TRAINING A HUMAN MINI-HEART:

Effect of dynamic preload on human-engineered ventricles

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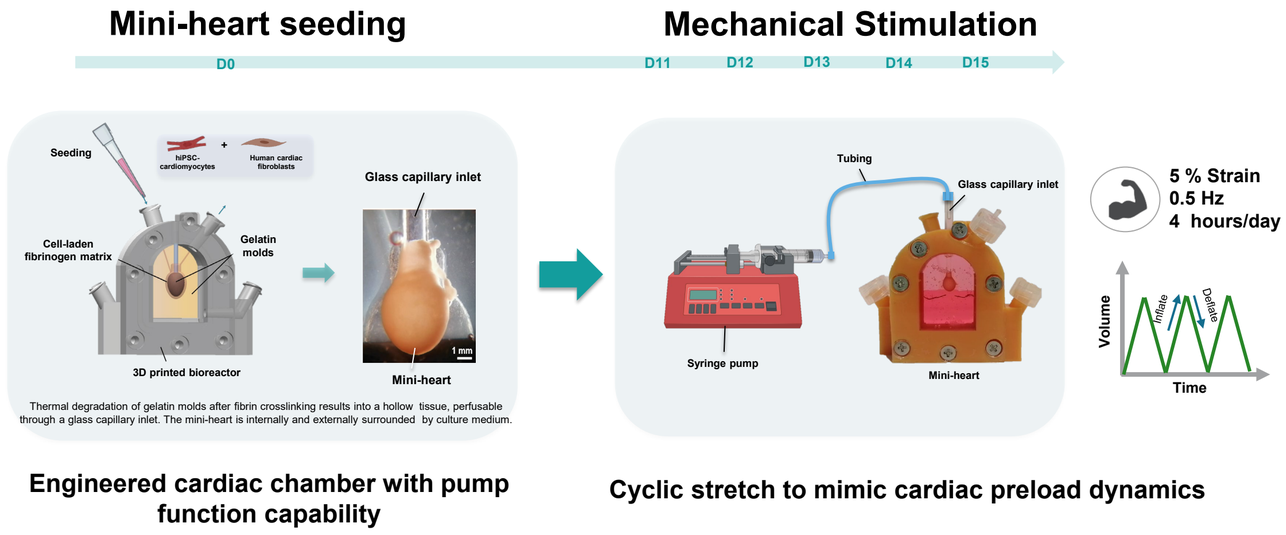
Introduction

- Ventricular filling with blood imposes a dynamic stretch (preload) on the myocardium, triggering mechano-regulatory responses that play an important role in cardiomyocyte maturation and the development of cardiomyopathies.
- Engineered 3D cardiac tissues used for *in vitro* disease modelling are typically subjected to static loads. Integration of dynamic preload in these platforms requires complex setups and often compromises the acquisition of contractility readouts.
- Instead, **engineered ventricles model the pump function of the heart,** enabling the **acquisition of hemodynamic parameters** under **controlled mechanical loading.**
- We engineered **hiPSC-based cardiac ventricles** and subjected them to **multi-axial cyclic stretch** to mimic physiological preload dynamics. We evaluated its impact on **pump performance** and **cardiomyocyte maturation**.

