Modelling Human Colonic Smooth Muscle Cell Electrophysiology

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Abstract—The colon is a digestive organ that is subject to a wide range of motility disorders. However, our understanding of the etiology of these disorders is far from complete. In this study, a quantitative single cell model has been developed to describe the electrical behaviour of a human colonic smooth muscle cell (hCSMC). This model includes the pertinent ionic channels and intracellular calcium homeostasis. These components are believed to contribute significantly to the electrical response of the hCSMC during a slow wave. The major ion channels were constructed based on published data recorded from isolated human colonic myocytes. The whole cell model is able to reproduce experimentally recorded slow waves from human colonic muscles. This represents the first biophysically-detailed model of a hCSMC and provides a means to better understand colonic disorders.

Keywords—Mathematical modelling, Cell model, Human colon, Ion channels.

INTRODUCTION

Gastrointestinal (GI) motility arises from the coordinated activity of smooth muscle cells, interstitial cells, and the enteric nervous system. Interstitial cells of Cajal (ICCs) are the pacemaker cells that generate spontaneous electrical activity, known as slow waves, while smooth muscle cells function as the ultimate effector targets that perform the mechanical response. ICCs are electrically coupled to smooth muscle cells (SMCs) *via* gap junctions.⁴⁹ The regular slow waves, originating from the ICCs, are passively propagated to neighbouring SMCs.⁵¹ SMCs respond to this electrical stimulus through the activation of voltage-dependent ion channels. The influx of calcium ions serves as an important intracellular second messenger to further regulate the response.⁴⁹

Many prevalent digestive problems, such as chronic constipation, irritable bowel syndrome, and intestinal pseudo-obstruction, have been correlated with colonic motility dysfunction.^{29,52} These disorders regularly inflict considerable and long-term morbidity in affected patients.⁴³ However, the diagnosis and treatment of these disorders remain challenging due to our limited understanding of their etiology.⁵² The emergence of computational modelling provides a promising tool to describe the underlying intricate cellular networks, which is a crucial step towards better understanding the complex physiology and pathophysiology of the colonic motility.

Previous modelling work attempted to describe human colorectal myoelectrical activity using a chain of relaxation oscillators.³⁵ These models were able to reproduce the three basic motility patterns (the low- and high-frequency oscillations and the zero activity) and reveal the relationship between the phase shift and mode frequency. However, this phenomenological approach has limited applications due to the lack of a direct physical interpretation of the model parameters. In recent years, biophysical models have been developed to describe the cellular electrophysiology of SMCs in the canine stomach and human jejunum with the input of slow waves from an ICC.^{15,44} These models have succinctly described the cell electrophysiology through descriptions of the pertinent ion channels and intracellular calcium dynamics, which are the primary regulators of SMC behaviour. Here, we present a novel biophysically-based human colonic SMC model (hCSMC) with descriptions of predominant components that can reproduce cellular electrical behaviour in response to a slow wave from an ICC. This model provides a fundamental framework for better understanding human colonic motility and the relevant disorders.

MATERIALS AND METHODS

Following a Hodgkin–Huxley (HH) approach, the cell membrane of the SMC was described by an

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equivalent circuit consisting of a capacitance connected in parallel with variable conductances representing the different ion channels.²⁴ The governing equation for the membrane potential, V_m , over time, t , is given by

$$\frac{dV_m}{dt} = -\frac{1}{C_m} (I_{ion} + I_{stim}) \quad (1)$$

where C_m is the cell capacitance, which was chosen to be 80 pF, within the range of 50–90 pF as reported in hCSMCs.^{18,32,65} I_{stim} is the stimulus current from an ICC, and I_{ion} is the sum of all ionic currents crossing the cell membrane, which can be represented as:

$$I_{ion} = I_{Kni} + I_{Kfi} + I_{CaL} + I_{CaT} + I_{Na} + I_{NCX} + I_{NaK} + I_{NSLC} \quad (2)$$

where I_{Kni} and I_{Kfi} are the non-inactivating and fast-inactivating potassium currents, I_{CaL} and I_{CaT} are the L-type and T-type calcium currents, I_{Na} is the sodium current, I_{NCX} and I_{NaK} are the currents generated by sodium–calcium exchanger and sodium–potassium pump, respectively, and I_{NSLC} is the non-selective leakage current. Figure 1 shows a schematic overview of the proposed hCSMC model. The ionic currents were described using the HH formalism.²⁴ The complete set of mathematical equations for the hCSMC model and the corresponding parameter values and current responses are available in Supplementary Material.

