Investigating the Role of Osteoclasts in Bone Tissue Engineering via nCP-Hydrogel Scaffolds

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Introduction: Bone regeneration within critically-sized bone defects remains a difficult challenge in orthopedics. Each year, an estimated two million people undergo bone grafting procedures, which amounts to billions in medical costs globally[1]. Although bone-grafting persists as the standard treatment, issues such as immune rejection of allografts and donor site morbidity in the case of autografts plague such procedures. Bone tissue engineering aims to circumvent the shortcomings of bone-grafting by developing constructs with osteoconductive and osteoinductive properties through the coordination of cells, biomaterials, and cellular signals. Cells of bone-forming lineage, including hMSCs and osteoblasts, have received much attention in the field as the cell choice for incorporation in biomaterial constructs. The role of osteoclasts, the hematopoietic bone resorbing counterpart, have not received as much attention in bone tissue engineering research. Although osteoblasts and osteoclasts have been thought to fulfill discreet roles in the bone remodeling process, recent research demonstrates that osteoclasts play an integral role in propagating osteoblast-mediated bone formation via extensive and intricate pathways of communication[2]. This study aims to investigate the differentiation and resorption of osteoclasts within bone tissue engineered constructs by varying calcium phosphate content and investigate the ability of osteoclasts to promote bone formation.

Materials and Methods: A preliminary 2D study was conducted to investigate culture conditions to be utilized in 3D studies. Co-cultures and monocultures of MSCs and Osteoclast precursors (OCp) were prepared in 48-well plates at a density of 30,000 cells/well. MSCs and OCps were seeded simultaneously at a 1:1 ratio in basal, osteogenic, or osteoclastogenic conditions. Cultures were supplied with fresh media every three days. GelMA was synthesized according to a previously established protocol. Calcium Phosphate nanoparticles (nCP) (hydroxyapatite and β-tricalcium phosphate) were incorporated in 1% (w/v) concentration separately, or equal concentrations of 0.5% (w/v) together as shown in Figure 1A. The gelMA-CP mixture was then casted into cylindrical hydrogel scaffolds (10 mm diameter x 2 mm height) following the encapsulation hMSCs and/or hPBMCs, a precursor to osteoclasts, at a density of 1×10^6 cells/mL. Cultures were maintained for 28 days in identical conditions. Fresh media was supplied every three days. Osteogenesis, osteoclastogenesis, and bone formation were assessed via gene expression analysis, mineralization assay, and immunofluorescence.

Results and Discussion: Our results suggest that hMSC organization and ECM deposition was assisted in the presence of osteoclast precursors in osteoclastogenic conditions. Incorporation of different calcium phosphate phases proved influential in *in-vitro* bone formation and cell behavior within the hydrogel scaffolds. Utilization of hMSC/hPBMC co-cultures in gelMA-CP bioinks for extrusion-based 3D printing will be explored in future work.

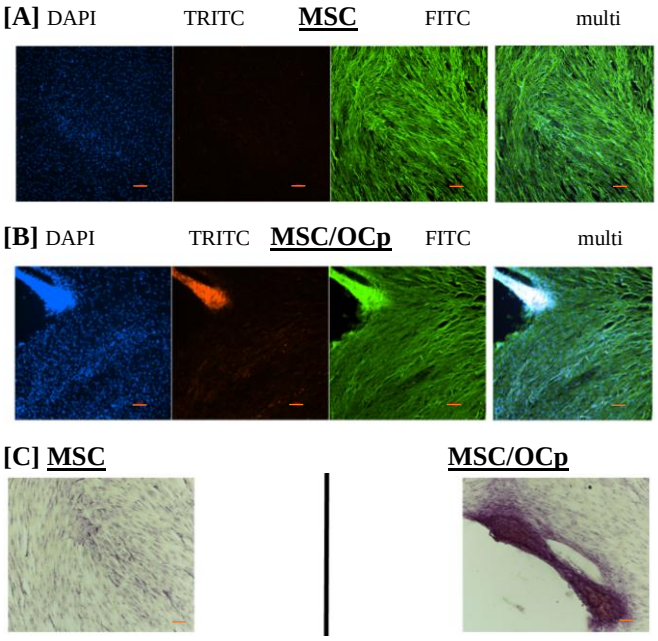

Figure 1: 2D MSC monocultures and MSC/OCp co-cultures after 14 days in OC conditions. [A&B] Immunofluorescence Imaging: blue indicates cell nuclei (DAPI), orange indicates COL-1 (TRITC), and green indicates actin (FITC). [C] Alizarin Red S Staining. 200 uM scale bars included.

Conclusion: The goal of this study is to investigate the ability of osteoclasts to promote bone formation within bone tissue engineered constructs. Ultimately, this study will serve to enhance future directions in bone tissue engineering, specifically 3D bone bioprinting.

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References:

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[2] Kim, J., et al. *Cells* 2020, *9*(9), 2073.