Optimising a Myocardium-like Hydrogel for Enhanced Cardiomyocyte Culture

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The Importance for Sources in In-Vitro Studies

Cardiovascular disease (CVD) has for long been cited as the leading cause of death globally [1]. The World Health Organization estimates that CVDs claim the lives of around 17.9 million annually [2].

Developing successful tissue regeneration and drug discovery strategies requires high levels of experimental control are made possible by the creation of three-dimensional in vitro models [3].

The two predominant methods for the extraction of cardiomyocytes for study are primary-source extraction and iPSC-derived Stem cells. Primary source extraction involves deriving cardiomyocytes (CMs) from biological heart tissue such as embryonic heart tissue or cardiac biopsies from adult hearts [4]. Induced pluripotent stem cells, which are reprogrammed adult somatic cells, produce induced pluripotent cardiomyocytes (iPSC-CMs), which are heart muscle cells. By offering patient-specific cellular models, these specialized cells are an effective tool for drug development, toxicological testing, and modelling heart disorders [5].

Objective of Research

The overarching aim of this study is to develop a hydrogel system that permits acoustic patterning of cardiomyocytes in the pre-crosslinked, fluid state, while providing sufficient structural integrity post-crosslinking to preserve alignment and support tissue maturation. These objectives will be achieved via an acoustic patterning setup including CMs embedded in various hydrogel concentrations as well as comparative mechanical testing of these hydrogels as a “synthetic cardiac slice” to closely mirror native characteristics.

Methodology: Synthesis of Hydrogels

I. Alginate-only Hydrogel

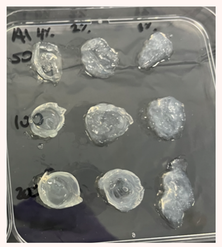

Figure 1. Alginate-only hydrogel

- A CaCl2 solution was prepared by mixing 40 mL of PBS with 1.76 g of CaCl2
- 1.6 g of amorphous alginate was weighed out and added slowly to 80 mL of PBS buffer solution
- The alginate was then added dropwise to CaCl2 in the compartments of the hydrogel dish.
- Concentrations 200, 100 and 50 mM of CaCl2 were attempted in order to determine the optimal consistency required to hold the cardiomyocytes during acoustic patterning.

II. Alginate-Gelatin Hydrogel

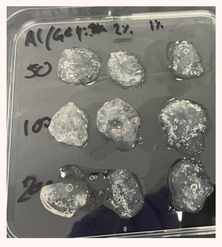

Figure 2. Alginate-gelatin hydrogel

- 4 different combinations of alginate and gelatin concentrations were synthesized
- 2% weight by volume of gelatin was prepared via combining with 80 mL of PBS buffer
- In this case, 4 alginate mixtures were prepared – with 4%, 3%, 2% and 1% concentrations respectively
- 2% gelatin solution were added to each of these to determine the effect of gelatin on the consistency of hydrogels synthesized via different alginates

Methodology: Mechanical Testing of Hydrogels

A. Hydrogels as Synthetic Cardiac Slices

This experiment partly hinges on the modelling and testing of hydrogels as synthetic cardiac slices, whereby they are synthesized with different chemical concentrations to find the closest match in mechanical properties. Unlike to isolated myocytes, cardiac slices allow for the study of heart function in a multicellular setting with an entire myofilament lattice.

B. Design and Working Principle of Tensile Test Device [6]

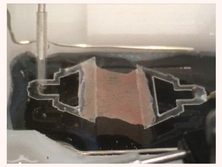

Figure 3. Cardiac slice loaded onto the IonOptix Slice System [6]

- Sturdy, stable triangular clips are used to attach the hydrogel samples that mount between a programmable length controller and a powerful force transducer.
- Temperature control is made possible by chamber fluid flow.
- Electrical field stimulation is made possible by fixed platinum electrodes.
- Platinum minimizes electrolysis, permits electrical conductivity, and is biologically inert.

Methodology: Acoustic Patterning

A. Design and Setup of Device

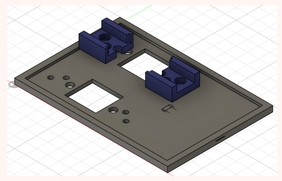

Figure 4. CAD Model (developed on Fusion) of the Acoustic Patterning device

- Two piezoelectric transducers are supplied with a sinusoidal voltage signal of approximately 20 VPP, tuned to its resonant frequency (≃2 MHz).
- When driven at resonance, each transducer produces transverse acoustic waves that form a standing wave field. Standing waves are characterized by periodic pressure nodes and antinodes.
- The primary acoustic radiation force F_{rad} acting on a spherical particle of radius a in a one-dimensional standing wave of wavelength λ can be described by:

$$F_{\text{rad}} = -\frac{4\pi a^3 \kappa_m E_{ac}}{\lambda} \Phi \sin(2kx)$$

For most mammalian cells suspended in aqueous medium, ϕ>0, causing them to migrate toward pressure nodes. As a result, cells accumulate at these nodal positions, forming well-defined, tightly packed bands.

