Design and Cytocompatibility of Hyaluronic Acid Hydrogels for Bone Regeneration

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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 allografts, which are grafts from a donor of the same species, are often used to heal large bone defects, but have a high chance of immune rejection and often fail to integrate with the host bone due to sterilization processes that remove viable cells and proteins. Autografts, a tissue graft from the same patient, are ideal because there is low host rejection, and the graft is not weakened from sterilization, but they are not widely available and can cause donor site morbidity. To combat this problem, biomaterials, composed of natural polymers like collagen and hyaluronic acid, can be used to deliver osteogenic (bone-forming) proteins that produce an osteogenic healing response.

Objective

This study describes the development, design and cytocompatibility of hyaluronic acid (HA) based hydrogels for bone regeneration applications.

Methods

HA hydrogels were formed by dynamic, covalent bonds between aldehyde functional groups on oxidized or pendant diol oxidized HA and HA functionalized with adipic acid dihydrazide or carbohydrazide groups at 2.5% w/v. The treatments tested for cell compatibility with green fluorescent fibroblasts are as follows:

- Oxidized HA + Carbohydrazide HA
- Pendant Diol Oxidized HA + Carbohydrazide HA
- Oxidized HA + Adipic Acid Dihydrazide HA
- Pendant Diol Oxidized HA + Adipic Acid Dihydrazide HA
- Tissue Culture Plastic (Positive Control)

Live and dead cells were evaluated using green fluorescence from GFP and red fluorescence from ethidium homodimer, respectively. The cells were then imaged and analyzed on days 0, 1, 3, and 5 where cell viability and count was recorded.

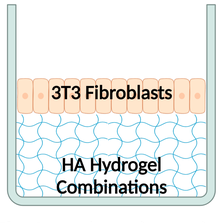

Fig. 1. Hydrogels seeded with fibroblasts

Fibroblast Cell Culture Images

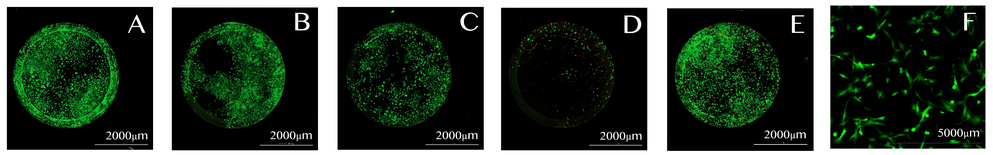

Fig. 2. Fibroblast Cell Culture Images 5 Days Post Treatment. Dead cells are indicated by red staining and live cells are indicated by green fluorescence (GFP). **A** Oxidized HA + Carbohydrazide HA. **B** Oxidized HA + Adipic Acid Dihydrazide HA. **C** Pendant Diol Oxidized HA + Carbohydrazide HA. **D** Pendant Diol Oxidized HA + Adipic Acid Dihydrazide HA. **E** Tissue Culture Plastic (Positive Control). **F** Close up image of 3T3 GFP fibroblast cells.

Fibroblast Viability and Growth

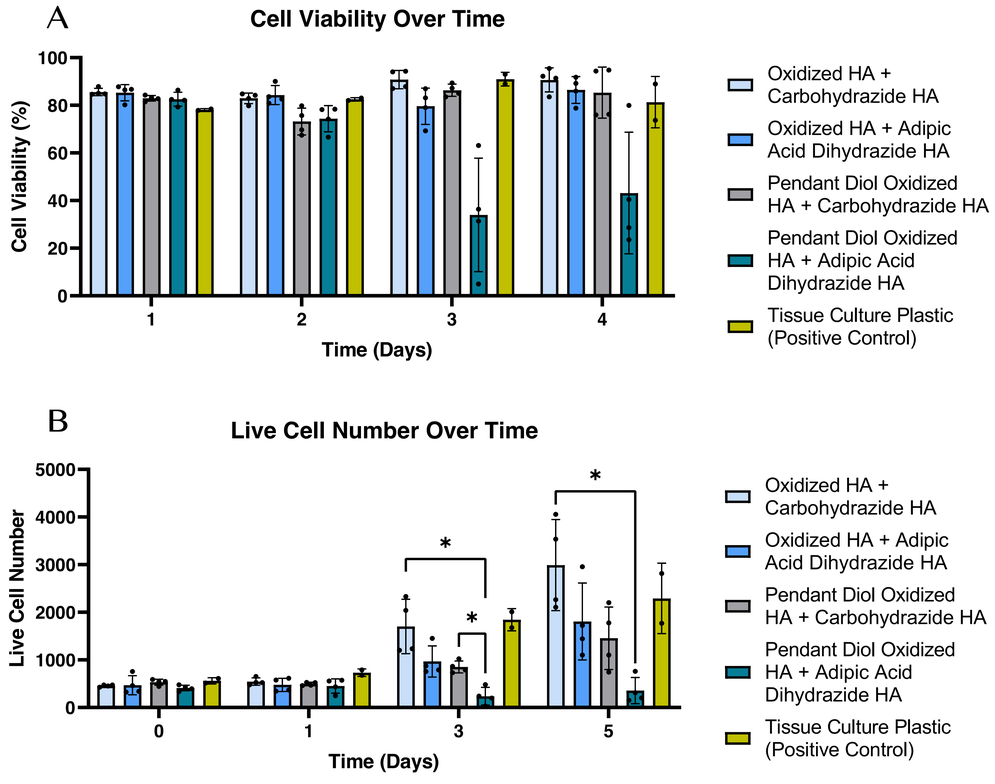

Fig. 3. A Fibroblast Cell Viability (%) Over Time. Apart from the pendant diol oxidized HA and adipic acid dihydrazide HA treatment, all other conditions tested maintained high cell viability over time. bars represent standard deviations. **B** Fibroblast Live Cell Number Over Time. The oxidized HA + carbohydrazide HA treatment appears to have a comparable growth rate to the tissue culture plastic treatment. Asterisks (*) indicate $p < 0.05$, 2-way ANOVA with Tukey's post-hoc test. Error bars represent standard deviations.

Conclusions

- The most noticeable differences in trends were observed when looking at cell count over time. oxidized HA and carbohydrazide HA supported a cell count increase of 554.2% whereas the pendant diol oxidized HA and adipic acid dihydrazide HA facilitated a decrease of 12.9%. In comparison, the control supported an increase in cell count of 308.2%.
- A combination of oxidized HA and HA-carbohydrazide at 2.5% (w/v) maintained high cell viability (>82.3% for all time points) and encouraged a rate of cell growth that surpassed all other conditions. We suspect that this difference in cytocompatibility is likely due to fewer free aldehydes or dihydrazides in this condition or gel degradation, that might be contributing to toxicity in the other present conditions.

Future Work

- Since the combination of **oxidized HA and HA-carbohydrazide at 2.5% (w/v)** showed high cell viability and a rate of cell growth that surpassed all conditions, it would be worthwhile to determine if different ratios of the oxidized HA and carbohydrazide HA would produce varying rates of cell viability or cell count.
- In the future, it would also be advantageous to determine if these hydrogels could be protein delivery vehicles for osteogenic healing proteins like bone morphogenetic protein 2, which could ultimately lead to the use of HA hydrogels as a biomaterial that rivals the healing response of bone autografts.

References

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Hettiaratchi, M, Krishnan, L, Rouse, T, Chou, C, McDevitt, T, & Guldberg, R 2020, 'Heparin Mediated Delivery of Bone Morphogenetic Protein-2 Improves Spatial Localization of Bone Regeneration, *Science Advances,* vol. 6, no. 1

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