Microscopic Modelling of the Non-Linear Gap Junction Channels

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Abstract

The usual way to model the propagation of the action potential through the cardiac tissue is to assume passive diffusive intracellular and extracellular domains, and ion channel dynamics on the cells' membrane. Gap junctions (GJ) are localised clusters of gap junction channels (GJCs) that connects electrically adjacent cells. The importance of GJCs and their modifications in the signal propagation has been demonstrated in the experimental studies (e.g. Beauchamp et al 2012). But, in the current mathematical models the behaviour of the GJCs is either neglected or assumed to be passive, i.e the conductance of GJCs is taken as a steady constant. On the other hand, the experimental results, obtained by the dual-voltage clamp technique, show that GJCs are time and voltage dependent. Here we focus on describing ventricular GJCs made of connexin Cx43 and Cx45. We use the Hodgkin-Huxley formalism to describe GJC conductance via one gating variable. We incorporate the non-linear GJC voltage dependence into the microscopic model of the tissue as a new boundary condition on specific parts of the cells' membranes.

Introduction

We are interested in mathematical models of the electrical propagation in the cardiac tissue. The main parameters that define the shape and speed of propagation are ionic properties of the cardiac cells, the geometric structure and the gap junction conductance between the adjacent cells.

We can approach the modelling of the signal propagation from two points of view. The more common one is to treat the cardiac tissue as the continuous domain, and write the model that is not dependent on the size of the single cardiac cell. These standard bidomain and monodomain models are convenient for the large scale numerical experiments (thousands of cells) as one can use the coarse meshes on the single domain.

The other way to model the signal propagation, that we will use here, is to look into the propagation of the signal on the scale of a few cardiac cells. Such models are numerically very challenging, and they should not be used for the simulations on the larger scales. But they provide closer insight into the contributions of different parameters of the model. More specifically, we are interested in understanding the non-static electrical properties of gap junctions. Additionally, we should note that the standard continuous models are derived from the microscopic models using the multi scale homogenisation techniques (Neu-Krassowska 1992).

The cardiac cells are surrounded by an extracellular matrix and neighbouring cells are electrically connected via gap junctions (GJ). Gap junctions are clusters of gap junction channels (GJCs) that are mainly localised on the longitudinal ends of the cells, where they compose the intercalated disks. These channels play a role of resistance pathways for electrical propagation, by providing the direct passage of molecules and ions from one cell to another.

Each gap junction channel is formed by two end-to-end hexameric structures called hemichannels or connexons, each of which is inserted in the membrane of adjacent cells. The hemichannels are composed of connexins, currently believed to be a protein family of approximately 20 isoforms (Harris 2001). The different isoforms compose channels that have diverse electrical properties (e.g. Desplantez et al, 2004).

Three different connexins, Cx43, Cx40, and Cx45, are expressed in heart. Cx43, the most abundant connexin, is found in ventricular and atrial myocardium. Cardiac diseases that lead to arrhythmias are associated with gap junction remodelling (Beauchamp et all 2004, 2012). Additionally, it has been observed in the experimental settings that the action potential (AP) propagation in the engineered strand of cells is highly dependent on the level of expression of Cx43, and the speed of propagation can be decreased to 7% of normal in knock-out Cx43 transgenic mouse model.

In the usual continuous models for the electrical propagation, the GJCs are tiny channels between cells which interior domains extend the intra-cellular domain, e.g. sim-

ple linear electrical conductors. However, the voltage clamp experiments (e.g. Harris 1981, Cristiane del Corso et al 2006, Desplantez et al 2004) have shown that GJCs exhibit a non-linear voltage and time dependence on the difference of membrane potential between adjacent cells.

In this ongoing work, we propose a 2D discrete microscopic mathematical model that takes into account the non-linear behaviour of GJCs. We use non-standard geometrical setting for the tissue micro structure, where we abandon the physical connection between adjacent cells (Figure 4). We keep the functional relation between the intracellular potentials between the adjacent cells via boundary conditions. Using this setting and the data on GJCs from Desplantez et al 2004, we would like to observe the decrease in propagation velocity due to different GJC, imitating the experiment in Beauchamp et al 2012.

1. 0-D Non-linear GJ model

1.1. Dual voltage clamp experiment data

The technique used to perform the dual voltage clamp experiment is described in del Corso et al 2006. It is a technique used to assess the behaviour of GJCs between a single pair of adjacent cells. The patch clamp electrodes are inserted in each cell and a constant transjunctional voltage V_j is applied, which provides the insight into the time evolution of the transjunctional current I_j, and junctional conductance $G_j = V_j/I_j$.

