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Development of Biophysically Detailed Electrophysiological Models for Pacemaking and Non-Pacemaking Human Pulmonary Vein Cardiomyocytes

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Abstract— **Ectopic foci originating from the pulmonary veins (PVs) have been suggested as the underlying cause for generating atrial arrhythmias that include atrial fibrillation (AF). Recent experimental findings indicate two types of PV cells: pacemaking and non-pacemaking. In this study, we have developed two mathematical models for human PV cardiomyocytes with and without pacemaking activities. The models were reconstructed by modifying an existing model of the human right atrium to incorporate extant experimental data on the electrical differences between the two cell types. Differences in their action potential (AP) profiles and automaticity were reproduced by the models, which can be attributed to the observed differences in the current densities of I_{NCX}, I_{to}, I_{Na} and $I_{Ca\text{-}L}$, as well as the difference in the channel kinetics of $I_{Ca\text{-}L}$ and inclusion of the I_f and $I_{Ca\text{-}T}$ currents in the pacemaking cells. The developed models provide a useful tool suitable for studying the substrates for generating AF.**

I. Introduction

Studies have shown that ectopic beats originating in the pulmonary vein (PV) may contribute towards the initiation [1-2] and maintenance [3] of atrial arrhythmias that include atrial fibrillation (AF). Two types of PV cells have been identified - one with pacemaking and the other without pacemaking activities [4-6]. The pacemaking PV cells can be further classified as either fast or slow pacemaking cells with the former referring to cells having a pacemaking rate faster than the primary cardiac pacemaking cells (the sinoatrial node; SAN), and the latter referring to cells having a pacemaking rate slower than the SAN cells [5,9]. PV cells with fast pacemaking rates may contribute to arrhythmogenesis [5]. Further experimental studies have also indicated that the pacemaking PV cells differ from non-pacemaking PV cells in the current densities of some ion channels that are responsible for generating cardiac action potentials (APs). These channels include the L and T-type calcium currents ($I_{Ca\text{-}L}$ and $I_{Ca\text{-}T}$), the hyperpolarization activated 'funny' current (I_f), the transient outward potassium current (I_{to}) and the sodium/calcium exchanger current (I_{NCX}) [4-7].

So far, two pacemaking currents (I_{CaT} and I_f) have been found in pacemaking PV cells [7,9]. It has been shown that expression level of $I_{Ca\text{-}T}$ in pacemaking PV cells is significantly higher than that in non-pacemaking PV cells [8]. The expression level of I_f is considerably lower in the slow pacemaking PV cells than in the fast pacemaking cells. This suggests a positive correlation between the I_f channel protein expression and the beating rate [5]. Blocking I_f by ivabradine has been found to decrease spontaneous activity of PV cells by 85% [10]. I_{NCX} has also been shown to contribute to cell automaticity as blocking I_{NCX} decreases the firing rates of PV cells [6]. Though these experimental studies provide insights towards understanding the automaticity in PV cells, the mechanisms underlying the genesis of pacemaking still remains to be elucidated.

The aim of this study was to develop mathematical models for the electrical APs of PV cells with and without pacemaking as these models may provide a suitable tool for further investigating the mechanisms for generating pacemaking activity in PV cells and the substrates responsible for the genesis of AF.

II. Method

The models for the pacemaking and non-pacemaking human PV cells were reconstructed by modifying the Courtemanche *et al.* (CRN model) [11] model of electrical action potential (AP) of human right atrium (RA) cells to incorporate available experimental data on the electrical properties of PV cells, especially their differences between the pacemaking and non-pacemaking cells. The CRN model was chosen because it has been used in several studies for simulating normal atrial electrical propagation and AF [12-14]. All current formulations were fit to experimental data following the same approach as used for modelling cardiac cells [11].

