Title: **Evaluating the Ability of Osteopontin Phosphoforms and Peptides Derived from Osteopontin and Perlecan to Support Bone Cell Adhesion**

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Objectives: 3D-printed bone scaffolds are promising surrogates for bony lattices needed for dental and orthopedic applications. Integration and function require stable bone cell adhesion and differentiation. We explored the potential of two phosphoforms of the extracellular matrix (ECM) bone protein, osteopontin (OPN), and two synthetic peptides from OPN and another from perlecan (PLN), in enhancing adhesion of two bone marrow stromal cells (BMSCs)[1,2,3]. We hypothesized that OPN would better support cell adhesion than other surface coatings due to the presence of two integrin binding motifs.

Methods: To identify the best adhesive coating, we used non-adhesive 96-well plates pre-coated with high or low phosphorylated rat OPN; two versions of RGD-containing OPN synthetic peptide; or PLN adhesive peptide (0.15 mg/mL). Rat-tail collagen type I (0.15 mg/mL) served as a positive control and bone serum albumin served as negative non-adhesive control. Sterilized plates were coated with test proteins and peptides. BMSC cells were seeded at 15,000 cells/well and cultured without fetal bovine serum. Adhesion was monitored over 24 hours and imaged using a Keyence microscope. Attachment and spreading with different coatings was quantified using ImageJ.

Results: Cells seeded on collagen type I formed a uniform monolayer on the cell plate. Cells seeded on OPN phosphoforms, Perlecan Domain IV peptide, OPN-derived peptide, or GRGDS attached as mixtures of single cells that attached and spread and attached, nonuniform sized clusters that resemble bone nodules able to mineralize. Cells on OPN protein phosphoforms exhibited cell adhesion and spreading and signs of migration. Quantification of these results is ongoing.

Conclusion: This study provides insights into the adhesion properties of BMSCs in response to different bone ECM proteins and peptides. OPN is a promising protein for further study. Future work will determine the impact treatment coatings have on adhesion and differentiation and their usefulness in 3D-printed bone scaffolds.

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References:

1. Safran, J., et al. Modulation of Osteopontin Post-translational State by 1,25-(OH)2-Vitamin D3. Journal of Biological Chemistry, 1998.
2. Dashdulam, D., et al. Osteopontin heptamer peptide containing the RGD motif enhances the phagocytic function of microglia. Biochemical and Biophysical Research Communications, 2020.
3. Shin, H., et al. Attachment, proliferation, and migration of marrow stromal osteoblasts cultured on biomimetic hydrogels modified with an osteopontin-derived peptide. Biomaterials, 2004.