HYDROPEP Therapeutics

Hydrogels for Targeted Bone Formation

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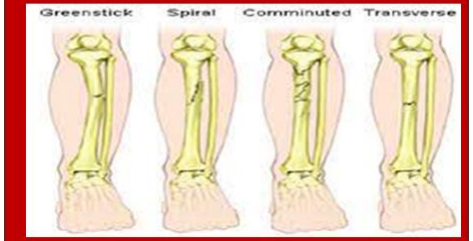
The Problem

1 in 2 women and **1 in 5 men** over 50 years old sustain **osteoporosis-related fracture**
- Vertebral compression and hip (femur) fractures are most prevalent

~2% of Americans sustain a **long-bone fracture** in their lifetime
- Femur (300K hospitalizations/yr) and tibia/fibula (77K hospitalizations/yr) are most prevalent

Osteoporosis is the progressive decrease in bone mineral density

Vehicle accidents, extreme sports, repetitive forces (e.g., running) can lead to long-bone fracture



Treatment Options
- **Surgery:** internal/external fixation, replacement

Treatment Outcomes
- **Disability** ~50% of hip fractures
- **Death** ~25% of hip fracture patients within a year post-surgery
- **Non-unions** ~10% of long-bone fractures

HYDROPEP Therapeutics

Mission: to develop bone-producing materials that prevent (1) fracture & (2) non-unions

The Team

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Education and Training

- B.S., ChemE, Carnegie Mellon University, 2006
- B.S., BME, Carnegie Mellon University, 2006
- Ph.D., Chem & BiocheE, Rutgers University, 2014
- Postdoctoral Trainee, Bioengineering, University of Pennsylvania

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Education and Training

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- M.D., Albert Einstein College of Medicine, 2008
- Orthopaedic Surgery Resident, Hospital of the University of Pennsylvania, 2013
- Orthopaedic Oncology Fellowship, Memorial Sloan-Kettering Cancer Center, 2014

Vega Lab Overview

- Over $1.7M in funding from NIH, NSF, and Foundation Grants
- Inventor/co-inventor on 5 patent filings
- Lab has produced 16 publications & 2 additional under peer review
- Lab has presented 46 abstracts at regional and national conferences & presented at 18 invited talks
- Mentored/graduated 15 undergraduate students, 4 Master students, 2 Ph.D. students
- Lab personnel: 1 postdoc, 4 Ph.D. students, 12 undergraduate students
- Select awards: NSF CAREER (2023), NEBEC New Innovator (2023), CMBE Young Innovator (2022), ORS NIRA Finalist (2022)
- Served on NSF, NIH, and DoD review panels

Orthopaedic Research Lab Overview

- Over $460k in funding from DoD, and Foundation Grants
- Inventor/co-inventor on 1 patent filings
- Lab has produced 32 publications
- Lab has presented 51 abstracts at regional and national conferences & presented at 10 invited talks
- Mentored/graduated 11 medical students
- Lab personnel: 1 postdoc, 1 research manager, 3 M.D. students, 1 research assistant, 15 medical students, 3 undergraduate students
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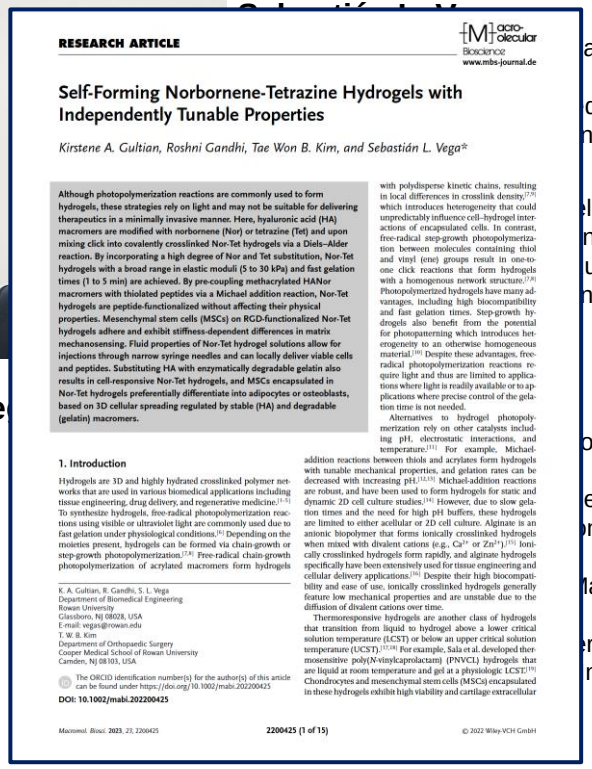

