Modelling Atrial Arrhythmia In vitro Using Pluripotent Stem Cell-derived Atrial Cardiomyocytes in Three-dimensional Culture

Yuexin Yu1,2, KokLeng Tan1, Bakiah Shaharuddin1, Zhikun Guo2 and Jun Jie Tan1,*

This refers to the article “Generating Ring-Shaped Engineered Heart Tissues from Ventricular and Atrial Human Pluripotent Stem Cell-Derived Cardiomyocytes”, by Goldfracht et al. (2020), doi: 10.1038/s41467-019-13868-x

Atrial fibrillation (AF) is the most common form of arrhythmias characterized by uncontrolled, rapid atrial contractions that can lead to atrial stunning, embolic stroke and heart failure. Anti-arrhythmic pharmacotherapy remains the first line of treatment for AF, but the approach is often challenged by the risk of inducing fatal ventricular tachyarrhythmias [1]. Experimental models ranging from in vivo, in vitro to in silico have been used for studying arrhythmogenesis, or for drug testing and discovery. However, the interpretation of findings from in vivo animal models can be complicated by significant inter-subject biological variations and differences between species. As for the most extensively used in silico model, despite its cost-effectiveness and high reproducibility that enable rapid testing, the model would still need validation in a biological system [2].

Successful creation of human-induced pluripotent stem cells (iPSCs) using cell reprogramming technology offers a new in vitro model using human atrial cells. Atrial differentiation can be directed from iPSCs based on the important timeline and signalling cues that are involved in embryonic development in vivo. Most protocols employed the generation of the embryoid body from iPSCs followed by activation with bone morphogenic protein-4 (BMP4) and activin A signalling to drive the mesodermal formation and direct cardiac atrial fate via the subsequent activation of retinoic acid signalling [3, 4]. These differentiated cells are more representative of human atrial electrophysiology and can easily be extrapolated to clinical use as compared to the previously used animal cells isolated from primary atrial tissue or immortalized animal atrial cardiomyocytes [5]. The iPSC-derived atrial cell model has first been used to demonstrate the increase in the distribution of $I_{\text{f}}$ and $I_{\text{CaL}}$ current in patients with an inherited form of AF [6].

Studies have shown that differentiated human iPSC-derived atrial cells expressed higher atrial-specific genes and proteins in three-dimensional engineered heart tissue (EHT) as compared to monolayer culture, while possessing contraction kinetic, action potential, response to atrial-selective, acetylcholine-regulated potassium current $I_{\text{kACh}}$ similar to the atrial heart muscle [7]. Most recently, Goldfracht and colleagues demonstrated the use of a ring-shaped, atrial EHT (EHTAtria) as a model to study re-entrant arrhythmias [8], in addition to the successful generation of ventricular EHT as a comparator in their study. In their study, the EHTAtria was engineered mainly from collagen and two million atrial cardiomyocytes differentiated from the HES3-Nkx2.5egfp/cre reporter human embryonic stem cell line, with an efficiency of 82% expressing both eGFP (indicative of Nkx2-5 expression) and cardiac troponin T (cTnT). Of those, less than 5% were MLC2v+, the ventricular isoform of the myosin light chain 2. The EHTAtria also revealed sarcolipin expression in addition to significant upregulation of multiple atrial-specific genes including protein connexin-40 ($GJA5$), potassium voltage-gated channel subfamily A member 5 ($KCNA5$), potassium inwardly-rectifying channel subfamily J member 3 ($KCNJ3$), atrial natriuretic peptide ($NPPA$), myosin regulatory light chain 2

1Regenerative Medicine Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, Penang, Malaysia
2Henan Key Laboratory of Medical Tissue Regeneration, Xinxiang Medical University, Henan, China

*Corresponding author:
Jun Jie Tan,
E-mail: jjtan@usm.my

Received: April 22 2020
Revised: June 6 2020
Accepted: June 11 2020
Published Online: August 3 2020

Available at: https://bio-integration.org/
spiral-wave loops around the EHT atria. These arrhythmias reported a success rate of 86.6% in converting EHT atria into its effectiveness in treating acute AF. In the experiment, they and the relatively novel drug vernakalant which is known for two anti-arrhythmic agents, the well-established flecainide EHT atria arrhythmias by field stimulation, as well as by using two anti-arrhythmic agents, the well-established flecainide and the relatively novel drug vernakalant which is known for its effectiveness in treating acute AF. In the experiment, they reported a success rate of 86.6% in converting EHT atria into a normal rhythm by field stimulation, followed by 77.7% using 10 μM flecainide and 52.9% with 30 μM vernakalant. Interestingly, the arrhythmogenic activity recurred in more than 80% atrial-EHTs spontaneously within 15 min after field stimulation, 41% of which exhibited different patterns of the re-entrant arrhythmias as compared to the original arrhythmic pattern. However, all flecainide-treated EHT atria remained in normal rhythm while only 23% of vernakalant-treated EHT atria resumed arrhythmias with a re-entry pattern similar to that of the original.

Notably, the amino-cyclohexyl ether, class III (recently defined as class I by the US Food and Drug Administration, FDA) anti-arrhythmic drug vernakalant was recently found to be a non-selective multichannel blocker that is not atrial-specific, and possibly cause of several severe adverse effects including hypotension, arrhythmias, bradycardia and death [9]. The effect of the drug in depressing ventricular dV/dt\textsubscript{max} was also previously reported in an in vitro model using atrial cells derived from human embryonic stem cells [3]. On the contrary, Goldfracht et al. did not observe significant changes in the action potential duration of ventricular EHT following vernakalant treatment, possibly owing to the immaturity of the ventricular EHT.

Taken together, this study involves multidisciplinary integration using advances in developmental and stem cell biology, reprogramming technology and tissue-engineering strategy in creating ventricular and atrial-specific EHT. The proposed atrial-EHT arrhythmia model can serve as a useful drug-testing platform to determine antiarrhythmic drug efficacy, its potential in preventing its recurrence, as well as studying novel drug safety and specificity. With the use of iPSCs, personalized EHT atria can be created for precision screening to tailor the most efficacious and safe pharmacological regimen for individual patients. Further studies to address the immaturity of EHT atria, either by introducing hormones [10], extracellular matrix [11], biophysical stimulation [12] or by metabolic modulation [13] (see review [14]), in order to acquire adult-like atrial construct with ionic fingerprint similar to the mature human atrium, are necessary to increase the reliability and accuracy in interpreting the drug response and predicting potential side effects prior to in vivo testing.

### References


### Acknowledgement

JJT is a recipient of the Fundamental Research Grant Scheme from the Ministry of Higher Education Malaysia (203.CIPT.6711640) and Universiti Sains Malaysia RUI Grant (1001.CIPT.8011102).

### Disclosure

JJT received grants from CryoCord Sdn Bhd and ALPS Global Holding. Others declare no competing interest.


