Development of an Electrically Conductive Heart-on-a-chip Model for the Formation of Functional and Mature Human iPSC-derived Cardiac Tissues

Hamid Esmaeili[1*], Keagan Neff[1], Raymond Q. Migrino[2], and Mehdi Nikkhah[1,3]
[1]School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ
[2]University of Arizona College of Medicine, Phoenix, AZ
[3]Biodesign Virginia G. Piper Center for Personalized Diagnostics, Arizona State University, Tempe, AZ

Introduction: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been shown as promising cell sources for cardiovascular disease modeling and regenerative medicine, but their true potential has yet remained to be fully exploited by developing novel culturing strategies. Microfluidic technologies have enabled the recapitulation of specific *in vivo* organ features, such as tissue alignment and cellular constituents, in an organotypic manner. In this regard, Heart-on-a-chip (HOC) models have shown a tremendous potential to promote the functionalities of cardiac cells in a physiologically relevant environment. However, compared to the native myocardial tissue, the traditional hydrogel scaffolds used in HOCs often lack integrated critical features, namely electrical conductivity in conjunction with an organized (i.e., anisotropic) structure to facilitate tissue function and propagation of electrical signals across cardiac cells. To that end, in this study, we investigated the influence of an electrically active microenvironment within a native-like HOC model on the functionality and maturation of the cardiac tissues. Isogenic human cardiac tissues were formed by injection of human cardiac cells encapsulated in a hydrogel scaffolds embedded with conductive gold nanorods (GNRs) into HOCs, following by in-depth biological and functional assessments.

Materials and Methods: hiPSC-CMs and hiPSC-Cardiac Fibroblasts (hiPSC-FBs) were differentiated from IMR90-4 stem cells (WiCell) as described elsewhere.[1] GNRs were synthesized using a customized protocol as described previously[2]. The HOCs with the embedded microposts were fabricated according to our previously published study[1] (**Fig. 1A**). The prepolymer solution of the electrically active hydrogel scaffolds were prepared by mixing various concentrations of GNRs including 0.0, 0.2, 0.5, and 1.0 mg/mL into a hydrogel cocktail comprising of Collagen-I and Matrigel. To form the cardiac tissues, a combination of hiPSC-CMs and hiPSC-FBs (4:1) with a seeding density of 35 M/mL were homogenously mixed in the prepolymer solutions, injected into the HOCs, and maintained for 14 days in culture. The cytotoxicity of the GNRs was assessed using a standard live/dead assay kit on day 2 of culture. Beating analysis including spontaneous beat rate and interbeat interval variability (IIV) (a measure of synchronicity) were extracted from the brightfield videos captured at day 14 of culture using a customized MATLAB code. Immunofluorescence (IF) staining was performed to stain the cardiac tissues with F-actin to probe the phenotype of the tissues. The nuclei were counterstained with DAPI. Calcium transient imaging was performed with calcium indicator (Fluo-4-AM) and five regions of interest (ROIs) were selected from the recorded videos to observe the traces of calcium spikes. Calcium images were then analyzed by ImageJ.