FIGURE 1. Schematic view of the hCSMC model. All pertinent components i.e., major ion channels and intracellular calcium dynamics are depicted. Intracellular calcium concentration is regulated by the influx through I_{CaL} and I_{CaT} , and extruded via I_{NCX} , which is usually located near the sarcoplasmic reticulum. Intracellular free calcium also modulates the kinetics of both potassium channels that are calcium-sensitive.

Non-inactivating Potassium Channels

Large outward potassium currents have been recorded from a wide variety of GI muscle cells.^{54,61} Distinct K⁺ channel populations have been reported to participate in regulating resting membrane potential, limiting the initial upstroke, maintaining the plateau phase, and initiating repolarization of a slow wave.²¹ The non-inactivating potassium currents (I_{Kni}) recorded in human colonic myocytes closely resemble those delayed rectifier components found in canine colonic smooth muscles.⁸ These currents also have similar characteristics to the calcium-activated K⁺ current, due to their voltage- and calcium-dependencies, and non-inactivating kinetics. The I_{Kni} was found to be selectively blocked by apamin and tetraethylammonium (TEA) in human colonic myocytes.¹⁸ This appears similar to the pharmacological properties of intermediate conductance K⁺ channels (IK) recorded from the mouse ileum.⁶² In this model, I_{Kni} is described by

$$I_{Kni} = \overline{G_{Kni}} d_{Kni} d_{CaKni} (V_m - E_K) \quad (3)$$

where $\overline{G_{Kni}}$ represents the maximum conductance, and d_{Kni} and d_{CaKni} denote the voltage-dependent and calcium-dependent activation gating variables. The model's parameter values were fitted to experimental recordings from hCSMCs.¹⁸ Figures 2a and 2b show the relative current amplitude of I_{Kni} over increasing test potentials at two different Ca²⁺ concentrations (pCa = 8.3 and 7.3; pCa = $-\log_{10}[\text{Ca}^{2+}]$) in comparison with the experimental data. The corresponding simulated current responses over time are provided in Supplementary Material.

Fast-inactivating Potassium Channels

A transient potassium current with fast kinetics, I_{Kfi} , has been recorded from isolated human colonic myocytes.¹⁸ Potassium currents with the same kinetic properties were also observed in myocytes from the murine GI tract.^{1,2} However, these so-called A-type currents were found to be 4-aminopyridine-sensitive, but not TEA-sensitive, which differs from those reported in human colon. The rapid-inactivating I_{Kfi} recorded in hCSMC was sensitive to both voltage and the Ca²⁺ concentration. I_{Kfi} is thus modelled as

$$I_{Kfi} = \overline{G_{Kfi}} d_{Kfi} d_{CaKfi} f_{Po} (V_m - E_K) \quad (4)$$

where $\overline{G_{Kfi}}$ is the maximum channel conductance, d_{Kfi} and d_{CaKfi} represent the voltage and calcium-dependent activation gating variables, while f_{Po} is used to describe the voltage and calcium-dependent inactivation kinetics that control the parallel shift of the steady-state inactivation curve in response to varying

FIGURE 2. *I-V* curve for ionic conductances (a, b) Normalized $I_{K_{Ni}}$ amplitude as a function of test potential for simulated (solid line) and experimental (dots) data at $pCa = 8.3$ (left) and 7.3 (right).¹⁸ (c) Simulated $I_{K_{Ni}}$ current density evoked by depolarizing steps from holding potential of -80 mV at 50 nM ($pCa = 7.3$) (solid line) and 5 nM ($pCa = 8.3$) (dashed line) in comparison with experimental data (dots).¹⁸ (d) Peak I_{CaL} current density vs. test potential for simulated (solid line) and experimental data (dots) recorded at a temperature of 37°C .³² (e) Peak I_{CaT} *I-V* plot for simulated (solid line) and experimental data (dots) adopted from Xiong *et al.*⁶⁶ (f) Normalized peak I_{Na} as a function of test potential for simulated (solid line) and experimental (dots) data recorded by Xiong *et al.*⁶⁵.

potentials or Ca^{2+} concentrations.¹⁰ This channel model was fitted to the experimental data reported by Duridanova *et al.*¹⁸ The activation and inactivation time constants were corrected for temperature using a Q_{10} value of 1.44 when assembled into the whole cell model of hCSMC.^{30,60} Figure 2c illustrates the peak current densities induced by depolarizing steps from a holding potential of -80 mV at two different Ca^{2+} concentrations. These currents are almost absent at low calcium concentrations and at more positive holding potentials. The transient current responses obtained through the voltage clamp simulations are given in Supplementary Material.