RESEARCH ARTICLE

Macromolecular Bioscience
www.mbs-journal.de

Self-Forming Norbornene-Tetrazine Hydrogels with Independently Tunable Properties

*Kirstene A. Gultian, Roshni Gandhi, Tae Won B. Kim, and Sebastián L. Vega**

Although photopolymerization reactions are commonly used to form hydrogels, these strategies rely on light and may not be suitable for delivering therapeutics in a minimally invasive manner. Here, hyaluronic acid (HA) macromers are modified with norbornene (Nor) or tetrazine (Tet) and upon mixing click into covalently crosslinked Nor-Tet hydrogels via a Diels–Alder reaction. By incorporating a high degree of Nor and Tet substitution, Nor-Tet hydrogels with a broad range in elastic moduli (5 to 30 kPa) and fast gelation times (1 to 5 min) are achieved. By pre-coupling methacrylated HANor macromers with thiolated peptides via a Michael addition reaction, Nor-Tet hydrogels are peptide-functionalized without affecting their physical properties. Mesenchymal stem cells (MSCs) on RGD-functionalized Nor-Tet hydrogels adhere and exhibit stiffness-dependent differences in matrix mechanosensing. Fluid properties of Nor-Tet hydrogel solutions allow for injections through narrow syringe needles and can locally deliver viable cells and peptides. Substituting HA with enzymatically degradable gelatin also results in cell-responsive Nor-Tet hydrogels, and MSCs encapsulated in Nor-Tet hydrogels preferentially differentiate into adipocytes or osteoblasts, based on 3D cellular spreading regulated by stable (HA) and degradable (gelatin) macromers.

1. Introduction

Hydrogels are 3D and highly hydrated crosslinked polymer networks that are used in various biomedical applications including tissue engineering, drug delivery, and regenerative medicine.[1–5] To synthesize hydrogels, free-radical photopolymerization reactions using visible or ultraviolet light are commonly used due to fast gelation under physiological conditions.[6] Depending on the moieties present, hydrogels can be formed via chain-growth or step-growth photopolymerization.[7,8] Free-radical chain-growth photopolymerization of acrylated macromers form hydrogels with polydisperse kinetic chains, resulting in local differences in crosslink density,[7,9] which introduces heterogeneity that could unpredictably influence cell–hydrogel interactions of encapsulated cells. In contrast, free-radical step-growth photopolymerization between molecules containing thiol and vinyl (ene) groups result in one-to-one click reactions that form hydrogels with a homogenous network structure.[7,8] Photopolymerized hydrogels have many advantages, including high biocompatibility and fast gelation times. Step-growth hydrogels also benefit from the potential for photopatterning which introduces heterogeneity to an otherwise homogeneous material.[10] Despite these advantages, free-radical photopolymerization reactions require light and thus are limited to applications where light is readily available or to applications where precise control of the gelation time is not needed.

Alternatives to hydrogel photopolymerization rely on other catalysts including pH, electrostatic interactions, and temperature.[11] For example, Michael-addition reactions between thiols and acrylates form hydrogels with tunable mechanical properties, and gelation rates can be decreased with increasing pH.[12,13] Michael-addition reactions are robust, and have been used to form hydrogels for static and dynamic 2D cell culture studies.[14] However, due to slow gelation times and the need for high pH buffers, these hydrogels are limited to either acellular or 2D cell culture. Alginate is an anionic biopolymer that forms ionically crosslinked hydrogels when mixed with divalent cations (e.g., Ca^{2+} or Zn^{2+}).[15] Ionically crosslinked hydrogels form rapidly, and alginate hydrogels specifically have been extensively used for tissue engineering and cellular delivery applications.[16] Despite their high biocompatibility and ease of use, ionically crosslinked hydrogels generally feature low mechanical properties and are unstable due to the diffusion of divalent cations over time.

