3D heart cell culture model from Zebrafish larvae for cardiac research

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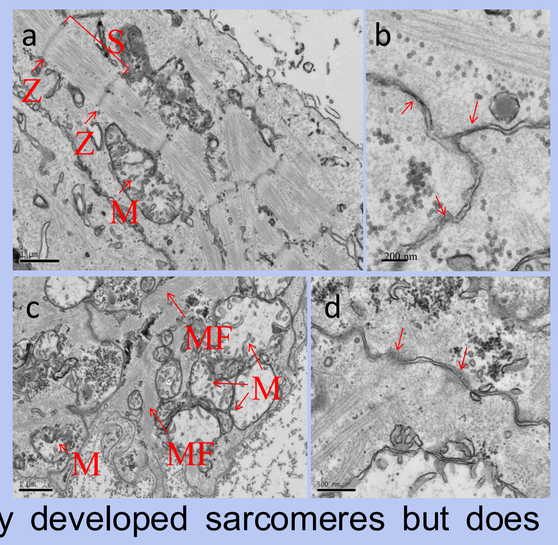
Background

Zebrafish (ZF) are an appropriate platform for disease modelling and pharmacology testing, due to their electrophysiological similarity to humans. ZF heart cells can be spontaneously propagated from embryonic heart progenitor cells to a mature 3D myocardium *in vitro*, termed ZF Heart Aggregates (ZFHAs). ZFHAs are a novel, cost effective and a relatively high throughput model which could help reduce the expenses required in *in vivo* testing, studying cardiac function and disease. ZFHA tissue seen in figure **(a),** displays developed sarcomeres (S) which are bordered by Z-Lines (Z), which are reminiscent of adult ZF tissue. Larval heart tissue Seen in figure (c) dose not show fully developed sarcomeres but does show myofilaments (MF). (b,d) shows cell membranes exhibiting cell-cell connections (arrows), indicating ZFHA & larval hearts are functional syncytia [(1)].

