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EVALUATION OF A COMPUTATIONAL MODEL FOR DRUG ACTION ON CARDIAC TISSUE

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Abstract. In this paper an electromechanical finite element model of cardiac tissue is evaluated with respect to its ability to simulate drug action. We compare experimental mechanical data of our partners with corresponding simulation results.

1 INTRODUCTION

Computational modeling of cardiac tissue has become important to study its behavior on different length scales aside from experiments which often are expensive, time-consuming and present difficulties in reproducibility. On the cellular level the models represent chemical and electrophysiological processes using a stiff system of ordinary differential equations. They are usually based on the original Hodgkin & Huxley model for cell membrane current [1]. Numerous electrophysiological cell models of different mammalian and human cell types in varying complexity have been established and used as summarized in [2]. Employing finite difference, finite volume or mass-lattice methods several groups have performed simulations to better understand the cellular processes, e.g. [3, 4, 5]. The Finite Element Method (FEM) has been employed to simulate excitation-contraction coupling in the whole heart, e.g. [6, 7].

Most recently, first simulation studies have been carried out to computationally investigate the effects of drugs on cardiac tissue, like in [8, 9]. In this paper we focus on the evaluation of a computational model that we presented in 2013 in [10] with respect to inhibitory drugs. The experimental basis on drugs is exceptionally large. Thus for

the parameterization of the model we mostly use literature data. We compare the computational results with mechanical data which we measure in an experimental setup of our project partner, the Lab of Medical and Molecular Biology at Aachen University of Applied Sciences, for the in vitro investigation of the contractibility of cardiac tissue.

Firstly, short introductions into the basic cellular processes and the related drug action are given. Then the experimental setup and the related computational model are described before the simulations of the effect of inhibitory drugs on cardiac tissue is compared to the experimental results.

2 CELLULAR PROCESSES

The adult human heart is composed of two major types of cardiac muscle cells, contractile myocytes and specialized pacemaker-conduction cells. Myocytes form the bulk of the heart and are specialized to perform mechanical work, triggered by the intracellular calcium concentration which activates their contractile machinery. Although the cells belonging to the pacemaker-conduction system contract weakly, their main task is to generate and spread electrical impulses, thereby initiating myocardiac action potentials that lead to contraction.

Here we describe the action potential of self-contractile purkinje fiber cells because this is the type of cell that the chosen computational electrophysiological model has been developed for. Purkinje cells belong to the pacemaker-conduction system and play a major role in electrical conduction and propagation of impulses to the ventricular muscle. Their action potential sequence is comparable to that of ventricular cells. Additionally they have the ability to generate the action potential themselves. Figure 1 schematically illustrates the cellular processes that take place during cell contraction and relaxation. The contents of this figure are very closely related to the computational model that is described later.

An action potential is the transient depolarization of a cell resulting from voltage- and time-dependent currents across the membrane. At rest (phase 4), cardiac muscle cells possess a negative membrane potential due to outward-directed potassium currents I_{K1}. Cells from the pacemaker-conduction system slowly depolarize due to pacemaker currents until a threshold potential is exceeded, followed by a full action potential sequence. The rate of spontaneous depolarization determines the beating frequency of the heart. Myocytes lack these pacemaker currents. Their action is initiated by propagating electrical impulses from the pacemaker-conduction system.

After the threshold potential is actively exceeded, the upstroke (phase 0) of ventricular cells is carried by a rapid inward sodium current I_{Naf}. When the cell is depolarized, a transient outward current I_{Cl} that is carried by chloride and potassium ions causes a quick repolarization (phase 1). This is followed by a second inward current I_{si} which is mainly carried by calcium ions and marginally carried by sodium ions. It counteracts the outward directed slow and fast delayed rectifier potassium currents I_{x1} and I_{x2}, leading to a depolarized plateau (phase 2). The calcium influx during the plateau phase triggers a