The behaviour of GJCs is described with the following parameters: $g_{j,0}$ represents the maximal junctional coupling between cells and $g_{j,inst}$ represents the instantaneous junctional conductance. Due to limitations of the experimental setup, these two are not necessarily the same. We use $g_{j,0}$ as a reference value for the maximal conductance that can be achieved in alive tissue. The dynamics is described by normalised $g_{j,\infty}$ that represents the steady state junctional conductance and $\tau_{j,\infty}$ that describes the time rate at which the GJCs go from the instantaneous to the steady state. $g_{j,inst}, g_{j,\infty}$ and $\tau_{j,\infty}$ are measured with respect to transjunctional voltage, V_j.

All parameters depend on type of connexins that form GJCs. In healthy ventricular myocytes the most expressed connexin is Cx43, and in much smaller amount Cx45. Hence we will be interested in GJCs formed by these two connexins. The experimental data (Desplantez et al. 2004, 2007, 2011), shown in Figure 1, suggest that the conductance of homomeric homotypic Cx43 (Cx45) GJCs is roughly symmetric with respect to V_j. While we know that GJCs can express significantly different behaviour in case of heteromeric or heterotypic GJCs, for the modelling purposes we will focus only on these two types of the GJCs.

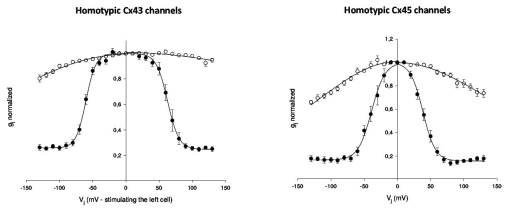

Figure 1: Dependence of the normalised gap junction conductance $g_{j,\infty}$ (lower curves) and $g_{j,inst}$(upper curves) on junctional potential Vj for homotypic Cx43 and Cx45 channels.

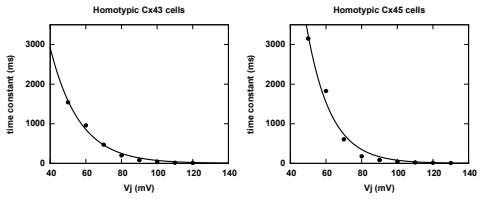

Figure 2: Kinetics of inactivation, $\tau_{j,\infty}$ as a function of V_j for homotypic Cx43 and Cx45 channels.

1.2. Data fitting into non-linear GJ model

Even though the behaviour of GJCs has long been observed and described as non-linear (Harris 1981), currently the common way to model GJCs in the tissue is to assume passive behaviour. This means that the electrical current for a given transjunctional voltage V_j,

$$I_j = G_j V_j, \tag{1}$$

is assumed to be linear, i.e the junctional conductance G_j is assumed to be constant. This simplification is useful and in the healthy tissue it is a good approximation, due to the very different time scales for GJCs (order 3s) compared to ionic channels (order 20-300ms). As we are interested in the pathological cases we want to observe the importance of the dynamical behaviour of GJCs. Hence, we use non-linear functions to fit the experimental data presented above.

We assume that GJCs behave as gates and the data are used to specify the nature and behaviour of the gating variables. The gates have three known states but we use only two, open and closed, for sake of simplicity. The rates at which the gates open or close depend on the transjunctional voltage. We write the conductance as follows

$$G_j(t, V_j) = g_{j,0} g_j(t, V_j), \tag{2}$$

where $g_{j,0}$ is the above mentioned maximal junctional coupling, and g_j represents the gating variable, that takes values between 0 and 1. The dynamics of the gating variable

is described by a simple ODE

$$\frac{dg_j}{dt} = \frac{g_{j,\infty}(V_j) - g_j}{\tau_{j,\infty}(V_j)}. \tag{3}$$

where $g_{j,\infty}$ and $\tau_{j,\infty}$ are fitted to the experimental data (Figures 1 and 2). These data are taken from the work of Desplantez et al 2004, 2007 and 2011. We fit the relationship between $g_{j,\infty}$ and V_j to a two-state Bolzmann equation (Cristiane del Corso 2006)

$$g_{j,\infty}(V_j) = \frac{1 - g_{j,min}}{1 + e^{A(V_j - V_{j,0})}} + g_{j,min}, \tag{4}$$

where $V_{j,0}$ is the voltage at which the conductance is half-minimal, $g_{j,min}$ is a normalised voltage-insensitive residual conductance and A is a parameter defining the steepness of voltage sensitivity.

