Macro-scale phenomena of arterial coupled cells: a massively parallel simulation

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Impaired mass transfer characteristics of blood-borne vasoactive species such as adenosine triphosphate in regions such as an arterial bifurcation have been hypothesized as a prospective mechanism in the aetiology of atherosclerotic lesions. Arterial endothelial cells (ECs) and smooth muscle cells (SMCs) respond differentially to altered local haemodynamics and produce coordinated macro-scale responses via intercellular communication. Using a computationally designed arterial segment comprising large populations of mathematically modelled coupled ECs and SMCs, we investigate their response to spatial gradients of blood-borne agonist concentrations and the effect of micro-scale-driven perturbation on the macro-scale. Altering homocellular (between same cell type) and heterocellular (between different cell types) intercellular coupling, we simulated four cases of normal and pathological arterial segments experiencing an identical gradient in the concentration of the agonist. Results show that the heterocellular calcium (Ca2⁺) coupling between ECs and SMCs is important in eliciting a rapid response when the vessel segment is stimulated by the agonist gradient. In the absence of heterocellular coupling, homocellular Ca2⁺ coupling between SMCs is necessary for propagation of Ca2⁺ waves from downstream to upstream cells axially. Desynchronized intracellular Ca2⁺ oscillations in coupled SMCs are mandatory for this propagation. Upon decoupling the heterocellular membrane potential, the arterial segment loses the inhibitory effect of ECs on the Ca2⁺ dynamics of the underlying SMCs. The full system comprises hundreds of thousands of coupled nonlinear ordinary differential equations simulated on the massively parallel Blue Gene architecture. The use of massively parallel computational architectures shows the capability of this approach to address macro-scale phenomena driven by elementary micro-scale components of the system.

Keywords: calcium oscillations; spatial gradient; intercellular coupling; Blue Gene; multicellular; arterial bifurcation

1. INTRODUCTION

The scientific method has been predicated on the thesis that by ‘breaking down’ complex phenomena into its component parts, we may be able to further understand the natural beauty of our world both surrounding us and inside us. Both animals and plants are formed from a collection of cells which in some cases numbers into the billions. Each cell within three-dimensional space connects to many providing a unit capable of complex interactions. We are now at the stage where through the use of supercomputing technologies, we are able to ‘rebuild’ those parts into a viable whole and thus compare with the phenomena normally seen with the naked eye. The vasculature of the human is an example of this ‘connectedness’; the model and results set out below emphasize the joining of both cellular biology and supercomputing, where we are able to build those complex units and further our understanding of how we as humans live and breath.

The inner arterial wall of vessels is in constant contact with the flow of blood. The resultant local mechanical and biochemical variations within that flow affect both the local and global vascular tone. Arterial geometries such as branching, bifurcations and curvatures occur systemically in the vasculature and influence the haemodynamics such as wall shear stress (WSS) and its derivatives along with the convective mass transport of blood-borne chemical species. The correlation between low WSS and preferential localization of pathological markers such as atherosclerotic lesions in these regions is certainly not a new observation [1,2]. Recent advances in experimental methods have enabled the demonstration of this correlation in vivo. Cheng et al. [3] demonstrated this by inducing lowered, increased and oscillatory shear stress by placing a perivascular cast in vivo on a straight
segment of carotid artery in an apolipoprotein-E-deficient mouse model. Their results showed that lowered and oscillating WSS created atheroprotective environments, whereas high WSS was atheroprotective. Oscillatory WSS favoured the formation of stable plaques, whereas lowered WSS caused vulnerable plaques susceptible to intraplaque haemorrhage.

Associated with the magnitude of the WSS is the thickness of the concentration boundary layer of blood-borne species enabled by the high Péclet number (ratio of advection to diffusive transport of a species, e.g., adenosine triphosphate (ATP), adenosine diphosphate or oxygen). In the regions with low WSS and flow detachment, the concentration boundary layer thicknesses for such molecules [4] but does not change significantly with pulsatile flow [5]—an effect owing to the different timescales in WSS variation compared with changes in the mass transfer of species [6,7]. This produces a concentration gradient of agonist such as ATP in these regions not only perpendicular to the endothelial surface but also importantly in the axial (streamwise) coordinate that can be treated by time-averaged concentration values.

Other unique properties of these vascular regions have also been identified, inclusive of an altered orientation of the endothelial architecture (axial rather than longitudinal cellular orientation) and a relative expansion of the interstitial space between the endothelial cells (ECs) close to areas of low WSS. These findings have each correlated strongly with atherosclerotic plaque formation (see fig. 3 in the study of Malek & et al. [8]). An important observation is that atherosclerotic lesions appear to grow ‘upstream’ (P. D. Weinberg 2010, personal communication) indicating a phenomenon independent of the motion and transport of LDL (such as cholesterol) by convection. This strongly suggests that cell-to-cell communication or ‘paracrine’-like influences may play an important role in atherosclerosis propagation along the vascular wall. An understanding of cellular ‘coupling’ through connexin gap junctions is therefore a crucial investigative target.