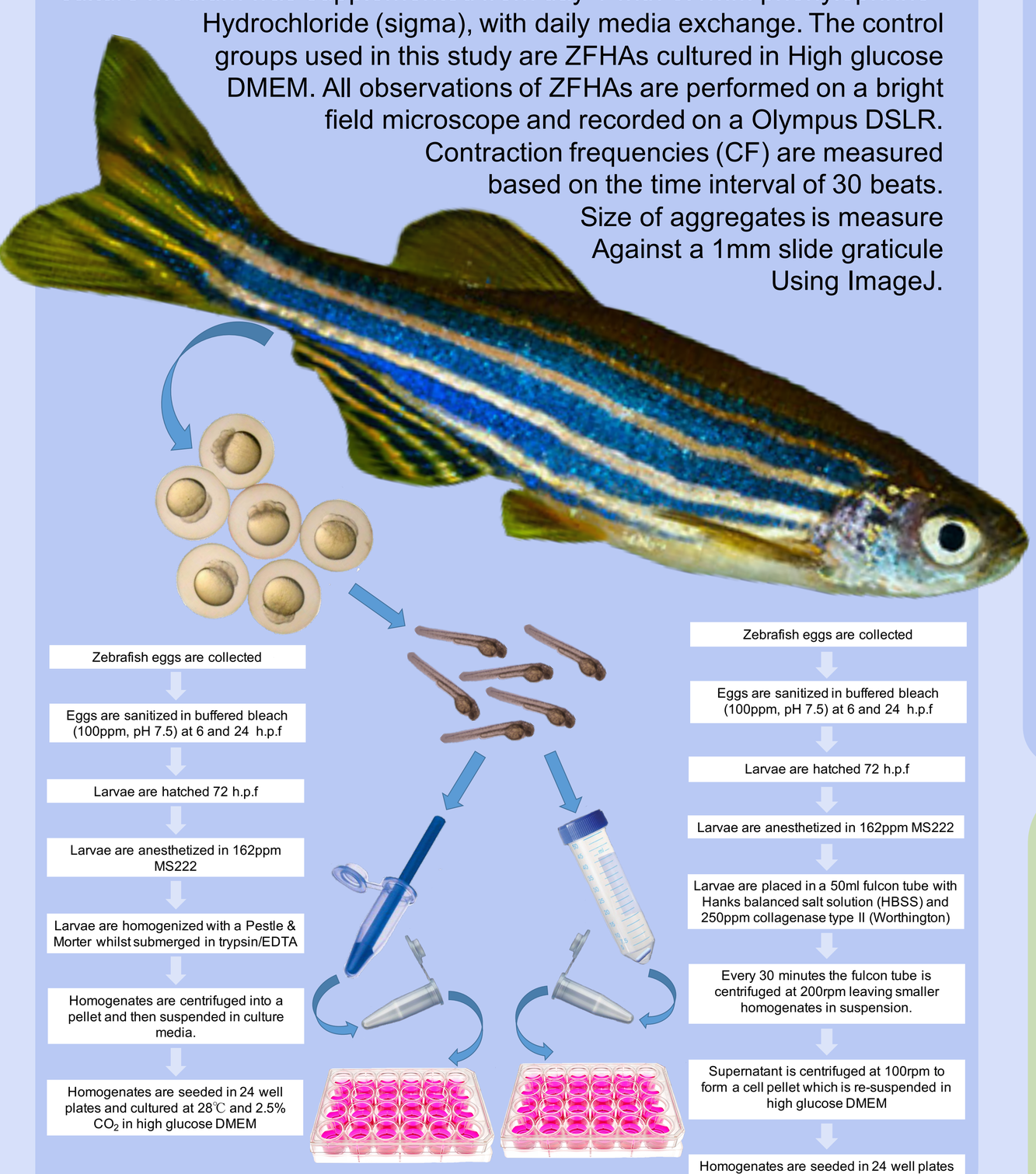


Project Aims

1. Can we make them bigger?
2. Can we make them in a better way?
3. Is ZFHA electrophysiology similar to larval or adult Zebrafish?

Materials and methods

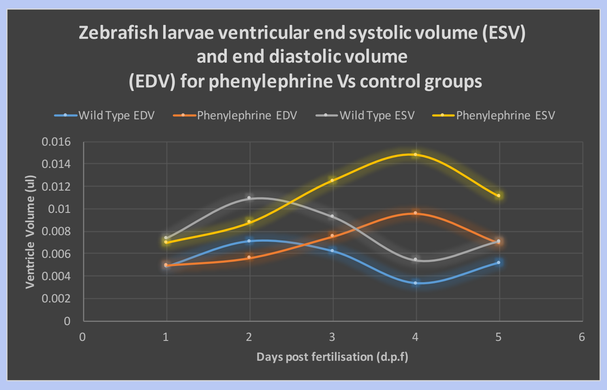
All ZFHAs are cultured in high glucose DMEM (sigma), supplemented with Foetal bovine serum 16% (v/v) (Gibco), Amphotericin (1000ppm) (PAA) and Penicillin/Streptomycin (1000ppm). Persistent achromobacter contaminations, are dealt with by supplementing culture media with Gentamicin (1000ppm), Pipricillin (1000ppm) and Ciprofloxin (10ppm). ZFHA can be observed after 24 hours in incubation. To assess hypertrophy, culture medium was supplemented from day 1 with 0.1mM phenylephrine-Hydrochloride (sigma), with daily media exchange. The control groups used in this study are ZFHAs cultured in High glucose DMEM. All observations of ZFHAs are performed on a bright field microscope and recorded on a Olympus DSLR. Contraction frequencies (CF) are measured based on the time interval of 30 beats. Size of aggregates is measure Against a 1mm slide graticule Using ImageJ.

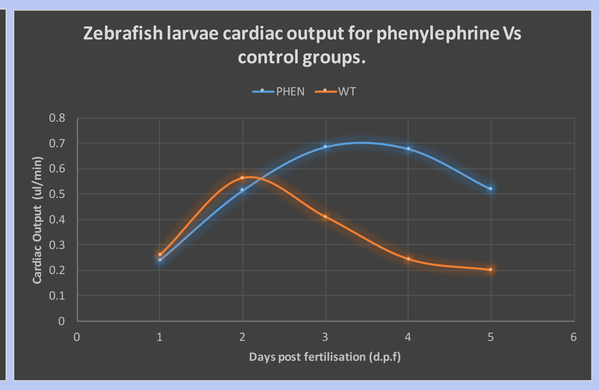


Results

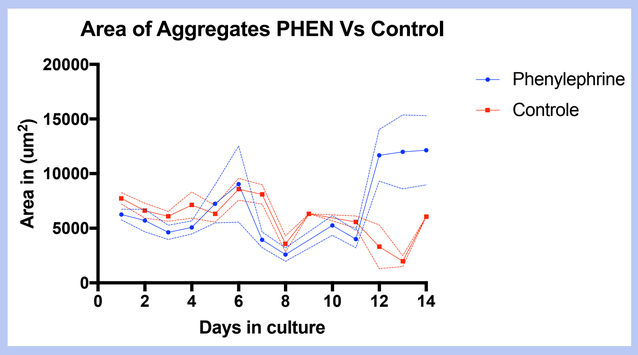
Can we make them bigger?

- We tested to see if cardiac hypertrophy could be induced in Zebrafish larvae when exposed to phenylephrine.
- End systolic (ESV) and end diastolic (EDV) volumes were increased when exposed to phenylephrine.
- Cardiac output was dramatically increased over five days when exposed to phenylephrine.