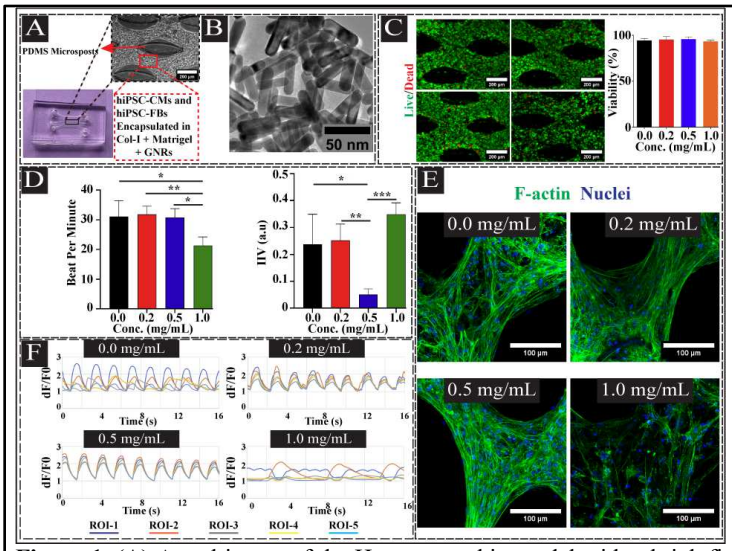

Figure 1. (A) A real image of the Heart-on-a-chip model with a brightfield image of the cardiac tissues formed between the embedded microposts. **(B)** TEM images of GNRs. **(C)** Live/Dead assay images of the cardiac tissues containing 0.0, 0.2, 0.5, and 1.0 mg/mL of GNRs (n=3). **(D)** Spontaneous beat per minute (left panel) and interbeat interval variability (IIV) of the cardiac tissues within the Heart-on-a-chip systems (n=4). **(E)** Immunofluorescence (IF) images of cardiac tissues formed within the hydrogel scaffolds with 0.0, 0.2, 0.5, and 1.0 mg/mL of GNRs for 14 days stained for F-actin (green), and nuclei (blue). **(F)** Spontaneous calcium transient traces of the cardiac tissues. Five regions were selected per image and the correlated signals of each region were shown on the graphs. The color codes correspond to the regions of interest within each analyzed video. GraphPad Prism software version 6 was used to evaluate the statistical analyses with P-values <0.05 considered significant.

Results and Discussion: TEM imaging showed the formation of highly purified GNRs with an average length of 42.7 nm ± 4.8 (mean ± S.D) (**Fig. 1B**). The cytotoxicity assay confirmed that even at very high concentration of

GNRs (i.e., 1.0 mg/mL) there was no significant cell death compared to the control group (0.0 mg/mL) (**Fig. 1C**). While the spontaneous beating numbers of 0.0, 0.2, and 0.5 mg/mL conditions were not significantly different from each other, the beating number of the cardiac tissues with 1.0 mg/mL GNRs was significantly lower than that of conditions with lower amount of GNRs (**Fig. 1D**). However, the IIV for 0.5 mg/mL condition was significantly lower than the rest of the groups suggesting a more synchronous beating across the cardiac tissues (**Fig. 1D**). The IF-stained samples with F-actin revealed the formation of tighter cardiac tissues in the 0.5 mg/mL condition (**Fig. 1E**). However, at 1.0 mg/mL group, the cardiac tissues did not form very well as the cardiac cells tended to form aggregates rather than elongated muscle-like cardiac tissues (**Fig. 1E**). Finally, the Ca^{2+} transient traces further confirmed the formation of more robust cardiac tissues as the peaks in different ROIs were matched to each other suggesting a more synchronous beating and release of Ca spikes (**Fig. 1F**). We speculated that increased amount of GNRs in the hydrogel scaffolds cocktail interfered with the natural ECM structure resulting in a less anchorage point for the cardiac cells to attach and grow. This could be the underlying reason for the decreased beating number, the higher IIV values, and the irregular calcium traces at very high concentrations of GNRs.

Conclusion: Our data suggests that GNRs could be incorporated into hydrogel scaffolds to a very high concentrations without significantly affecting the viability of cardiac cells. However, an optimum concentration of GNRs was required for the formation of functional cardiac tissues to result in lower beating irregularity and synchronous calcium spikes. Further assays such as gene expression analysis through qPCR and RNA-seq are currently underway to mechanistically investigate the interactions of GNRs with stem cells within the HOC model system.

References: 1. Veldhuizen, J. et al. Biomaterials **2020**, 256 (120195). **2**. Navaei, A. et al. Acta Biomaterialia **2016**, 41(133-146). **Acknowledgments:** This project was supported by the NSF Award # 2016501