Title: Design and Cytocompatibility of Hyaluronic Acid Hydrogels for Bone Regeneration

Authors: Ireland Johnson[1,2], Veronica Spaulding[2,3], Andres Guerrero[3], Marian Hettiaratchi[2, 3]
[1] Department of Biology, University of Oregon, [2] Phil and Penny Knight Campus for the Accelerating Scientific Impact, University of Oregon, [3] Department of Chemistry, University of Oregon

Introduction: Large bone defects and fractures caused by trauma or disease remain a serious challenge for orthopedic surgeons, and there is a need for more effective treatment strategies to repair injured bone. Bone autografts, a tissue graft from the same patient, are an ideal treatment strategy because there is a low chance of host rejection, and the graft is not weakened from sterilization. However, bone autografts are not widely available, and their harvest can cause donor site morbidity. As an alternative strategy, biomaterials composed of natural polymers like collagen and hyaluronic acid can be used to deliver osteogenic proteins that stimulate an osteogenic healing response[1]. The objective of this study was to develop hyaluronic acid (HA) hydrogels and test their cytocompatibility for bone regeneration applications.

Materials and Methods: 2.5% (w/v) HA polymer-based hydrogels[2] were formed by dynamic, covalent bonds between aldehyde functional groups on oxidized (OX HA) or pendant diol oxidized HA (PD HA) and HA functionalized with adipic acid dihydrazide (AD HA) or carbohydrazide (CH HA) groups. Hydrogel cytocompatibility was evaluated by measuring fibroblast (NIH3T3-GFP) cell viability and proliferation, where live cells were quantified using green fluorescence from green fluorescent protein (GFP) and dead cells were quantified using red fluorescence from ethidium homodimer. The cells were imaged and analyzed on days 0, 1, 3, and 5 after seeding on top of the hydrogels.

Results and Discussion: HA hydrogels fabricated with OX HA and CH HA supported a 5.5-fold increase in cell number over 5 days; in comparison, tissue culture plastic (positive control) supported a 3-fold increase in cell number. OX HA + CH HA hydrogels maintained high cell viability (>82%) for all time points. Hydrogels fabricated with PD HA and AD HA demonstrated a 13% decrease in cell number (Figure 1). We hypothesized that the differences observed in cell survival and proliferation were likely due to differences in the amounts of free aldehydes/hydrazides in the hydrogels, which will be the subject of future testing. We did additional testing on the best performing hydrogel platform, the OX HA + CH HA hydrogel, to determine if optimization was possible. We found that adjusting the w/v% of the carbohydrazide HA had no effect on the cell viability and growth, indicating that the physicochemical properties of this platform could be tuned to support cell survival and proliferation in a wide range of potential applications.

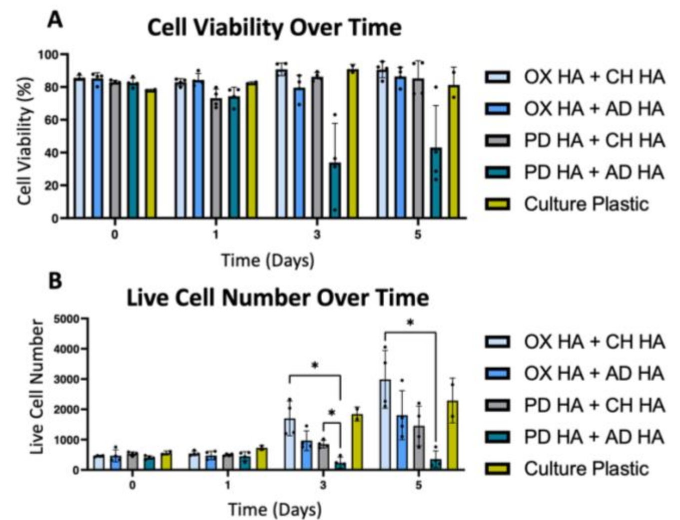

Figure 1. A) Live Fibroblast Number in HA Hydrogels. Live and dead fibroblasts were quantified using GFP and ethidium homodimer respectively. Asterisks (*) indicate $p<0.05$, 2- way ANOVA with Tukey's post hoc test. Error bars represent standard deviations. **B) Fibroblast Viability in HA Hydrogels.** Except for the PD HA + AD HA hydrogel, all other hydrogels maintained cell viability of > 80%, similar to the tissue culture plastic control, over five days. Data plotted as mean +/- standard deviation.

Conclusion: Since the results from this study demonstrated that the oxidized HA and carbohydrazide HA (OX HA + CH HA) hydrogel supported cell growth better than other hydrogels, we are currently optimizing this hydrogel for use as a protein delivery vehicle for osteogenic protein delivery. Results from this project could lead to the development of a biomaterial that rivals the healing response of an autograft.

Acknowledgements: This project was supported by the Knight Campus Undergraduate Scholars Program, a UO-OHSU Collaborative Seed Grant, and Collins Medical Trust Grant.

References: 1. Hettiaratchi, et al. Sci Adv 2020, 6(1): eaay1240 2. Muir and Burdick, Chemical Reviews 2020.