A Population-of-Models Capturing Vascular Smooth Muscle Cell Variability

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Abstract

Vascular smooth muscle cells (SMCs) play a central role in regulating vascular tone and hemodynamics. Their variability and its contribution to system-level behavior is, however, neglected in current models. The ability to capture SMC biological variability and boundary condition complexity in-silico, would allow for more accurate representation and, later in integrated models, prediction of coronary perfusion and cardiovascular dynamics. With this work we aim to address SMC biological variability with the development, and extensive validation of a population of computational SMC models. A population of 10,000 electrophysiological models (POMs) was generated using latin hypercube sampling to vary key ionic and contractile parameters within physiologically constrained ranges. The population was then calibrated against experimental data and subjected to four distinct physiological challenges; testing key SMC electro-chemo-mechanical responses to various stimuli and thus challenging their stability, responsiveness, and functional plausibility. All steps were performed using open-source POMtool software. We then demonstrated the use of this population in blood pressure drug testing by applying nifedipine as a calcium channel blocker to the calibrated population, and observing their altered response to the same physiological challenges used in the population pruning. The POMs framework captures inter-subject variability in SMC electrophysiology, whilst providing a computationally tractable approach for multiscale integration and drug testing.

1. Introduction

Vascular smooth muscle cells (SMCs) control the flow of blood by contracting or relaxing and, in turn, altering the diameter of blood vessels. SMCs respond and adjust to numerous stimuli, including local blood pressure, flow-induced endothelial signals, neural vasoconstrictors and metabolic demand [1–3]. If these mechanisms are altered, it can directly translate into abnormal vessel behavior and impaired blood flow. Vascular SMC dysfunction and remodeling, for example, contribute to coronary microvascular dysfunction in cardiomyopathies such as hypertrophic cardiomyopathy (HCM). This makes the SMC a key therapeutic target for regulating cardiovascular function [4, 5]. Mechanistic modeling allows us to understand how these cells work and how they are altered by diseases and drugs. The SMC model, developed for the SMASH-HCM project, combines existing models to capture the membrane electrophysiology, intracellular calcium dynamics and contractile behavior of the cell [6]. In this paper we create a population of SMC models (POMs) to capture their biological variability in-silico. The population was then pruned to ensure that each accepted cell was biologically plausible. This was done by testing the models against several biophysical and chemical 'challenges' representing different aspects of SMC functional control in response to various stimuli. We further demonstrate a use of our POMs by administering, in-silico, varying dosages of nifedipin, a general blood pressure management medication, to the population. We then explore how the models' response to our challenges are altered owing to drug administration.

2. Methods

A well-established electrochemical SMC model was selected as the base model [7,8]. Then, as detailed by Anderton et al. in [6], stretch and shear control mechanisms and a calcium driven contractile element, were further integrated into the model based on the work of Murtada and Yang [9, 10]. A schematic of the resulting integrated electro-chemo-mechanical SMC model is shown in Figure 1. Following this, the open-source POMtool framework (availible at github.com/TAU-CBIG/POMtool) was used to create and prune our SMC population. The population was then introduced to nifedipine and re-subject to the pruning challenges. The population production and drug demonstration procedures are described in detail below.

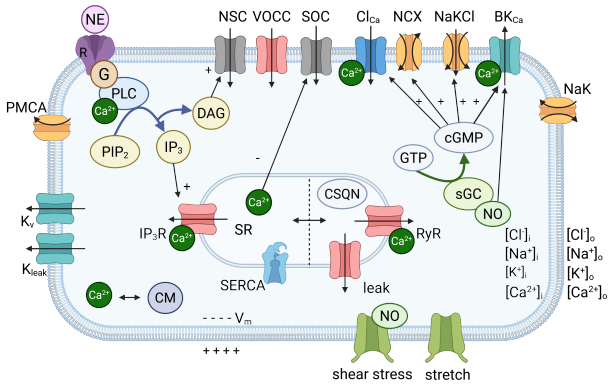

Figure 1: Schematic of the integrated electrophysiological smooth muscle cell model (created with Biorender) [7, 11].

2.1. Population production

Model parameters were selected and varied to create a population of 10000 models. The parameters were initially selected to include a combination of common parameters described in both Kapela's, [7], and Morotti's [8] model sensitivity analysis, with know uncertainty ranges. Further, additional parameters were selected to account for at least one parameter in each of the ion channels and transport mechanisms of the model. The population was then subjected to a set of four comprehensive physiological challenges, to determine model variants' ability to reproduce expected qualitative behaviors across a range of conditions. The responses were quantified with biomarkers that were specific to each challenge and based on either experimental or validated computational fluctuations in SMC calcium concentration or membrane potential variation in response to the various challenge stimuli. These biomarkers were used to 'prune' the POMs, resulting in a smaller population of physiologically validated models that could be used in investigating SMC variation on vascular function. The challenges and their respective biomarkers are described below.