A. Model for Non-Pacemaking PV cells

Experimental data on the ionic channel currents of I_{Kr}, I_{Ks}, I_{K1}, I_{to}, I_{Na} and $I_{Ca\text{-}L}$ [15-17] of PV cells were used for

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modifying the CRN model. In the model development, voltage clamp experiments following the same protocols as used in these experimental studies were implemented to derive parameters for the I_{Kr}, I_{Ks}, I_{K1}, I_{to}, I_{Na} and $I_{Ca\text{-}L}$ model equations. The derived parameters for each of the current equations were validated by matching the simulated current-voltage (I-V) relationships to respective experimental data. As the CRN model is for a RA cell, a scaling factor of 1.6 to the I_{Kr} channel conductance was implemented to convert it to a left atrium (LA) cell as observed in a previous experimental study [18] before the modified equations for I_{Kr}, I_{Ks}, I_{K1}, I_{to}, I_{Na} and $I_{Ca\text{-}L}$ were integrated into the CRN model, to produce a PV model.

Fig. 1 shows ratios of the current densities of the individual ionic currents between the LA and PV cells calculated from the model, which match to the experimental data of [15-17].

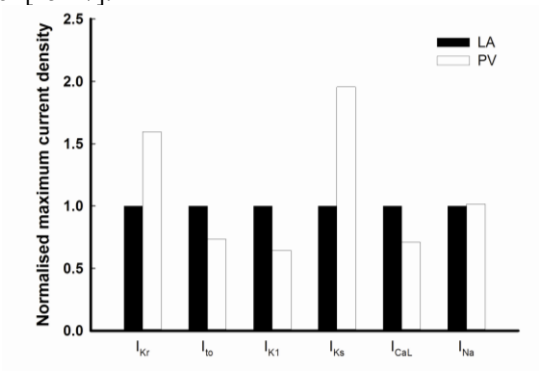

Figure 1. Ionic channel heterogeneity between LA and PV cells. Ratios in the maximum current densities between the LA *(black)* and PV *(white)* cells.

B. Model of Pacemaking PV cells

1. $I_{Ca\text{-}L}$, I_{Na}, I_{NCX}

Based on experimental observation [4], the current formulations of $I_{Ca\text{-}L}$, I_{Na} and I_{NCX} from the CRN model were adjusted for pacemaking PV cells by adjusting the parameters in the voltage clamp simulations. The maximal $I_{Ca\text{-}L}$, I_{Na} and I_{NCX} current densities were scaled to pacemaking PV cells according to the ratios shown in Fig. 2 as suggested by experimental data [4].

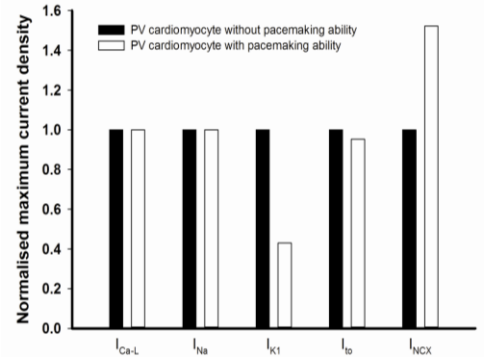

Figure 2. Differences in the maximum current densities in the myocytes with *(black)* and without *(white)* pacemaker ability in PV cells, normalised to the values in the non-pacemaking cells.

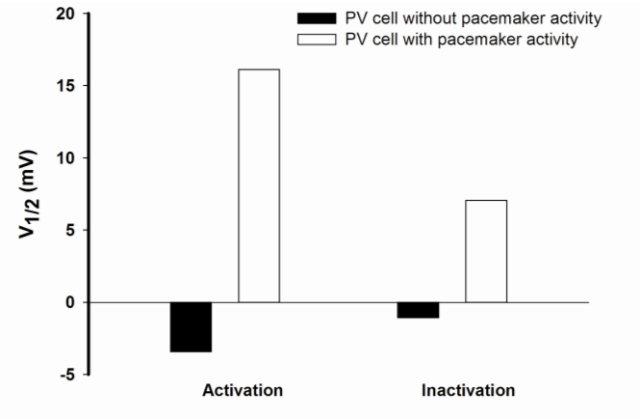

Figure 3. Values for half-activation and half-inactivation ($V_{1/2}$) curves for pacemaking (white) and non- pacemaking (black) PV cells.

Fig. 3 shows the $I_{Ca\text{-}L}$ half maximal activation voltage ($V_{1/2}$) for $I_{Ca\text{-}L}$ for the pacemaking and non-pacemaking PV cells.

