

## Altering the nutrient supplement and stability of hydrogel for cardiac therapy

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**Statement of Purpose:** Heart disease related deaths are among the top causes every year. Current treatment options to replace the necrotic tissue rely on cell-based therapy using injectable hydrogels. In particular, adult stem cells are explored to regenerate the tissue. However, implantation of stem cells by many approaches is shown to result in significant attrition of injected cells, attributed to cell death. For complete engraftment of injected stem cells, hydrogels that could support cell survival and stability during the regenerative process is essential. Growth media is required for cells to survive in nutrient deprived environment. We hypothesized that the combination of hydrogel and growth media would help with the cell's nutrient requirement. To test the possibility, we utilized chitosan-gelatin injectable hydrogels. Further, we tested the effect of transglutaminase on the durability of the gels with different types and molecular weights of gelatin and chitosan. These concoctions were tested with adipocyte stem cells for viability.

**Methods:** Gelatin (type A, type B, ~100, 175, 300 bloom), Chitosan (low to high molecular weight), growth powdered media that supports adipocyte stem cells, transglutaminase, and  $\beta$ -glycerophosphate were used in different combinations. Solutions were formed and tested for gelation and compressive characteristics at 37°C. After 2 hours of incubations, samples were subjected to compression testing using the INSTRON 5542 and the compressive modulus were determined from the initial slope of the stress-strain plot. To investigate the stability of the hydrogels in a 7 day culture, hydrogels were seeded with cells and incubated at 37°C with 5% CO<sub>2</sub>. Adult human adipocytes from Life Technologies were cultured on these hydrogels and tested for viability and distribution via histology.

**Results:** Presence of growth medium did not hinder the gelation characteristics. Further, addition of low amount of TG also did not affect the gelation. Type A (acid treated) 175 bloom had a larger compressive modulus than type B (base treated) 225 bloom. The effect of variable bloom gelatin was also addressed by having hydrogels with ~100 bloom, 175 bloom, and 300 bloom. The gelatin with the highest bloom number had a stronger compressive modulus than the other two gelatin. Next, the effect of chitosan with different molecular weights was determined. High molecular weight chitosan had the stronger compressive modulus of the others and medium molecular weight had the lowest. Next, the optimal concentration of growth media was determined by varying its concentration; 2% had the highest compressive modulus and that at higher concentration (4%) compressive modulus would decrease.

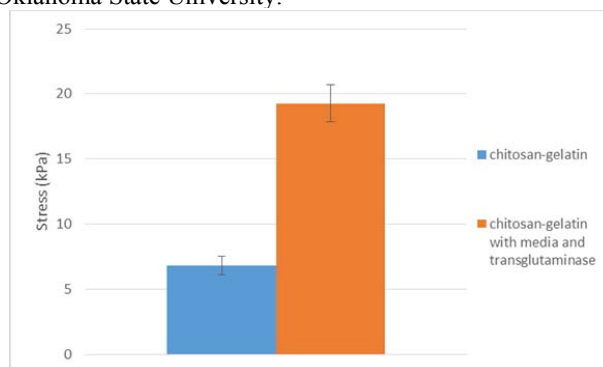


Figure 1. Comparison of compressive modulus.

Finally, the effect of adding growth media and transglutaminase was determined. Presence of growth medium alone and TG alone increased the compressive modulus marginally. However, the combination of both growth medium and TG at similar concentration nearly tripled the compressive modulus (Figure 1). An additional effect of adding TG was determined while conducting seven day cell culture experiments. It was observed that the hydrogel with TG (Figure 2) was more stable even when exposed to proteolytic enzyme TrypLE utilized for cell harvestation from the hydrogels. On the contrary hydrogels without TG (Figure 2) were not stable despite the presence of cells.

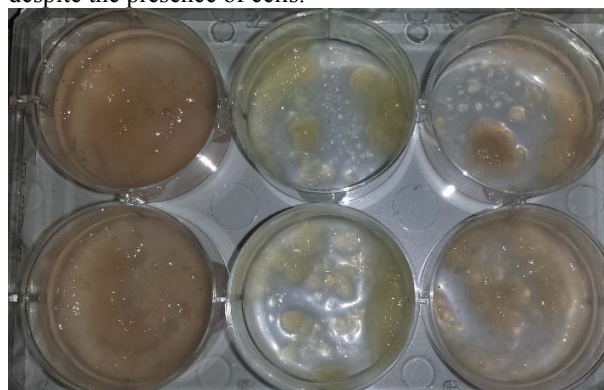


Figure 2. Stability of hydrogels after seven days of incubation with human adipocyte stem cells. Chitosan-gelatin with media and TG, chitosan-gelatin, and chitosan-gelatin with media hydrogels from left to right.

**Conclusions:** Chitosan -gelatin injectable hydrogels can be prepared by blending growth medium to support the growth of cells. Molecular weight and type of gelatin used have an effect on the compressive modulus. Addition of TG improved the durability of the hydrogels in the seven day cell culture.

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