L-type Calcium Channels

L-type Ca^{2+} channels, encoded by the CACNA1C gene, have been identified in hCSMCs.³² These channels are the major route for Ca^{2+} influx to activate a contractile response in GI smooth muscles.^{4,5} This slow-inactivating calcium current is sensitive to both nifedipine and BAY K 8644,⁶⁶ similar to those observed in the canine and murine colon.^{37,64} I_{CaL} is described as

$$I_{CaL} = \overline{G_{CaL}} d_{CaL} f_{CaL} f_{CaCaL} (V_m - E_{Ca}) \quad (5)$$

where $\overline{G_{CaL}}$ is the maximum conductance with a value of 8.3 nS, chosen to reproduce experimental data recorded from the human colon.³² The gating variables d_{CaL} and f_{CaL} represent the voltage-dependent activation and inactivation, while f_{CaCaL} describes the calcium-dependent inactivation, whose formulation was adopted from the Corrias & Buist model.¹⁵ The time constant of f_{CaCaL} was chosen to be 12 ms, which is similar to the fast inactivation time constant recorded by Li *et al.*³⁴ Parameter values for the voltage-dependent gating variables have been fitted to replicate the kinetics of human colonic myocytes.³² Figure 2d shows the peak current density as a function of test potential for the simulation result and the corresponding experimental data adopted from human colonic myocytes.³² The current densities were computed through dividing the peak currents by the membrane capacitance of 50 pF, which was adopted from the same experimental study.³² The simulated current responses over time are demonstrated in Supplementary Material.

T-type Calcium Channels

Low-voltage activated T-type currents have been identified in the rat colon,⁶⁴ and human colonic myocytes,⁶⁶ but not in the canine colon.⁴⁷ These currents were sensitive to Ni^{2+} , but resistant to nifedipine and diltiazem.⁶⁶ The subtype of the Ca^{2+} channel responsible for the T-type currents has been suggested to be of alpha 1H subunit, which is the $Ca_v3.2$ isoform.⁵³ I_{CaT} has been reported to contribute about 30% of the total Ca^{2+} current in smooth muscle cells from the human colon.⁶⁶ These channels are activated at about -70 mV, and usually inactivate within 50 ms. The transient behaviour of these channels suggest its possible role in the initial upstroke phase of slow wave, but not the long-lasting plateau phase. I_{CaT} is modelled as

$$I_{CaT} = \overline{G_{CaT}} d_{CaT} f_{CaT} (V_m - E_{Ca}) \quad (6)$$

where $\overline{G_{CaT}}$ is the maximum channel conductance and its value, 1.72 nS, was selected to reproduce the I - V curve observed in human colonic myocytes.⁶⁶ d_{CaT} and f_{CaT} are the voltage-dependent activation and inactivation gating variables. The steady-state activation curve was adopted from the same experimental data from the human colon.⁶⁶ The inactivation parameters were fitted to experimental data from human urethra myocytes in the absence of a detailed kinetic description from human colonic muscles.²⁵ The representative I - V relationship of I_{CaT} has been replicated according to the experimental data presented by Xiong *et al.*⁶⁶

Our model was subjected to a holding potential of -100 mV and clamped to test potentials ranging from -80 to $+70$ mV for a duration of 300 ms. Figure 2e shows the I - V profile of I_{CaT} , which matches well with the experimental data from hCSMCs with a peak current recorded at -20 mV. The corresponding current responses are given in Supplementary Material.⁵⁷

Sodium Channels

Fast Na^+ currents with various isoforms have been identified throughout the GI tract.^{26,40,65} The variety of isoforms are usually distinguished by their different half maximal inhibitory concentrations (IC_{50}) towards the sodium channel blocker, tetrodotoxin (TTX). The TTX-sensitive Na^+ current has much faster time courses in activation and inactivation compared to TTX-resistant current. Less TTX-sensitive or TTX-resistant sodium currents were observed in rat fundus and human jejunum smooth muscle cells,^{40,42} whereas a highly TTX-sensitive sodium current with an IC_{50} of 14 nM has been identified in the human colon,⁶⁵ resembling those recorded from the rat ileum, and guinea-pig ureter.^{40,55} Intriguingly, a recent study by Neshatian *et al.* (2015) has unveiled the molecular identity of the human colonic sodium channels to be SCN5A-encoded $Na_v1.5$ using reverse transcription-polymerase chain reaction (RT-PCR) and western blots.⁴¹ These channels are similar to those voltage-gated, mechanosensitive sodium channels discovered in human jejunum smooth muscle cells. I_{Na} is represented by

