si ions from both materials into the culture medium since the expression of genes.in SPCL-CaSi and SPCL-Si scaffolds was higher in general, as it can be seen in Fig. 1 (c) and (d). Nevertheless, the expression of ALP and RUNX2 was more pronounced for SPCL-CaSi scaffolds in a-MEM indicating the stimulatory effect of Si and Ca, on the osteogenic differentiation of hASC.

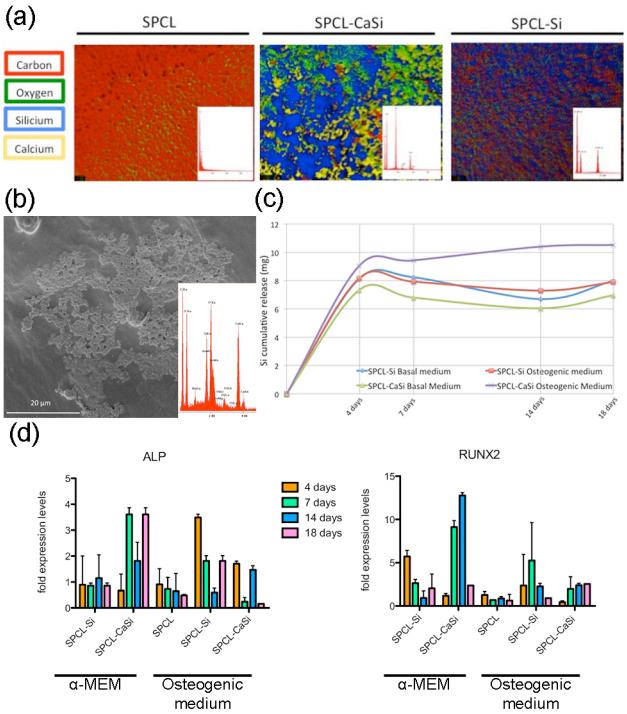

Figure 1. (a) X-Ray elemental mapping and EDX analysis (inset) SPCL, SPCL-CaSi and SPCL-Si scaffolds. X-ray elemental mapping of carbon (C, red), oxygen (O, green), silicon (Si, blue) and calcium (Ca, yellow). (b) SEM imaging and EDS analysis (inset) of mineralization nodules formed on SPCL-Si scaffolds at 18 days of culture. (c) Si release into the culture medium as function of time, measured by ICP (d) expression of osteogenic genes, namely ALP and RUNX2, by real time RT-PCR.

Conclusions: The present work shows that combining wet-spinning technology with a calcium silicate solution as a non-solvent allows designing scaffolds with key cues to render osteoconductive behaviour as the classic ceramic materials for bone regeneration. Additionally, these results suggests that Si and Ca ions may relevantly contribute to properly drive hASC towards the osteoblastic phenotype and highlight the potential of those ions together in the control of cellular response such as cell differentiation and/or in stem cells recruitment upon implantation of a cell-free scaffold.

References:

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