2.1.1. Description of challenges

Challenge 1: Rest In rest state, the simulation is initialized and kept at rest, with no external stimuli or parameter perturbations were applied. The model's state variables were allowed to evolve freely until a steady state was reached, the steady state calcium and membrane potential were then evaluated as a biomarkers [12, 13].

Challenge 2: NENO The Norepinephrine (NE) and nitric oxide (NO) challenge mimics neuronal control and shear stress induced nitric oxide (NO) release. The cells were initialized at rest, administered 3 μM norepinephrine (NE) from 10 ms onward, and administered 1 μM of nitric oxide (NO) at 70 ms onward. This protocol was informed by the response dynamics reported in [7], which is based on [14–16]. The steady-state calcium and voltage values before and at the time of NE administration, as well as just prior and 40 s after NO administration were used at biomarkers.

Challenge 3: Stretch SMCs respond to mechanical stretch by increasing intracellular calcium concentration to promote contraction. This stretch can be caused by a number of factors, including increased blood pressure. To manage computational cost in population simulations, five stretch levels were selected: 0%, 20%, 40%, 60% and 30%, with the simulation run-time being enough for each subsequent stretch change to reach equilibrium in the base model. The steady-state calcium and voltage values for each stretch were used as biomarkers. While there was no data to compare this exact incremental change in stretch to, the general pruning range of intracellular calcium concentration was kept near experimental physiological data ranges, but large enough to allow room for uncertainty [17].

Challenge 4: V-Clamp Patch clamp recordings are key experimental outputs that allow us to understand cell membrane potential, and cell ionic currents and concentrations. The V-Clamp challenge follows the V-Clamp experiment by [18], where calcium concentrations emerging from different clamped membrane potentials were monitored. The biomarkers included the calcium concentration for voltage clamp membrane potential values held at -50, -40, -30, -20, -10, and 0 mV (response curves not shown here).

Challenge notes: Where experimental data wasn't available, previous models [10, 19, 20] were used to set acceptable biomarker ranges.

The shear-stress induced production of NO was assumed negligible and thus set to zero for all challenges. This was primarily to allow for the comparison of our models output to previous models and experimental data.

2.2. Demonstration of Drug effects

To demonstrate a use of our SMC POMs, we simulated the effect of varying nifedipine concentrations on the final accepted population. Nifedipine administration was simulated by applying a L-type calcium current block to each model (the voltage dependence of the block or interactions with other channels was not considered in this implementation). The blocking effect of nifedipine was implemented using the Hill equation to scale the conductance of the channel based on the concentration of the drug 'administered'. Nifedipine concentrations of 0 μM, 0.001 μM, 0.01 μM, 0.1 μM, 1 μM, and 10 μM were used. The Hill equation used was based on the drug response curve in [21], which has been previously utilized to inform computational simulations in the Hernandez-Hernandez model [22].The IC50 value of L-type calcium current was 20 nM

and the Hill coefficient was determined to be 0.99 from the IC50 curve in [21]. The simulations followed the same protocol for the Rest, NENO, and Stretch challenges, with only the accepted models tested.

3. Results

Of the 10000 POMs, 1897 cells passed both the Rest and Stretch challenges. Once the NENO challenge was considered, only 557 of those cells met all conditions. Of those, only 222 cells passed the V-Clamp challenge. Figure 2 shows an example of the population's responses to the NENO challenge, with the membrane potential and calcium curves produced by cells that did not pass the NENO challenge in grey, cells that did meet the NE-NO challenge criteria (challenge accepted) in red, cells that passed all the challenges (globally accepted) in blue.