$$I_{Na} = \overline{G_{Na}} d_{Na} f_{Na} (V_m - E_{Na}) \quad (7)$$

where $\overline{G_{Na}}$ is the maximum conductance, while d_{Na} and f_{Na} denote the gating variables for voltage-dependent activation and inactivation, respectively. Their steady-state kinetics were fitted to experimental data from the human colon.⁶⁵ The time constants for the gating variables were chosen to reproduce the current response curves recorded by Xiong *et al.*⁶⁵ Figure 2f illustrates the normalized peak amplitude of I_{Na} as a function of test potential from a holding potential of -100 mV. The simulation results agree well with the experimental data adopted from the human colon, with the maximum current recorded at 0 mV. The time constants for both activation and inactivation were corrected to physiological condition from the experimental temperature ($22^\circ C$) using a Q_{10} value of 1.7, which was chosen based on the temperature-sensitive sodium channel kinetics reported by Han *et al.*²³ The current waveforms of I_{Na} during voltage clamp simulations are illustrated in Supplementary Material. The current behaviour resembles the representative experi-

mental $\text{Na}_v1.5$ current recordings from human colonic myocytes as reported by Neshatian *et al.*⁴¹

Sodium–Potassium Pump

Previous studies have suggested a significant contribution from the Na^+/K^+ pump to the resting potential in the canine proximal colon and guinea-pig taenia-coli.^{7,12} A relatively constant resting membrane potential, averaging -50 mV, was recorded throughout the human colonic circular muscle layer.⁴⁶ In the absence of quantitative data from the human colon, a formulation was adopted from the model proposed by ten Tusscher *et al.* (2004) to describe the behaviour of I_{NaK} .⁵⁹ A maximum current of 0.3082 nA was selected to maintain ionic homoeostasis with a low intracellular Na^+ concentration of 10 mM.

Sodium–Calcium Exchanger

The influx of Ca^{2+} ions through the activation of L-type and T-type Ca^{2+} channels are primarily expelled from the cell by the sodium–calcium exchanger, with the sarcoplasmic reticulum (SR) acting as an important intermediary.⁶ This role is well supported by the close anatomical apposition of the SR and NCX.³⁹ Due to the lack of quantitative data from human colonic myocytes, a formulation similar to the model of ten Tusscher *et al.* (2004) was used to describe the current through the sodium–calcium exchanger (I_{NCX}).⁵⁹ However, instead of adding an extra factor, as used in ten Tusscher's model, to account for the higher concentration of Ca^{2+} near the subspace, here we adopted a slightly different approach. Considering the low affinity of NCX and its role as the only calcium removal mechanism in our case, we use the total Ca^{2+} concentration in place of the small subspace Ca^{2+} concentration factor, with a maximal current value of 1.6575 nA. This is important to ensure calcium homoeostasis and to attain the physiological Ca^{2+} concentrations during slow waves.

Non-selective Leakage Currents

Dwyer *et al.* (2011) have identified the presence of basally-activated non-selective cation channels in human and monkey colonic myocytes, which contributed significantly to the regulation of resting membrane potential.²⁰ These currents may have a significant role in determining the basal excitability of colonic muscles. Na^+ ions have been shown to be the main charge carrier for these basal currents to support the relatively depolarized resting potential of human colon. We thus include a non-selective leakage conductance (NSLC) in our model, which is described by

$$I_{\text{NSLC}} = G_{\text{NSLC}}(V_m - E_{\text{NSLC}}) \quad (8)$$

Here, a constant conductance G_{NSLC} with a value of 0.845 nS, was adopted to obtain the normal resting membrane potential of human colonic myocytes at -50 mV.