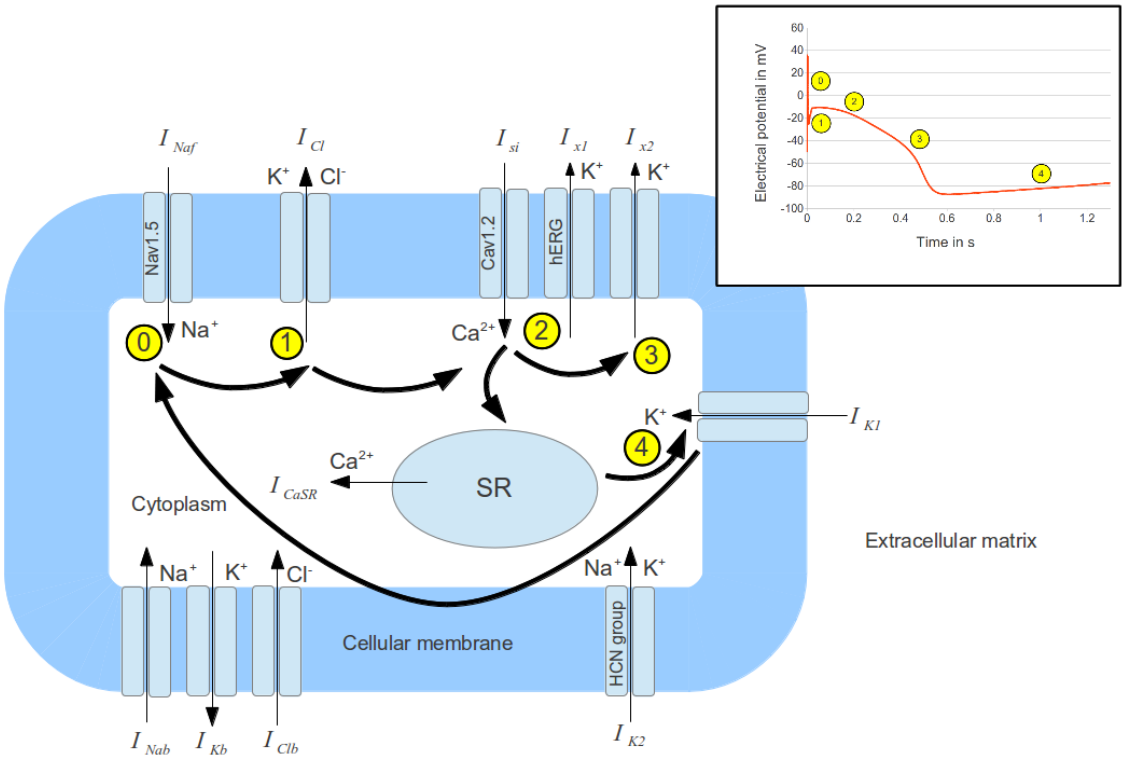

Figure 1: Electrophysiology of purkinje cells and a typical action potential with phases 0–4

massive release of calcium as indicated in the figure by I_{CaSR} from the sarcoplasmic reticulum. The sarcoplasmic reticulum is an intracellular calcium store. Some of the calcium ions then bind to troponin C, thereby initiating the contraction process. Total action potential repolarization (phase 3) to the resting state occurs when the L-type calcium channels (Cav1.2) close. Further there are three background currents, a sodium current I_{Nab}, a potassium current I_{Kb} and a calcium current I_{Clb} that have only little influence on the action potential. Ion pumps and exchangers, e.g. the sodium-calcium exchanger and sodium-potassium ATPase, help regulating the ionic concentration gradients across the membrane in order to allow action potential sequences to occur physiologically identical.

It has to be emphasized that the cellular processes are much more complicated than described here and that they are far from entirely understood. Nevertheless the provided overview is detailed enough to understand the way how cardiac cells are activated and inactivated in accordance with the generally accepted theory.

3 DRUG ACTION

The variety of known drugs is huge. Thus we limit the investigations to drugs that selectively influence specific ion channels with different intensity and depending on their concentration. We distinct between inhibitory and stimulating behavior that changes the beating force and the heart rate (beating frequency) and again we limit ourselves to three inhibitory drugs, namely Lidocaine, Veratridine and Verapamil.

3.1 Lidocaine

The common local anesthetic drug Lidocaine is clinically used as an antiarrhythmic drug for emergency-treatment of ventricular arrhythmias. As a Class IB antiarrhythmic agent, it binds to fast sodium channels (Nav1.5) in their inactivated state, thereby inhibiting their activation. The block of fast sodium channels results in a decreased sodium influx I_{Na} during depolarization that subsequently causes a lowered inner calcium concentration in ventricular myocytes during the action potential plateau due to reverse mode of the sodium-calcium-exchanger [11]. Lidocaine thus lowers ventricular contractile force.

