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A Computational Approach to Understanding Gastrointestinal Motility in Health and Disease

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Abstract— **Gastrointestinal (GI) motility disorders are not well understood, resulting in patient management that typically controls symptoms. Patients suffer from reduced quality of life and incur large costs from chronic GI disorders. It is imperative to elucidate underlying mechanisms causing GI motility disorders that, in turn, can facilitate development of treatment such as drug therapeutics. To this end, we seek to use multi-scale computational models to better understand GI motility in health and disease. An initial computational framework was established to study genetic perturbation in causing a phenotypical change at the GI tissue level. Computer models describing a couple of genetic perturbations were developed and examined in the multi-scale framework. Preliminary findings suggest alterations to phenotype that may adversely affect GI motility. However, much work remains, given the tissue complexity and uncertainties in our knowledge of the GI organs. A future direction will be to incorporate multi-scale mechanical models in the current framework.**

I. Introduction

The gastrointestinal (GI) tract provides for the nutritional, electrolyte and water requirements of the body. Ingested food and fluids get processed and absorbed, and waste materials are removed. Motility, that is the mechanical or contractile behaviour of the GI tract, is what moves and mixes contents along the tract. Motility is a result of coordination of several players such as the smooth muscle cells (SMC), interstitial cells of Cajal (ICC) and the nervous systems. The dominant view is that the self-excitable ICC are pacemakers in the GI tract (e.g. the stomach and intestines), which generate periodic electrical signals received by the SMC. In turn the SMC contract rhythmically, generating basal motility. The nervous systems and other agents modulate the basal motility. In the anomalous state of GI organ motility, either hypermotility or hypomotility, a variety of symptoms and disorders can occur. These include early satiety, nausea, and abdominal pain. Nutritional uptake, and even drug uptake can be adversely affected. Given the critical roles of the ICC and SMC, GI dysmotility can arise due to abnormality in the electrics and/or mechanics of these cells in the GI tissue.

Despite major advancement in GI knowledge, much remains unknown or debatable about the GI physiology in health and disease. Although not life-threatening, GI disorders afflict a significant portion of the population and can cause sustained lower quality of life and incur huge costs. Given the poor understanding of most GI motility disorders, treatments typically target symptoms with lifestyle adjustment, instead of rectifying underlying causes. Drug therapeutics for the GI tract and interest from pharmaceutical companies are thus limited. It is in the public's interest for better patient management and GI healthcare, and hence it is imperative to place emphasis on and advance our understanding of the GI tract.

GI motility disorders, such as irritable bowel syndrome and intestinal pseudo-obstruction are observed to be heterogeneous and multi-factorial. Genetic perturbation is one potential factor conferring disorder susceptibility. Studies have examined ion channels associated with the GI tract. An epidemiological study by Locke et al [1], established a strong correlation between cardiac voltage-gated sodium (Na^+) channel mutation and GI symptoms such as abdominal pain. In the same study, cardiac voltage-gated potassium (K^+) channel mutations did not correlate significantly with GI symptoms. Further statistical study was suggested to support the findings. The same cardiac ion channels of the heart are also expressed and perform essential functions in the GI ICC and SMC.

Mazzone et al found a novel and rare missense R76C mutation of a Na^+ channel interacting protein, telethonin, in a patient with idiopathic intestinal pseudo-obstruction [2]. Co-transfection of the Na^+ channel and telethonin genes was performed on HEK cells. Voltage clamp study on these HEK cells suggested that the electrical behaviour of the Na^+ channel was affected by the mutation. However, this does not mean that the R76C mutation does indeed contribute to intestinal pseudo-obstruction in the native environment.

A variety of voltage-gated K^+ channels are found in the ICC and SMC which typically constitute a significant and important efflux of electrical current of K^+ ions that regulates excitability of these cells. There is no known experimental study on K^+ channel mutations in causing GI motility disorder, and earlier statistics have suggested the lack of correlation between K^+ channel mutations and GI symptoms. Nonetheless, there is an interest to examine the impact of a known K^+ channelopathy from other body systems, in the context of the GI tract. An I177N mutation of the $K_v1.1$ voltage-gated potassium channel, known to cause episodic ataxia, a disorder that affects the central and peripheral nervous systems, was chosen for this study [3]. The same channels are found in the GI ICC and it will be

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interesting to investigate how the mutations affect GI behaviour.

To examine the link between genotype at the sub-cellular level to phenotype at tissue or organ level, a computational approach can be used to complement traditional clinical and experimental methods. An initial multi-scale model of the stomach was established. Computer models describing the mutations were created, verified and integrated into the multi-scale framework to examine the electrophysiological consequences, if any.