Stimulus Current from ICC

In colonic muscle, spontaneous slow waves are generated by ICC residing in the submucosal layer and are passively conducted to smooth muscle cells.^{46,48} The slow waves from the ICC are injected as a stimulus current, I_{stim} , into SMC via gap junctions, which are represented by a constant conductance. I_{stim} is described as

$$I_{\text{stim}} = G_{\text{couple}}(V_m - V_m^{\text{ICC}}) \quad (9)$$

Here, G_{couple} is the coupling conductance between the ICC and SMC, V_m represents the membrane potential of the hCSMC while V_m^{ICC} represents the membrane potential of the ICC. In the absence of a biophysical model description of a colonic ICC, a phenomenological model was used to describe a single slow wave from an ICC, which can be represented as

$$V_m^{\text{ICC}} = \begin{cases} V_{\text{rest}}^{\text{ICC}} + V_{\text{amp}}^{\text{ICC}} \left(\frac{t}{t_2} \right) & \text{for } t < t_{\text{peak}}^{\text{ICC}} \\ V_{\text{rest}}^{\text{ICC}} + V_{\text{amp}}^{\text{ICC}} \left(\frac{1}{1 + \exp\left(\frac{t - t_1}{t_{\text{slope}}}\right)} \right) & \text{for } t_{\text{peak}}^{\text{ICC}} \leq t \leq t_{\text{period}} \end{cases} \quad (10)$$

where $V_{\text{rest}}^{\text{ICC}}$ and $V_{\text{amp}}^{\text{ICC}}$ signify the resting potential of ICC and the amplitude of slow wave. The parameters $t_{\text{peak}}^{\text{ICC}}$ and t_{period} denote the duration of the upstroke and the period of a single slow wave, respectively. t_1 and t_2 are the conditioning factors while t_{slope} is the slope factor. All parameters were chosen to reproduce a representative slow wave recording from the human colon.¹⁴ The values of the corresponding parameters were provided in Supplementary Material.

Intracellular Ion Concentrations

Changes in the intracellular concentrations of the three major ions were tracked over time to ensure ion homoeostasis. A large fraction of calcium influx through the L-type and T-type Ca^{2+} channels is buffered upon entering the cell. Only a small proportion of the unbound free Ca^{2+} ions is able to exert regulatory responses. Previous studies estimated the ratio of bound-to-free Ca^{2+} concentration in cytoplasm to be approximately 40.¹⁶ To this end, a calcium buffering factor, β with a constant ratio of 1:41 was used to describe the buffering effect on the calcium influx. The

free intracellular Ca^{2+} concentration is thus governed by

$$\frac{d[\text{Ca}^{2+}]_i^{\text{free}}}{dt} = \frac{-\beta}{2FV_{\text{cell}}} (I_{\text{CaL}} + I_{\text{CaT}} - 2I_{\text{NCX}}) \quad (11)$$

where F is Faraday's constant, and V_{cell} is the total cytoplasmic volume. The value of V_{cell} was chosen to be 5 pL, by assuming 52% of a cylindrical cell volume with a length 83 μm and radius of 6 μm , consisted of cytoplasm after subtracting the volume fraction of the nucleus and other organelles.^{3,13} Changes in the intracellular Na^+ and K^+ concentrations were also tracked over time by the following equations

$$\frac{d[\text{Na}^+]_i}{dt} = \frac{-1}{FV_{\text{cell}}} (I_{\text{Na}} + 3I_{\text{NCX}} + 3I_{\text{NaK}} + I_{\text{NSLC}}) \quad (12)$$

$$\frac{d[\text{K}^+]_i}{dt} = \frac{-1}{FV_{\text{cell}}} (I_{\text{Kni}} + I_{\text{Kfi}} - 2I_{\text{NaK}} + I_{\text{stim}}) \quad (13)$$

Here I_{stim} , the stimulus current from ICC, is incorporated into the total $[\text{K}^+]_i$ to prevent a potential non-physiological drift caused by a nonconservative implementation of the stimulus.²⁸

RESULTS

Slow Waves and Whole Cell Current Responses

Integrating all of the ion channels into a cell model with an ICC stimulus produces a series of slow waves resembling those recorded from human colonic smooth muscles.¹⁴ The simulated slow waves shown in Fig. 3b have a resting potential of -55 mV after coupling with the more hyperpolarized resting potential of ICC and indicate an amplitude of 16 mV with frequency of 5 cycles/min (cpm), which are in agreement with those published in literature and depicted in Fig. 3a.^{14,46} In view of the large variation of frequencies across the human colon, our model can be calibrated to emulate varied conditions under different frequencies by tuning the parameters in Eq. (10) that describes the input slow-wave characteristics from the ICC.