Recent studies provide evidence for the inhibitory effect of Lidocaine on HCN channels which are assumed to be responsible for the pacemaker current thus the drug lowers the heart rate [12].

3.2 Verapamil

Verapamil is a multi-ion-channel blocker classified as a class IV-antiarrhytmic agent. In cardiac tissue, this agent especially inhibits calcium ion influx through L-type calcium channels (Cav1.2) during the early plateau phase as well as potassium ion efflux through rapidly activating delayed rectifier potassium channels (hERG) during the late plateau and repolarization phases by dose-dependent blocking of the activation of the respective channels. This causes a slower repolarization-rate that in turn leads to an action potential prolongation. The action potential prolongation leads to the expectation that Verapamil induces a negative chronotropic effect, i.e. a reduction of the heart rate [13]. The decreased calcium influx yields a negative inotropic effect of Verapamil, i.e. a reduced beating force, since calcium ions play a major role in the contraction process. The drug additionally blocks fast sodium channels (Nav1.5), but with a very low potency [14].

3.3 Veratridine

Veratridine is a sodium channel toxin that solely prolongates the permeability of fast sodium channels (Nav1.5) by drug-induced inhibition of channel inactivation, resulting in an increased inner sodium ion concentration [15, 16]. This consequently triggers an increase in intracellular calcium ions by stimulation of the sodium-calcium exchange [11] thus leads to an increased beating force. By an extension of the plateau phase, Veratridine additionally induces an action potential prolongation due to the lowered calcium influx which results in a decreased beating frequency [13].

4 EXPERIMENTAL SETUP

For the mechanical investigation of cardiac tissue we employ the so-called CellDrum^{TM} [17]. This experimental setup consists of a circular, 4 μm thin silicone membrane with a diameter of 1.6 cm = 16000 μm. On top of the membrane myocardiac cells are cultivated and chemically fixed in a collagen monolayer that has a thickness of around 4 μm too. A culture medium keeps the cells alive and as in the real heart the tissue is beating autonomously. This composite material is clamped in a fixed ring. The CellDrum^{TM} can be placed into an inflation setup as depicted in Fig. 2. A syringe pump generates an inflating pressure. The deflection is measured using a laser sensor resulting in a pressure-deflection curve as shown in the same figure.

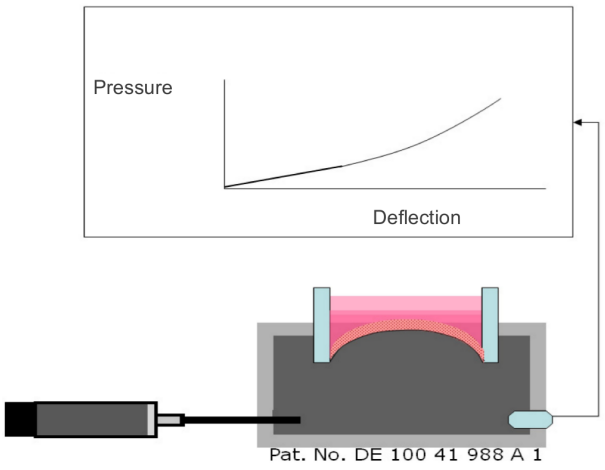

Figure 2: Schematic drawing of the inflation experiment

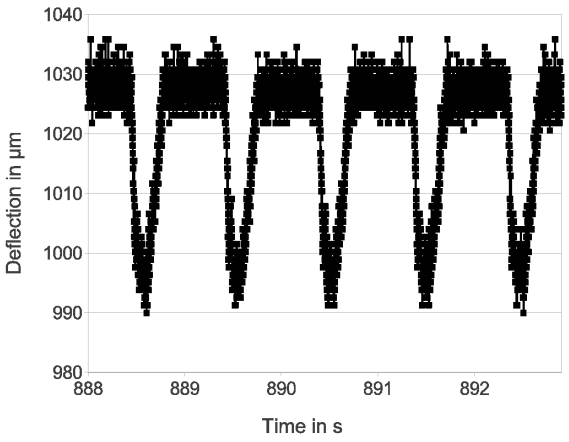

Figure 3: Change in deflection due to beating

Figure 3 shows a typical curve of the deflection of the composite in time at constant pressure. In this case the cells are beating with a frequency of approximately 1 Hz and during contraction they change the deflection of the composite. Depending on the medication that is applied to the tissue, the change in deflection due to the contraction of the cells will be different and the beating frequency might change because the action potential of the cells differs from the normal situation.

