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Studying Cardiovascular Disease Using Human Stem Cell-derived 3D Cardiomyocytes

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What are cardiomyocytes?

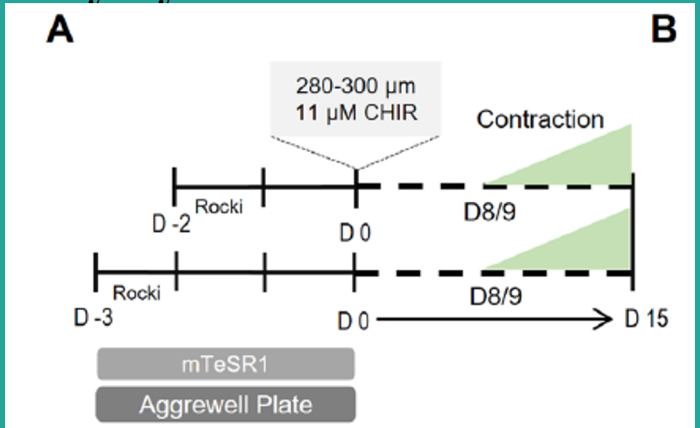
Cardiomyocytes are the cells that make the heart contract. They are essential for the heart to beat.

Why this experiment?

Heart disease is the leading cause of death in both men and women in the United States. This experiment provides insight on how using metabolites derived naturally from the microbiome can influence cardiac inflammation.

Procedure:

3D cardiomyocytes were derived from human induced pluripotent stem cells through the utilization of small molecules and growth factors. Quantitative PCR was performed to verify that the organoids expressed genes associated with cardiomyocyte cells. Primers for the genes FOXa2, TNNT2, MYH7, AHR, CACNA1, and KCNH2 were used for the qPCR, as these genes are expressed more prominently in cardiomyocyte cells than the undifferentiated iPSCs.



Procedure (cont.):

Cardiovascular disease was modeled by inducing inflammation in the organoids using lipopolysaccharide (LPS), a gram-negative bacteria membrane product associated with inflammation and septic shock, indoxyl-3-sulfate (I3S), which is representative of uremic toxins build-up, and poly (I:C), which simulates a viral infection. Cell viability was analyzed by a luminescence (Cell Titer Glo) assay.

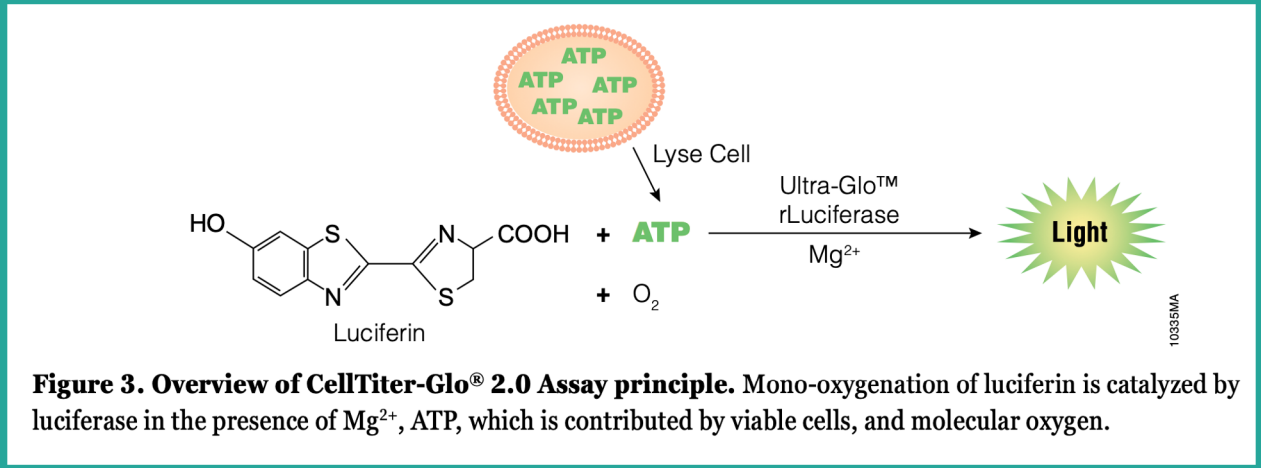
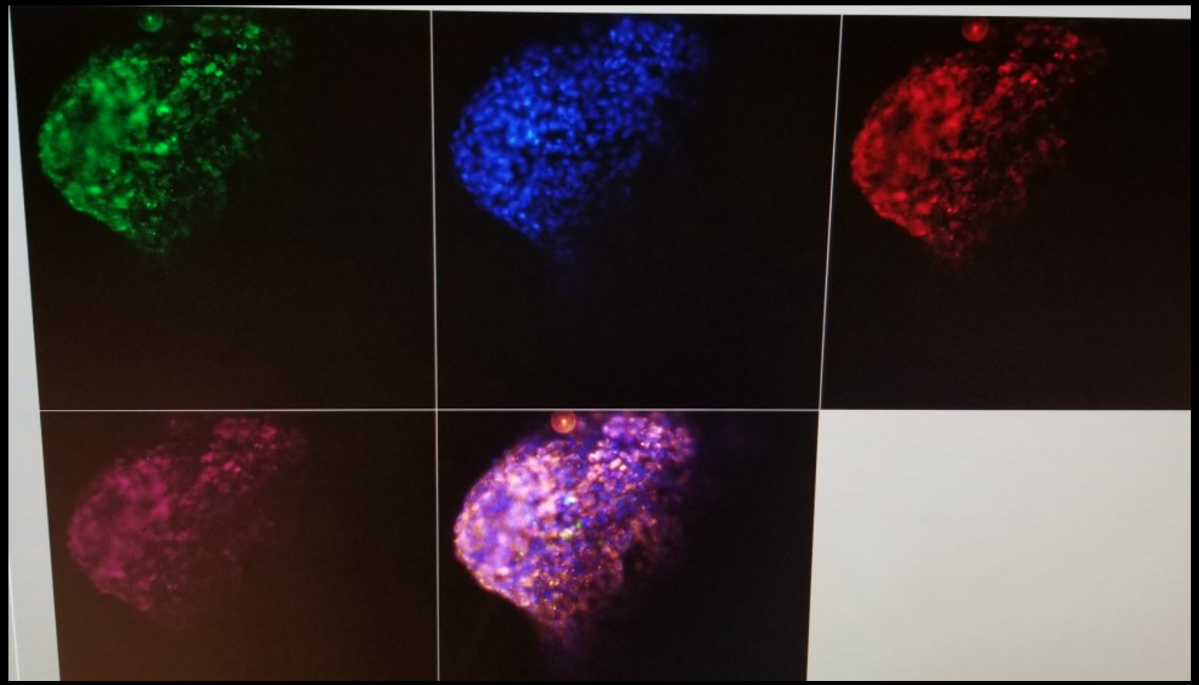


