# Complex Hydrogels for Enhances Spinal Fusion

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BioMEL

## Abstract

Musculoskeletal diseases are the second leading cause of disability globally and the leading cause in the United States for people over 50 years of age. One of the current gold standards for orthopedic surgical materials is poly(methyl methacrylate) (PMMA), which has a significantly higher compressive strength than native bone and does not facilitate tissue regeneration. Degradable bioactive materials are a desirable alternative to promote native bone regeneration which will help decrease the need for secondary surgeries. Our lab has developed a complex hydrogel that incorporates osteoinductive calcium ($Ca^{2+}$) and phosphate ($P_i$) ionic signaling molecules to help promote bone regeneration. Materials-associated local ion delivery has been found to facilitate mesenchymal stem cell (MSC) osteoblastic differentiation while providing mechanical stability closer to native bone tissue than PMMA. In order to implant these hydrogels *in vivo*, a safe sterilization procedure must be established. Using autoclaved chitosan and sterile filtered hydrogel cross-linking solution, complex hydrogels were created and evaluated by mechanical testing (uniaxial compression and swelling assessment). Mechanical characteristics were not significantly altered with sterilization. With the mechanical characteristics unaltered, the hydrogel sterilization can proceed to a cell study to determine efficacy of sterilization. After cell studies, the complex hydrogels can move forward into animal models for immunology/toxicity studies and surgical experiments and eventually utilized in human medicine to promote a faster and stronger bone regeneration than currently available options.

## Background

- According to WHO, Musculoskeletal conditions are the leaders in global disability
- Pitfalls of Current Orthopedic Implants
  - Significantly higher yield strength than bone
    - Stress Shielding- ⇧ likelihood of secondary fracture
  - Nonbiodegradable
- Our Complex Hydrogel
  - Able to stimulate osteoblastic differentiation of MSCs with $Ca^{2+}$ and $P_i$
  - Biodegradable
  - Closer representative yield strength to native bone

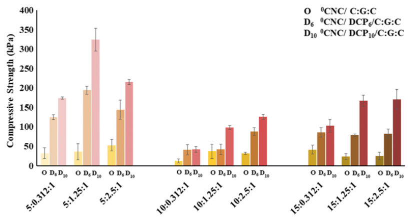


**Figure 1**: Compressive strength of hydrogels containing varying amounts of Dibasic Calcium Phosphate (DCP). Hydrogel were prepared with 0%, 6%, or 10% DCP. The 5:1.25:1 (carbonate: genipin: chitosan) with 10% DCP had the highest compressive strength.[1]

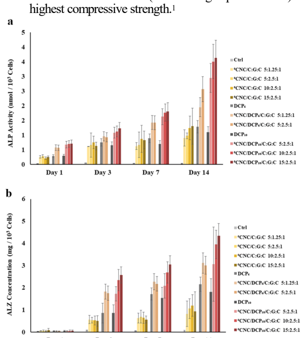


**Figure 2:** Differentiation of MSCs to different gel concentrations with varying amounts of DCP. (a) MSC alkaline phosphatase (ALP) activity and cell-based mineralization (b) ALP enzyme activity. Alizarin red (ALZ) stain was used to indirectly measure mineralization of the various hydrogels. ALP activity and mineralization were normalized on a per cell basis.[1]

## Results

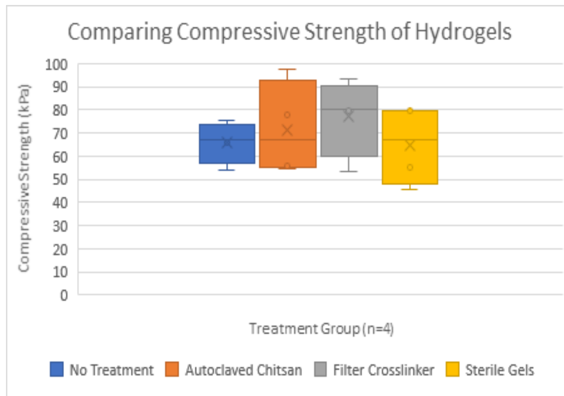


**Figure 3:** Comparing compressive strengths of sterilized hydrogels. Blue: No treatment, orange: Autoclaved chitosan with no treatment cross linker, grey: Filter cross linker and no treatment chitosan, yellow: sterilized gels (autoclaved chitosan and filtered cross linker). Hydrogels showed no significant change in strength post treatment.

