Microenvironment Changes in the Stem Cell Niche Influence the Differentiation Pathway to Bone

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Introduction: The field of bone regeneration is highly dependent on the development of biomaterials which can facilitate stem cell differentiation to bone. One problem associated with current bone substitutes is the lack of osteogenic properties i.e., the substitute's ability to attract and maintain stem and progenitor cells at the site of the defect. Despite *in vitro* assays which may indicate that a specific material is suitable for osteoblast attachment and differentiation, downstream *in vivo* testing often fails. The failures of these substitutes may be linked to inherent limitations in the commonly used *in vitro* osteogenesis assays including cell source and growth conditions as well as a lack of emphasis on incorporating materials native to bone repair and regeneration. Specifically, many substitute materials are tested using immature osteoblasts or osteoblast progenitors as the cell source, however during the normal physiological bone healing process, mesenchymal stem cells (MSC) home to the site of injury and differentiate to form new bone. More importantly however, MSCs are extremely important in the bone healing process and rather than providing clues to whether a material supports differentiation, these cells may contain the necessary information needed to direct their own differentiation. ***We hypothesize that differentiating MSCs secrete a cell-instructive extracellular matrix which provides cues to guide the differentiation process.*** In this study, we defined osteogenic changes occurring in stem cell-produced extracellular matrix during the differentiation process.

Methods: Mesenchymal stem cells were derived from human adipose tissue (adipose-derived stem cells or hADSC), seeded onto tissue culture plastic (TCP) and induced to differentiate using a dexamethosone, ascorbic acid, and beta-glycerol phosphate supplemented media. At days 3, 6, 9, 12, 15, 18, 21, 24, and 27 cells were removed using a decellularization kit (Sigma), leaving dishes coated with intact extracellular matrix (ECM) that had been secreted by the cells.

To access the potential of the cell-produced ECM to enhance hADSC differentiation to an osteoblast-like phenotype, undifferentiated hADSC were seeded onto the dishes containing the intact, cell-secreted ECM and induced to differentiate using the same supplemented media. At day 16, calcium deposition was visualized using alizarin red staining. Enhancement of the cells' ability to display osteoblast-like phenotype was assessed by qPCR, ELISA, and immunohistochemical staining for osteogenic markers.

Results: Cells seeded onto day 3, 6, 9, 12, 15, 18, 21, 24, and 27 ECM showed differential alizarin red staining. Robust calcium deposition was clearly evident by day 15, but not before. Matrices from later time points appear to display similar calcium levels, but cell detachment was prevalent. (Figure 1). qPCR results showed earlier and increased levels of osteocalcin gene expression in cells grown on day 15 ECM compared to cells grown on plain TCP. (Figure 2).

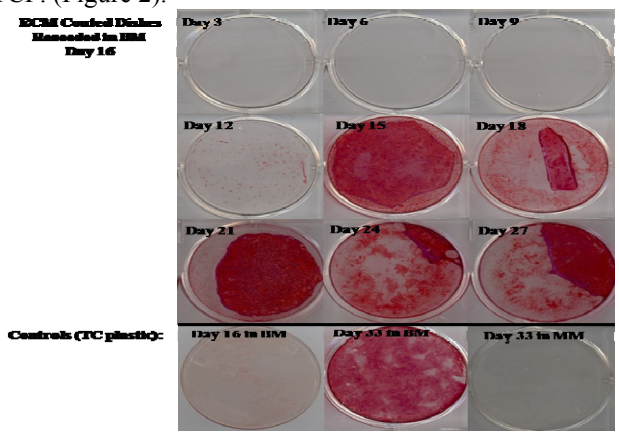

Figure 1. Calcium deposition visualized with alizarin red. Top squares (above the black line) show calcium deposition by hADSC on ECM from progressive time points in differentiation. Day 15 ECM appears to have a significant effect on calcium deposition compared to earlier timepoints. Similar staining in cultures grown on TCP does not occur until day 33 (bottom panel).

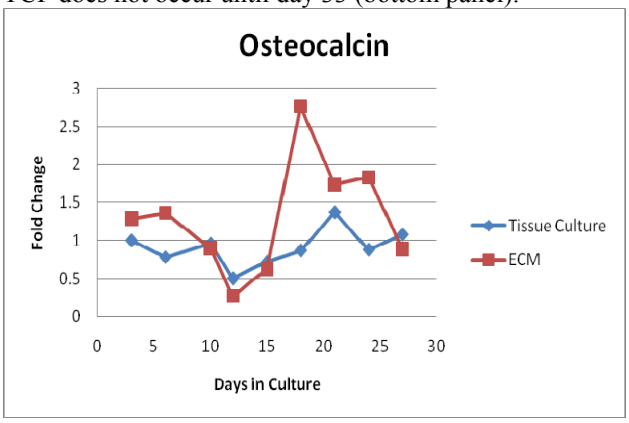

Figure 2. Bone marker osteocalcin mRNA expression. Cells grown on day 15 ECM show earlier and significantly more osteocalcin expression compared to cells grown on TCP in supplemented media.

Conclusions: The normal time course of hADSC differentiation in supplemented media is 25-30 days. By this time point, heavy calcium staining is typically evident in cultures grown on TCP. However, expression of osteogenic genes is typically low. In contrast, when cells are grown on the ECM secreted by differentiating cells, calcification is evident at much earlier time points and expression of osteogenic genes is significantly enhanced. This suggests that differentiating MSC secrete osteoinductive compounds that become incorporated into the ECM and are recognized by native MSC. This finding suggests that cell-secreted matrices can provide a model for discovery of osteogenic compounds which could play an important role in driving the differentiation process and may eventually be able to be incorporated into bone graft substitutes.