

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

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**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  $I_f$  and  $I_{CaL}$  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  $I_{k,Ach}$  similar to the atrial heart muscle [7]. Most recently, Goldfracht and colleagues demonstrated the use of a ring-shaped, atrial EHT (EHT<sup>Atria</sup>) 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 EHT<sup>Atria</sup> was engineered mainly from collagen and two million atrial cardiomyocytes differentiated from the HES3-Nkx2-5<sup>egfp/w</sup> 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<sup>+</sup>, the ventricular isoform of the myosin light chain 2. The EHT<sup>Atria</sup> 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

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(MYL7) and nuclear receptor subfamily 2 group F member 2 (*NR2F2*), confirming its atrial identity.

While assessing the electrophysiological attribute of EHT<sup>Atria</sup>, they observed different types of rhythm disarray at baseline, most of which were re-entrant waves of varying complexity, ranging from large single circular to multiple spiral-wave loops around the EHT<sup>Atria</sup>. These arrhythmias could be corrected by applying electrical shock with prolonged field stimulation pulses. They further evaluated the EHT<sup>Atria</sup> for its usefulness as an AF-like model for drug screening and testing, by examining the ability to reverse EHT<sup>Atria</sup> 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<sup>Atria</sup> into a normal rhythm by field stimulation, followed by 77.7% using 10  $\mu$ M flecainide and 52.9% with 30  $\mu$ M 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<sup>Atria</sup> remained in normal rhythm while only 23% of vernakalant-treated EHT<sup>Atria</sup> 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  $dV/dt_{max}$  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<sup>Atria</sup> 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<sup>Atria</sup>, 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.

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## Disclosure

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