**Table 1:** Percent water of each gel with average for each treatment group. Hydrogels showed significant change in percent water with the sterilized gel having the highest water content.

| Treatment Group/Gel | % Water of Gel | Average % Water of treatment Group |
|---|---|---|
| No Treatment 1 | 90.9 | 90.6 |
| No Treatment 2 | 90.3 | |
| No Treatment 3 | 90.5 | |
| No Treatment 4 | 90.5 | |
| No Treatment 5 | 90.6 | |
| Autoclaved Chitosan 1 | 92.1 | 92.0 |
| Autoclaved Chitosan 2 | 91.7 | |
| Autoclaved Chitosan 3 | 91.8 | |
| Autoclaved Chitosan 4 | 91.7 | |
| Autoclaved Chitosan 5 | 92.4 | |
| Filter Cross linker 1 | 91.4 | 91.2 |
| Filter Cross linker 2 | 90.8 | |
| Filter Cross linker 3 | 91.0 | |
| Filter Cross linker 4 | 91.2 | |
| Filter Cross linker 5 | 91.7 | |
| Sterilize 1 | 91.5 | 92.6 |
| Sterilize 2 | 94.4 | |
| Sterilize 3 | 93.0 | |
| Sterilize 4 | 92.6 | |
| Sterilize 5 | 91.7 | |

## Conclusions

1. Compressive Strength is not significantly affected with sterilization.
2. Percent Water is significantly affected with sterilization.

## Methods

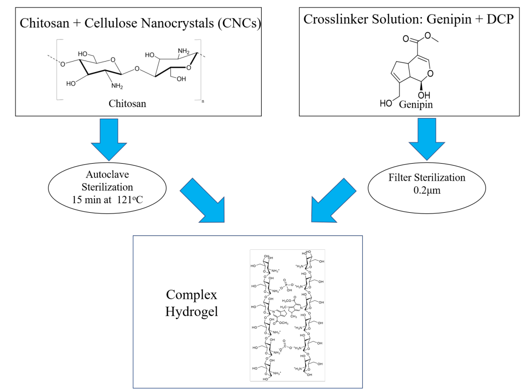


**Figure 4:** Flow chart of constructing complex hydrogels. Complex hydrogels are made by mixing chitosan and CNCs for 24hrs prior. Then sonicating genipin and DCP in water. When solution is completely dissolved combine crosslinker and chitosan backbone at 1:5 ratio. Gently vortex solution and incubate at 37°C for 24 hours.

- Uniaxial Compression Test
  - Hydrogels compressed to 60% deformation
  - Maximum yield strength calculated

$$\sigma_{yield} = F_{max}/Area$$

- Swelling Test
  - Measure initial weight of hydrogels
  - Lyophilize for 3days
  - Measure new weight
  - Calculate % Water

$$\%H_2O = \frac{initial\ weight - dry\ weight}{total\ weight}$$

**Figure 5:** Complex Hydrogel composed of Chitosan with Genipin: Bicarbonate crosslinker at 5:1.25:1 concentration.

## Discussion

- Compression
  - No significant change- mechanical characteristics of the gel are preserved with sterilization
- Swelling
  - With the significant difference, future studies will need to identify if the swelling affects the function of the hydrogel.
- Future Directions
  - Cell Studies
    - Embed cells in untreated hydrogel
    - Undergo sterilization and assess metabolic activity 0, 3, 5, and 7 days after sterilization
  - Dissociation Study
    - Determine if sterilization affects dissociation rate of hydrogel

## Acknowledgments

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## Citations

[1] S. Ali Akbari Ghavimi, E.S. Lungren, J.L. Stromsdorfer, B.T. Darkow, J.A. Nguyen, Y. Sun, F.M. Pfeiffer, C.L. Goldstein, C. Wan, and B.D. Ulery. "Effect of Dibasic Calcium Phosphate Incorporation on Cellulose Nanocrystal / Chitosan Hydrogel Physical Properties and Bioactivity." *The AAPS Journal*. 21: 41 (May 2019)