Optimization of Live Bi-Ventricular Slice Culture Platform for Embryonic Ventricular Morphogenesis Investigation

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Abstract:

Congenital heart defects (CHDs) affect approximately 1 in 100 live births, making understanding embryonic cardiac development essential for identifying mechanisms underlying abnormal ventricular morphogenesis. This project focused on optimizing a live bi-ventricular slice culture platform using Hamburger-Hamilton stage 36 (HH36, D10) chicken embryonic hearts for dynamic imaging of ventricular development.

Heart slices were prepared using vibratome sectioning and evaluated for physiological and cellular viability during ex vivo culture. A 5.5% agarose concentration was identified as optimal for producing stable 200-µm heart slices. Tissue viability was assessed through spontaneous beating frequency and immunohistochemical analysis of proliferation and apoptosis. Beating frequency increased significantly between 1 and 2 hours, demonstrating that the tissue required an initial recovery period following mechanical stress from isolation and vibratome sectioning followed by a consistent beating throughout 8 hours post slicing.

Expanded immunohistochemical analysis demonstrated significantly lower $pHH3^{+}/DAPI^{+}$ proliferation in cultured slices compared with in vivo hearts, while $Caspase\text{-}3^{+}/DAPI^{+}$ apoptosis did not significantly differ across culture conditions. These results establish a viable platform for live imaging while identifying cellular changes associated with ex vivo culture.

Summary of Research:

The embryonic ventricular wall consists of compact myocardium, a dense outer layer, and trabecular myocardium, an inner network of muscular projections.

Coordinated development and remodelling of these regions are necessary for normal ventricular formation. Conventional histology provides static images of cardiac structure but cannot capture the dynamic cellular movements responsible for tissue remodelling. Therefore, this project aimed to establish a live embryonic heart-slice culture system that could preserve tissue function while enabling confocal time lapse imaging of ventricular morphogenesis.

Chicken embryos were incubated to HH36 (D10) and embryonic hearts were isolated, embedded in agarose, and sectioned into approximately 200-µm slices using a vibratome. Agarose concentration was optimized to provide sufficient structural support while allowing successful sectioning. A 6% concentration was too rigid, whereas 5% did not provide adequate support; 5.5% produced stable slices suitable for imaging. Confocal microscopy was then used for live imaging, while fixed slices were analysed using immunohistochemistry. MF20 was used to identify cardiomyocytes, DAPI to identify nuclei, phosphorylated histone H3 (pHH3) to identify proliferating cells, and Caspase-3 to identify apoptotic cells. Positive cells were quantified using ImageJ, and marker positive cells were normalized to total nuclei to generate $pHH3^{+}/DAPI^{+}$ and $Caspase\text{-}3^{+}/DAPI^{+}$ ratios.

Physiological viability was initially assessed by measuring spontaneous beating at 0, 1, 2, 4, 6, and 8 hours after sectioning. Beating frequency was significantly lower at 1 hour than at 2 hours after sectioning (paired t-test, $t(16) = -5.27$, $p = 7.63 \times 10^{-5}$), indicating that the tissue required an initial recovery period following mechanical stress from isolation and vibratome sectioning.. Following recovery, beating remained stable from 2–8 hours (repeated-measures ANOVA, $F(3,48) = 0.601$, $p = 0.617$). However, no significant difference in beating frequency was observed from 2–8 hours, indicating that cardiac function remained stable following recovery. This supported approximately 2 hours as an appropriate recovery period before long-term live imaging.

The expanded immunohistochemical analysis provided additional information about cellular activity. $pHH3^{+}/$

DAPI^{+} ratios decreased from 0.0351 in vivo to 0.0187, 0.0169, 0.0141, and 0.00925 at 2, 4, 6, and 8 hours, respectively. A one-way ANOVA demonstrated a significant difference among groups ($F(4,48) = 4.110$, $p = 0.00606$). Compared with in vivo hearts, proliferation was significantly lower at 2 hours ($p = 0.0231$), 4 hours ($p = 0.0100$), 6 hours ($p = 0.00353$), and 8 hours ($p = 0.000821$). Thus, although the slices remained contractile, their proliferative activity was reduced compared with normal in vivo tissue.

In contrast, Caspase-3 analysis did not demonstrate a significant overall difference among groups ($F(4,53) = 2.071$, $p = 0.0976$). Comparisons with in vivo hearts were also not significant at 2 hours ($p = 0.132$), 4 hours ($p = 0.918$), or 6 hours ($p = 0.108$). These findings suggest that ex vivo culture did not produce a statistically detectable increase in apoptosis within the available dataset.