5 COMPUTATIONAL MODEL

The finite element based computational model has already been described in [10] and we only recall its general structure. Due to symmetry, we represent a quarter of the thin circular tissue construct and discretize it with second-order triangular heterosis shell elements in a geometrically nonlinear framework.

Following the cardiac tissue model of Hunter et al. [18] the Cauchy stress tensor $\boldsymbol{\sigma}$ is described by equation (1) as an additive split of the passive and active part $\boldsymbol{\sigma}_p$ and $\boldsymbol{\sigma}_a$

respectively

$$\boldsymbol{\sigma} = \boldsymbol{\sigma}_p + \boldsymbol{\sigma}_a = 2J^{-1}\boldsymbol{B}\frac{\partial \Phi}{\partial \boldsymbol{B}} - p\boldsymbol{I} + T(t, \boldsymbol{B}, Ca_i)\boldsymbol{I} \ , \tag{1}$$

with Φ a hyperelastic strain energy density, J the determinant of the deformation gradient, $\boldsymbol{B}$ the left Cauchy-Green tensor, p the hydrostatic pressure, $\boldsymbol{I}$ the identity tensor, T a scalar related to the active stress, t the time and Ca_i the inner calcium concentration that mainly drives the contractibility of cardiac cells. We implement this model at the level of the constitutive equation.

Internally, T is described by a system of ordinary differential equations (ODEs). The McAllister-Noble-Tsien model [19] is a model that describes the action potential of self-contractile purkinje fiber cells. Although we are investigating myocardiac monolayers this model was our first choice because of its relative simplicity, low complexity and manageability. The system of ODEs is solved on each Gaussian point of the finite element mesh and as it is a stiff system we chose a singly-diagonal implicit Runge-Kutta method with adaptive time stepping to solve it. The overall structure of the system of ODEs reads as

$$\frac{\partial V_m}{\partial t} = \frac{1}{C_m}\left(I_{stim} - \sum_{i=1}^{9} I_i(g_{x_1}, g_{x_2}, ...)\right) \tag{2}$$

$$\frac{\partial g_x(V_m)}{\partial t} = \alpha_x^+(V_m)(1 - g_x) + \alpha_x^-(V_m)g_x \ , \tag{3}$$

which prescribes the time course of the action potential V_m and the ionic gates g_x, which in turn control the ionic currents I_i. The parameters α_x^+ and α_x^- are opening and closing rates of the respective gate g_x and they can be determined experimentally.

In order to fully determine T in equation (1) one still needs to introduce an equation for the computation of the inner calcium concentration Ca_i. As explained with the help of figure 1, Ca_i is driven by slow diffusion of sodium and calcium ions through the cell membrane and by a calcium-induced calcium release. In the McAllister-Noble-Tsien model those components are abstracted within a slow inward current I_{si}. Beeler and Reuter [20] proposed another ordinary differential equation to determine the calcium concentration from this current

$$\frac{\partial Ca_i}{\partial t} = 1.848 \cdot 10^{-4} I_{si} + 0.07(10^{-4} - Ca_i) \ , \tag{4}$$

which is scaled to a minimum and maximum Ca_i of $0.1\,\mu$M and $1\,\mu$M, respectively.

It is important to note and it can be seen in equation (1) that we assume an isotropic tissue. Although in the heart cardiac tissue is always anisotropic the circular structure of the membranes in fact results in an overall isotropic structure of the tissue.

In the next section we present the simulation results in comparison with experimental data. Inflation experiments have been conducted for different concentrations of inhibitory drugs. As already explained in section 3 drugs are acting more or less selectively on specific

ion channels. In the proposed model we therefore introduce a scaling factor [8]

$$\frac{1}{1+\frac{D}{IC_{50}}} \tag{5}$$

into the respective ODE (3) where D is the applied concentration of the drug and IC_{50} is the half-blocking concentration, i.e. the concentration of the drug that is needed to half-block a specific gate.