II. METHODS

A. Ion channel models

Available voltage-clamp experimental data for the aforementioned mutations were used to construct multi-state Markov models of the Na^+ and K^+ channels and their mutations. Fig. 1 shows the general topology of the Na^+ channel model that includes three clusters of open, closed, and inactivated states. Each cluster can contain a number of states and their transitions. Clancy and Rudy's topology [4] was used to create two Na^+ models describing wild-type and R76C telethonin modulated channel electrics. Similarly, in Fig. 1, the states contained in the dashed box, shows the general topology of the $K_v1.1$ channel, and unlike the Na^+ channel, the $K_v1.1$ channels are known not to inactivate. A common topology based on the Monod-Wyman-Changeux model was chosen, given the tetrameric nature of the $K_v1.1$ protein channel [5]. Likewise, two $K_v1.1$ models describing wild-type and I177N mutated channel electrics were created.

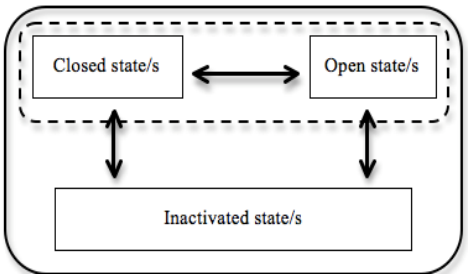

Fig. 1. General topology of a multi-state Markov model. Contained within the solid box are the closed, inactivated and open states that describe a Na^+ channel; while the states contained within the dashed box are representative of $K_v1.1$ channels which are known not to inactivate. Arrows indicate transitions between states that are dependent on conditions such as transmembrane voltage that influences these voltage-gated ion channels.

The opening and closing of voltage-gated ion channels depend on the host cell's transmembrane voltage. Channels in the open state conduct its ions across the cell membrane, thereby shaping cellular electrical behaviour. In the aforementioned Na^+ and $K_v1.1$ models, the rate transition between states depends on transmembrane voltage. These transitions are described by single exponential type of equation that has a thermodynamical basis, where the parameters correspond to the energies involved in transition of one state of the ion channel to another. The general equation form is shown in Equation 1, where A and B are parameter values to be determined and V_m is the transmembrane voltage.

$$k = A\exp(B * V_m) \tag{1}$$

A set of ordinary differential equations (ODE) describing the change in probability of each state in a Markov model can be set up based on its rate equations (Equation 2 shows an example of an ODE, where P_i refers to state i's probability, k refers to the transition rate of a state, j and l denote states that transit into and away from state i respectively). The set of ODE will allow the probability of the ion channels in the open state to be solved.

$$\frac{dP_i}{dt} = \sum_{j \neq i} k_j P_j - P_i \sum_{l=i, \neq j} k_l \tag{2}$$

For instance, solving the open probability, O_{Na}, of a Na^+ channel then allows the whole cell sodium current, I_{Na} to be calculated (Equation 3, where G_{Na} and E_{Na} are Na^+ whole cell conductance and reversal potential respectively).

$$I_{Na} = G_{Na} O_{Na} (V_m - E_{Na}) \tag{3}$$

To completely define the wild-type and mutation models of Na^+ and $K_v1.1$, voltage-clamp experimental data [2, 3] were used in conjunction with a suitable procedure to fit for the parameter values of the rate transition equations (based on the format earlier described for Equation 1). The simplex method was used to estimate the parameter values through minimization functions of sum of squared error (between experimental and model predicted data). Sensitivity was examined by random variation of the parameters, within a 10% range, that possibly corresponds to experimental variation. The outputs exhibited the general behaviour expected of these channels.

B. Single cell models

The verified models of the Na^+ and $K_v1.1$ channels can then be integrated into established biophysical single cell models of the gastric ICC [6] and SMC [7] electrics. Each cell model is formulated using the traditional Hodgkin-Huxley approach,

$$\frac{dV_m}{dt} = -\frac{1}{C_m} \sum I_{ion} \tag{4}$$

where V_m is the transmembrane voltage, C_m is the cell membrane capacitance, and I_{ion} are the critical ionic currents that shape the cellular electrical behaviour. I_{ion} will include currents carried by Na^+ and $K_v1.1$ channels. Note that the ICC model is designed to be self-excitatory, akin to actual ICC while a realistic ICC stimulus acts as the input to excite the SMC model. Na^+ channels and telethonin are found in the human GI SMC, while $K_v1.1$ channels are found to exist in the GI ICC. The R76C mutation was examined by replacing the existing Na^+ descriptions from the SMC model with the Na^+ Markov models, followed by in silico experiments with 100% wild-type Na^+ channels and 100% R76C affected Na^+ channels. The existing $K_v1.1$ model in

the ICC model was replaced with the $K_v1.1$ Markov models to examine the I177N's impact in a similar manner. The models were solved using the forward Euler method.