Regional analysis also showed no significant overall difference between compact and trabecular myocardium for either marker. For pHH3, the combined compact mean was 0.0183 compared with 0.0116 in trabecular myocardium ($p = 0.0712$). For Caspase-3, the combined means were 0.0238 and 0.0233, respectively ($p = 0.907$). Therefore, regional differences in proliferation or apoptosis alone do not appear sufficient to explain ventricular remodelling at this developmental stage.

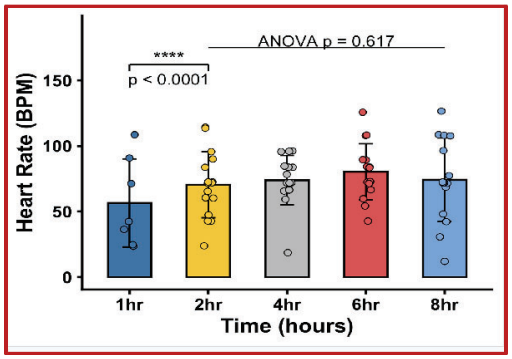

Figure 1: Heart rate (BPM) over a time period of 8 hours. Heart rate increased significantly from 1 hr to 2 hr ($p < 0.0001$) then remained stable from 2–8 hr (ANOVA, $p = 0.617$).

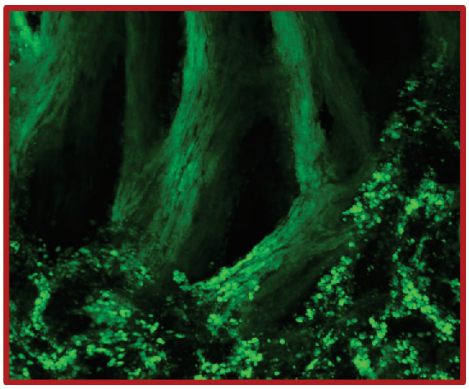

Figure 2: Immunohistochemical stained picture of compact and trabacular region of a H.H stage 34 embryonic heart ventricle.

Conclusions and Future Steps:

The optimized D10 heart-slice culture platform successfully maintained spontaneous cardiac activity for at least 8 hours following an approximately 2-hour recovery period. This supports its use for long-term live confocal imaging and dynamic investigation of ventricular morphogenesis. However, the immunohistochemical results demonstrate that while beating remained stable and apoptosis did not significantly increase, proliferation was significantly lower in cultured slices than in normal in vivo hearts which can be an indication of active remodelling sacrificing the rate of proliferation.

The lack of significant regional differences in proliferation and apoptosis between compact and trabecular myocardium suggests that ventricular wall remodelling may involve mechanisms beyond regional changes in cell division or cell death. Dynamic cellular movement, tissue deformation, and trabecular compaction may contribute substantially to ventricular morphogenesis and can be investigated using the established live imaging platform.

Future work will focus on HH38/D12 heart slices analyses to determine whether tissue viability and cellular remodelling at later developmental stages. Comparing D7, D10 and D12 hearts will allow developmental changes in ventricular morphogenesis to be characterized. Further analysis of compact versus trabecular regions will help determine their respective contributions to ventricular wall remodelling. Ultimately, this platform can be applied to congenital heart disease models, including Hypoplastic Left Heart Syndrome (HLHS), to investigate how abnormal cellular morphogenesis contributes to defective ventricular development.

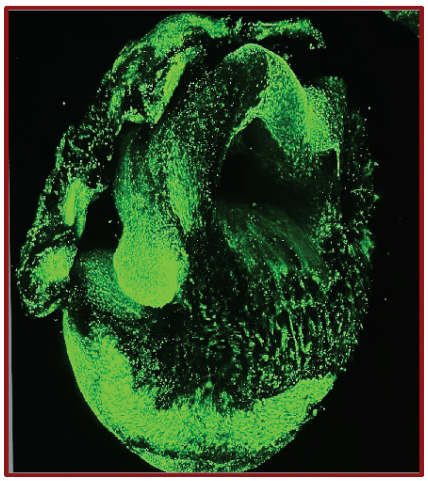

Figure 3: Overview of immunohistochemically stained image of H.H stage 34 embryonic heart.