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 have potential as a novel, cost effective and relatively high throughput model for studying cardiac function and disease which could help reduce the amount of expensive *in vivo* testing. Cardiomyocytes have vast metabolic capabilities which indicates the possibility for purification of ZFHA cardiomyocyte progenitor cells from whole embryo tissue(1). As well as purifying ZFHAs, exploiting α-Adrenergic receptors to influence cardiac maturation in culture. α-Adrenergic receptors play a major role in controlling contraction frequency and cardiac rhythm in larval fish as they mature(2) and could have similar importance in the maturation of in ZFHAs.

Method

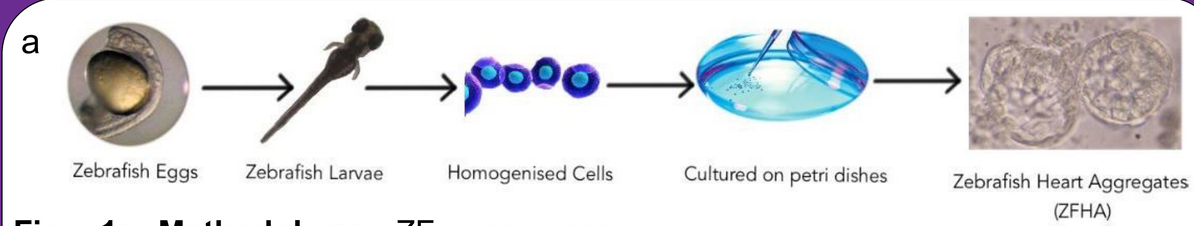
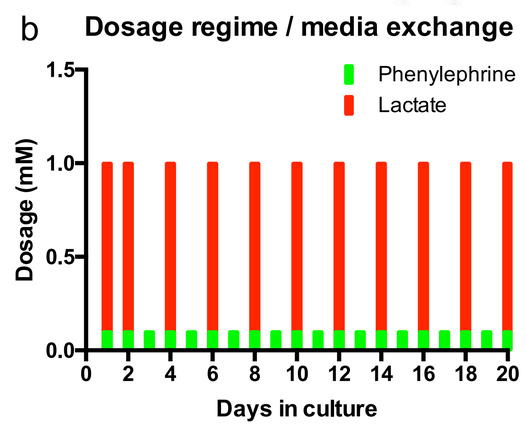
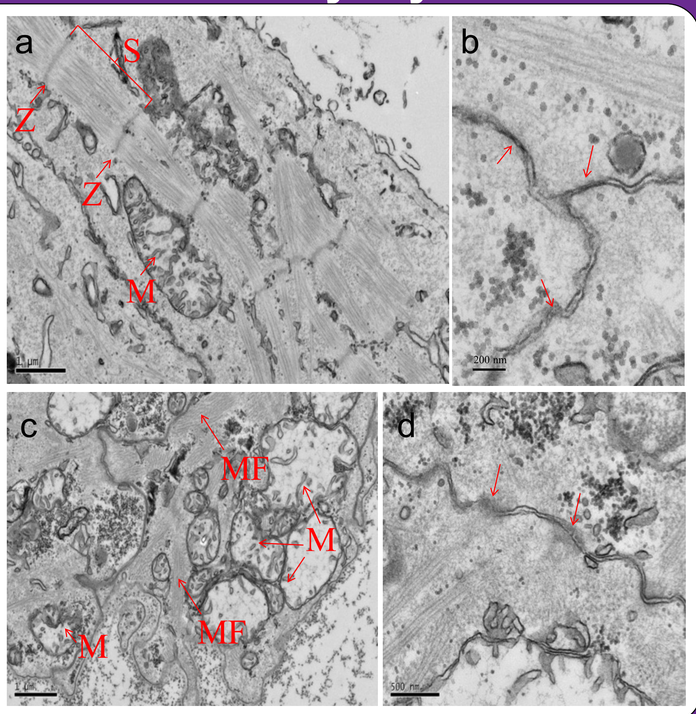