Thermoresponsive hydrogels are another class of hydrogels that transition from liquid to hydrogel above a lower critical solution temperature (LCST) or below an upper critical solution temperature (UCST).[17,18] For example, Sala et al. developed thermosensitive poly(*N*-vinylcaprolactam) (PNVCL) hydrogels that are liquid at room temperature and gel at a physiologic LCST.[19] Chondrocytes and mesenchymal stem cells (MSCs) encapsulated in these hydrogels exhibit high viability and cartilage extracellular

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Injectable hydrogel with immobilized BMP-2 mimetic peptide for local bone regeneration

Kirstene A. Gultian[1], Roshni Gandhi[1], Kayla DeCesari[1], Vineeth Romiyo[2], Emily P. Kleinbart[2], Kelsey Martin[2], Pietro M. Gentile[2], Tae Won B. Kim[1,2] and Sebastián L. Vega[1]*

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Osteoporosis is a disease characterized by a decrease in bone mineral density, thereby increasing the risk of sustaining a fragility fracture. Most medical therapies are systemic and do not restore bone in areas of need, leading to undesirable side effects. Injectable hydrogels can locally deliver therapeutics with spatial precision, and this study reports the development of an injectable hydrogel containing a peptide mimic of bone morphogenetic protein-2 (BMP-2). To create injectable hydrogels, hyaluronic acid was modified with norbornene (HANor) or tetrazine (HATet) which upon mixing click into covalently crosslinked Nor-Tet hydrogels. By modifying HANor macromers with methacrylates (Me), thiolated BMP-2 mimetic peptides were immobilized to HANor via a Michael addition reaction, and coupling was confirmed with ^{1}H NMR spectroscopy. BMP-2 peptides presented in soluble and immobilized form increased alkaline phosphatase (ALP) expression in MSCs cultured on 2D and encapsulated in 3D Nor-Tet hydrogels. Injection of bioactive Nor-Tet hydrogels into hollow intramedullary canals of Lewis rat femurs showed a local increase in trabecular bone density as determined by micro-CT imaging. The presented work shows that injectable hydrogels with immobilized BMP-2 peptides are a promising biomaterial for the local regeneration of bone tissue and for the potential local treatment of osteoporosis.

KEYWORDS
osteoporosis, injectable hydrogels, hyaluronic acid, BMP-2, DWIVA peptides, bone regeneration

1 Introduction

Osteoporosis is characterized by a reduction in bone mineral density and disruption of bone microarchitecture (Wright et al., 2014). Osteoporosis is the most common chronic metabolic bone disease with an estimated 200 million people affected worldwide (Sozen et al., 2017). According to the International Osteoporosis Foundation, 1 in 3 women above the age of 50 and 1 in every 5 men will experience fragility fractures resulting from osteoporosis in their lifetime (Cooper, 1999). Osteoporosis increases the

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Research Lab Overview

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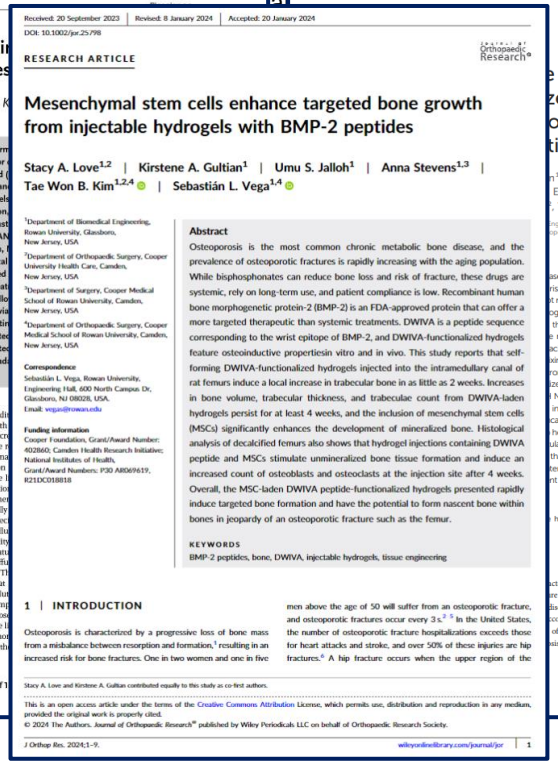

