Utilizing 3D printed scaffold to generate a novel Cardiac toxicity Model for screening potential drug candidates.

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Introduction

The introduction of 3D bio-printing is expected to revolutionize the field of tissue engineering, regenerative and transplant medicine. In this study, we utilized adult human cardiac stem cells to seed onto a 3D printed flexible Cardiac scaffold. The cardiac stem cells were able to differentiate to myocardium tissue within 7 days. The myocardium tissue was comparable to human heart with measureable physiological and electrophysiology parameters when perfused through the aorta. We tested drug toxicity [Fluorouracil (5-FU), Cyclophosphamide (Cytoxan®), CEP1340, CEP1601, CEP1603 and Doxorubicin (Adriamycin®)] with the 3D printed heart. The results were comparable to published human studies with the following drugs: epinephrine increased cardiac output and the heart rate; Atenolol (Tenormin) decreased cardiac output and heart rate. On the basis of these results, we can conclude that the 3D printed heart is a suitable model system to study drug toxicity. This study demonstrates the potential use 3D printed hearts for screening potential drug candidates.

Methods:

The human cardiac stem cells 36099-26 were cultured in T225 flasks that were pre-coated with Celprogen's Xeno-free ECM, E36099-26-T225 and cultured in a humidified 37^{0}C incubator for 7 days. After 7 days in culture the cells were trypsinized and seeded onto a 3D printed scaffold heart. The 3D printed heart was maintained in the humidified 37^{0}C incubator for 14 days. The cardiac stem cells were induced to differentiate into adult cardiac cells by seeding the adult human Cardiac cells 36044-15 on to the 3D printed scaffold after 3 days with the cardiac stem cells (36099-26). For the first 3 days the cardiac stem cells were incubated with M36099-26S media and after the third day when the adult cardiac cells (36044-15) were added the media was changed to M36044-15DS. The cells were cultured for 14 additional days and then perfused with M36044-15S , with a peristaltic pump at 1ml per minute rate with continuous perfusion. The 3D printed heart was ready for toxicity studies at day 20. Baseline measurements were established for the 3D printed heart; heart rate, electrophysiology, cardiac out-put and flow rate, measurements were takes with and without electrical stimulation.

In-vitro study:

The human cardiac stem cells were established in culture utilizing standard cardiac cell culture condition established by Celprogen. The human cardiac stem cells (36099-26) were cultured in M36099-26S media with serum on pre-coated T225 flask (E36099-26-T225).
The human adult cardiac cells (36044-15) were cultured in M36044-15S media with serum on pre-coated T225 flask (E36044-15-T25). After inducing the cardiac cells to the 3D scaffold with the human cardiac stem cells the media for replenishing the cells was changed to one differentiation media M36044-15DS with serum.
After 14 days in culture the 3D printed heart was moved to the tissue culture hood and perfused with M36044-15DS media for 6 days at 1 ml /minute, pre-warmed at 37^{0}C at 21% oxygen and atmospheric carbon dioxide and nitrogen concentrations.

3D printing Scaffold:

The 3D printed scaffold was generated with a flexible Poly Lactic Acidic (PLA) with a 3D printed [mono-print] modified with a syringe [smart syringe pump Parker] for dispensing cells into the scaffold. The scaffold was sterilized and then pre-coated with the cardiac stem cell's ECM (extracellular matrix), prior to seeding the cells . Once the cardiac cells were engrafted within the 3D printed heart, baseline cardiac measurements were performed to ensure that we were capable to generating cardiac physiology parameters . The following drugs were tested to establish the drug discovery parameters: epinephrine –increase cardiac rate, Atenolol –decrease cardiac rate, and drug toxicity with Fluorouracil (5-FU), Cyclophosphamide (Cytoxan®), CEP1340, CEP1601, CEP1603 and Doxorubicin (Adriamycin®) .

Results:

The results are indicated in the figures and graphs below:

Figure 1: Model for 3D printed Human heart Scaffold.

Figure 2. A. Real scale 3D printed heart of 17 year old female. **B.** Human 3D printed heart approximately 1/5 reduction of real scale heat as seen in A. **C.** Interior of the 3D printed heart.

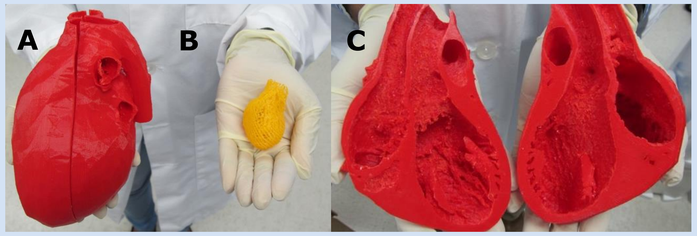


Figure 3: A. 3D printed heart in culture with human cardiac stem cells. **B**. Cardiac Cells engrafting in the 3D heart scaffold, in Tissue culture conditions. **C &D**. Engrafting Cardiac cells in the inner chambers of the 3D scaffold.