Figure 3. Overview of CellTiter-Glo® 2.0 Assay principle. Mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg^{2+}, ATP, which is contributed by viable cells, and molecular oxygen.

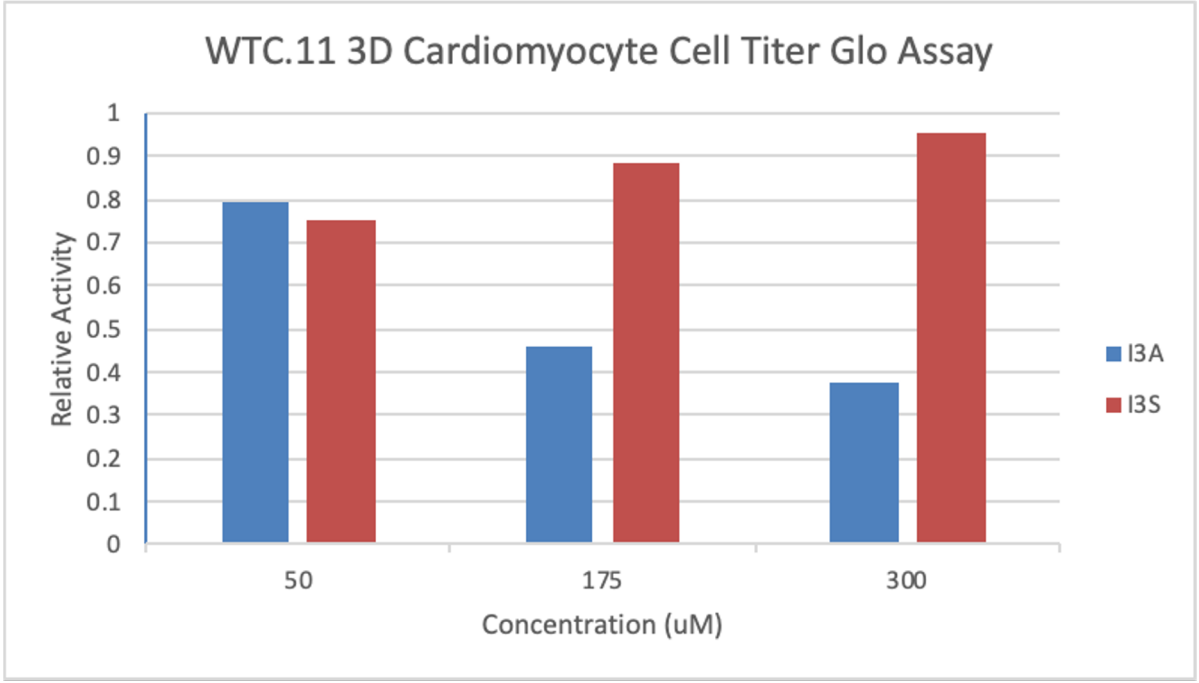
Video of Cardiomyocytes Contracting

Confocal Microscope Image

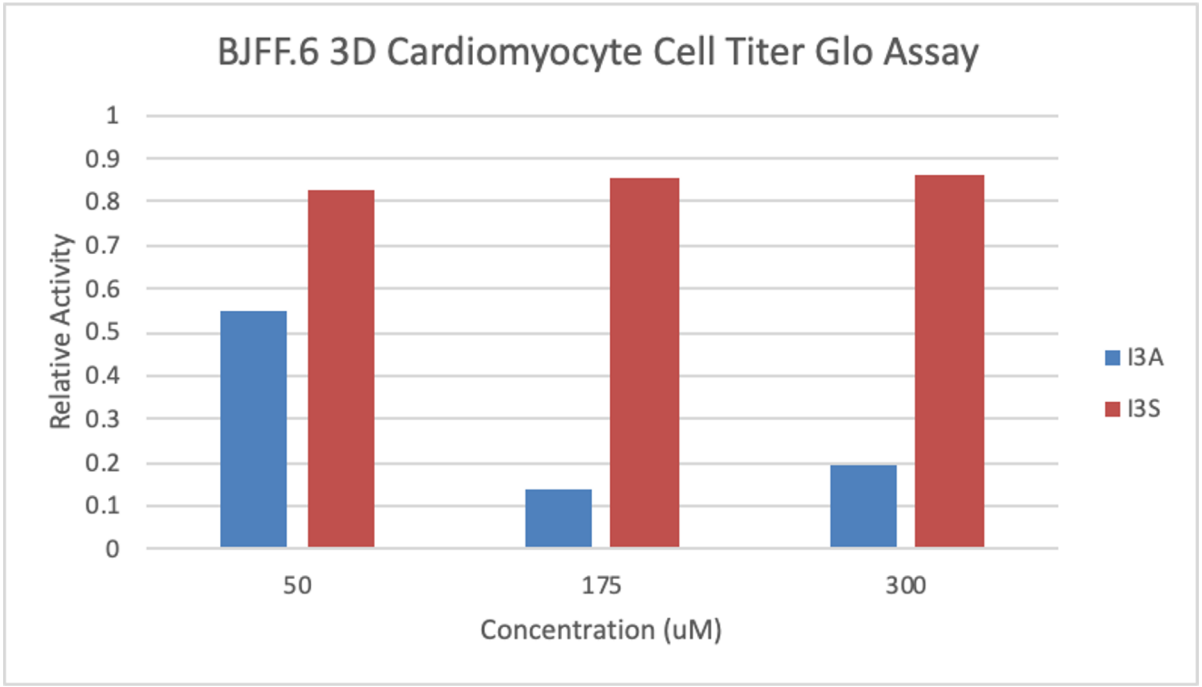


Green: GATA-6
Red: TBX5
Blue: DAPI
Magenta: NKX2.5

Results


WTC.11 3D Cardiomyocyte Cell Titer Glo Assay
Relative Activity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
50
175
300
Concentration (uM)
I3A
I3S

Results


BJFF.6 3D Cardiomyocyte Cell Titer Glo Assay
Relative Activity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
50
175
300
Concentration (uM)
I3A
I3S

Discussion

We have shown that it is possible to create 3D cardiomyocytes utilizing iPSCs. The qPCR results showed increased expression of all the cardiac markers (FOXa2, TNNT2, MYH7, AHR, CACNA1, and KCNH2) compared to the undifferentiated iPSCs.

The cardiomyocytes were killed when treated with indole-3-acetate (I3A). When performing the Cell Titer Glo Assays, both the WTC.11 and BJFF.6 cardiomyocytes were killed in the presence of increasing concentrations of I3A. Some of the data of the Cell Titer Glo Assays deviated from expected values due to our inability to ensure equal amounts of cells in every well. Without the ability to do this, it may appear that treating the BJFF.6 with 300 μM of I3A did not kill the 3D cardiomyocytes as effectively as the 150 μM. This may have resulted from there being more cells in the 300 μM well to begin with. This is an important finding because I3A concentrations have been shown to be higher in patients with chronic kidney disease, which is associated with cardiovascular issues.

This experiment showed the ability to effectively differentiate cardiomyocytes from human iPSCs and addressed the role of the microbiome in cardiac inflammation.

Acknowledgements

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References

Branco, M. A., Cotovio, J. P., Rodrigues, C. A., Vaz, S. H., Fernandes, T. G., Moreira, L. M., ... & Diogo, M. M. (2019). Transcriptomic analysis of 3D cardiac differentiation of human induced pluripotent stem cells reveals faster cardiomyocyte maturation compared to 2D culture. *Scientific reports*, *9*(1), 1-13.

CellTiter-Glo® 2.0 Cell Viability Assay Technical Manual, Promega Corporation.