LIPUS-Derived Mechanical Stimulation of Collagen Hydrogels for Bone Repair

Fayekah Assanah[1], Hanna Anderson[1,3], Kevin Grassie[1,3] and Yusuf Khan[1, 2, 3]
[1]Department of Biomedical Engineering, University of Connecticut, Storrs, CT; [2]Department of Orthopedic Surgery; [3]Connecticut Convergence Institute for Translation in Regenerative Engineering, UCONN Health, Farmington, CT

Statement of Purpose: Regenerative engineering has tremendous potential as an alternative to over 2 million bone grafting procedures performed annually [1]. Toward that end, we have merged regenerative engineering techniques with existing clinical therapies to develop a treatment strategy for large-scale bone defects. This approach combines hydrogel-based cell delivery with low intensity pulsed ultrasound (LIPUS), an FDA approved treatment for fracture repair. Our strategy involves the delivery of cell-laden hydrogels to bone defects followed by the application of transdermal, LIPUS-derived acoustic radiation force (ARF). Physical forces applied to cell-hydrogel constructs would stimulate the cells within the defect site to promote bone formation without disrupting the unstable bone defect. We have demonstrated the efficacy of this technique by observing the upregulation of load-sensitive markers in cell-loaded collagen hydrogels [2, 3]. Here, we determined the optimal combination of hydrogel stiffness and LIPUS intensity that initiates an immediate upregulation of prostaglandin E_2 (PGE_2) and cyclooxygenase-2 COX-2), both well-established markers of bone formation, and downstream mineralization of hydrogels by encapsulated marrow-derived stem cells. We also evaluated this *in vivo* using mouse calvarial defects.

Methods: *Hydrogel Synthesis:* Type I collagen hydrogels were fabricated using different collagen concentrations, 0.1, 0.3 and 0.5%. *Cell Analysis:* Bone Marrow Stromal Cells (BMSCs) were harvested from the long bones of wild-type mice and cultured for 7 days on TCP. Adherent cells were then encapsulated in hydrogels ($5x10^5$cells/gel) and maintained in 37^0C and αMEM media (10% FBS 1% pen/strep). LIPUS-derived ARF was applied to cell-hydrogel constructs (0.3, 0.5% collagen) using a 1 MHz unfocused immersion transducer, by submerging the transducer in media and placing directly above the cell-hydrogel construct. A one-time ARF treatment (300 mW/cm^2) was applied to the cultures for 20 minutes. After an hour, media and cells were extracted to measure PGE_2 level and gene expression of COX-2 using ELISA and qRT-PCR, respectively. For long-term studies cell-hydrogel constructs were treated with ARF for 5 out of 7 days per week for 3 weeks and stained with alizarin red stain to evaluate mineral formation. One-way analysis of variance with Tukey post-hoc test was used to determine statistical significance between experimental groups ($p<0.05$, n=3). For the *in vivo* studies, a unilateral critical size calvarial defect (size: 4mm) was created in NSG mice and a collagen hydrogel (0.3%) encapsulating $5x10^5$ reporter BMSCs per hydrogel was placed inside the defect. ARF was applied transdermally to the defect site once per day for 20 minutes, 5 days per week, for 4 weeks.

Results: ARF applied to cell-hydrogel constructs increased the levels of (B) COX-2 and (C) PGE_2 of mechanically stimulated, hydrogel-encapsulated BMSCs compared to control (no ARF). Interestingly just encapsulating cells in the 0.3% hydrogel (no ARF) upregulated COX-2 and PGE_2, but the combination of both encapsulation and ARF increased these expressions further. (D) ARS staining shows that cells encapsulated within 0.3% gels increased mineral formation over the other groups with ARF treatment after 3 weeks. I*n vivo* analysis revealed that mechanically loading encapsulated cells resulted in better bone healing after 4 weeks. X-ray and DIC images show more bone formation with ultrasound (F,H) than without (E,G). Viable cells were present within the defect site (I) that co-localized with mineralized tissue (J) and osteoclast activity (K), suggesting active bone remodeling.

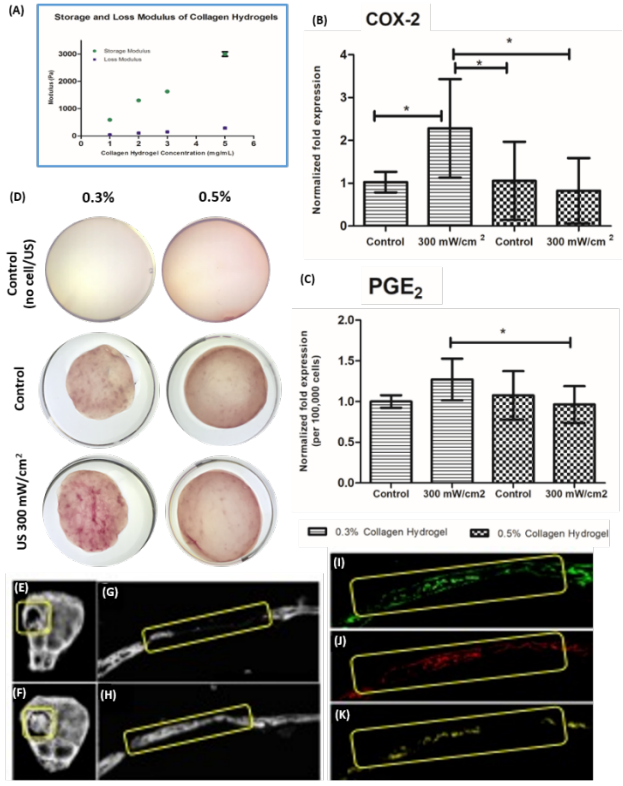

Figure 1: (A) Storage and Loss Modulus of varying concentrations of collagen hydrogels. (B) Cox-2 (C) PGE_2 upregulation of encapsulated cells in collagen hydrogels after ARF exposure (n=3). (D) Alizarin Red Staining of collagen hydrogels. Mouse Calvarial Defect: X-ray images (E) no ARF (F) ARF. DIC image of (G) no ARF (H) ARF (I) Presence of Donor cells. Enhanced (J) Alkaline Phosphatase (K) Osteoclasts in defects with ultrasound.

Conclusions: Our data suggests that hydrogel stiffness influences cell behavior, specifically the upregulation of COX-2 and PGE_2 (critical to downstream bone formation), mineralization of hydrogels, and in vivo bone formation.

References: [1] Giannoudis PV. Bone substitutes 2005; 36; S20-S2. [2] Veronick JA. Exp Biol Med 016; 241: 1149–1156. [3] Veronick JA. Tiss Eng Part A 2017; 00 1-10.