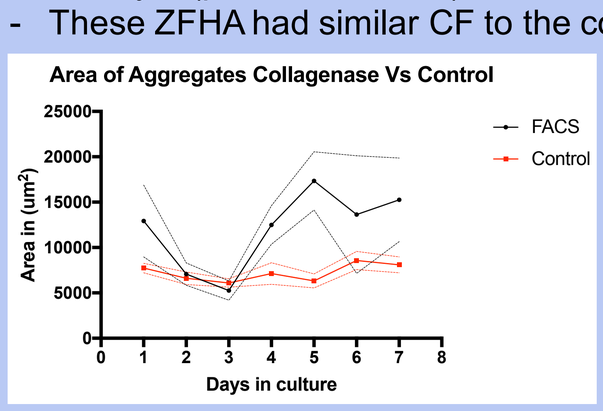


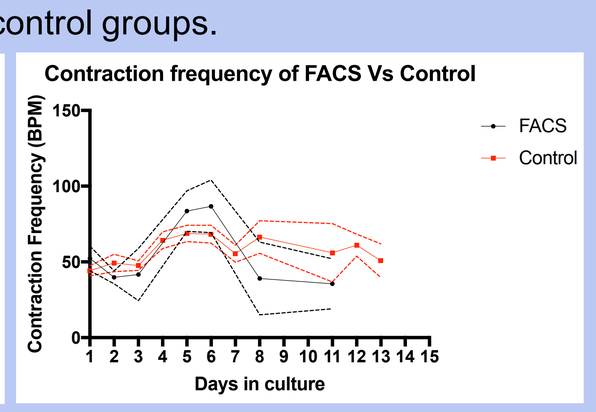
- Phenylephrine shows a significant increase in size on day 12 in culture ($p=0.00058$, $N=4$).



Can we make them in a better way?

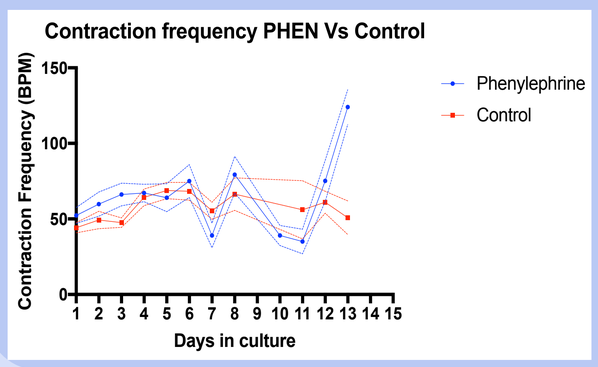
- A new method using collagenase digestion and gentle vortex forces was used to create ZFHA.
- ZFHA produced with the FACS method had a significant increases in size on day 5 ($p=0.00006$, $N=4$).
- These ZFHA had similar CF to the control groups.

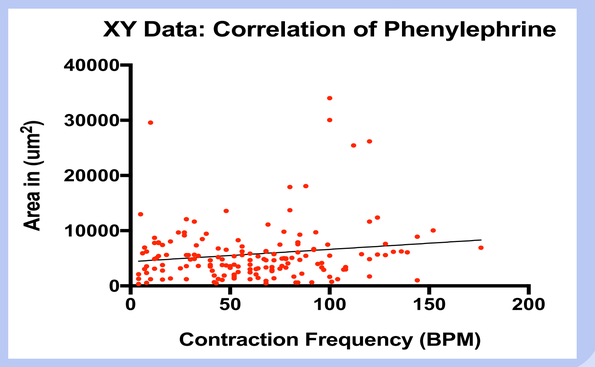




Electrophysiology

- Phenylephrine acts as an α-Adrenergic receptor (α-AR) agonist.
- Aggregates exposed to phenylephrine, had significantly higher CF on day 13 when compared to the control group ($p=0.0026$,$N=4$).
- When exposed to phenylephrine, a significant positive correlation was observed between size and CF ($r=0.02372$, $p=0.0497$, $N=163$).





Discussion

ZFHAs show organised cardiomyocyte structures with similar CF to adult ZF. There is a significant correlation between ZFHA size and contraction frequency when phenylephrine is added to the culture media. This suggests that there is a consistent ratio between size and the amount of available α-AR on the cell surface of the ZFHAs. It is also seen that contraction frequency and size are slightly increase when in the presence of a phenylephrine compared to control cultures. Collagenase digestion showed no significant difference in contraction frequency compared to control indicating cardiomyocytes don't have altered electrophysiology, however Significant increases in size are seen when the FACS method is adopted though.

Zebrafish Heart aggregates

Watch a ZFHA beating on YouTube:
Just download Quick Scan from the App store.
Scan the code to the left and enjoy watching the culturing of a ZFHA and the incredible progression of a functional ZFHA over 5 days.

Sources

1. Grunow, B., Mohamet, L., & Shiels, H. A. (2015). Generating an in vitro 3D cell culture model from zebrafish larvae for heart research. *Journal of Experimental Biology*, *218*(8), 1116–1121.

About the author

This research was done in support of my MRes in Tissue Engineering and Regenerative Medicine at the University of Manchester. I am currently seeking a PhD in a cardiovascular related field or employment as a research assistant in a similar field.

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