6 SIMULATION RESULTS

In this section we show simulation results for the three drugs named in section 3 and discuss them by comparison to CellDrum results and to findings from literature.

The results need to be regarded with respect to the fact that, although a lot of experiments have been performed all over the world, the gathered information varies. The experimental data strongly depends on the cell type, their maturity, the production batch, the investigated species, the experimental setup, the environment of the cells and many more. Therefore the data differs in the reported beating force, cell stresses, beating frequency and IC_{50} values. We try to select information that fit our setup the most but often even this very carefully selected information varies quite much. Even under relatively well-controlled and well-known conditions the CellDrum results themselves vary for the named reasons. Beating force and frequency, both are quite different depending on whether the experiment is performed two weeks or threee months after cultivation. Therefore our current results can only show trends qualitatively.

6.1 Lidocaine

Figures 4 and 5 show the simulated influence of Lidocaine to the change in deflection of the membrane and to the beating frequency of the cells at different concentrations and corresponding experimental results obtained from CellDrum experiments. We apply an IC_{50} value of 50 μM to the activation gate of the fast sodium channel and to the activation gate of the pacemaker current in order to model the drug's action.

Clearly both, the simulated dose-dependent change in beating force and in heart rate, are in good agreement with the experimental results. Within the range of 0–100 μM of applied Lidocaine, the beating force seems to be linearly dose-dependent. The model even captures the nonlinear slope of the experimental curve for the influence of Lidocaine on the beating frequency shown in figure 5. Although, qualitatively, the simulation results show a good agreement with the experiments the untreated cells (0 μM of Lidocaine) show half of the change in deflection of the membrane and half of the beating frequency the cells have had in standard pressure-deflection experiments. The reason is unknown but probably is one of those quoted in the introduction to this section.

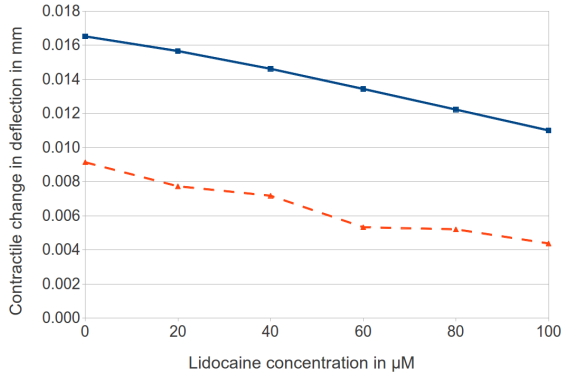

Figure 4: Lidocaine affecting contractibility in experiment (*triangular markers*) and simulation (*rectangular markers*); sample size $m = 3$

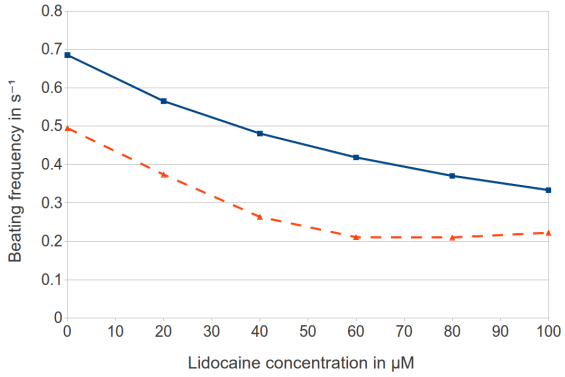

Figure 5: Lidocaine affecting beating frequency in experiment (*triangular markers*) and simulation (*rectangular markers*); sample size $m = 3$

6.2 Verapamil

To appropriately model Verapamil we take $IC_{50} = 40\,\mu$M to modify the activation gate of the fast inward sodium channel. The secondary inward channel of the model is, amongst others, responsible for calcium influx during the plateau phase. Its activation is modified by $IC_{50} = 0.1\,\mu$M. Lastly the activation of the delayed rectifier potassium current needs to be modified by $IC_{50} = 0.14\,\mu$M. The simulation and experimental results are shown in figures 6 and 7.