Figure 3c presents the corresponding intracellular free calcium fluctuation $[\text{Ca}^{2+}]_i^{\text{free}}$ during slow wave activity. The calcium transients resemble the shape and the typical phases of slow waves.³³ The predicted variation of the intracellular calcium level is in line with the experimental calcium transients observed in human colon, with amplitudes within 150 nM.³¹

The whole cell current responses of the hCSMC were examined by subjecting the model to the voltage clamp conditions presented in the experimental study

FIGURE 3. Slow waves in human colonic smooth muscles. (a) Experimental recordings from the human colon with similar characteristics in both left and right colon, reproduced from Choe *et al.* (2010) with permission.¹⁴ (b) Simulated hCSMC slow waves at 5 cpm. (c) Variation in intracellular free Ca^{2+} concentration during slow waves.

on human colonic myocytes by Duridanova *et al.*¹⁸ Figure 4a shows the whole cell current waveforms evoked by depolarizing steps from -80 to $+40$ mV in 10 mV increments. The resulting current waveforms demonstrate a good reproduction of the experimental observations.¹⁸ The rapidly developing large outward current peaks within 50 ms and declines to a steady-state level, which is about 80% of the peak current at a $+40$ mV clamping voltage. In the same experimental study, the effect of calcium currents (I_{Ca}) blockade on the whole cell current waveforms was also investigated after the application of NiCl_2 and nifedipine. This experimental condition was simulated by setting both Ca^{2+} conductances to zeros. The lack of calcium influx through L-type and T-type Ca^{2+} channels attenuates a large part of the potassium currents that are highly calcium-sensitive, particularly I_{Kni} . The changes in the current waveforms demonstrated in Fig. 4b agree with the experimental current waveforms.¹⁸

Potassium and Calcium Channel Blockers

Activation of Ca^{2+} -sensitive K^+ currents is believed to contribute to the repolarization of the slow

FIGURE 4. Whole cell current waveforms over time during voltage clamp conditions. (a) Modelled hCSMC whole cell currents with 400 ms depolarizing pulses. (b) Current responses after blocking the two major inward currents (I_{CaL} and I_{CaT}). (Note: Both simulation conditions were in the absence of I_{Na} to mimic the presence of tetrodotoxin in the experimental setting.¹⁸).

waves.¹¹ This is supported by an inhibition of the slow wave repolarization, with an increased plateau potential and duration, in the canine colon after the application of tetrapentylammonium (TPeA), a potent blocker of Ca^{2+} -activated K^+ currents.⁹ Tetraethylammonium (TEA), a well-known K^+ channel blocker, also produced similar effects on the slow waves and caused a significant depolarization of the resting membrane potential.⁴⁵ To simulate the effect of adding TEA at its IC_{50} , the maximum channel conductance of I_{Kni} was reduced by half and the resulting slow waves are shown in Fig. 5a. As expected, a depolarized resting membrane potential is observed, along with a significantly higher plateau potential. These findings are in agreement with the proposed role of I_{Kni} in maintaining the resting potential and plateau phase due to its non-inactivating behaviour.^{18,45} The unchanged plateau duration is due to the constant phenomenological description of slow waves used in our model that cannot account for the implications of the intervention on the ICC.

The rapidly-inactivating potassium currents observed in GI SMCs have been found to be sensitive to 4-AP and flecainide.^{1,2} These studies have presented a depolarized membrane potential in the presence of 4-AP. In an experiment with myocytes isolated from the murine antrum, the membrane potential between slow waves was elevated by approximately 10% by flecainide at a concentration near to its IC_{50} .¹ Due to the similar channel kinetics, this blocking effect was simulated by halving the maximum conductance of I_{Kfi} . The resulting slow waves in Fig. 5b demonstrate a 6.25% rise in the resting membrane potential. No significant change was observed at the plateau phase, which is consistent with the proposal that the I_{Kfi}

component seems to be mainly activated at the initial upstroke during a depolarization and is almost completely inactivated before reaching the maximum upstroke amplitude.¹⁸

L-type Ca^{2+} currents are the main contributor that regulates the intracellular Ca^{2+} concentration. It has been suggested that Ca^{2+} entry through voltage-dependent Ca^{2+} channels is the key driver to couple slow waves to muscle contraction.^{4,63} Experimentally, blocking half of the L-type Ca^{2+} channels in canine colon by nifedipine caused an 8% depolarization in membrane potential.²² Here, this effect was simulated by reducing the channel conductance to half of its original value. The result in Fig. 5c indicates a rise of 8% in membrane potential between slow waves, which is in line with the aforementioned study. The plateau phase is regulated by the interplay of I_{CaL} and the sustained outward currents I_{Kni} . In theory, a decrease in Ca^{2+} channel conductance should reduce the plateau phase.⁶³ However, the Ca^{2+} -sensitive I_{Kni} have a larger conductance compared to the I_{CaL} . It is therefore suggested that a reduction in calcium influx through the L-type channels decreases the I_{Kni} , leading to a rise in the plateau phase.