As ATP causes vasoconstriction in neighbouring smooth muscle cells (SMCs) followed by their relaxation via release of endothelial-derived hyperpolarization factor and nitric oxide, the gradients in ATP concentration in these areas become of greater significance. We thus attempt to investigate both the time and spatial cellular dynamics in a computationally designed arterial segment simulated by extremely large coupled populations of ECs and SMCs forming a segment of an artery. These dynamics are modelled by systems of non-linear ordinary differential equations (ODEs) simulating time-varying spatial gradients (on a scale much larger than the cell) in the agonist concentration of ATP on the luminal side of the segment known to elicit an EC-mediated vasoconstrictor response in the underlying SMCs.

The adhesion and subsequent chemical binding of ATP onto the surface of the EC starts a complex cascade of reactions both inside the EC and the SMC, which is schematically described (in a numbered manner in the caption) in figure 1. Essentially, the binding of ATP on the surface of the EC induces the formation of the molecule inositol trisphosphate (IP3) allowing the release of calcium (Ca2+) from internal stores in the cell called the endoplasmic reticulum (ER). The release of calcium itself causes a further release; a reaction called calcium-induced calcium release (CICR). In addition the Ca2+ concentration gradient between the inside and the outside of the cell allows an ion channel to open and allow the influx of Ca2+ into the cell. This is called capacitative calcium entry (CCE). As Ca2+ carries a positive valence of 2, the voltage difference (membrane potential) in the cell changes as a result of either efflux or influx. CICR and CCE alter the membrane potential and enables further an influx of extracellular Ca2+ ions through L-type voltage-operated Ca2+ channels (VOCCs) into the SMCs lying beneath the EC [9]. The important consequence of an adequate concentration of agonist (ATP) and the resulting variations in IP3 and the membrane voltage is that the Ca2+ concentration within the cell can oscillate in time. The amplitude of these oscillations can depend on the availability of the agonist (ATP); at low concentrations of ATP, the amplitudes are relatively large while at high concentrations, the amplitudes are conversely small. If the concentration increases further, the oscillations cease as they do if the concentration is particularly low. This dynamic is shown in figure 2. Finally, the experiment seems to show that WSS can also increase Ca2+ influx via shear-gated plasma membrane Ca2+ channels.

In the ECs, membrane depolarization (cell potential increasing towards zero) encourages an influx of a mix of cations from the extracellular space via certain non-selective ion channels [10]. This progressive increase and then depletion of the internal Ca2+ stores enable an efflux of potassium K+ via calcium-activated potassium channels (KCa) dependent on the cytosolic calcium concentration (see 9 and 10 in figure 1). This potassium efflux pushes the membrane potential back to its resting state thus closing VOCCs in SMCs (via 19 in figure 1). Ca2+ is constantly removed from the cytosol by three main active and passive pathways: (i) an Na/Ca exchanger (number 15 in figure 1), (ii) extrusion via plasmalemal Na+Ca ATPase (number 8 in figure 1), and (iii) and the refilling of intracellular stores through a sarco/plasmemic/ER CaATPase (SERCA) pump (number 6 in figure 1). The existence of voltage-activated calcium channels (VOCCs) in SMCs and the positive feedback of IP3 thus sets up the cytosolic calcium oscillator in the SMCs, with the amplitude and frequency of these oscillations having an inverse relationship.

The efflux of K+ in the space outside of the cell makes the membrane potential of the EC more negative [11]. In both cell types, Ca2+ constantly leaks out of the cell stores (ER/SR) into the cytosol under a concentration gradient and is compensated, in part, by the SERCA pump uptake [12].

Apposing cells of the same or different types can exchange metabolites, including Ca2+ by allowing ionic currents to move through gap junctions. Gap junctions are intercellular channels formed by the docking of a pair of hemichannels from each participating adjoining cell. Hemichannels or connexons are composed of six homogeneous and heterogeneous
units of proteins called connexins and in arterial vascular beds Cx37, Cx40 and Cx43 are most common [13]. Depending on the properties of the constituent connexins, the gap junctions can exhibit selective ionic permeability, thus allowing only certain molecules to pass from one cell to another.
Homotypic gap junctions that are composed of Cx40 and Cx43 favour the passage of cationic ions and molecules [14], such as Ca$^{2+}$ and IP$_3$ depending on the physical size of the channel and electrostatic constitutency. In contrast, homotypic Cx37 channels are highly selective and hence preferentially allow the passage of monovalent cationic currents. However, these Cx37 junctions have the highest conductance of the three vascular connexins and thereby have the most influence on the membrane potential [15]. Cx40 in favouring divalent cations is deemed here to be the major contributor to a concentration gradient-driven Ca$^{2+}$ current. Cx43 has been shown to be the least selective allowing passage of a whole range of large and small molecules such as ATP and IP$_3$ although it has the lowest conductance of the three [15–18].

