3D Bioprinted Spheroidal Droplets with Cardiomyocytes and Cardiac Fibroblasts for Drug Cytotoxicity Testing

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Statement of Purpose: A 3D bioprinted spheroidal cell model is expected to provide an enhanced habitat for tissue formation as it enables sufficient distribution of oxygen, media, growth factors, nutrients and ions into the scaffold maintaining cell growth and proliferation [1]. We hypothesized that 3D cardiac cell spheroidal droplets will serve as a platform for enabling the heterocellular coupling between human cardiac myocytes and fibroblasts for studying drug interactions [2]. In order to develop this 3D cardiac cell-based model, AC16 cardiomyocytes (CMs) and adult cardiac ventricular fibroblasts (CFs) were pre-mixed in alginate-gelatin hydrogels in equal densities and printed. The cell-spheroidal droplets were analyzed for cell viability and the onset of heterocellular coupling was quantified for up to one month *in-vitro*. This newly developed cardiac spheroid model was adopted to test the effects of doxorubicin (DOX), an anti-tumor agent that is known to have cardiotoxicity. After confirmation of cardiac toxocty with DOX, we aimed to mitigate these cytotoxic effects using two well-known antioxidants; n-acetylcysteine (NAC) and Tiron. This study will yeld a high throughput cardiac model for drug screening and evaluation.

Methods: A 3D spheroidal design of diameter 2 mm was designed using SolidWorks® whereas a 2-layered flattened disc structure served as a control design. The spheriodal structure was chosen for high-throughput printing of droplets inside a 96 round-bottom well plate using a CELLINK BIO X or a BioAssembly Bot printer where the temperature controlled printer head was used to deposit the droplets within wells. After deposition within wells, the droplets were crosslinked with 80mM $CaCl_2$ solution for 15 min. Biocompatibility studies focussed on measurement of cell viability via an MTS assay. Heterocellular coupling between CMs and CFs was probed by immunostaining using Troponin-I for the CMs while fibroblast surface protein (FSP) was used as a biomarker for the CFs. In order to elucidate the dose responsive range of DOX on the cardiac spheroids containing both CMs and CFs, different concentrations of DOX were tested to determine the half-maximal inhibitory concentration (IC50). Additionally, varying concentrations of antioxidants were used to verify their IC50 to diminish the toxic effects of DOX in the cardiac spheroids.

Results: Results showed that the spheroidal droplets were stable in 1X PBS and did not disintegrate for upto 42 days in-vitro (Figure 1A). SEM imaging revealed sufficient porosity with an average pore diameter of 228.67±92.07 µm (Figure 1B), while rheological studies showed that the elastic modulus of the scaffolds was within the range of 11.6±1.1 kPa. High porosity enabled the paracrine interaction between the cells and exchange of nutrients for prolonged survival in the scaffolds. MTS results showed significant cell viability during long-term in-vitro culture. Heterocellular coupling between the cells was significantly higher in the 3D spheroidal droplets compared with the controls (Figure 1C-G). This formed as a basis for further drug testing. The effective IC_{50} for DOX in our cardiac model was found to be 60uM while the IC_{50} for the antioxidants was found to be 8mM for both NAC and Tiron (Figure 1 H, I). The maximum dose of 15mM for antioxidant's showed higher cell viability when administered in combination with DOX ($p>0.05$) eliminating the toxic effects imposed by DOX, in our cardiac model.

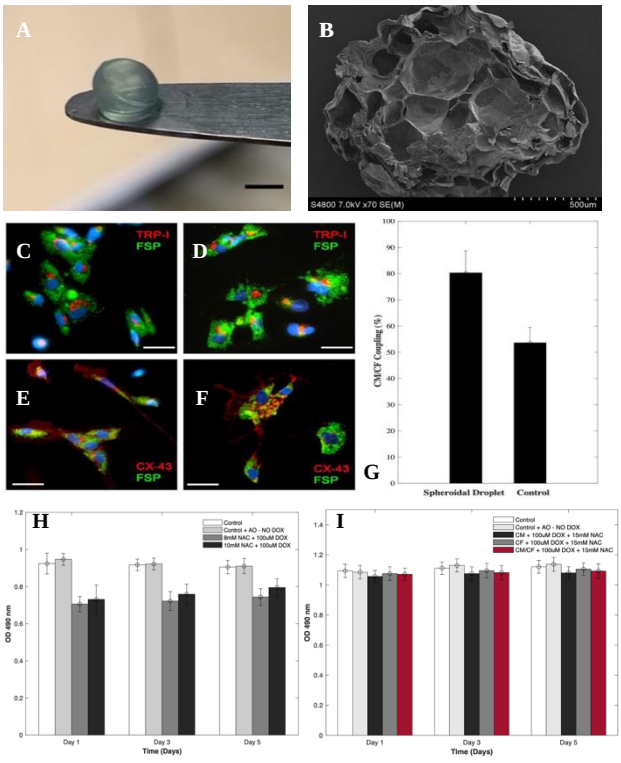

Figure 1. (A) 3D printed spheroidal droplet scaffold (B) SEM of crosssection of the 3D printed spheroidal droplet scaffold. (C-G) Confirmation of heterocellular coupling and quantitative analysis for CMs and CFs in the spheroidal droplets in comparison with the control (G). The immunocytochemical studies show hetero-cellular coupling of CM and CF after 7 days of culture (scale bar 80µm). (H,I) Optical density measurements for MTS assay of 3D spheroidal droplets testing for the dose responsive range of doxorubicin (DOX) and the antioxidant NAC.

Conclusion: The key objective in this study was to create a 3D cardiac tissue model which is capable of mimicking *in-vivo* conditions allowing for a better understanding of cardiac biology and facilitate the study of cardiac biomarkers as targeted therapeutic strategies. Optimization of high-throughput printing parameters led to the production of a 3D spheroidal droplet design that exhibited an interpenetrating network hydrogel with sigifincant porosity and no necrotic core yielding higher cell viability and superior proliferation in comparision with the control structures. Heterocellular coupling between CMs and CFs was significantly higher in the 3D spheroidal droplets than in 2D control due to the higher vicinity of cells in a 3D hydrogel scaffold. Strong scientific premise further enabled the adoption this 3D cardiac model for a complete dose screening of doxorubicin, with n-acetylcysteine and Tiron to study the effects of these drugs.

References:

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