Determining Effects of Pressure of Hydrogel Formation in Vitro

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ABSTRACT

After a myocardial infraction, also known as a heart attack, myocardial cells die and are replaced by a thick scar, which cannot pump blood like a normal tissue. Advancements in cardiac tissue engineering have lead to the development of a biomaterial called a hydrogel to prevent scar formation and help the heart heal and function as it normally would.

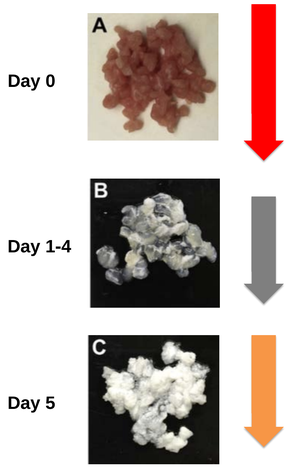
The hydrogel is formed using extracted porcine Extracellular Matrix to create a medium for cell growth and differentiation. The resulting biomaterial is injected into the target region of the heart. The material appeared to encourage healthy muscle and blood vessel formation in the infarcted areas. Data and animal trials from various experiments show that the myocardial ECM-derived material not only improves function outcome after a heart attack, but is also safe and non-toxic.

It has been observed that gelation occurs much faster in vivo(~30 seconds) than in vitro(~6 hours) at 37° C. Understanding why this occurs can make in vitro studies much more effective, help us understand more about its properties and simulate the in vivo environment more accurately in future research.

Hydrogel Creation Process

Day 0: Initial Tissue Processing: Left Ventricle, right Ventricle and Atria are isolated. Adipose tissue, papillary muscles, fascia and chordae tendinea are also removed. Remaining myocardium is then cut into 3-5mm bits

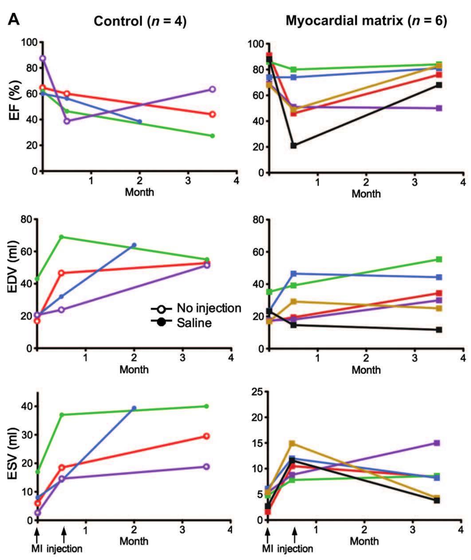
Day 1-5: SDS solution changes: Tissue is strained in a beaker with magnetic stir bar. 1% SDS solution is replaced every 24 hours to ensure complete decellularization. 4 mL PenStrep is added to avoid bacterial growth.



Myocardial matrix improves cardiac muscle and reduces infarct fibrosis

Myocardial matrix

Pre-MI	Pre-injection	Pre-euthanasia
79.3 ± 4.2	53.5 ± 8.7	73.7 ± 5.3*
4.3 ± 0.7	10.9 ± 1.0	8.1 ± 1.6*
22.2 ± 2.9	27.8 ± 5.2	33.5 ± 6.2**



Characterization and Quality Check

H&E Stain

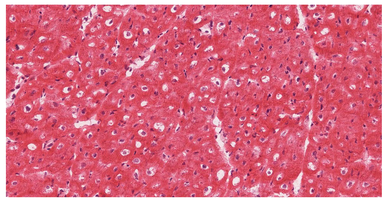
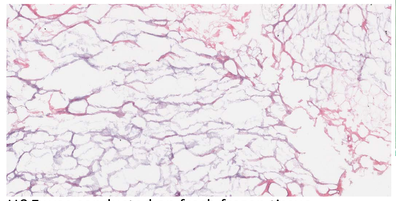


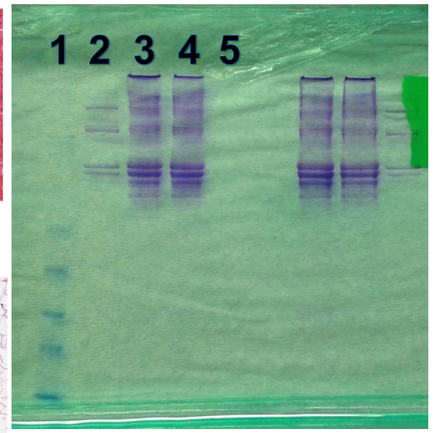
Fig. Above: Porcine Fresh Tissue-Left Vent.