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RESEARCH ARTICLE

Mesenchymal stem cells enhance targeted bone growth from injectable hydrogels with BMP-2 peptides

Stacy A. Love[1,2] | Kirstene A. Gultian[1] | Umu S. Jalloh[1] | Anna Stevens[1,3] | Tae Won B. Kim[1,2,4] | Sebastián L. Vega[1,4]

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Abstract

Osteoporosis is the most common chronic metabolic bone disease, and the prevalence of osteoporotic fractures is rapidly increasing with the aging population. While bisphosphonates can reduce bone loss and risk of fracture, these drugs are systemic, rely on long-term use, and patient compliance is low. Recombinant human bone morphogenetic protein-2 (BMP-2) is an FDA-approved protein that can offer a more targeted therapeutic than systemic treatments. DWIVA is a peptide sequence corresponding to the wrist epitope of BMP-2, and DWIVA-functionalized hydrogels feature osteoinductive propertiesin vitro and in vivo. This study reports that self-forming DWIVA-functionalized hydrogels injected into the intramedullary canal of rat femurs induce a local increase in trabecular bone in as little as 2 weeks. Increases in bone volume, trabecular thickness, and trabeculae count from DWIVA-laden hydrogels persist for at least 4 weeks, and the inclusion of mesenchymal stem cells (MSCs) significantly enhances the development of mineralized bone. Histological analysis of decalcified femurs also shows that hydrogel injections containing DWIVA peptide and MSCs stimulate unmineralized bone tissue formation and induce an increased count of osteoblasts and osteoclasts at the injection site after 4 weeks. Overall, the MSC-laden DWIVA peptide-functionalized hydrogels presented rapidly induce targeted bone formation and have the potential to form nascent bone within bones in jeopardy of an osteoporotic fracture such as the femur.

KEYWORDS
BMP-2 peptides, bone, DWIVA, injectable hydrogels, tissue engineering

1 | INTRODUCTION

Osteoporosis is characterized by a progressive loss of bone mass from a misbalance between resorption and formation,[1] resulting in an increased risk for bone fractures. One in two women and one in five men above the age of 50 will suffer from an osteoporotic fracture, and osteoporotic fractures occur every 3 s.[2,5] In the United States, the number of osteoporotic fracture hospitalizations exceeds those for heart attacks and stroke, and over 50% of these injuries are hip fractures.[6] A hip fracture occurs when the upper region of the

Stacy A. Love and Kirstene A. Gultian contributed equally to this study as co-first authors.

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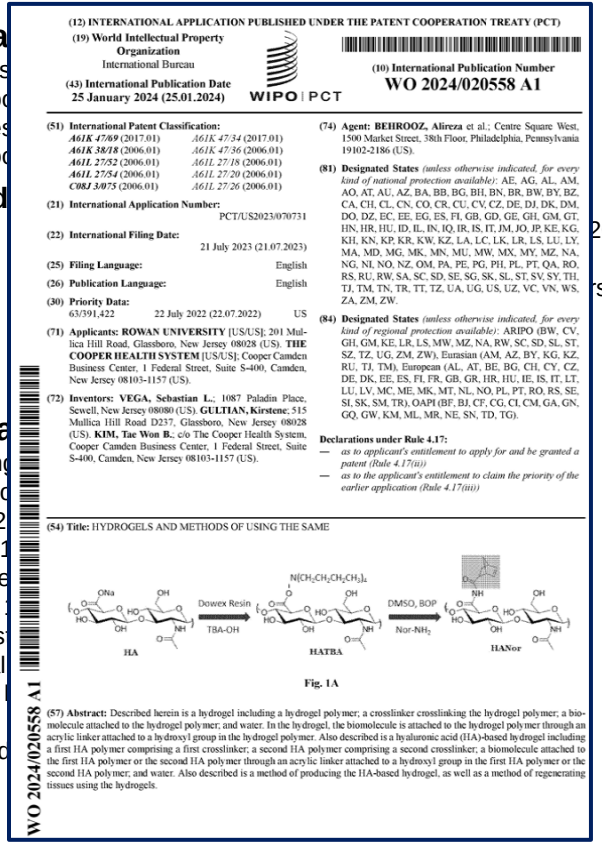