Fig. 1: Methodology. ZF eggs are washed and bleached to inhibit protozoa and bacterial contamination. Homogenization of three day old larvae yields homogenates. Seeding of homogenates in 24 well plates with culture media is incubated at 28℃ and 2.5% CO_2 for 20 days. ZFHAs were cultured in high glucose DMEM (sigma) comprising 80% FBS (Gibco) and 1% of ampotericin (PAA), penicillin/streptomycin and gentimicin (Sigma). ZFHA can be observed after 24 hours in incubation. Lactate cultures were fed with no glucose DMEM (Gibco) supplemented with 1mM sodium DL-lactate >99% (Sigma). Medium was replenished daily for 48h and every 2 days thereafter. To assess hypertrophy, culture medium was supplemented from day 1 with 0.1mM phenylephrine-hydrochloride (sigma) with daily media exchange. High glucose DMEM cultures used as the control.



ZFHAs Consist of Cardiomyocytes

Fig. 2: Electron microscopy ZFHAs (a&b) and 3 day old ZF larval cardiac tissue (c&d). (a) ZFHAs consist of cardiomyocyte tissue that displays sarcomeres (S) which are bordered by Z-Lines (Z). (c) Larval hearts do not show fully developed sarcomeres but do show myofilaments (MF). Mitochondria (M) are present in both samples indicating high metabolic activity. (b,d) Cell membranes exhibit cell-cell connections (arrows), indicating ZFHA & larval hearts are functional syncytia (3).



ZFHA Optical ECG

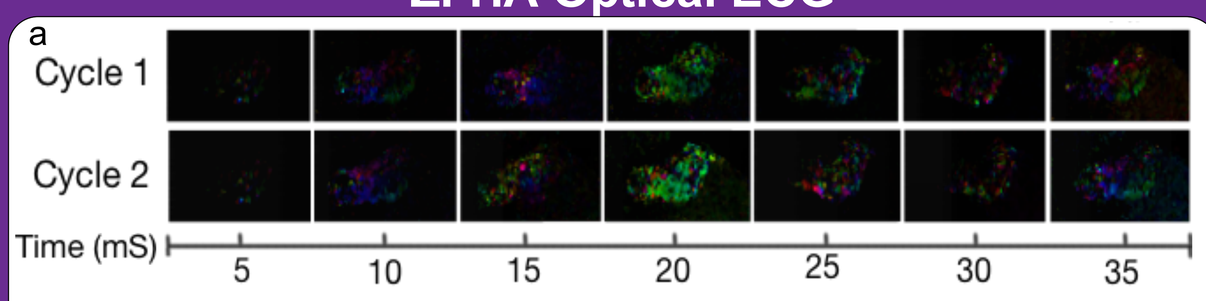
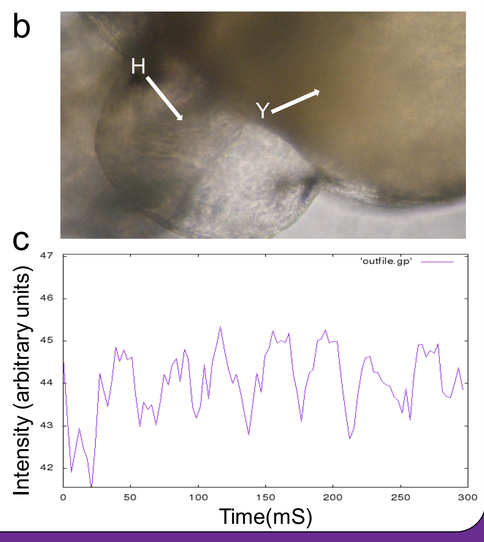


Fig. 4: Larval Heart ECG from Video. ZF larvae were used in conjunction with a program allowing electrocardiogram (ECG) mapping. This method is being developed to aid in the ECG mapping of ZFHAs without the use of toxic tracking dyes. a) the larval heart imaged using Bright field microscopy showing the heart (H) next to the yolk sack (Y). b) ECG formed from the video. c) Video trace of 2 cycles of contraction displaying electrical activity across the myocardium, green indicating maximum contraction.



