Silicate-substituted Calcium Phosphate With Enhanced Strut Porosity Stimulates Osteogenic Differentiation Of Human Mesenchymal Stem Cells

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Introduction: Some bone graft substitutes are osteostimulative, meaning that they actively upregulate mesenchymal stem cell proliferation and stimulate differentiation into osteoblast-like cells. The purpose of this study was to verify the osteostimulative potential of SiCaP with enhanced porosity (SiCaP EP). Although SiCaP EP has the same chemical composition of SiCaP, it has increased strut porosity which mimics the microporous osteocyte lacunae network present in physiological bone.

Methods: The osteostimulative properties of SiCaP EP were evaluated in-vitro with STRO-1+ immunoselected human bone marrow derived mesenchymal stem cells (HBMSCs). Osteostimulative materials (silicate-substituted calcium phosphate (SiCaP) and Bioglass 45S5 (Bioglass)) were also assessed as positive controls along with non-silicate substituted hydroxyapatite (HA) as a negative control. HBMSCs were also assessed on Thermanox (TMX) discs cultured in basal and osteogenic media to determine when osteogenic differentiation could be significantly detected with this in-vitro cell system. HBMSC viability and necrosis (n=6), total DNA content (n=6), alkaline phosphatase expression (ALP) (n=6), and osteocalcin expression (n=12) were evaluated after 7, 14, 21, and 28 days.

Results: The results of cell viability and necrosis indicated that all test groups were able to sustain live human bone marrow derived mesenchymal stem cells out to 28 days. The DNA assay demonstrated a trend for increasing cell proliferation in the basal and osteogenic controls over the 28 day period. The amount of cells proliferating on SiCaP EP was significantly greater (p<0.05) than SiCaP and Bioglass. The total amount of ALP detected for the SiCaP EP test group increased over the 28 day period. Peak production from cells was between 21-28 days, although ALP expression per unit cell DNA was generally flat across the time course. The amount of ALP detected was significantly higher (p<0.05) in the SiCaP EP test group compared to the SiCaP and Bioglass test groups (Figure 1). There was a general trend for an increase in Osteocalcin production over the 28 day period for cells cultured in osteogenic media whereas for cells cultured in basal media there was a general trend for a decrease in Osteocalcin over the same period. Peak production of Osteocalcin was between 3-7 days for SiCaP and between 7-11 days for SiCaP EP. Osteocalcin production was significantly higher (p<0.05) in the SiCaP EP group than the non-silicated HA and Bioglass groups (Figure 2).

Discussion: The total amount of ALP being expressed by cells in the Thermanox control groups followed a similar trend to the total amount of DNA detected in the same groups and corresponded to the observations made by Cameron et al. who found an increase in ALP expression over the period 0-21 days when human bone marrow derived stem cells were cultured on tissue culture plastic (Cameron, Travers et al. 2013). When analyzing total ALP, the cells appeared to be up-regulated by the osteogenic media but only after 28 days of culturing. This effect was less apparent when normalizing the amount of ALP expressed per unit DNA. However, when taking into account the amount of cell DNA there seemed to be a peak in ALP expression at 14 and 28 days when cells were cultured in either medium. This sequential pattern of differentiation into mature osteoblasts was similar to the temporal pattern observed in hBMSCs in a previous study (Arpornmaeklong, Brown et al. 2009).

Significance: SiCaP EP was able to promote the proliferation of an equivalent number of human bone marrow derived mesenchymal stem cells compared to the positive controls (Bioglass and SiCaP) as demonstrated through cell DNA analysis. SiCaP EP promoted early-stage differentiation of human bone marrow derived mesenchymal stem cells as demonstrated through equivalent or better up-regulation of ALP expression compared to negative and positive controls. SiCaP EP promoted late-stage differentiation of human bone marrow derived mesenchymal stem cells as demonstrated through up-regulated production of Osteocalcin compared to the negative control and equivalent temporal up-regulation compared to the positive controls. SiCaP EP is osteostimulative based on its propensity to support human bone marrow derived mesenchymal stem cell proliferation and promote the differentiation of human mesenchymal stem cells down the osteoblastic lineage from ALP-expressing, matrix-producing osteoblasts to Osteocalcin-producing pre-osteocytes.

Acknowledgments:
Figure 1. Alkaline phosphatase expression as p-nitrophenol normalized for DNA comparing: A) TMX basalt control vs. TMX estrogenic control. B) SiCalP EP vs. Hx, C) SiCalP EP vs. SiCalP D) SiCalP EP vs. Bioglass (*p < 0.05, Mann-Whitney).
Figure 2: Osteocalcin expression comparing A) TMX basal control vs. TMX osteogenic control, B) SiCaP EF vs. IIA, C) SiCaP EF vs. SiCaP D) SiCaP EF vs. SiCaP + EGF (* p<0.05 Mann-Whitney).