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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

(54) **Title:** HYDROGELS AND METHODS OF USING THE SAME

Fig. 1A

(57) **Abstract:** Described herein is a hydrogel including a hydrogel polymer; a crosslinker crosslinking the hydrogel polymer; a biomolecule attached to the hydrogel polymer; and water. In the hydrogel, the biomolecule is attached to the hydrogel polymer through an acrylic linker attached to a hydroxyl group in the hydrogel polymer. Also described is a hyaluronic acid (HA)-based hydrogel including a first HA polymer comprising a first crosslinker; a second HA polymer comprising a second crosslinker; a biomolecule attached to the first HA polymer or the second HA polymer through an acrylic linker attached to a hydroxyl group in the first HA polymer or the second HA polymer; and water. Also described is a method of producing the HA-based hydrogel, as well as a method of regenerating tissues using the hydrogels.

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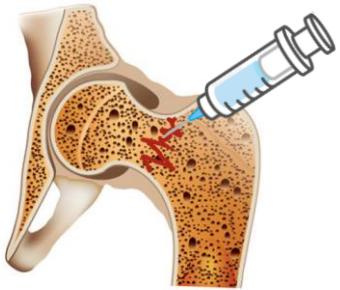
Our Solution

Objective

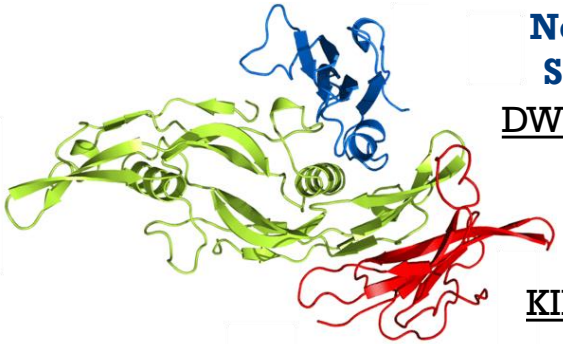
To deliver <u>therapeutics</u> via an injection that locally form bone at the injection site.



- **Preventative:** strengthen bone prone to fracture due to osteoporosis and/or prevent non-unions
- **Accelerated healing:** Improve healing of non-unions

Bone morphogenetic protein-2 (BMP-2)

FDA-approved protein used for spine fusion and non-union surgeries.



Non-Smad-Signaling
<u>DWIVA</u> peptide

Smad-Signaling
<u>KIPKA</u> peptide

BMP-2 peptides are robust, specific & can reduce off-target effects of BMP-2 proteins

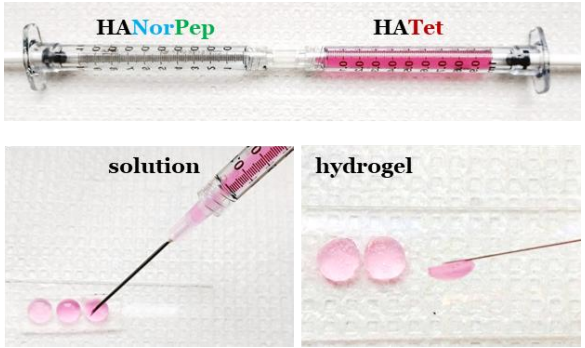
Bone Morphogenetic Protein-2
(osteoinductive protein = causes bone formation)

- Postoperative inflammation
- Ectopic bone formation
- Hyperactive osteoclast-mediated bone resorption

