Self-Organizing Human Cardiac Organoids from iPSCs show Matured Cardiomyocytes and Vascular-like Endothelial Networking Structures

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Background

Human induced pluripotent stem cell (hiPSC) derived somatic cells, engineered tissues, and organoids are increasingly important in personalized medicine[1, 2]. Here, we generate free-floating multi-cellular cardiac organoids (COs) from hiPSC.

Aims & Methods

Mesodermal and cardiac cell differentiation protocols[3] are applied on hiPSCs-aggregates via temporary biphasic Wnt signaling pathway modulation by small molecules and additional growth factor supplementations, respectively.

Results

This new COs generation procedure is robust, highly efficient, and reproducible, as well as generates COs under fully defined serum-free conditions. COs contain more than 80% cardiomyocytes and 20% non-myocytes, including about 17% endothelial cells **(Fig.1)**.

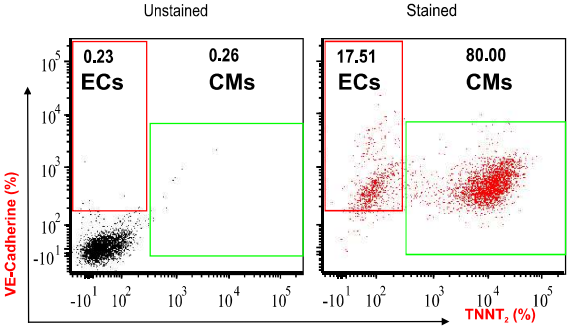

Fig. 1: Flow cytometric analysis of dissociated cardiac organoids.
Representative flow cytometric scatter plots of negative control and single cells stained for $TNNT_2$-FITC cardiac marker : VE-Cadherin-PE endothelial marker.

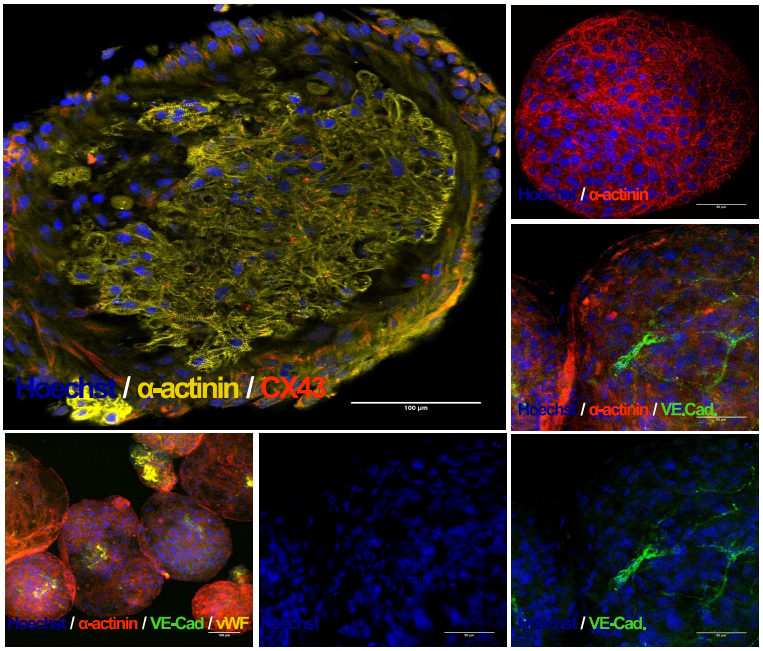

Fig. 2: Whole mount immunostaining of cardiac organoids.
Cardiac organoids were stained without prior dissociation with antibodies against α-actinin (red and yellow), CX43 (Red), VE-Cadherin (green), vWF (yellow). Nuclei were counterstained with Hoechst 33342 (blue). Scale bars: 100 µm.

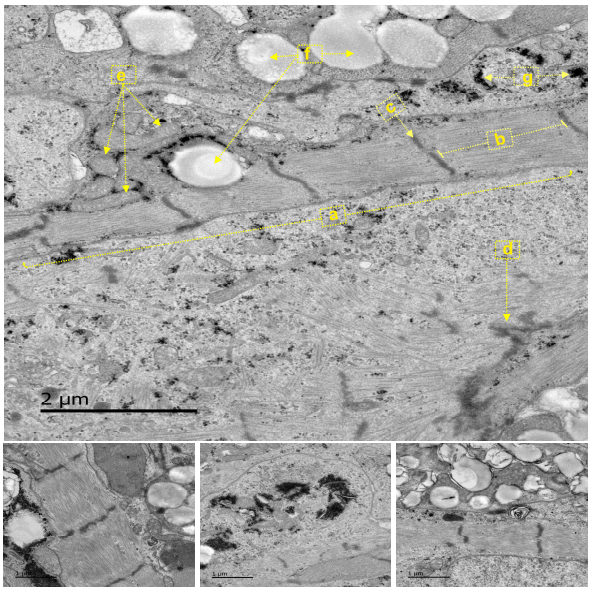

Fig. 3: Electron Microscopic images of cardiac organoid.
Myofibrils (a), Sarcomere length (b), Z-disc (c), intercalated disc (d), Mitochondria (e), Lipid droplets (f), and Glycogen (g).

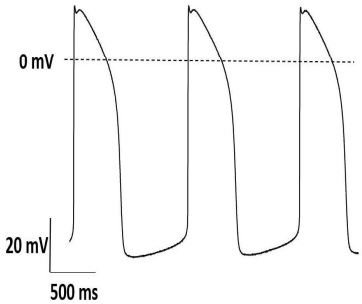

Fig. 4: Sharp electrode electrical activity of cardiac organoid.

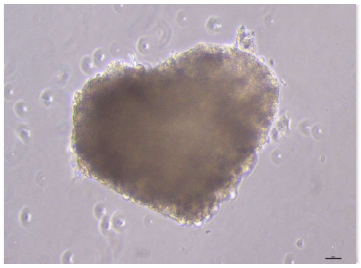

Fig. 5: Bright Field of cardiac organoid.
Scale bar: 100 µm.

Conclusions

Cardiac organoids show formation of a complex network of endothelial cells. Altogether, we present a method to generate COs that contain matured cardiomyocytes intertwine with other cardiac cells such as endothelial cells. COs are providing a model to study human cardiogenesis and cardiac (patho)physiology, performing pharmacological drug validations, and generating cells for cell based therapies.

References

1- Kim, H., Kamm, R. D., Vunjak-Novakovic, G. & Wu, J. C. Progress in multicellular human cardiac organoids for clinical applications. Cell Stem Cell 29, 503-514, doi:https://doi.org/10.1016/j.stem.2022.03.012 (2022).

2- Hofbauer, P. et al. Cardioids reveal self-organizing principles of human cardiogenesis. Cell 184, 3299-3317.e3222, doi:10.1016/j.cell.2021.04.034 (2021).

3- Hamad, S. et al. Generation of human induced pluripotent stem cell-derived cardiomyocytes in 2D monolayer and scalable 3D suspension bioreactor cultures with reduced batch-to-batch variations. Theranostics 9, 7222-7238, doi:10.7150/thno.32058 (2019).

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