Activation of T-type Ca^{2+} channels has been revealed to initiate the upstroke phase, which is important for the entrainment of slow waves.³³ Moreover, in human cultured colonic SMC, inhibition of T-type Ca^{2+} channels by otilonium bromide (OB) exerted an inhibitory effect on muscle contraction.³⁸ The effect of inhibiting I_{CaT} on the slow waves was simulated by varying the channel conductance. Figure 5d shows slight depolarization of 6.25 and 12.5% in the membrane potential of slow waves after a half- and full-block of I_{CaT} . This finding is consistent with the data

from canine colon with a small membrane depolarization ranging from 7 to 12% after the application of 0.5–2.0 mM of Ni^{2+} (a potent inhibitor of T-type Ca^{2+} channels).²⁷ No effect was observed in the plateau phase due to the fast inactivation of I_{CaT} .

Effects of Extracellular Ionic Concentrations

Extracellular ionic concentrations are a prerequisite in setting the Nernst potentials of ions across the cell membrane. Increasing the extracellular K^+ concentration reduces the driving force for generating out-

ward currents, leading to smaller potassium current amplitudes. Figure 6a delineates the change in the slow wave profile after elevating $[\text{K}^+]_o$ from 6 to 10 mM. Variations in the resting membrane potential and plateau phase suggest the involvement of K^+ conductances. Resting potentials were depolarized by 3 mV in 10 mM of $[\text{K}^+]_o$, which is close to the 5 mV variations recorded from the canine colon.³⁶

Altering the Ca^{2+} gradient across the cell membrane was found to have significant impact on the generation of slow waves.⁶³ The upstroke and plateau phase have been shown to be partly attributed to the

FIGURE 5. The implications of ion channel blockers on the slow wave profile. (a) Simulated 50% blockade of I_{Kni} (dashed line) vs. slow waves under the control condition (solid line). (b) Simulated presence of I_{Kfi} blocker (dashed line) in comparison with the control condition (solid line). (c) Simulated 50% blockade of I_{CaL} (dashed line) vs. controlled slow waves (solid line). (d) Simulated presence of I_{CaT} blocker during half-blocking (dashed line) and full-blocking (dotted line) conditions in comparison with the control slow wave activity (solid line).

FIGURE 6. The effects of extracellular ionic concentrations on the slow wave profile. (a) Simulated slow waves with increased $[\text{K}^+]_o$ (dashed line) vs. slow waves under the control condition (solid line). (b) Simulated effect of reduced $[\text{Ca}^{2+}]_o$ on slow waves (dashed line) compared to the control slow wave activity (solid line).

activation of Ca^{2+} channels. Experimentally, decreasing $[\text{Ca}^{2+}]_o$ to 0.1 mM depolarized the membrane potential by about 30%.⁶³ This effect was simulated by setting $[\text{Ca}^{2+}]_o$ equal to 0.1 mM in our model. The resulting slow waves (dashed line) together with the original slow waves (solid line) are shown in Fig. 6b. The membrane potential was observed to depolarize by 28%, which is in line with the aforementioned experimental study. The slow waves were not abolished, as was reported in the experimental study. This discrepancy is probably caused by the inhibitory impact of low $[\text{Ca}^{2+}]_o$ on ICC in experiment, recalling that here a phenomenological ICC model has been adopted. In the absence of an ICC stimulus, the membrane potential was observed to depolarize by 7.5 mV.

DISCUSSION

In this study we have developed and validated a model of human colonic smooth muscle cell electrophysiology. To the best of our knowledge, this is the first colonic SMC model, and second human GI model following the human jejunal SMC.⁴⁴ To maintain a balance between accuracy and simplicity in the whole cell model, our model incorporates only those components that were deemed to be major players in setting the cellular electrical response during slow wave activity. Model development and validation were performed in four steps. First, the predominant ionic currents were constructed and validated against individual experimental data from isolated human colonic myocytes. Thereafter, the hCSMC whole cell model was assembled by incorporating the other currents necessary to achieve cell homeostasis. Next, a phenomenological model of an ICC slow wave was applied to the hCSMC model to reproduce experimental recordings from the human colon. Finally, the cell behaviours under different pharmacologically-altered conditions were examined.

