Basic Science - Arrhythmias

# Modelling of atrial fibrillation at physiologically relevant scales enabled by massive expansion of native human atrial cardiomyocytes

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**Background:** Current in vitro models of atrial fibrillation have limited translational potential due to a lack of relevant human physiology or the inability to reach the high activation frequencies present in human atrial fibrillation. Absence of relevant models is the result of a general deficit of readily available and standardized sources of well-differentiated human atrial cardiomyocytes. Therefore, we aimed to immortalize native human atrial cardiomyocytes to produce natural and standardized lines of these cells.

**Methods:** Human fetal atrial cardiomyocytes were transduced with a lentiviral vector directing myocyte-specific and doxycycline-inducible expression of simian virus 40 large T antigen. Addition of doxycycline to the culture medium pushed cardiomyocytes towards a highly proliferative phenotype (proliferation up to 10^12 cells). These cells were labelled hiAMs (human immortalised Atrial Myocytes). After differentiation upon doxycycline removal, hiAM cells were characterized using various molecular, biological and electrophysiological assays.

**Results:** Following cardiomyogenic differentiation, hiAMs no longer expressed the proliferation marker Ki67, revealed striated α-actinin and troponin T staining patterns and displayed synchronous contractions. Optical voltage mapping of hiAM monolayers revealed excitable cells showing homogeneous spreading of action potentials at 22.5 ± 3.1 cm/s with a mean APD80 of 139 ± 22 ms. Addition of flecainide (10 μM) to hiAM monolayers decreased the conduction velocity by 35% and increased the APD80 by 107%. Dofetilide (10 nM) addition had no effect on the conduction velocity, but did increase the APD80 by 81%. Due to their scalability, monolayers of hiAMs as big as 10 cm2 showing homogenous action potential propagation could easily be created. Following high-frequency electrical pacing, rotors could be induced with an average activation frequency of 7.5 ± 0.9 Hz. Infusion of flecainide during arrhythmic activity resulted in termination of the rotor in 18 of 24 attempts (75%), whereas addition of 0.1% DMSO (vehicle control) did not result in termination in any of the attempts. Dofetilide infusion did not result in termination. However, it did lower the average activation frequency to 2.1 ± 0.7 Hz.

**Conclusion:** We have generated first-of-a-kind lines of human atrial cardiomyocytes, allowing massive cell expansion under proliferation conditions and robust formation of cross-striated, contractile and excitable cardiomyocytes after differentiation. These characteristics allow, for the first time, the modelling, at a large-scale, of human atrial arrhythmias with frequencies similar to human atrial fibrillation. With the generation of hiAMs, a user-friendly, clinically-relevant and much-anticipated human atrial research model has been produced.

Abstract Figure. hiAM AF Model