PROBING THE INTEGRATION BETWEEN THREE-DIMENSIONAL "SPARK-CELL" SPHEROIDS AND HUMAN CARDIAC SYNCYTIA

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INTRODUCTION

Three-dimensional "spark-cell" assemblies were obtained using opto-genetically-transformed live cells to enable remote control of heart tissue by light. Such structures can be utilized as effective optical pacemakers in several high-throughput applications, including drug screening, cardiotoxicity testing, and maturation of stem-cell technology towards personalized medicine. This study follows from prior developments by our group based on the "tandem-cell-unit" (TCU) approach. When light-sensitized non-excitable cells, e.g. ChR2-HEKs, are put into contact with excitable cells, such as human stem-cell derived cardiomyocytes (hiPS-CMs) *in vitro*, light-mediated changes in ChR2-HEK membrane potential can elicit action potentials (APs) in the non-modified cardiomyocytes. Confining these "spark-cells" into easy-to-handle 3D structures is desirable for future automation of the process.

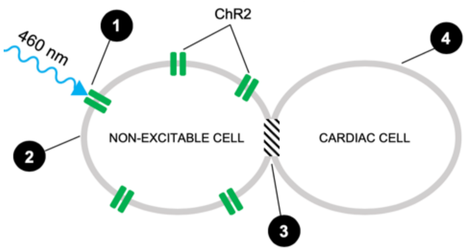


Figure 1 | TCU approach. (1) Blue light induces conformational change in ChR2 expressed in a non-excitable cell resulting in (2) an elevated membrane potential in the non-excitable cell. Because there exists (3) some form of coupling between the non-excitable and cardiac cell, (4) the system evokes an action potential and thus contraction in the cardiomyocyte.

3D "SPARK-CELL" SPHEROID ASSEMBLY

- ***Seeding:*** ChR2-expressing 293T HEK cells were seeded at multiple densities (from 2 x 10^4 cells/well to 10 x 10^4 cells/well) onto 96-well microplates designed to deter cell attachment to surfaces and promote spheroid formation.
- ***Imaging:*** Spheroids were imaged every 24 hours under bright-field and fluorescence to quantify shape and size over time.
- ***Growth:*** Medium was replaced following imaging using a 50/50 approach.
- ***Handling:*** Standard micropipettors and wide-mouthed tips were used to lift and deposit spheroids onto monolayers of iPS-CMs.
- ***Functional Testing:*** Blue light pulses (460nm) were applied at different frequency for optical pacing. Genetically encoded calcium indicators and all-optical electrophysiology were used in conjunction with the optogenetic stimulation to register the response of the hiPS-CMs.

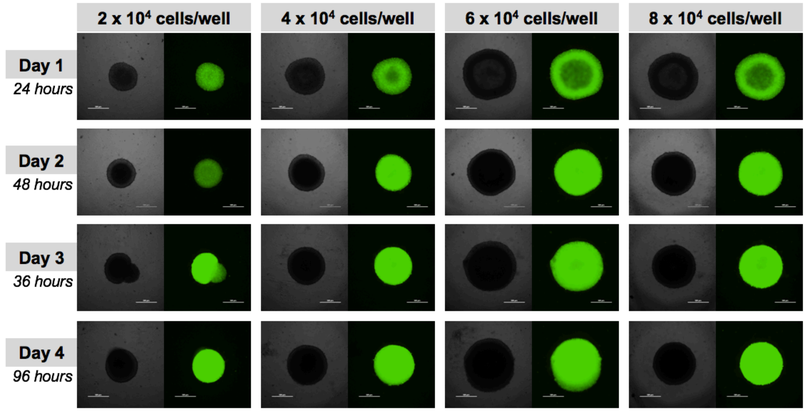


Figure 2 | Sample set of spheroids. Shown are assembled constructs seeded from 2 x 10^4 cells/well to 8 x 10^4 cells per well in increments of 2 x 10^4 cells/well (left to right) imaged under brightfield and fluorescence over 4 days (top to bottom).

CHARACTERIZATION OF CELL SPHEROIDS

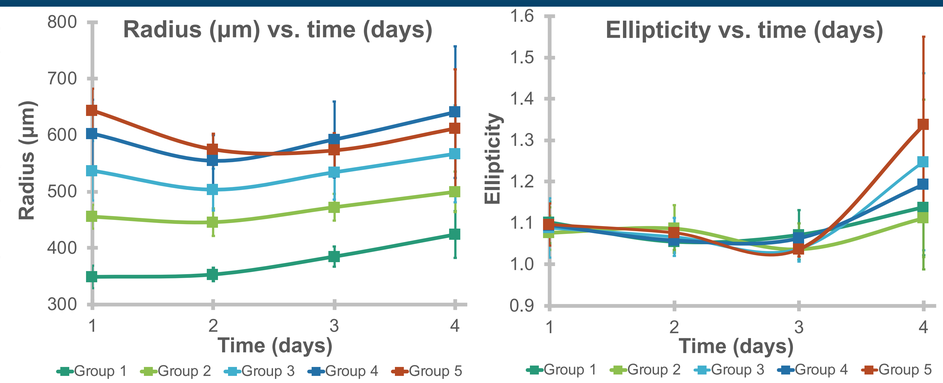
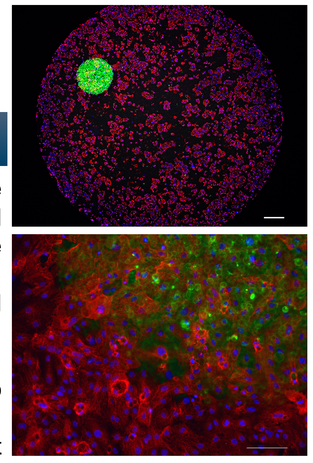


Figure 3 | HEK-cell spheroid measurement data. We quantified size and shape of HEK-cell spheroids as functions of time. Left: size (major axis/2) as a function of time; right: shape (major axis/minor axis) as a function of time. Sample size n = 6 for each point on the plot.

