

Conference Proceedings

3D Bioprinted GelMA-Gelatin-Hydroxyapatite Osteoblast Laden Composite Hydrogels for Bone Tissue Engineering.

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Introduction

3D bioprinting offers a novel solution to critical sized bone defects by allowing the placement of osteogenic cells, additive biomaterials and bioactive signaling that mimic native tissue. We describe the suitability of an extrusion-based 3D bioink composed of gelatin methacryloyl (GelMA), gelatin, hydroxyapatite (HA), and osteoblasts for bone tissue engineering.

Methods

A mouse calvarial osteoblast-laden GelMA-gelatin-LAP bioink consisting of various concentrations of HA was 3D-bioprinted into a porous hydrogel construct. After days 1, 14, and 28, five constructs from each HA concentration were analyzed. The water weight percent differences of the hydrogels and the degradation behavior in enzyme solution were characterized. An ALP assay and histological analysis were performed. Cell survivability was determined using a LIVE/DEAD Viability/Cytotoxicity Kit and AlamarBlue Cell Viability reagent. Real-time polymerase chain reaction (RT-qPCR) was performed to measure expression levels of bone morphogenetic protein-7 (BMP-7) and Osteocalcin (BGLAP).

Results

The addition of 5, 10, and 20 mg/ml of HA significantly reduced hydrogel swelling ($p \leq 0.01$) from baseline GelMA-Gelatin hydrogels (Figure 3A). HA significantly decreased hydrogel breakdown in a concentration dependent manner (Figure 3B, $p \leq 0.001$). A significant increase in cell proliferation at day 28 was noted in all groups (Figure 3D). ALP activity (Figure 3E) significantly increased with the addition of 5mg/ml and 20mg/ml of HA at days 7 and 28 ($p \leq 0.05$). Live/dead staining at 1, 14, and 28 days showed high chondrocyte viability. The addition of 20mg/ml of HA demonstrated significantly greater BMP7 and BGLAP gene expression at both 14 and 28 days over the hydrogels without HA (Figure 5, $p \leq 0.05$).

a [Conflicts of Interest Statement for Lindsey Johnson](#)

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b [Conflicts of Interest Statement for Dr. Allen](#)

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d [Conflicts of Interest Statement for Dr. Danilkowicz](#)

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e [Conflicts of Interest Statement for Dr. Adams](#)

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Conclusion

The addition of HA to GelMA-gelatin hydrogels significantly decreased hydrogel swelling, improved the ability of the hydrogel to resist enzymatic degradation, increased osteoblastic differentiation and mineralization, and increased osteogenic gene expression while maintaining equal cell viability and proliferation to non-HA hydrogels.



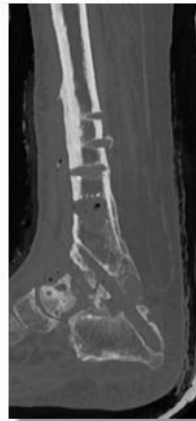
Critical Sized Bone Defects



High-Energy Trauma



Congenital Deformity



Non-Union



Implant Failure



Charcot

Introduction

One of the unmet needs in orthopedic surgery is the critical-sized bone defect. These defects can be the result of congenital deformity, high energy trauma, non-union, failed arthrodesis or arthroplasty or the degenerative pathology we see in Charcot.

This is the third of four award-winning presentations from the inaugural Medical School Orthopedic Society (MSOS) symposium. MSOS is a medical student-run initiative with a mission to support “research and educational opportunities for students interested in orthopedic surgery.”

This presentation was given by Lindsey Johnson, a rising fourth-year medical student at Campbell University who recently completed an orthopedic surgery research year at Duke University.




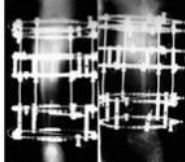
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DISCLOSURES

The authors do not have anything to disclose pertaining to this presentation.


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Limitations of Current Technology

Allograft Bone	Vascularized Bone Graft	Metal Augments	Bone Transport
			
<ul style="list-style-type: none"> - High nonunion rate - Graft collapse - Dead bone 	<ul style="list-style-type: none"> - Limited size - Patient suitability 	<ul style="list-style-type: none"> - Limited by size & shape - Infection - Stress shielding 	<ul style="list-style-type: none"> - Patient tolerance - Pin site infection

The current treatment options for these bone voids are limited in their size, risk of rejection or infection. They often require multiple operations that are burdensome for the patient.

3D Printing



3D-printing has partially addressed these concerns through patient-specific implants often made of titanium or cobalt chrome, but these are still foreign materials that are subject to the same risk of infection and non-union.

3D Bioprinting



Hypothesis

In our study, we described the suitability of an extrusion-based 3D bioink composed of gelatin and gelatin methacryloyl with various concentrations of hydroxyapatite (HA). Osteoblasts were added to this bioink formula for bone tissue engineering. We hypothesized that the addition of HA would (1) decrease hydrogel swelling and degradation and (2) increase osteoblast cell proliferation and markers of bone matrix deposition.