C. Multi-cellular models

The single cell models can be linked up into a continuum syncytium to form a multi-cellular model described by bidomain equations which are widely used in cardiac tissue modeling. Such a continuum model can be formulated at various scales such as a 1-dimensional strip, 2-dimensional tissue block, and a 3-dimensional organ structure. Here, an extended version of the bidomain equations [8] was used to form a 1-dimensional strip of gastric tissue (Equations 5 to 7).

$$\sigma_e \frac{d^2\phi_e}{dx^2} + \sigma_i^{ICC} \frac{d^2\phi_i^{ICC}}{dx^2} + \sigma_i^{SMC} \frac{d^2\phi_i^{SMC}}{dx^2} = 0 \qquad (5)$$

$$\sigma_i^{ICC} \frac{d^2\phi_i^{ICC}}{dx^2} = A_m^{ICC}(C_m^{ICC} \frac{dV_m^{ICC}}{dt} + \sum I_{ion}^{ICC}) + A_m^{gap} I_{gap} \qquad (6)$$

$$\sigma_i^{SMC} \frac{d^2\phi_i^{SMC}}{dx^2} = A_m^{SMC}(C_m^{SMC} \frac{dV_m^{SMC}}{dt} + \sum I_{ion}^{SMC}) - A_m^{gap} I_{gap} \qquad (7)$$

New symbols in Equations 5 to 7 include σ for conductivity, ϕ for electrical potential, A_m for membrane area to volume ratio; subscripts i and e refer to intracellular and extracellular spaces respectively. I_{gap} refers to the current that flows between ICC and SMC via connecting protein channels known as gap junctions. I_{gap} is currently defined using a simple relation:

$$I_{gap} = g_{gap}(V_m^{ICC} - V_m^{SMC}) \qquad (8)$$

In a similar fashion, mutations of Na^+ and $K_v1.1$ models can be examined in separate 1-dimensional models. The single cell models containing the Markov models can be integrated into the 1-dimensional models through the I_{ion} term seen in Equations 6 and 7. A forward-time and central-space finite difference method was used to solve the models. The tissue parameter values (e.g. A_m) follow the GI extended bidomain equations in [8].

III. Results and Discussion

A. Ion channel models

The Na^+ and $K_v1.1$ models were verified against experimental data, for their adequacy in describing its channel behaviours. Fig. 2 shows that the steady-state activation kinetics of both wild-type and R76C affected Na^+ channel behaviour which matched well with experimental data. Fig. 3 shows that the normalized $K_v1.1$ currents of both wild-type and mutation were able to agree well with experimental data.

B. Single cell models

It was found that the presence of R76C mutation caused an increase in the resting transmembrane voltage of the SMC membrane depolarization (Fig. 4). In the ICC, the presence of I177N mutation resulted in a significant change in the transmembrane voltage, where there is a loss of phasic behaviour associated with strong depolarization (Fig. 5).

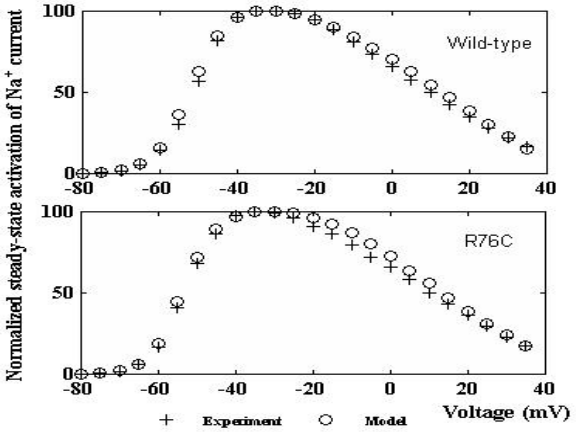

Fig. 2. A comparison of normalized steady-state activation kinetics of both wild-type and R76C affected Na^+ channels. A good agreement between experimental data [2] and model prediction is observed. Note that there is a leftward shift of 3.5mV ($V_{0.5}$) and other kinetic changes (not shown) due to the R76C mutation.

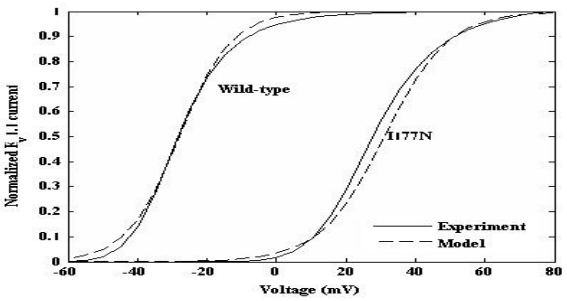

Fig. 3. A comparison of normalized current from both wild-type and I177N $K_v1.1$ channels. A good agreement between experimental data [3] and model prediction is observed.