Methods

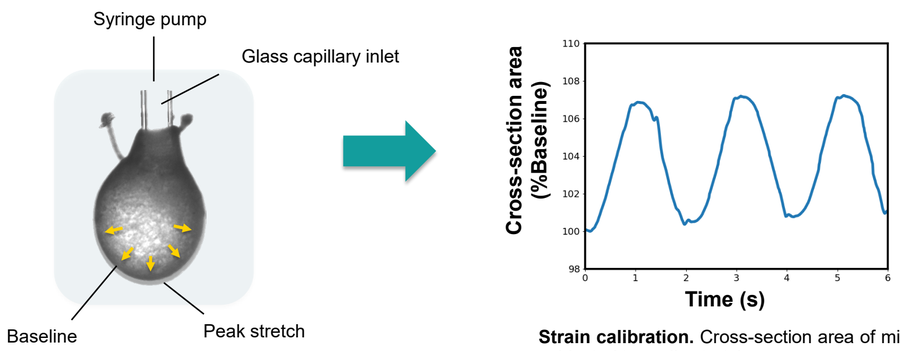


Engineered cardiac chamber with pump function capability

Cyclic stretch to mimic cardiac preload dynamics

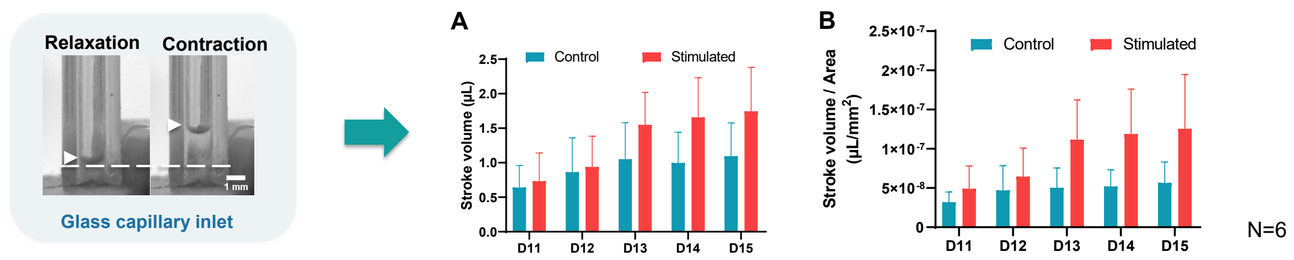
Results

Strain calibration



Strain calibration. Cross-section area of mini-heart subjected to cyclic stretch.

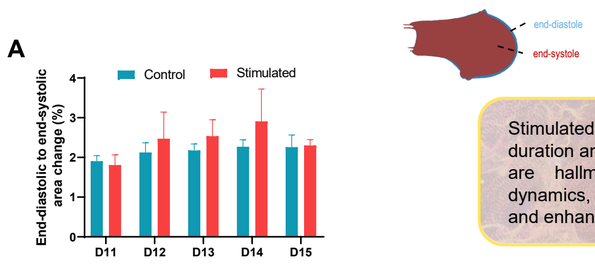
Dynamic preload conditioning enhances mini-heart pump performance



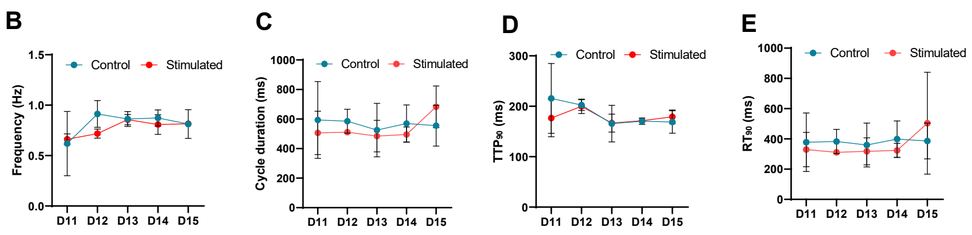
Displacement of liquid during beating cycles enables estimation of stroke volumes.

Pump performance increases with cyclic strain stimulation. A) Stroke volumes estimated from liquid displacement in the glass capillary inlet between day 11 and day 15. B) Stroke volume per end-diastolic cross-section area, as equivalent of ejection fraction, between day 11 and day 15.

Effect of mechanical stimulation on contractile kinetics

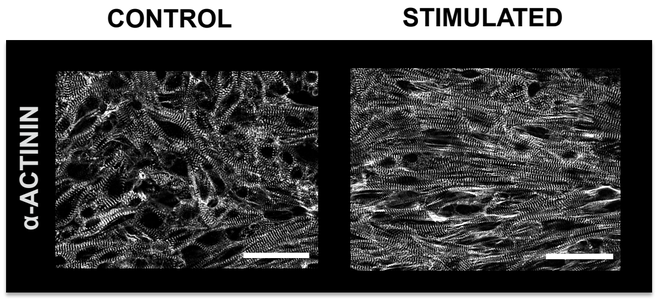


Stimulated samples exhibited shorter cycle duration and shorter relaxation times, which are hallmarks of improved relaxation dynamics, more efficient calcium reuptake, and enhanced cardiomyocyte maturation.