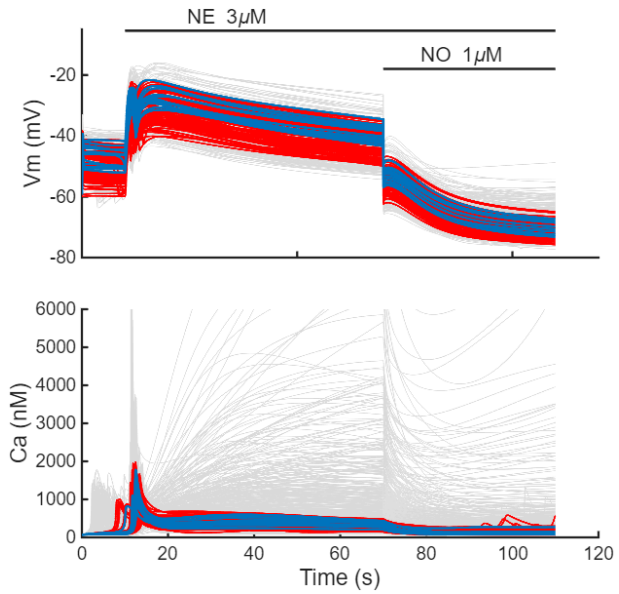

Figure 2: NENO challenge voltage (top) and calcium (bottom) curves for a sample of 10 % of the POMs produced when the POM was initialized at rest, administered 3 μM norepinephrine (NE) from 10 ms onward, and administered 1 μM of nitric oxide (NO) at 70 ms onward. The gray curves (–) were produced by cells that did not pass the NENO challenge, the red curves (–) were produced by cells that did meet the NE-NO challenge criteria, and the blue curves (–) are the outputs of those cells that met the criteria for all four challenges.

The effect of varying nifedipine dosages on the POMs' resting membrane potential and calcium concentration (a and b respectively), and membrane potential and calcium concentration at 60 % stretch increase (c and d respectively) is described by the box plots in figure 3. The figure also includes the populations' full membrane potential (E) and calcium concentration (F) response to NE-NO stimulus.

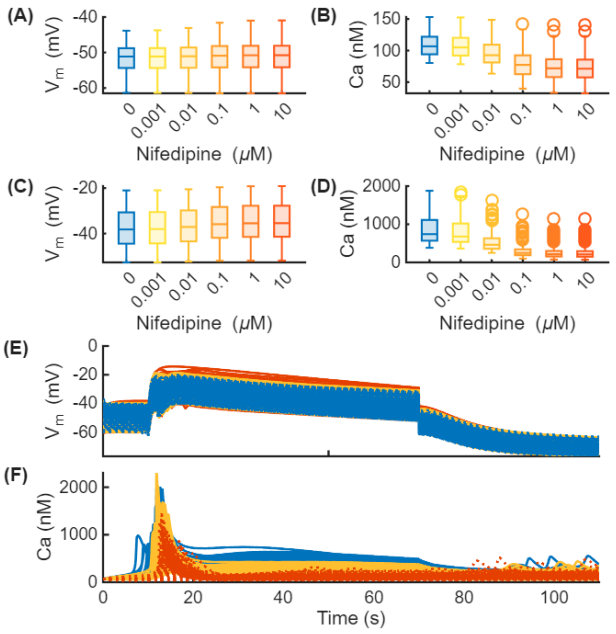

Figure 3: The effect of Nifedipine on average membrane potential (A & C) and intracellular calcium (B & D) in resting simulation (A & B) and in the highest stretch region of the stretch simulation (C & D). The populations' full membrane potential (E) and calcium concentration (F) responses to NE-NO stimulus are also given; where the baseline accepted cell responses without nifedipine are shown in blue (–), the accepted cell responses with 10 nM nifedipine are shown in yellow (–), and the dark orange curves (- -) are those of accepted cell responses with 1 μM of nifedipine.

4. Discussion

Our POMs approach captures substantial variability in SMC electrophysiology. This is especially clear from the variation in calcium response curves visible in Figure 2. Further, the comprehensive physiological challenges produce a robust pruned population of SMC models for use in various applications. The low acceptance rate of 2.2 % during population pruning however, underscores the necessity of rigorous population calibration and validation to ensure physiological plausibility.

We also demonstrate that our POMs framework further supports the in-silico evaluation of therapeutic interventions, particularly those targeting calcium handling and contractile dynamics. In Figure 3, a trend of reduced calcium response, and corresponding changes in cell membrane potential, with increased nifedipine dosage can be

seen. Given that calcium is the main driver for contraction, this trend is consistent with the drug results presented in [23]. Additionally, we see a substantial reduction in intracellular Ca with nifedipine concentrations higher than 0.1 μM as in [22]. Physiologically, this reduced contraction will reduce vessel wall tension, resulting in greater vessel diameter and reduced blood pressure.

With future work in mind, the physiological challenges used in model calibration provide a computationally tractable approach to SMC model validation that could be used as a benchmark against which to test future SMC models. The incorporation of these models into coupled vessel and cardiovascular system simulations will further enable the investigation of the impact of SMC variability on coronary perfusion, hemodynamics, disease phenotypes and therapeutic interventions.

5. Conclusion

This work lays the foundation for incorporating biological variability into vascular system simulations, and highlights the importance of rigorous model validation and variability quantification in advancing predictive cardiovascular modeling.

Acknowledgments

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