We know that $\tau_{j,\infty}$ depends on the opening and closing rates, and these depend on V_j in an exponential way (Harris 1980). It is easy then to derive the simple relationship between $\tau_{j,\infty}$ and V_j used for data fitting:

$$\tau_{j,\infty}(V_j) = ae^{-bV_j}. \tag{5}$$

The parameters obtained from the data fitting in the case of homotypic Cx43 and Cx45 GJCs are presented in Table 1.

Table 1: Parameters obtained from the experiments and the data fitting. The units in the table are the following: $g_{j,0}$ in nS, $V_{j,0}$ in mV, A and b in mV^{-1} and a in ms.

	$g_{j,0}$	$g_{j,min}$	$V_{j,0}$	A	a	b
Cx43	68.3	0.27	60.5	0.098	33880	-0.06
Cx45	2.0	0.16	38.6	0.1	132330	-0.07

We perform 0D simulation for the GJ current (1) with the non-linear model for the GJ conductance (2) - (5). In Figure 3 we plot the current w.r.t. time, for each fixed V_j. The numerical results correspond to the experimental observations (Desplantez et al 2004).

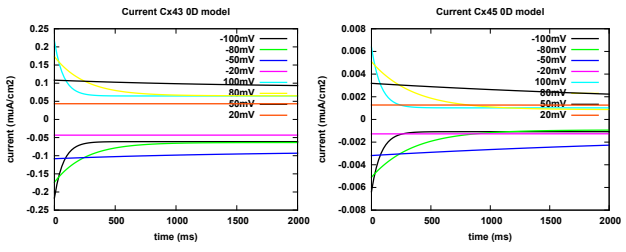

Figure 3: Transjunctional current simulated with the parameters obtained from the experiments and the data fitting.

2. Tissue model with non-linear GJCs

2.1. Experimental results on the strand

The main reference in this section will be Beauchamp et al 2012. In the study they quantify the relation between the degree of heterogeneity in Cx43 expression and disturbances in electric propagation. The full details of the experiment can be found in their paper. They engineered patterns of murine strands (4-5 mm in length and 50, 100 or 200 μm in width) from various mixtures of Cx43 and Cx43KO cells (knockout of Cx43, i.e. cells that do not express Cx43).

The propagation velocity was measured from the time difference in average activation and the distance between 2 regions of interest in the various cell mixtures. The original velocity of 30.5 cm/s in the strands with only Cx43 cells, dropped to 76%, 55% and 19% of the original velocity when the ratios of Cx43 and Cx43KO cells were 80%:20%, 50%:50% and 20%:80% respectively. Finally it drops to 2.1 cm/s for the strands with only Cx43KO cells.

For the modelling purposes we assume that Cx43KO cells behave as homotypic Cx45 cells.

2.2. 2-D Discrete mathematical model

In order to reproduce the results of the previously described experiment, we write a microscopic mathematical model for the signal propagation in the tissue. We use a non-standard geometrical setting for the tissue micro structure. Namely, we represent each myocyte as a separate rectangular domain, where the boundary represents the cell membrane. (Figure 4). Furthermore, we split the boundary into "ionic" and "junctional" boundaries and we apply the ionic model on the former and the previous non-linear GJ model on the latter. In this way, even if we do not have the physical connection, as is the case in the standard settings, we keep the functional relation between adjacent cells via boundary conditions.

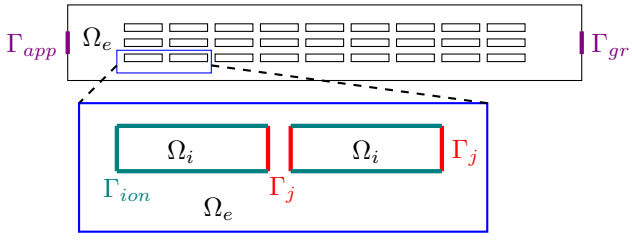

Figure 4: Geometric settings for 2D mathematical model. Ω_i, Ω_e - intra and extracellular domains, Γ_{ion} - "ionic" boundary, Γ_j - "junctional" boundary.