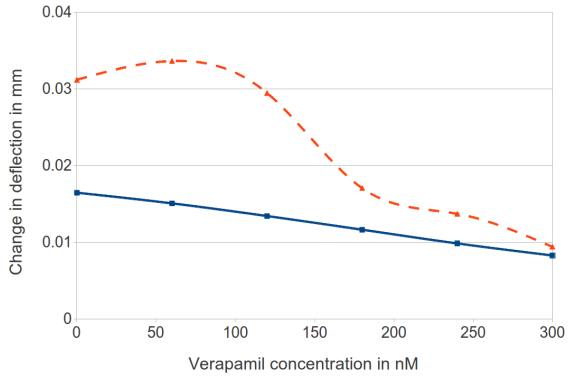

Figure 6: Verapamil affecting contractibility in experiment (*triangular markers*) and simulation (*rectangular markers*); sample size $m = 3$

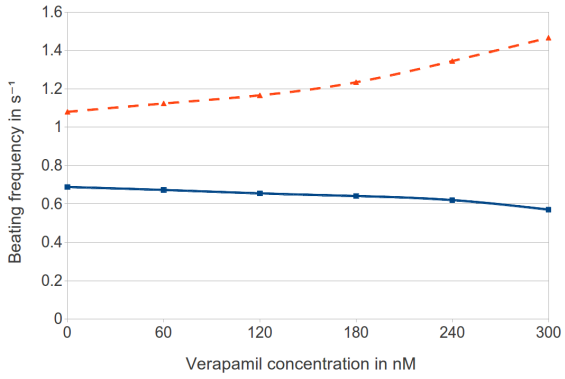

Figure 7: Verapamil affecting beating frequency in experiment (*triangular markers*) and simulation (*rectangular markers*); sample size $m = 3$

The experimental results show concentration-dependent negative inotropic and positive chronotropic effects. The negative inotropic effect of Verapamil confirms recent findings

[21, 22, 23] and the increase in contractibility for very low concentrations of Verapamil has also been observed in [21] leading to the suspicion that Verapamil affects further ion channels or that non-investigated actions take place. Despite this effect the model appropriately reproduces the negative inotropic effect.

The positive chronotropic effect of Verapamil, i.e. the increase in beating frequency, obtained in CellDrum experiments, is contradictory to recent findings on stem-cell derived cardiomyocyte clusters (hiPSC-EBs and hESC-EBs). The simulation shows a negative chronotropic effect that qualitatively fits the experimental findings in the literature, although the increasing nonlinearity for Verapamil concentrations above 180 nM cannot be verified due to a lack of data.

6.3 Veratridine

Veratridine is entirely accounted for effecting fast sodium channels and its half maximal inhibitory concentration for modeling the inhibition of the fast sodium inactivation is $IC_{50} = 0.4\,\mu\text{M}$. The increased calcium influx due to a reversal mode of the sodium-calcium-exchanger is considered by the inhibition of the opening transition rate of the slow inward inactivation gate by $IC_{50} = 1.2\,\mu\text{M}$.

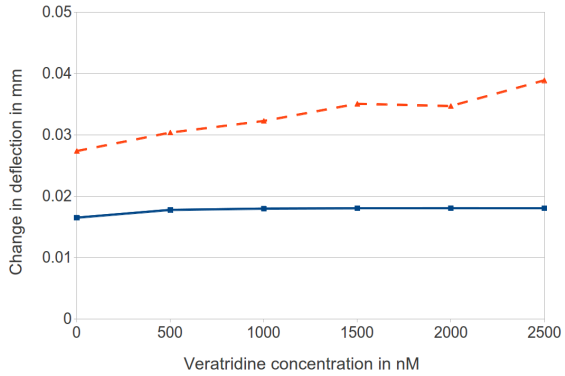

Figure 8: Veratridine affecting contractibility in experiment (*triangular markers*) and simulation (*rectangular markers*); sample size $m = 3$