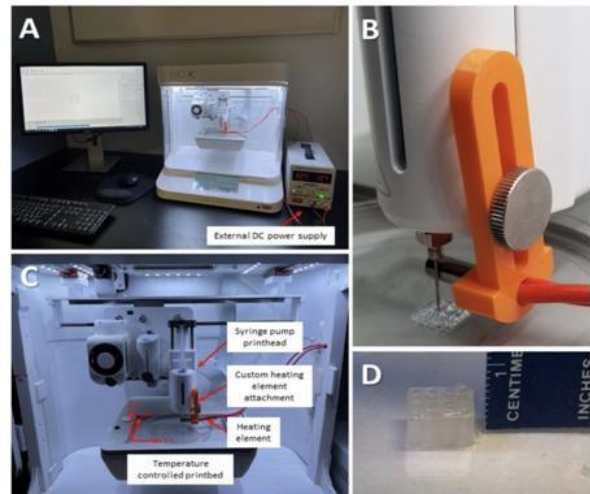
Hypothesis

3D Printed Bioink for Bone Tissue Engineering

We describe the suitability of an extrusion-based 3D bioink composed of *Gelatin*, Gelatin Methacryloyl (*GelMA*) with various concentrations of Hydroxyapatite (*HA*), and *Osteoblasts* for bone tissue engineering.

We hypothesized that hydroxyapatite would:

- 1) Decrease hydrogel swelling and degradation
- 2) Increase osteoblast cell proliferation and markers of bone matrix deposition

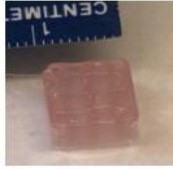


This is where bioprinting comes into play! The ultimate goal is that the bioprinted construct will be implanted in the body, but ultimately degrade as the patient's bone grows in its place.

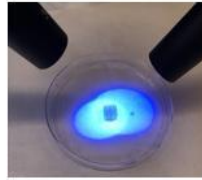
A micro-CT image is created often from the contralateral limb. This file is translated into a form readable by the bioprinter, which lays down a layer-by-layer construct from the bioink components. The printed construct then undergoes a crosslinking process for stability and is placed into osteogenic culture media to allow the cells to grow into the hydrogel.

Materials & Methods

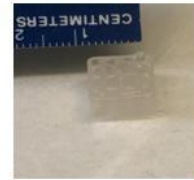
3D-bioprinted porous, cell-laden cubes



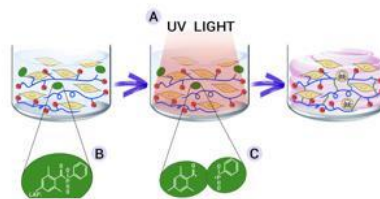
Crosslink with Photoinitiator (LAP)



Culture for 1, 14, 28 day(s)



Cells	Mouse calvarial osteoblasts
GelMA (10%)	100 mg/mL
Gelatin (5%)	50 mg/mL
Hydroxyapatite	0, 5, 10, 20 mg/mL
Other	LAP photoinitiator, cell culture media, HEPES buffer

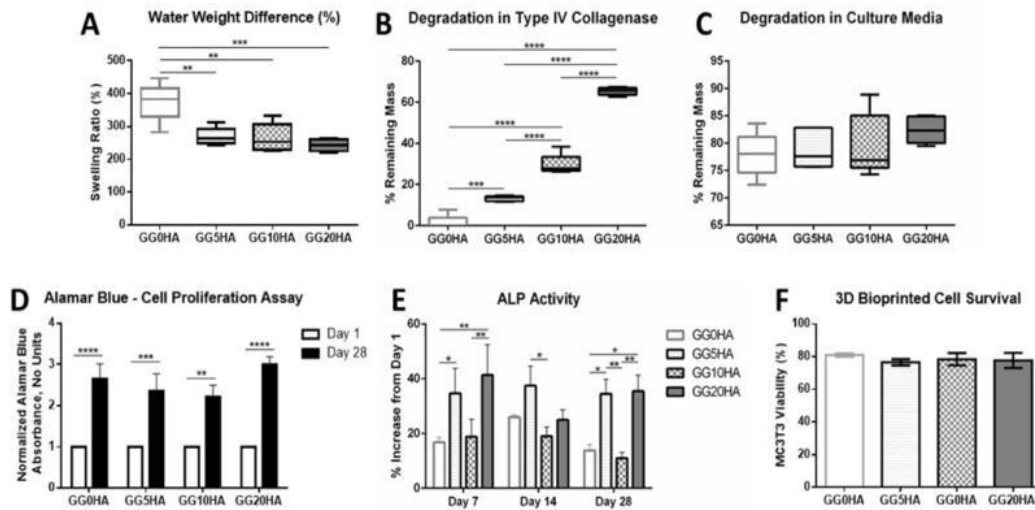


Degradation Analysis
Swelling Characterization
RNA Expression (RT-qPCR)
Alkaline Phosphatase Activity
Histology
Cell Viability

Materials and Methods

We printed our hydrogel construct in a porous cube with the respective components previously described. The construct then underwent crosslinking via photopolymerization under UV light, which was then cultured in media for 1, 14 and 28 days. After which we performed a swelling and degradation analysis, cell viability and proliferation assay, and routine histology.