2. I_{to}

The formulation for I_{to} in the CRN model was fitted to the data taken from fast pacemaking PV cells. The parameters in the equations were adjusted in order to have the simulated I-V relationship matched the experimental data [19]. The current was scaled by the ratio shown in Fig. 2.

3. I_f and $I_{Ca\text{-}T}$

I_f and $I_{Ca\text{-}T}$ are absent in the original CRN model. We incorporated these two currents in the modified models for the pacemaking PV cells based on experimental data [7, 9].The formulations for these currents were taken from [19].

4. I_{K1}

I_{K1} has less expression in pacemaking PV cells as compared to the non-pacemaking cells as shown in Fig. 2. The formulation for I_{K1} was fitted to experimental data from [12].

III. Results

Fig. 4 shows the simulated APs for pacemaking and non-pacemaking PV cells. The computed APD_{90} of the non-pacemaking model is 94ms and that of the pacemaking model is 129ms. The difference in APD_{90} between the two models can be explained theoretically by a smaller I_{K1} in the pacemaking model which prolongs the action potential [18].

In the pacemaking PV cell model, spontaneous action potentials were generated. However, in the non-pacemaking PV cell model, a stable resting potential at -60 mV was observed without an external stimulus (Fig. 5A).

We investigated the effect of the basic cycle length (BCL) on the pacemaking frequency and found a positive correlation (Fig. 5). With the BCL changed from 1000ms to 500ms and 300ms, the measured pacemaking frequency changed from 0.91Hz to 0.87Hz and 0.78Hz respectively.

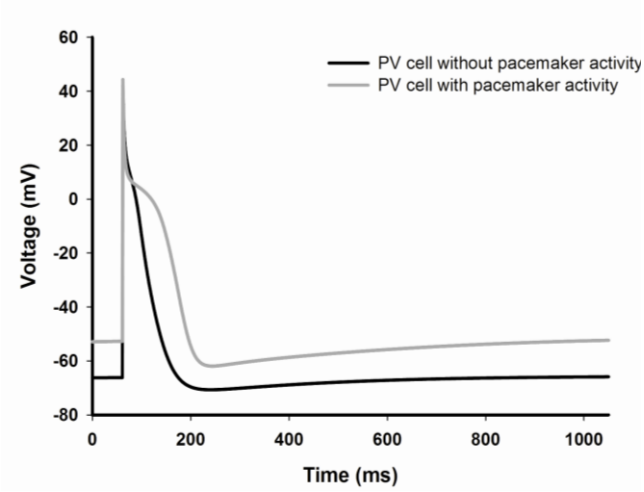

Figure 4. Action potential from the pacemaking (grey) and non-pacemaking (black) PV cell models. The pacemaking cell has a less negative resting potential and gradually depolarises during the diastolic phase. The resting potential in the non pacemaking cell remains stable at -60mV.

IV. Discussion

We have developed two single cell models for human PV cells: a pacemaking PV cell model and a non-pacemaking PV cell model. Both are based on experimental data on the expression of ionic currents [4,7,9,15-17]. The models demonstrate that inclusion of pacemaking currents can enable the cell to generate spontaneous action potentials without the need for an external stimulus. Removal of either of the currents $I_{Ca\text{-}T}$ and I_f prevented the cell from exhibiting spontaneous activity. The cell model also ceased pacemaking when the values of the half-activation and half-inactivation curves of $I_{Ca\text{-}L}$ were the same as used for non-pacemaking cell model. However when the updated I_{to}, I_{Na}, and I_{NCX} were replaced with their counterparts from non-pacemaking cells, the cell still exhibited pacemaking activity.

The difference in $I_{Ca\text{-}L}$ is found to be key to the pacemaking ability of the pacemaking PV cell model. This is in accordance with experimental results which have found $I_{Ca\text{-}L}$ to be crucial to the pacemaking ability of cells where it plays a key role during depolarization [20,21]. The model also shows the necessity of both I_f and $I_{Ca\text{-}T}$ for pacemaking ability. $I_{Ca\text{-}T}$ has been found in every cell exhibiting automaticity, possibly due to a relation between $I_{Ca\text{-}T}$ and the intracellular calcium cycle driving spontaneous depolarizations but this has not yet been validated [22].