Our Solution: HydroBone

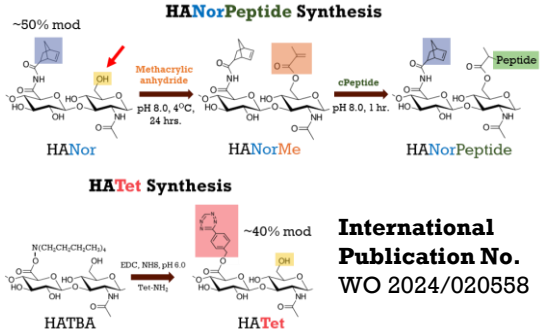
- **HydroBone** is a **self-forming hydrogel injection** that <u>locally</u> delivers BMP-2 peptides
- **HydroBone** can control BMP-2 signal dosing/presentation and thereby reduces off-target effects of BMP-2 proteins

Self-forming hydrogel injection



Gultian+ *Macromolecular Bioscience* 2023

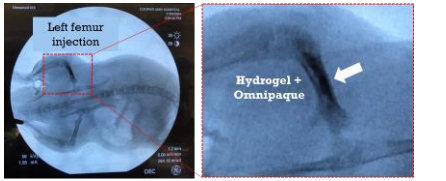
Hydrogel functionalization with BMP-2 peptides



International Publication No. WO 2024/020558

Gultian+ *Macromolecular Bioscience* 2023

Hydrogels induce bone formation at injection site (femur)



Gultian+ *Frontiers in Biomaterials Science* 2022
Love+ *Journal of Orthopaedic Research* 2024

Market Opportunity

- **Preventative (osteoporosis) & interventional (long-bone fracture) market: $28+ billion/yr**
  - **Osteoporosis therapeutics market** valued at $13.06 billion in 2022 & predicted to reach $20.53 billion by 2032, growing at a CAGR of 4.7% (Emergen Research)
  - **Fracture fixation market** was valued at $15 billion in 2022 and is predicted to reach $22.51 billion in 2028, growing at a CAGR of 7.0% (Business Research Insights)

- **Serviceable Available Market: $5.7+ billion/yr**
  - 1.3 million long bone fractures * $2500 per case = $ 3.25 billion/yr
  - 600,000 delayed healing (non-union) fractures * $2500 per case = $ 1.5 billion/yr
  - 400,000 arthrodesis (fusion) procedures per year * $2500 per case = $1 billion/yr

Summary

HYDROPEP Therapeutics

We developed **HydroBone**, an injectable hydrogel that delivers BMP-2 peptides

Three applications:

1. **Prevent osteoporosis-related bone fracture** by injecting HydroBone to areas prone to fracture (femur, lumbar vertebra)
2. **Prevent non-unions** by injecting HydroBone to long-bone fractures during surgery
3. **Improve healing** of non-unions of long bone fractures

Received pre-seed funding from **Foundation Venture Capital Group** to evaluate the effects of HydroBone in bridging a non-healing femur segmental defect

Experimental Design

Protocol at a Glance

1. **Stabilize femur** with external fixator plate (RISystem)
2. **Create non-healing defect** in the femur (5 mm)
3. **Treat defect** with one of 5 groups:
   - **Sham** (defect, no intervention)
   - **Gel** (no peptides)
   - **Gel +KIPKA** peptide [at dose shown to maximize Smad-dependent signaling in vitro]
   - **Gel +DWIVA** peptide [at dose shown to maximize Smad-independent signaling in vitro]
   - **Gel +rhBMP2 protein**

Measurable Outcomes: goals are to (1) confirm signaling pathway in vivo and (2) demonstrate endochondral ossification during bone healing.

1. **1-week post-op:** targeted BMP-2 mediated signaling **[histology]**
2. **2-weeks post-op:** soft callus formation **[histology]**
3. **4-weeks post-op:** hard callus formation **[histology, µCT]**
4. **8-weeks post-op:** bone remodeling/homeostasis **[histology, µCT, biomechanical testing]**

Thank You & Happy to Take Questions!

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HYDROPEP Therapeutics