Results & Discussion



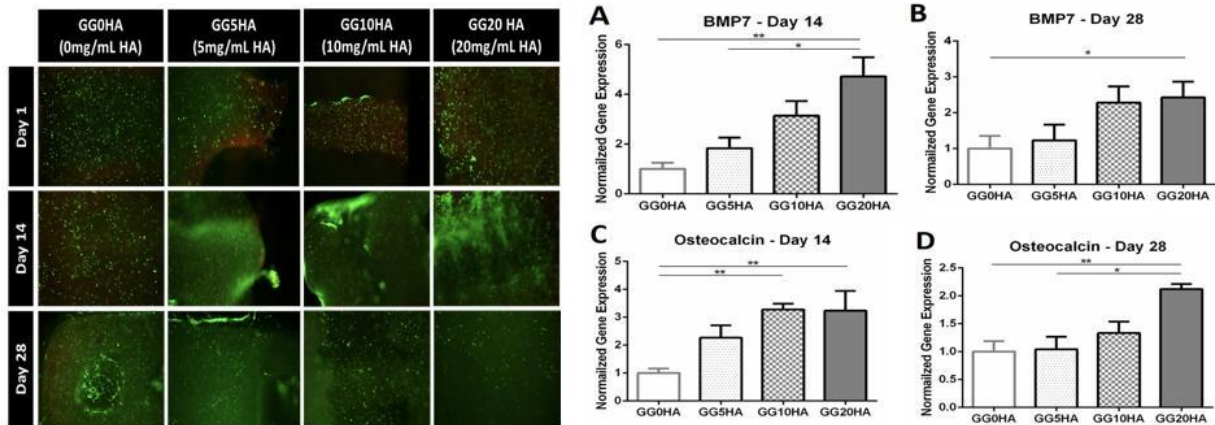
Results

The addition of HA at any concentration significantly reduced hydrogel swelling in comparison to the same hydrogel without HA (Figure A). This is clinically relevant to future in vivo implantation as minimizing hydrogel swelling would help ensure the construct does not significantly change size when implanted in the body. HA significantly decreased hydrogel breakdown in the presence of type IV collagenase, an enzyme present in the body that serves as surrogate for how quickly the construct would degrade in the body (Figure B).

The results in Figure C are critical to the overall tissue engineering process. Printed constructs will require a time in media before implantation to allow the cells to proliferate. However, HA did not decrease hydrogel degradation as was seen with collagenase (Figure C). A significant increase in cell proliferation at day 28 was noted in all groups, indicating that HA was not cytotoxic at any concentration (AlamarBlue) (Figure D).

Alkaline phosphatase (ALP) activity is an early marker of osteogenic differentiation. In our study, ALP activity significantly increased with the addition of 5 mg/mL and 20 mg/mL of HA at days 7 and 28. This suggests that HA plays a role in osteoblast differentiation and mineralization (Figure E). Figure F shows the results of post-printing cellular surviving, indicating that the cells could withstand the stress of the printing process (Figure F).

Results & Discussion



Live/dead staining on the left visually confirms the results that the cells were alive at each time point, post-printing with the green cells being the live ones and the red being the dead cells.

Finally, we looked at levels of gene expression of BMP-7 and Osteocalcin, a bone protein and an osteoblast-derived hormone, respectively. In all cases, the addition of 20 mg/mL of HA resulted in significantly greater gene expression of BMP-7 and Osteocalcin over hydrogels without HA. This indicates 20 mg/mL is the lowest threshold HA concentration to support osteogenic gene expression.

Conclusion

First Steps Toward A Viable 3D Bioprinted CSD Implant



The addition of HA to GelMA-gelatin hydrogels:

- Decreased hydrogel swelling
- Improved resistance to enzymatic degradation
- Increased osteoblastic differentiation and mineralization
- Increased osteogenic gene expression
- Maintained equal cell viability and proliferation



Conclusion

The addition of HA to GelMA-gelatin hydrogels (1) decreased hydrogel swelling, (2) improved resistance to enzymatic degradation, (3) increased osteoblastic differentiation and mineralization, (4) increased osteogenic gene expression, and (5) maintained equal cell viability and proliferation. These are some of the first steps toward a viable, 3D bioprinted implant.



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