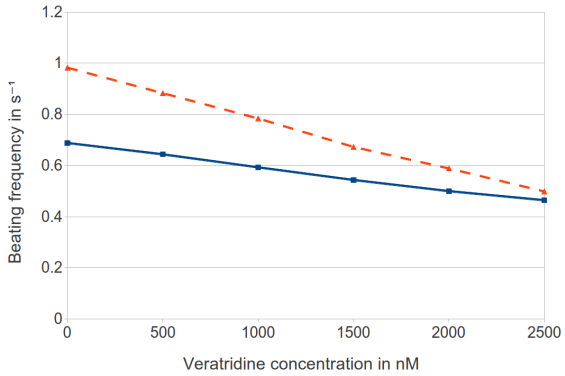

Figure 9: Veratridine affecting beating frequency in experiment (*triangular markers*) and simulation (*rectangular markers*); sample size $m = 3$

The simulations for Veratridine show dose-dependent positive inotropic and negative chronotropic effects, as shown in figures 8 and 9, respectively. The simulation results fit the expectations that arise from the description of the drug effects in section 3.3: an increased calcium concentration leads to a higher beating force and the prolonged action potential reduces the beating frequency.

In the simulation result in figure 8 there seems to be some saturation effect as the change in deflection stays nearly constant for Veratridine concentrations larger than $0.5\,\mu\text{M}$. This observation is misleading as in fact it can be explained by inappropriate parametrizations

of equation 4 and the Hunter model. The point of reference for the maximum inner calcium concentration is set to $1\,\mu$M although more than $10\,\mu$M can be reached. Thus maximum activation is already reached for comparably low Veratridine concentrations which leads to the necessity of a future model adjustment.

On the other hand the experimentally determined negative chronotropic effect can be simulated as shown in figure 9. Here the major reason for the different slopes is supposed to be an inaccurate IC_{50} value.

7 SUMMARY AND PROSPECTS

The computational model has been shown to be capable of appropriately simulating inhibition of activation and inactivation gates using the examples of Lidocaine, Verapamil and Veratridine. The simulation results are in fair agreement with experimental results that have been obtained in an inflation setup.

Although generally the results are very promising, we already indicated that still a lot of experiments, modeling and simulations has to be done in order to achieve more accurate and quantitatively comparable results. Concerning a proper quantitative investigation of the inotropic effect of drugs we need to adjust the model parameters to the respective tissue constructs by first conducting pressure-deflection experiments with the exact same untreated tissue constructs. This way we exclude most of the already listed numerous influences that are difficult or impossible to quantify and the contraction force in the untreated case will be the same in the simulations and the experiments.

An adjustment of the beating frequency of untreated tissue to experimental results is much more difficult because the pacemaker models have an inherent frequency that cannot be changed in a simple way because each single ion current influences the beating frequency.

From the modeling point of view in the near future we will employ more detailed and more accurate cell electrophysiology models that better fit the cell types occurring in the tissue. Most importantly those models need to represent in more detail the processes that are important for the calcium balance. Further we will model action potential propagation and simulate drugs that also have stimulating effects on the gates which can be done using action potential shifts.

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REFERENCES

[1] Hodgkin, A.L. and Huxley, A.F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* (1952) **117**(4):500–544.

[2] Fenton, F. and Cherry, E. *Models of cardiac cell.* Scholarpedia (2008).

[3] Nash, M.P. and Panfilov, A.V. Electromechanical model of excitable tissue to study reentrant cardiac arrhythmias. *Prog. Biophys. Mol. Bio.* (2004) **85**(2–3):501–522.

[4] Niederer, S.A. and Smith, N.P. An improved numerical method for strong coupling of excitation and contraction models in the heart. *Prog. Biophys. Mol. Bio.* (2008) **96**(1–3):90–111.

[5] Weise, L.D. and Panfilov, A.V. A discrete electromechanical model for human cardiac tissue: effects of stretch-activated currents and stretch conditions on restitution properties and spiral wave dynamics. *PloS One* (2013) **8**(3):e59317.

[6] Nickerson, D., Smith, N. and Hunter, P. New developments in a strongly coupled cardiac electromechanical model. *J. Work. Gr. Card. Pac. Arrhythm. Card. Cell. Electrophysiol. Eur. Soc. Cardiol.* (2005) **7**:118–127.

[7] Göktepe, S. and Kuhl, E. Electromechanics of the heart: a unified approach to the strongly coupled excitationcontraction problem. *Comput. Mech.* (2009) **45**(2–3):227–243.

