Cell Maturity Modulates Osteogenic Differentiation Responses to Ca Releasing Biomaterials through Ca Channels

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Introduction: To present an alternative to allo- and auto grafts, bone tissue engineering (TE) strives to regenerate biological substitutes. Ideally, biomaterials are designed to provide biological cues to guide cell behavior. Although cells with different osteogenic maturity have been used for bone tissue engineering, there are no criteria indicating which cell type best facilitates regeneration. In addition, it is not known how cell maturity affects cell response to extracellular cues associated with a biomaterial, limiting our ability to optimally design TE systems.
Calcium is a ubiquitous intracellular messenger in many cells. This inorganic element is involved in intracellular signaling that regulates cell adhesion, proliferation and differentiation. Meanwhile, many types of biomaterials have the ability to affect extracellular Ca concentration through surface precipitation and dissolution[1]. Here, we hypothesize that under exposure to a Ca signal generated by biomaterial, osteogenic maturity of cells affects their differentiation through differences in Ca channel activation.

Methods: *Material synthesis*: amorphous calcium phosphate (ACP) nano particles were synthesized based on a published protocol[2]. *Cells*: two types of cells with different osteogenic maturity were used. The less mature cell type was human induced pluripotent stem cell (iPS)-derived mesenchymal stem cells (MSC)[3]. The more mature cell type was human iPS derived-preosteoblasts (PO) obtained by culturing iPS-MSC in osteogenic medium for two weeks. *Cell culture and cell differentiation:* Cells were cultured with ACP-conditioned growth medium or growth medium with or without 5mM $CaCl_2$ supplemented for 10 days. ACP-conditioned medium was obtained by suspending either 0.1 or 0.5 g of ACP particles in a transwell and incubating particles in growth medium under cell culture conditions for two days. Real time-PCR was used to quantify expression of three genes (OCN, Runx2, and collagen type I). *Investigation of calcium channels:* with both MSC and preosteoblast incubated with Ca channel blockers, Nifedipine (L-type channel) and Thapsigargin (Ca channels in endoplasmic reticulum), intracellular calcium concentration was monitored using Fluo-4 Direct™ Calcium Assay Kits (Invitrogen, Cat # F10471).

Results and Discussions: Expression of collagen type I was significantly higher in PO than MSC in the absence of ACP (Fig. 1, left column). With ACP-conditioned medium, collagen I expression between two cell types was no longer different. Runx2 expression was not different between MSC and PO in the absence of ACP, but showed significant differences after culturing with ACP-conditioned media. These results indicate that the introduction of ACP changes gene expression of MSC and PO differently, highlighting a cell maturity effect.

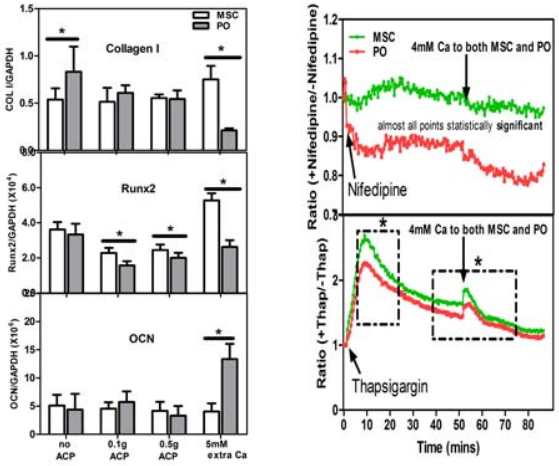

Figure1. Gene expression (left column) and intracellular Ca normalized to non blocker control (right column) * indicates a significant difference

Differences in relative gene expression between MSC and PO reflects an effect of cell maturity. Correlations between relative expression of each gene and extracellular Ca concentration leads to r-values of -0.96, -0.87, and 0.96 for collagen I, Runx2 and OCN, respectively, suggesting that Ca release from a biomaterial may be an important factor that gives rise to those different gene responses. Consistent with these observations, MSC and PO responses to the same Ca extracellular signal are driven by different mechanisms, with L type channels more important for PO but Ca channels in ER more important for MSC (Fig. 1 right).

Conclusions: Cell maturity affects osteogenic differentiation responses to Ca releasing biomaterials. These effects are due to different roles of Ca channels in regulating intracellular Ca signaling. Our results highlight that cell maturity is an important cell property that needs to be considered to maximize the ability for extracellular cues from biomaterials to effect cell response.

References:1. Zhang NL. Biomaterials. 2010. 31:7653-7665. 2.Syed-Picard FN.Biomaterials.2013.34:3763-3774. 3.Villa-Diaz LG. Stem Cells.2012.30:1174-1181.

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