Each key individual ionic current plays a distinct role in shaping the slow wave profile. The whole cell current waveforms show that large outward potassium currents are the main conductance over the physiological voltage range. However, minor variations in the calcium currents have a significant impact on the slow waves due to the potassium conductances that are calcium-sensitive. The transient potassium and calcium components are suggested to contribute to the rising phase of the slow waves, while the sustained (slow) components of both potassium and calcium are believed to participate in maintaining the plateau phase. There was no noticeable change in the slow wave

morphology when the sodium channel conductance was reduced (result not shown). The physiological role of the fast Na^+ current has not been well established in these cells, but it has been suggested that the presence of this current may assist in the propagation of excitation.⁶⁵ Recent findings have also demonstrated the mechanosensitivity of the fast Na^+ channels and their potential roles in regulating excitability and contractility of human jejunal and hCSMCs.^{41,56}

Electrophysiological studies have recorded spontaneous electrical slow wave activity across many regions of the GI tract. In general, slow waves, initiated by the pacemaker ICCs residing at the myenteric plexus, have been recorded in the stomach and the small intestine.⁵⁰ However, two discrete electrical events have been found in the colonic musculature; one is the slow waves (2–8 cpm) that mainly propagate along the circular smooth muscle layer, and the other is myenteric potential oscillations (MPO) that occur at a much higher frequency (16–28 cpm), and are largely recorded from the outer circular muscle layer and longitudinal muscle layer.¹⁴ In this study, we only focused on the slow wave component. Another limitation is the availability of data from colonic ICCs. The intrinsic electrical behaviour of the colonic smooth muscles is dependent on the slow wave input from ICCs. However, the details concerning the ionic conductances of the colonic ICC are still lacking. A prescribed phenomenological model has thus been used to emulate ICC slow wave activity as an input to the hCSMC model. The model is readily extensible to accommodate more details as it comes to hand.

Parameter selection is a constant challenge in biophysical modelling due to the inherent biological information carried by each parameter. Patch-clamp experiments have been widely implemented on SMCs to examine the behaviour of different regions of the GI tract in different species. Here our model was developed using experimental data primarily adopted from the human colon. The maximum conductances and kinetic parameters for the individual ionic channels were chosen to reproduce experimental recordings from isolated human colonic myocytes. In the absence of slow wave information from the intact human colon, the whole cell data was adopted from experiments on surgical waste tissue coming from patients with non-obstructive colonic diseases.^{14,46} To date there have been no pharmacological studies that identify the contributions of the major ionic channels in the human colon. The predicted effects of the different blockers have been validated using data from murine or canine colon, or from other GI regions. Despite the fact that some currents seem to be unique and found exclusively

in the human colon, the currents in general share many common kinetic properties with currents characterized from other species or other GI regions. Hence, it is quite conceivable that they have similar contributions in shaping the slow waves that are ubiquitously found throughout the GI tract.

In conclusion, a quantitative model of a human colonic smooth muscle cell has been constructed. This biophysically-based model incorporates the predominant physiological mechanisms active during basal electrical activity with descriptions of major ion channels and intracellular ionic homeostasis. These components are believed to contribute significantly to the electrical response of the hCSMC during a slow wave. The major ion channels have been reconstructed based on data recorded from isolated human colonic myocytes. The whole cell model is able to reproduce experimental slow waves recorded from human colon. This represents the first biophysically-detailed model of a hCSMC and provides a means to better understand colonic motility and the relevant motility disorders. This model is suitable for larger scale model development, i.e., a multicellular tissue model. Moving forward, incorporation into a tissue-level model will allow further validation against data from *in vivo* studies using manometry or extracellular recordings, which are important in gaining insights into the underlying motility patterns.^{17,19,58} Notably, the influence of enteric nervous system is an indispensable domain in regulating contractility and the complex motor patterns of GI smooth muscles. Integrating neuronal modulation to the model is one direction that can promise to bring exciting outcomes in the future.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (doi:[10.1007/s12195-017-0479-6](https://doi.org/10.1007/s12195-017-0479-6)) contains supplementary material, which is available to authorized users.

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CONFLICT OF INTEREST

Jing Wui Yeoh, Alberto Corrias, and Martin Lindsay Buist have declared that no conflict of interest exists.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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