Bone Engineering: Mimicking the In-vivo Calcium Phosphate-Enriched Environment

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Introduction

The use of calcium phosphate (CaP) based carriers in bone engineering is a promising approach for a local in-vivo enrichment of calcium (Ca$^{2+}$) and phosphate (P$_i$) to trigger progenitor/stem cells towards bone formation. The aim of this study was to mimic the Ca$^{2+}$ and P$_i$ ion-enriched environment in-vitro to assess the effect of these ions on the proliferation and osteogenic differentiation of human periosteum derived cells (hPDCs).

Materials and Methods

Culture medium containing 1% Ca$^{2+}$ or P$_i$(0, 2, 4, 6, 8 and 10 mM) in hepes buffered solutions were added to hPDCs cultures and incubated for 1, 3, 7, 14, 21 and 28 days. Cell proliferation, cell cycle progression, alkaline phosphatase (ALP) activity, expression of osteogenic marker genes (osteocalcin (OCN), osteopontin (OPN), bone morphogenetic protein-2 (BMP-2) and Runx2) and mineralisation were evaluated. Statistical significance was established at \( \alpha < 0.05 \) by using unpaired student t-test (two-tailed) or one-way ANOVA analysis.

Results

The Ca$^{2+}$ and P$_i$ treatment caused a time- and dose-dependent phenotypic change and aggregation of hPDCs, without any cell death after 28 days of culture. 4 to 8 mM of Ca$^{2+}$ and P$_i$ enhanced cell growth significantly from day 3 till 28 similar to mitogenic effect of osteogenic medium. Dissolved Ca$^{2+}$ and P$_i$ did not induce ALP production, but we observed a dose-dependent upregulation of osteogenic genes expression, important for the regulation of extracellular matrix mineralisation. This study implies a potential method to differentiate stem cells towards the osteogenic lineage for effective bone engineering.

Discussion and Conclusions

By mimicking the in-vivo CaP-enriched environment in-vitro, we show that Ca$^{2+}$ and P$_i$ induced cell proliferation by regulating cell cycle progression in a dose- and time-dependent manner. This is believed to be essential for achieving a critical cell mass that would initiate bone formation. Dissolved Ca$^{2+}$ and P$_i$ did not induce ALP production, but we observed a dose-dependent upregulation of osteogenic genes expression, important for the regulation of extracellular matrix mineralisation. This study implies a potential method to differentiate stem cells towards the osteogenic lineage for effective bone engineering.

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