The governing equations for the potentials in both intra

and extracellular spaces are Laplace equations.

$$\begin{aligned} \sigma_i \Delta u_i &= 0, && \text{in } \Omega_i, \\ \sigma_e \Delta u_e &= 0, && \text{in } \Omega_e, \end{aligned}$$

The ionic properties of the membrane are taken into account as the boundary conditions on the "ionic boundary".

$$\left.\begin{aligned} \partial_t V_m + I_{ion}(V_m, \mathbf{h}) &= -\sigma_i \nabla u_i \cdot \mathbf{n}, \\ \partial_t V_m + I_{ion}(V_m, \mathbf{h}) &= -\sigma_e \nabla u_e \cdot \mathbf{n}, \\ \partial_t \mathbf{h} &= f_{ion}(V_m, \mathbf{h}), \end{aligned}\right\} \quad \text{on } \Gamma_{ion}.$$

Here, $V_m = u_i - u_e$ is transmembrane potential, σ_i and σ_e are intra and extracellular conductivities and $\mathbf{h}$ is a vector of variables in the ionic model. For this study we choose Beeler Reuter ionic model (Beeler Reuter 1977).

The non-linear gap junctions are taken into account as a boundary condition on the "junctional boundary".

$$\left.\begin{aligned} G_j(V_j) \cdot V_j &= -\sigma_i \nabla u_i \cdot \mathbf{n}, \\ \partial_t g_j &= (g_{j,\infty}(V_j) - g_j)/\tau_{j,\infty}(V_j). \end{aligned}\right\} \quad \text{on } \Gamma_j.$$

Here, V_j represents the transjunctional jump of potential in the adjacent cells, G_j is given as 2, $g_{j,\infty}$ is 4 and $\tau_{j,\infty}$ is 5. We assume that the system is isolated, i.e. we have the homogeneous Neumann boundary conditions on the external boundary and Γ_j.

$$\sigma_e \nabla u_e \cdot \mathbf{n} = 0, \qquad \text{on } \partial\Omega_e \setminus \Gamma_{ion},$$

We set initial conditions such that the system is in the steady state. In order to observe the signal propagation we need to provide a stimulus. This is done as follows: on one side we keep the "ground" and on the other we apply the difference of potential, V_{stim}, that can trigger AP in the first cells that are affected. In other words, during the stimulation time $t \in [t_0, t_0 + t_{stim}]$, where t_0 is the beginning of stimulation, we solve the system with Dirichlet boundary conditions

$$\begin{aligned} u_e &= 0, && \text{on } \Gamma_{gr}, \\ u_e &= V_{stim}, && \text{on } \Gamma_{stim}, \end{aligned}$$

and we keep the homogeneous Neumann boundary conditions on the rest of the external boundary. Using the variational formulation of the problem and the standard techniques of functional analysis we can prove that the problem is well posed.

2.3. Numerical analysis and test case

To perform the numerical simulation we use the finite element approach with semi-implicit time discretisation scheme. Additionally, we decouple intracellular and extracellular problems and use the iterative scheme for each time step. As we expected, this problem is numerically very challenging. Even for a very small number of cells (4-10) the CPU time to simulate $10 - 20ms$ is of order of 10hrs.

The first thing we were interested in was to investigate the behaviour of our model by assuming that there are no gap junctions, i.e. we would apply the ionic model on both Γ_{ion} and Γ_j. The size of the myocytes used in simulations are $100\mu m \times 20\mu m$. This correspond to the average length and width of human ventricular myocytes. The space between adjacent cells is taken to be $1\mu m$. The mesh step is $1\mu m$ and the time step in the simulation is $0.02ms$.

In the first numerical experiments we used only one myocyte embedded in the extracellular space. By stimulating extracellular space we were able to trigger the AP in the cell and observe its propagation as expected. We observed all four phases of the AP in a single cell simulation.

On the other hand in two-cell experiments we were not able to observe the propagation of AP from one cell to another using only ionic model. Although, our current numerical experiments are very limited, GJ coupling seems necessary for the AP propagation. There are some possible explanations that we still need to test, i.e. smaller mesh step or the distance between the cells.

In this moment we do not have conclusive results from the simulations with gap junctions.

Discussion

Gap junctions play an important role for signal propagation in cardiac tissue. They have a non-linear dynamics that is neglected in the current mathematical models. There are experimental evidences that alternations in the expressions of their connexins affect the propagation velocity. In this project we use the experimental data to fit the non-linear 0D model for homotypic Cx43 and Cx45 GJCs. Finally, we propose a discrete spatial model for studying effects of non-linear GJCs in the tissue. In on going work we perform some 2D numerical test.

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