B. Procedure: Acoustic Patterning [7][8]

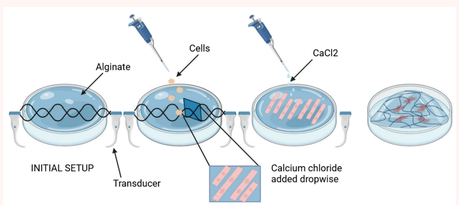

Figure 5. Graphic schematic of the step-by-step procedure for acoustic patterning created via BioRender [9]

Observations and Results

A. Mechanical Testing: Hydrogel Stress-Strain Curves

Displacement	Max Force	Relax Force	Stress Max (N)	Stress Relax (N)	Strain
0	-0.54	-0.54	-415.38	-415.38	0
1	2.92	1.96	2245.38	1507.69	0.017
2	6.32	4.94	4861.54	3803.08	0.033
3	10.306	8.36	7927.69	6430.77	0.05
4	14.65	11.4	11269.23	8769.23	0.067

Table 1. Data Obtained from Mechanical Testing of 200 mM 4 percent Alginate Hydrogel

$$\sigma = \frac{F}{A}$$

$$\varepsilon = \frac{\Delta L}{L_0}$$

$$E = \frac{\sigma}{\varepsilon}$$

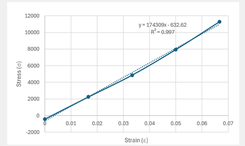

Figure 6. 200 mM 4%

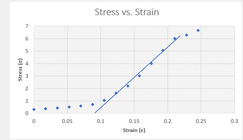

Figure 7. 200 mM 8%

B. Acoustic Patterning: Cell Arrangement and Results

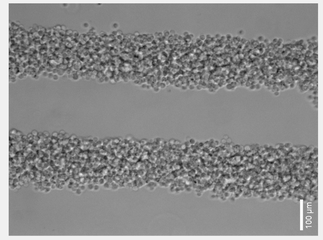

Figure 8. 2 MHz Frequency Standing Waves

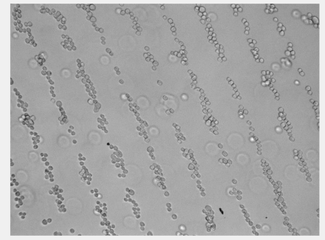

Figure 9. 6.7 MHz Frequency Standing Waves

Conclusion and Future Developments

This study demonstrated the importance of optimizing hydrogel structural properties and strategically pattern-ing cardiomyocytes for developing physiologically relevant in vitro models and environments for cardiac tissue. Mechanical testing of alginate hydrogels at different concentrations revealed that higher concentrations (8%) produced moduli well above the physiological stiffness range of native myocardium (10,000–15,000 N m^{-2}).

Future developments should therefore focus on improving the precision and reproducibility of mechanical testing, including viscoelastic characterization at 37 °C in physiologic media, and on

References

[1] World Health Organization. *Cardiovascular Diseases*. https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1. 2025.

[2] Mariachiara Di Cesare et al. “The Heart of the World”. In: *Global Heart* 19.1 (Jan. 2024). DOI: 10.5334/gh.1288. URL: https://pmc.ncbi.nlm.nih.gov/articles/PMC10809869/.

[3] Ignasi Jorba et al. “In Vitro Methods to Model Cardiac Mechanobiology in Health and Disease”. In: *Tissue Engineering Part C: Methods* 27.3 (Mar. 2021). Accessed 12 Sept. 2022, pp. 139–151. DOI: 10.1089/ten.tec.2020.0342.

[4] Science Direct. *ScienceDirect.com | Science, Health and Medical Journals, Full Text Articles and Books*. https://www.sciencedirect.com/. 2025.

[5] Eline Simons et al. “IPSC-Derived Cardiomyocytes in Inherited Cardiac Arrhythmias: Pathomechanistic Discovery and Drug Development”. In: *Biomedicines* 11.2 (Feb. 2023). Accessed 29 Mar. 2024, p. 334. DOI: 10.3390/biomedicines11020334. URL: https://www.mdpi.com/2227-9059/11/2/334.

[6] IonOptix. *Cardiac Slice System - IonOptix*. https://www.ionoptix.com/products/systems/cardiac-slice-system/. Accessed 12 Sept. 2025. 2025.

[7] Zhuhao Wu et al. “Acoustofluidics for Cell Patterning and Tissue Engineering”. In: *Engineered Regeneration* 3.4 (Dec. 2022). Accessed 15 Nov. 2022, pp. 397–406. DOI: 10.1016/j.engreg.2022.08.005.

[8] Mengxi Wu et al. “Sound Innovations for Biofabrication and Tissue Engineering”. In: *Microsystems & Nanoengineering* 10.1 (Nov. 2024). Accessed 19 Feb. 2025. DOI: 10.1038/s41378-024-00759-5. URL: https://www.nature.com/articles/s41378-024-00759-5.

[9] BioRender. *BioRender Illustrations*. https://app.biorender.com/illustrations/canvas-beta/68821c92816cf5ff89ba866b. Accessed 16 Sept. 2025. 2025.