[8] Obiol-Pardo, C., Gomis-Tena, J., Sanz, F., Saiz, J. and Pastor, M. (2011). A multiscale simulation system for the prediction of drug-induced cardiotoxicity. *J. Chem. Inf. Model.* **51**(2):483–492.

[9] Zemzemi, N., Bernabeu, M.O., Saiz, J., Cooper, J., Pathmanathan, P., Mirams, G.R. and Rodriguez, B. Computational assessment of drug-induced effects on the electrocardiogram: from ion channel to body surface potentials. *Brit. J. Pharmacol.* (2013) **168**(3):718–733.

[10] Frotscher, R., Gossmann, M., Raatschen, H.-J., Temiz-Artmann, A. and Staat, M. Simulation of cardiac cell-seeded membranes using the edge-based smoothed FEM. In H. Altenbach and G. Mikhasev (Eds.), *Shell and Membrane Theories in Mechanics and Biology: From Macro- to Nanoscale Structures.* Springer (2015).

[11] Saleh, S., Yeung, S.Y.M., Prestwich, S., Pucovsky, V. and Greenwood, I. Electrophysiological and molecular identification of voltage-gated sodium channels in murine vascular myocytes. *J. Physiol.* (2005) **568**(1):155–169.

[12] Meng, Q.T., Xia, Z.Y., Liu, J., Bayliss, D.A. and Chen, X. Local anesthetic inhibits hyperpolarization-activated cationic currents. *Mol. Pharmacol.* (2011) **79**:866–873

[13] Arnold, L., Page, J., Attwell, D., Cannell, M. and Eisner, D.A. The dependence on heart rate of the human ventricular action potential duration. *Cardiovasc. Res.* (1982) **16**(10):547–551.

[14] Kramer, J., Obejero-Paz, C.A., Myatt, G., Kuryshev, Y.A., Bruening-Wright, A., Verducci, J.S. and Brown, A.M. MICE models: superior to the HERG model in predicting Torsade de Pointes. *Sci. Rep.* (2013) **3**:2100.

[15] Brill, D.M. and Wasserstrom, J.A. Intracellular sodium and the positive inotropic effect of veratridine and cardiac glycoside in sheep Purkinje fibers. *Circ. Res.* (1986) **58**(1):109–119.

[16] Honerjäger, P. and Reiter, M. The relation between the effects of veratridine on action potential and contraction in mammalian ventricular myocardium. *N.-S. Arch. Pharmacol.* (1975) **289**(1):1–28.

[17] Linder, P., Trzewik, J., Rüffer, M., Artmann, G.M., Digel, I., Kurz, R. and Temiz Artmann, A. Contractile tension and beating rates of self-exciting monolayers and 3D-tissue constructs of neonatal rat cardiomyocytes. *Med. Biol. Eng. Comput.* (2010) **48**(1):59–65.

[18] Hunter, P.J., McCulloch, A.D. and ter Keurs, H.E.D.J. Modelling the mechanical properties of cardiac muscle. *Prog. Biophys. Mol. Bio.* (1998) **69**(2–3):289–331.

[19] McAllister, R.E., Noble, D. and Tsien, R.W. Reconstruction of the electrical activity of cardiac Purkinje fibres. *J. Physiol.* (1975) **251**(1):1–59.

[20] Beeler, B.Y.G.W. and Reuter, H. Reconstruction of the action potential of ventricular myocardial fibres. *J. Physiol.* (1977) **268**:177–210.

[21] Mehta, A., Chung, Y.Y., Ng, A., Iskandar, F., Atan, S., Wei, H. and Shim, W. Pharmacological response of human cardiomyocytes derived from virus-free induced pluripotent stem cells. *Cardiovasc. Res.* (2011) **91**(4):577–586.

[22] Yokoo, N., Baba, S., Kaichi, S., Niwa, A., Mima, T., Doi, H. and Heike, T. The effects of cardioactive drugs on cardiomyocytes derived from human induced pluripotent stem cells. *Biochem. Biophys. Res. Co.* (2009) **387**(3):482–488.

[23] Rosen, M.R. Consequences of the Sicilian Gambit. *Eur. Heart J.* (1995) **16**:32–36.