SPHEROID INTEGRATION WITH CARDIAC SYNCYTIA



- Monolayers of hiPS-CMs were transfected with a red-shifted genetically-encoded calcium indicator (R-GECO) 4 days post-thaw and 2 days before functional tests.
- ChR2 293T spheroids, in culture for 24 hours at seeding density 2 x 10^4 cells/well, were deposited onto the cardiac tissue 6 days after the hiPS-CMs were thawed.
- To investigate general structure of the system, allocated samples were labeled with fluorescent dyes to construct the three-dimensional image shown to the left.
  - *Alpha-actinin* (red) identifies the actin filaments in the sarcomeres of the myocytes.
  - *Hoechst* (blue) labels the cell nuclei.
  - *eYFP* (green) reporter identifies the expression of ChR2 which is localized in the HEK cell membranes.

Figure 4 | Spheroid on stained monolayer of iPS-CMs. Top: scalebar 0.5mm; bottom: higher magnification, scalebar 0.1mm.

SPHEROID-MEDIATED OPTICAL STIMULATION

- All-optical electrophysiology (OptoDyCE) was used for functional experiments within 12 hours after the spheroid was seeded on top of the hiPS-CM.
- Blue light (20ms at 460 nm, <1mW/mm^2) pulsed at frequencies between 0.5-2.0 Hz was used to trigger APs and thus contractions in the cardiac tissue.
- Genetically-encoded calcium sensor R-GECO was used to record responses by an EMCCD camera.
- A 1:1 relationship between blue light pulses and calcium transients was observed in successfully-paced samples.

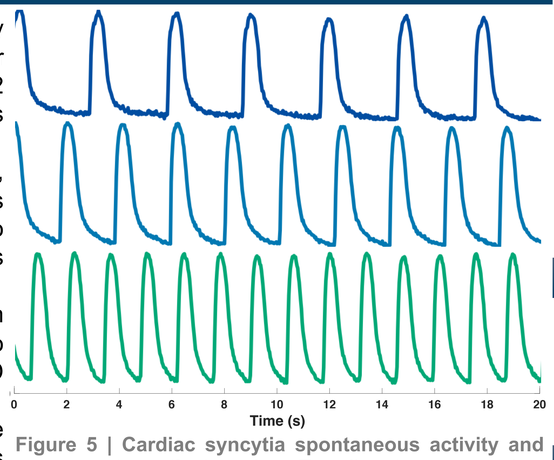


Figure 5 | Cardiac syncytia spontaneous activity and response to optical pacing. Top: Spontaneous calcium transients, middle: optical pacing blue light pulses at 0.5 Hz; bottom: optical pacing blue light pulses at 0.75 Hz.

PROBING SPHEROID/SYNCYTIA COUPLING

- Optical stimulation to modulate cardiac rhythm has distinct advantages over electrical stimulation, including scalability, cost-effectiveness and easy deployment in high-throughput drug testing applications.
- However, optogenetics-based stimulation methods require at least a 48-hour timeline to allow for expression of light-sensitive proteins.
- Hence, commercial attraction exists for a robust solution for optical stimulation that significantly reduces the timeframe within which such pacing is possible.
- Using the functional experimental protocol previously discussed, we set out to register the emergence of TCU-based coupling by probing a set of 18 x iPS-CM samples with ChR2-HEK spheroids over 10 hours.

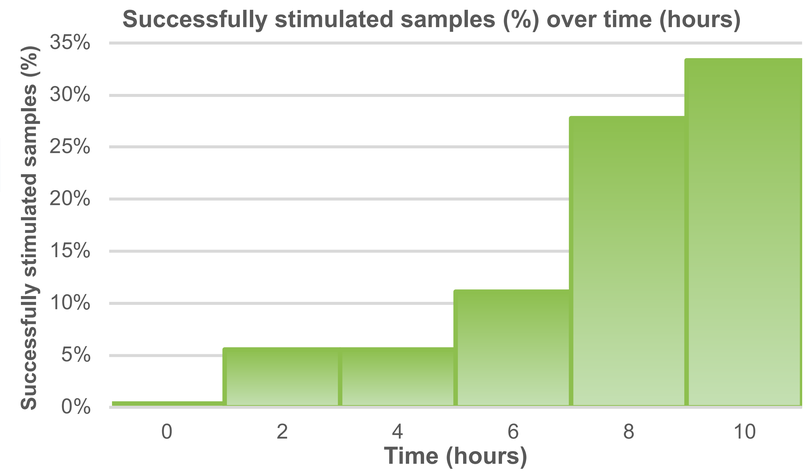


Figure 6 | Probing the emergence of TCU coupling between ChR2-HEK spheroids and iPS-CM syncytia over time. A total of 18 samples were tested and the percentage of those samples from which we observed response was plotted at time probes spaced 2 hours apart. These data indicate a preliminary run of the experiment.

CONCLUSIONS

- We demonstrate that three-dimensional "spark-cell" spheroids can confer cardiac optical pacing within a shorter time frame compared to direct optogenetic transformation of cardiomyocytes via plasmid transfection or viral infection, which require at minimum 48 hours.
- Our group has pursued several pathways to increase the attractiveness of our system such as attempting the method with cardiac progenitor cells to achieve greater biocompatibility and further reducing the integration timeline by incorporating nanomagnetic particle technology.
- Because our spheroids are easily handled and transferrable, they are compatible with robotics and automation. Overall, we envision our work to improve high-throughput pharmacological testing in cardiomyocytes.

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