ZFHA Lactate Exposure

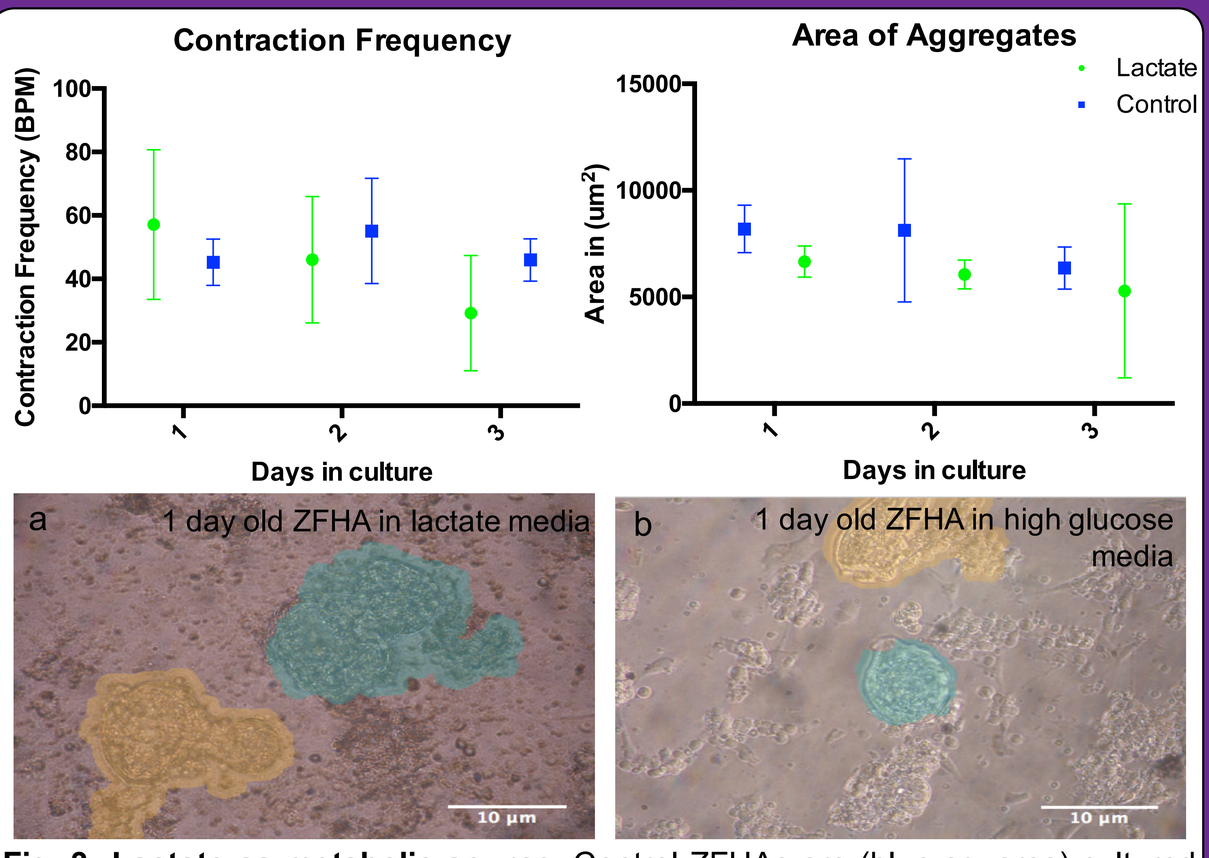


Fig. 3: Lactate as metabolic source. Control ZFHAs are (blue squares) cultured in high glucose DMEM, while the lactate group (green circles) are cultured in glucose free DMEM supplemented with 1mM Sodium-L-lactate. Independent T-tests show no significant difference in contraction frequency or size between lactate and control. a) Lactate group showing darker single cells surrounding ZFHA due to necrosis. b) Control group in high glucose media shows ZFHAs surrounded by healthier cells.