I_f has also been shown as integral to automaticity in the PV cells as well as in the SAN and AVN cells [23]. The exact source of the ability for spontaneous depolarisation is still a debated issue. Whether the "calcium clock", funny current or the NCX are responsible for cardiac pacemaking is not fully clear [24]. Our model clearly shows an important role of I_f and $I_{Ca\text{-}T}$ in pacemaking.

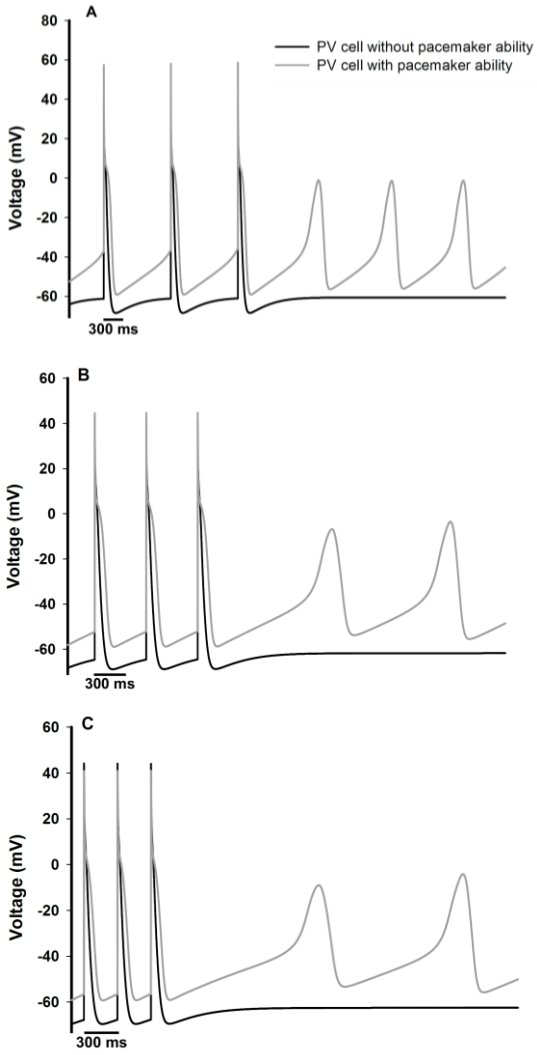

Figure 5. Automaticity and non-automaticity in the pacemaking cells, with varying BCL. Variation of membrane potential when a stimulus is applied for 30 beats. A) BCL = 1000ms, pacemaking frequency = 0.91Hz, B) BCL = 500ms, pacemaking frequency = 0.87Hz, B) BCL = 300ms, pacemaking frequency = 0.78Hz

Our model has also shown an integral action of a number of currents responsible for automaticity. $I_{Ca\text{-}L}$, $I_{Ca\text{-}T}$ and I_f have been shown to be integral to pacemaking whereas
dependence on I_{to}, I_{Na} and I_{NCX} was not shown. Heterogeneities in expression of I_{to} and I_{Na} between pacemaking and non-pacemaking myocytes was not found to be crucial to pacemaking ability.

Although $I_{Ca\text{-}T}$ has been detected in numerous animal studies, its presence in the human PV is yet to be validated [25]. A decrease in current density with the size of animal hearts has been seen. Hence, further research is required into $I_{Ca\text{-}T}$'s role in automaticity of human myocytes [25].

Stretch activated currents (SAC) have been detected in the PV cells [26]. When these SACs are blocked spontaneous activity of the stretched vein drops significantly [27].

Stretching of veins may be age related and these currents could play a role in the positive increase of cases of

AF with age [28]. Incorporation of these SACs in the model to view their overall impact on arrhythmogenesis would be of great interest.

In conclusion, we have developed two novel cell models for simulating the electrical action potential of PV cells, with and without pacemaking activity. As of this writing, there is no extant mathematical model that simulates pacemaking activities in the human PV cells. These models add two new members to the family of biophysically detailed computer models of human cardiac cells, which can be incorporated into the anatomical structure of the heart to form a virtual whole heart.

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