In vitro model of atrial fibrillation: investigating the initiation and maintenance mechanisms of atrial remodeling using hiPSC-derived atrial cardiomyocytes

M. Casini¹, C. Fambuena Santos², R. Emig³, R. Peyronnet³, U. Ravens³, I. Ontoria Oviedo¹, A. Climent², P. Sepulveda¹

¹Hospital Universitario y Politecnico La Fe, Valencia, Spain
²Polytechnic University of Valencia, Valencia, Spain
³University Heart Center Freiburg-Bad Krozingen, Freiburg, Germany

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Despite atrial fibrillation (AF) being a common, clinically important, and economically significant public health problem, the initiation mechanisms of atrial remodeling during AF is still unclear. Furthermore, really few experimental models have the power to investigate these mechanisms in a human invitro two-dimensional (2D) monolayer of atrial cardiomyocytes.

The purpose of this work is to use human induced pluripotent stem cell (hiPSC)-derived atrial cardiomyocytes to develop a new human in vitro atrial model of AF able to reproduce and evaluate the arrhythmia initiation and maintenance mechanisms.

Two different hiPSC lines were differentiated into atrial cardiomyocytes using 1 µM retinoic acid from day 3 to day 8. Protocol to differentiate atrial cells was compared with the classical method to generate ventricular hiPSC to demonstrate atrial speciﬁcity. On day 20 of the differentiation cells were seeded into plates with different sizes: 0.32 cm² and 9.6 cm², a larger size that allows rotor formation. After 10 days qPCR and optical mapping experiments were performed at 37°C. These experiments aimed to evaluate the electrophysiologic and genetic remodeling the

Cells undergo during AF initiation and maintenance.

The atrial phenotype was assessed by patch clamp recordings that showed cells treated with RA had significantly shorter action potential duration at 90% repolarization compared to the no RA treated, 182.3.5 ± 68 ms and 371.6 ± 82 ms, faster depolarization rate and higher spontaneous beating frequency (n=14, ± SD). Optical mapping experiments allowed the evaluation of activation rate depending on the size of the dish: larger wells activated spontaneously at 1.6 ± 1.9 Hz, whereas smaller wells presented 0.9 ± 0.5 Hz of activation rate. The fast activation rate on larger wells was associated higher number of reentrant wavefronts: 11.0 ± 8.2 reentries/cm², vs 0.9 ± 0.5 reentries/cm² in the smaller wells, demonstrating an AF phenotype. In fact, depending on the culture conditions cells showed different reentries occurrence: 100% of large dishes exhibited fibrillation compared to only 10% of small dishes. Furthermore, qPCR studies demonstrated that the spontaneous initiation and maintenance of rotors in the larger well sizes lead to an expression remodeling of the channels SCN5A, KCNJ2, GJA5, ATP2A2, and RYR2 as previously demonstrated on isolated tissue from AF patients.

We conclude that atrial cardiomyocytes obtained from hiPSC can recapitulate the remodeling that atrial tissue undergoes during AF initiation in patients. Providing a unique opportunity to study the initiation of fibrillatory activities and their maintenance in a human-relevant in-vitro model.
Electrophysiologic & genetic remodeling

**Electrophysiologic remodeling**

- **Disturbance Frequency**
  - **Control** vs. **AF**
  - Significance: 0.0040
- **Phase Shift Angle (°)**
  - **Control** vs. **AF**
  - Significance: 0.0125
- **Rhythm Period (s)**
  - **Control** vs. **AF**
  - Significance: 0.0020

**Gene expression remodeling**

- **SCN5A**
  - Relative Expression
  - **Control** vs. **AF**
  - Significance: 0.0033
- **KCNJ2**
  - Relative Expression
  - **Control** vs. **AF**
  - Significance: 0.0000
- **GJA5**
  - Relative Expression
  - **Control** vs. **AF**
  - Significance: 0.0049
- **ATP2A2**
  - Relative Expression
  - **Control** vs. **AF**
  - Significance: 0.0038
- **RYR2**
  - Relative Expression
  - **Control** vs. **AF**
  - Significance: 0.0000

*Control = small dishes, AF = large dishes*