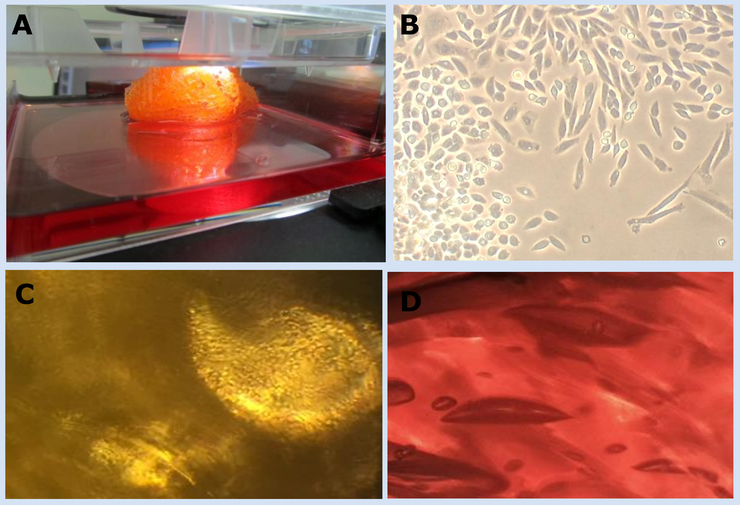


Figure 4: A. 3D Scaffold without engrafted cells **B.** 3D printed Scaffold heart continuously perfused with M36044-15DS media .

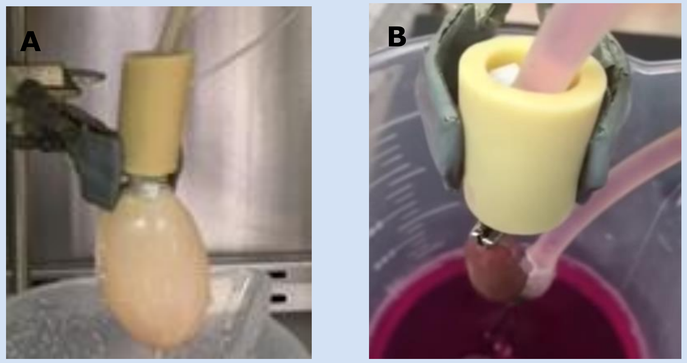


Figure 5: Electrophysiological readings of the 3D printed heart. A. Spontaneous activity. B. Baseline activity. C. Response to 60mA stimulus. D. Average response to 60mA stimulus

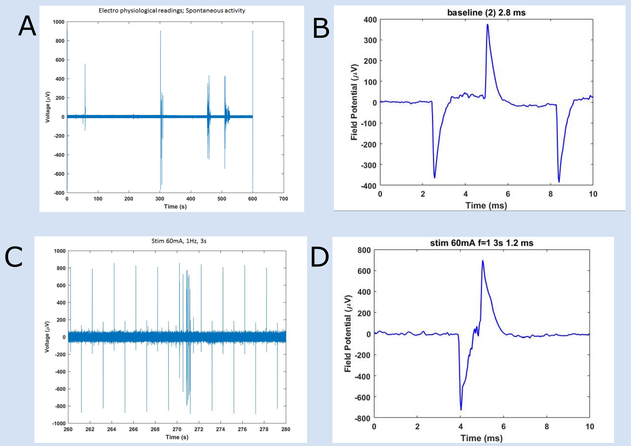
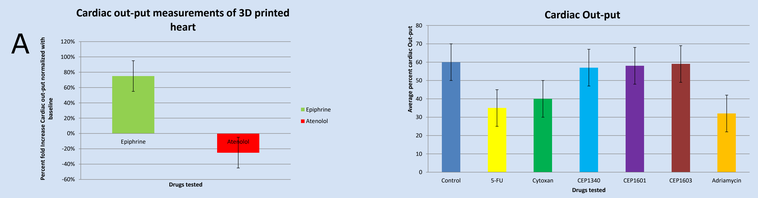


Figure 5: A. Cardiac Out-put measurements with different drugs. B. Cardiac out-put measurement



Conclusions:

On the basis of these results, we can conclude that the 3D printed heart is a suitable model system to study drug toxicity. This study demonstrates the potential use 3D printed hearts for screening potential drug candidates.

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