Ionic exchanges modulate the in vitro response of mesenchymal stem cells to biomimetic hydroxyapatite

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INTRODUCTION: In vitro assays are a potent tool for the evaluation of biomaterial performance. However, in the case of bioactive materials, like calcium phosphates (CaPs) the ionic exchange with the cell culture medium can make cell cultures very tricky. This is particularly relevant in the case of biomimetic hydroxyapatite, which has high specific surface area (SSA) and high surface reactivity. These features support its excellent in vivo performance, but can make the in vitro results difficult to interpret. The aim of this study was to analyse to what extent the outcome of cell culture studies on biomimetic calcium deficient hydroxyapatite depend on cell culture conditions, and more specifically on the ratio between cell culture and material volume, a parameter often overlooked.

METHODS: Calcium deficient hydroxyapatite (CDHA) discs were obtained from α-tricalcium phosphate (α-TCP) paste. To obtain β-tricalcium phosphate (β-TCP) the ionic exchange with the disc was sintered at 1100°C, and used as control. Two dimensions of discs were prepared: i) Large discs (~12 mm Ø x 2 mm height): CDHA -L and β-TCP-L; ii) Small discs (~5.5 mm Ø x 300 µm height): CDHA-S and β-TCP-S. SSA and porosity were determined by N2 adsorption and mercury intrusion porosimetry respectively. Rat mesenchymal stem cells (rMSCs) were seeded (300 cells/mm²) on the large and small discs, using always 2 mL of advDMEM complete medium, resulting in two cell culture conditions: i) low ratio between culture medium and biomaterial volume (VCM/VB ~ 10); and ii) high ratio (VCM/VB ~ 100). rMSCs proliferation was determined at 6h, 3, 7 and 14d by LDH quantification. Ca²⁺ and Pi concentration were monitored at the same time points. Quality of cell adhesion was assessed by visualization of phosphorylated focal adhesion kinase (pFAK) and actin stress fibres formation on CDHA-L and CDHA-S samples. For CDHA-S gene expression of RUNX2, ALP, COLL I, OC, ON, OP and BMP-2 was analysed by RT-qPCR..

RESULTS: Ca²⁺ and Pi concentrations in the culture medium were significantly altered only in CDHA-L (Fig. 1). Cells were able to proliferate on β-TCP (SSA=0.71 m²/g), irrespective of the size of the discs. In contrast, cell behaviour on CDHA (SSA=19.13 m²/g) was strongly dependent on disc size; even if cells adhered on both, they proliferated only on CDHA-S. pFAK was not detected and actin stress fibres did not form on CDHA-L. In contrast, both features were observed on CDHA-S. Noteworthy, upregulation of genes related to osteogenesis was observed on CDHA-S.

DISCUSSION & CONCLUSIONS: Although rMSCs were able to attach both on small and large CDHA discs, the changes induced in the ionic extracellular environment by CDHA-L resulted in a decrease of cell number over time. This effect of VCM/VB was not significant for β-TCP, due to its smaller reactivity and SSA. The non-maturation of pFAK and actin stress fibres was an indication of a deficient cell adhesion on CDHA-L, which could lead to cell death via anoikis, a type of apoptosis that is known to be triggered by the deficiency of calcium [1]. In fact, when ionic fluctuations were reduced by increasing VCM/VB (CDHA-S), more mature focal adhesions and actin fibres were found. This demonstrates the need of adjusting cell culture parameters considering material/cell culture medium interactions.

REFERENCES: 1B. Zhivotovsky et al. (2011), Cell Calcium 50(3), 211-221


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