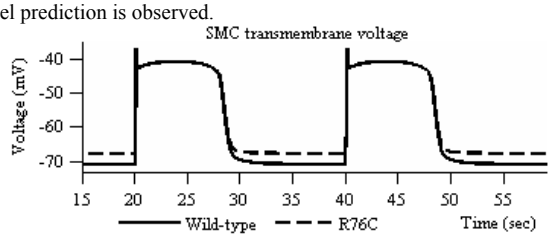

Fig. 4. Simulation result for SMC transmembrane voltage. The presence of R76C mutation has caused an increase in the resting phase transmembrane voltage.

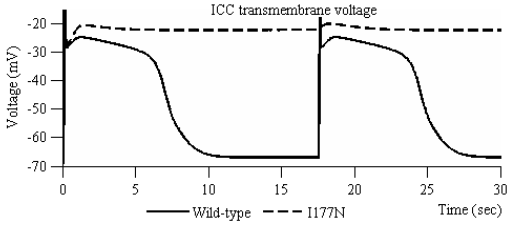

Fig. 5. Simulation result for ICC transmembrane voltage. The presence of

I177N mutation has caused general increase in depolarization resulting in loss of phasic voltage behaviour.

C. 1-dimensional model

The result for the R76C mutation in a 100mm strip of the 1-dimensional model is presented in Fig 6. The R76C mutation has caused an apparent reduction of transmembrane voltage frequency of about 0.2 cycles per minute from 13 cycles (wild-type) to 12 cycles (R76C) within the same time period.

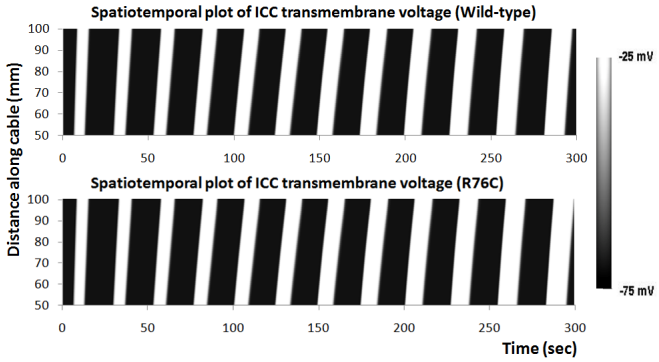

Fig. 6. The spatiotemporal plots of ICC transmembrane voltage, for the case of wild-type Na^+ channel (top) and R76C affected Na^+ channel (bottom). These results were obtained from simulations using a 100mm 1-dimensional gastric strip model. The R76C mutation has caused a reduction in frequency of the periodic transmembrane voltage behaviour.

D. Discussion

The macroscopic ion channel models were verified to adequately describe the experimental data. As better techniques appear, and as more experimental data becomes available, these ion channel models can be refined and the effects at the higher spatial scale models updated. For instance, biophysical characterization through single channel and patch clamp studies can shed light on how single channel conductance is affected by a mutation and how voltage sensitivity is modulated by mechanical stimulus (e.g. Na^+ channels are mechanosensitive [9]).

The simulation results of the single cell models indicated that the electrical behaviour of both the SMC and ICC was altered by the presence of the R76C mutation and I177N mutation respectively. At the multi-cellular level where ICC and SMC can communicate via intracellular and extracellular continuum spaces, as well as gap junctions, it was shown that the R76C mutation has caused a change in electrical frequency. This change possibly emerged due to nonlinear interactions that changed intracellular handling of calcium (Ca^{2+}), an important ion species for ICC pacing frequency.

Thus, an alteration in electrical behaviour can bring about consequences such as altered calcium regulation within the ICC and the SMC. Ca^{2+} in the SMC acts as a critical link between electrics and mechanics, where Ca^{2+} complexes with calmodulin to activate myosin light chain kinase (MLCK). MLCK phosphorylates myosin light chains which then elicit cross-bridge cycling and hence contraction in the SMC. Therefore, a change in calcium regulation over time, due to a change in cellular electrical behaviour can induce adverse change in timing and intensity of the SMC contractions. This preliminary work thus demonstrated that the applicability of a computational framework to understand the link between genotype and phenotype, where initial results suggest the potential of R76C and I177N mutations in disrupting motility. As experimental techniques expand and improve, the predictions of such a framework can be better validated.

IV. FUTURE WORK

The modeling framework put forth in this paper predominantly involves stomach electrics in a single strip. To better study how mutation altered electrics translate into mechanical perturbations, mechanical models have to be developed and integrated into the electrical framework of a whole stomach. This should be a bi-directional framework (i.e., electrics affect mechanics and vice-versa) that also includes description of the deformation of the GI organ geometry (and thus motility). A similar framework of the lower GI organs can also be set up to study motility disorders concerning the small and large intestines.

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