ZFHA Phenylephrine Exposure

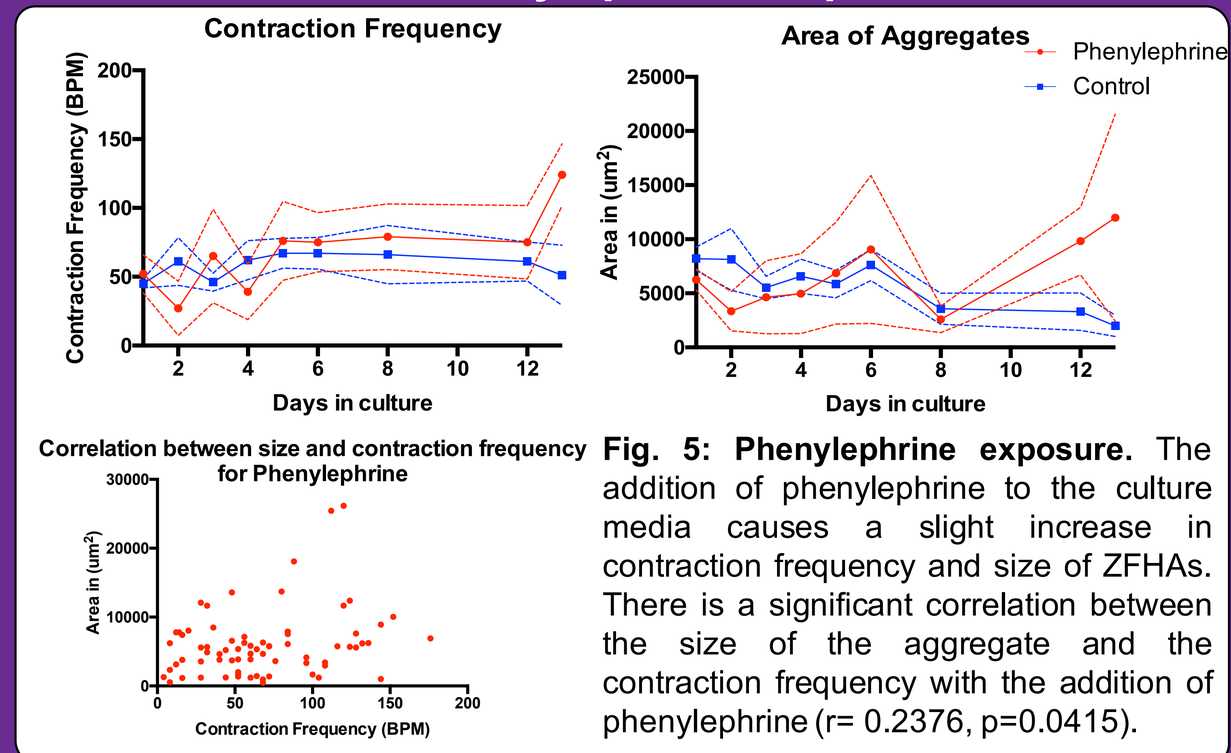


Fig. 5: Phenylephrine exposure. The addition of phenylephrine to the culture media causes a slight increase in contraction frequency and size of ZFHAs. There is a significant correlation between the size of the aggregate and the contraction frequency with the addition of phenylephrine (r= 0.2376, p=0.0415).

Conclusions

ZFHAs show organised cardiomyocyte structures and similar contraction rhythms to adult ZF. There is a significant correlation between ZFHA size and contraction frequency when phenylephrine is added to the culture media. Implications being that the size of the ZFHA is related to the amount of avalable α-Adrenergic receptors on the cell surface avalable to be acted on phenylephrine. It is also seen that contraction frequency and size are slightly increase when in the presence of a phenylephrine compared to control cultures. During lactate exposure no significant difference in contraction frequency and size of ZFHAs was observed. However, lactate exposure helped survival cardiomyocyte with the necrosis of other tissues. Using lactate as an alternative metabolic source could help, cost effectively, purify cardiomyocytes. Using optical mapping, analysis of contraction patterns can be observed without the use of toxic tracking dyes or radioactive isotopes.

References

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3. Grunow B, Mohamet L, Shiels HA. Generating an in vitro 3D cell culture model from zebrafish larvae for heart research. Journal of Experimental Biology. 2015 Apr 15;218(8):1116–21.