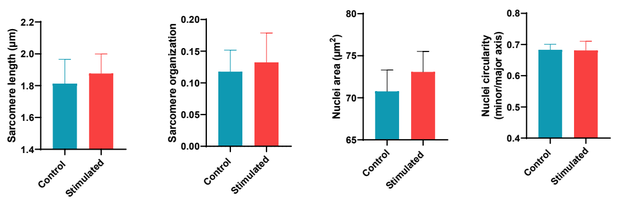
Effect of dynamic preload on contractile kinetics. A) Difference between end-systolic and end-diastolic mini-heart areas during beating cycles. Evolution of beating frequency (A), cycle duration (B), time-to-peak (TTP) (C) and relaxation time (RT) (E) between day 11 and day 15 based on cross-section area analysis.

Cyclic stretch promotes cell alignment and increases sarcomere length

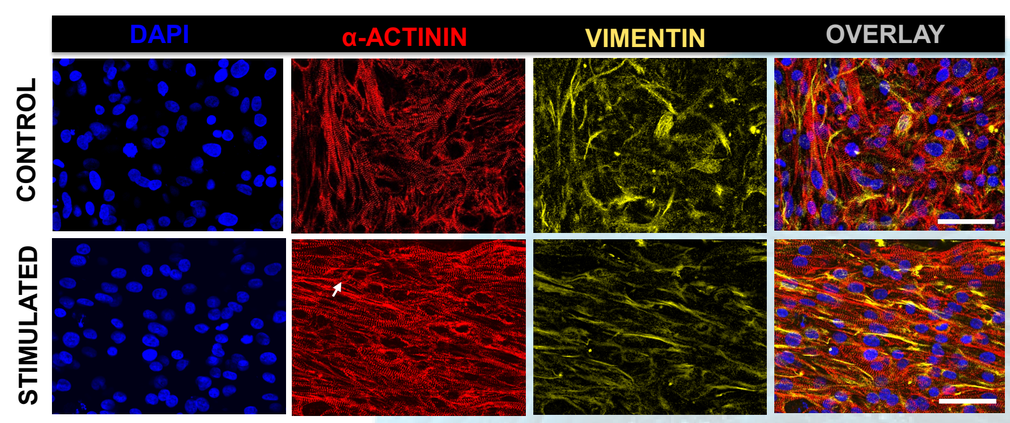


An enhanced degree of cell alignment was observed on stimulated samples, notably at the top of the tissues.

Effect of mechanical stimulation on sarcomere structures. Immunohistochemical staining of hiPSC-cardiomyocytes with α-actinin in control and stimulated samples. *Scale bars= 50 µm*



Sarcomere length and nuclei size increased (n.s.) on mechanically stimulated mini-hearts, suggesting an enhanced degree of cardiomyocyte maturation upon mechanical stimulation



Cyclic stretch promotes cell alignment. Immunohistochemical evaluation of control and stimulated samples stained for DAPI, α-actinin (hiPSC-cardiomyocytes) and vimentin (cardiac fibroblasts). α-actinin⁺ fibers (arrow) were observed on mechanically stimulated mini-hearts. *Scale bars= 50 µm*

Conclusions

- We successfully established a mechanical stimulation protocol to mimic preload dynamics on human engineered ventricles.
- The pump performance of engineered ventricles was enhanced upon mechanical stimulation.
- We observed an increase in sarcomere length and improved cell alignment in response to cyclic stretch, suggesting an enhanced degree of hiPSC-cardiomyocyte maturation.
- Further gene expression analysis is needed to evaluate the distinction between mechanically-induced maturation and possible pathological effects.

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