Fig. Below: Porcine De-celled "final" Tissue Atria



H&E was conducted on fresh frozen tissue embedded in 10 um sections and final ECM for each sample to verify that the decellurization process was thorough. The Hoechst stain gives nuclei a blue color and therefore a lack of this color in the final sample is indicative complete decellurization.

PAGE Gel for Protein Content



ECM solution is run on SDS-polyacrylamide gel electrophoresis gel to assess protein fragment content. 7.5 µl ECM, 7.75 µl NuPAGE SDS Running buffer, 1.5 µl reducing agent and 2.25 µl DI were added to each running sample. The gel was run at a constant voltage(200V) for approximately 50 minutes.(**1 = Collagen Ladder, 2 = Collagen, 3 = ECM, 4 = ECM, 5 = Elutant)**

Effect of Pressure on Gelation Hypothesis

In trying to explain for the reasons and factors behind faster gelation in vivo than in vitro, we hypothesized that sub-cutaneous pressure(506 Pa to 866 Pa) is the key factor. We set up an experiment using 5 mL syringes containing porcine ECM solution(6 mg/mL conc.) with pressure induced on all the experimental ones using rubber bands, where the pressure was calculated using spring constant and geometric arrangement. All samples were placed in incubator, had two 4 looped rubber band(except for control) and checked after 30 minute intervals between 1 hour and 3.5 hours. A sample was set up for each specific time interval along with one overnight sample. Pressure induced for each sample was approximately 20000 Pa. We added micro filters to the end of the syringe as well and analyzed the elutant in the PAGE gel

ACKNOWLEDGMENTS

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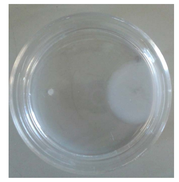
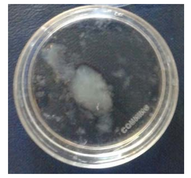
2. Ungerleider, J. L., Johnson, T. D., Rao, N. & Christman, K. L. Fabrication and Characterization of Injectable Hydrogels Derived from Decellularized Skeletal and Cardiac Muscle. Methods , accepted with minor revisions. (2015).

3. Wang, R. M. & Christman, K. L. Decellularized myocardial matrix hydrogels: In basic research and preclinical studies. Adv. Drug Deliv. Rev. doi:10.1016/j.addr.2015.06.002

Results and Analysis from Pressure Hypothesis Experiment



Above:
24 hour Experimental



Above:
24 hour Control

Above:
2.5 hour Experimental

3 hour Experimental

3.5 hour Experimental

3.5 hour Control

Comparing both 24 hour samples, a distinct difference in the gel formation can be observed. The experimental sample has a much more viscous and gel-like appearance whereas the control is more broken up and aqueous. The progression in the formation of gel can be seen from interval to interval. Comparing the 3.5 hour control and 3.5 hour experimental, it can be observed that the 3.5 hour control has a much more divided appearance whereas the 3.5 hour experimental seems to be more intact and viscous. Hence it can be seen that, overall, the pressure induced samples seem to have gelled more effectively than the non-pressure induced controls.

CONCLUSIONS

We demonstrated that pressure does in fact have a stimulating effect on gelation. The increase in pressure upon injection and pressure induced by the heart in vivo may also account for this phenomenon.

FUTURE RESEARCH

The overall goal of my lab was to further the understanding of the hydrogel's properties. The specific mechanism by which pressure advances gelation is still unknown. Understanding this mechanism is potentially a further area of research in order to gain a better understanding of how the hydrogel works. Other factors such as ionic concentration, material concentration, pH and structure can be tested in the future as well. Using more quantitative means to examine the gel, for example, by using a spectrophotometer, may help qualify results.