The gap junctions occur either as homocellular (EC ↔ EC or SMC ↔ SMC) or heterocellular (EC ↔ SMC) junctions.

Here, we consider that the cells are coupled via both homocellular and heterocellular junctions and have electrical, Ca$^{2+}$ and IP$_3$ couplings. We attempt to understand how these coupled populations of cells react to a spatially varying agonist concentration (ATP in this case) on ECs and the effects of different coupling combinations on the Ca$^{2+}$ dynamics of SMCs.

Cx37, Cx40 and Cx43 expressions have been reported in SMCs [19–21] although the reports are few and in specific vascular beds. In ECs however, the evidence of Cx37 and Cx40 expression is more systemic [22]. We thus consider homocellular Ca$^{2+}$ and membrane potential coupling in both ECs and SMCs, and IP$_3$ coupling between SMCs only which provides a model of a non-pathological arterial segment. The presence of myoendothelial gap junctions (MEJs) between SMCs and ECs has been demonstrated in several studies [23,24].

Coupled cell models comprising computationally coupled single EC and SMC models have been used to study the physiological basis of vasoreactivity in small arteries and arterioles. Diep et al. [25] coupled ECs/SMCs where each cell was modelled as a capacitor coupled to a nonlinear resistor and the intercellular gap junction was modelled by an ohmic resistor. Using this multicellular architecture simulating a resistance artery, they investigated the spread of the electrical signal following a local agonist stimulation, initiating either in the endothelium or in the SMC layer. Their results revealed that membrane potential changes did not spread equivalently to all unstimulated cells but depended heavily on the orientation and the strength of coupling. Membrane potential responses originating in the endothelium conducted more efficiently (i.e. to farther distances) than the SMC layer-initiated signal. In a subsequent study by Tran et al. [26], using the same multi-cellular infrastructure modelling a skeletal muscle feed artery, a dominant mechanism was proposed for the poor conduction of membrane potential change initiating in the SMC layer. The authors attributed this to the loss of charge owing to intercellular electrical coupling between SMCs for which the local agonist stimulation could not elicit a global membrane potential change. Furthermore, the spread of focal phenylephrine (a potent vasoconstrictor) stimulation which resulted in a global vasoconstriction, was proposed to be independent of membrane potential. The dominant mechanism in the spread of this vasoconstrictive response was suggested to be the SR Ca$^{2+}$ mobilization and the activation of IP$_3$ receptors on the SR in SMCs.

Similarly, Kapela et al. [27], coupled very detailed models of ECs and SMCs to construct a multi-cellular unit of rat mesenteric arteriole coupled by non-selective gap-junctional transfer. The study was aimed at the conducted vasoreactivity and the role of myoendothelial junctions. With respect to the membrane potential-dependence of the spread of the signal, their results were in agreement with those of Diep et al. [25] and endothelium-dependent spread was more efficient in the case when myoendothelial coupling was strong. In the case of weak myoendothelial coupling, the SMCs showed poor conductivity but the unstimulated cells became sensitive to any further extracellular current. They also suggested that the conduction among ECs was favoured by IP$_3$ coupling rather than the intercellular Ca$^{2+}$ diffusion between ECs.

It is the streamwise communication between both ECs and SMCs that is of particular interest in this case and the ability of the presented model to simulate macro length scales using massively parallel simulations enabled on architectures such as the IBM Blue Gene. With this model, we are able to ‘upscale’ micro-scale cellular phenomena using tens, hundreds of thousands and even millions of coupled cells into a macro-scale world, where we may compare directly with large-scale laboratory experimentation and importantly clinical findings. This has not been attempted before to this particular magnitude. Our results show phenomena not seen at the cellular scale and therefore provide an insight into the possible mechanisms of atherosclerotic lesion growth and other pathological phenomena. Importantly, the model provides a framework for ‘numerical experimentation’: the testing of cellular mechanisms otherwise impossible in the wet laboratory.

2. METHOD

In order to ensure good comparisons with other published data we first simulate a single EC or SMC with...
a system of first-order ODEs, using the model of Koenigsberger et al. [28]. This is a sufficiently detailed model accounting for the essential mechanisms of IP$_3$-induced cytosolic calcium release and the cascade of events following it, in both ECs and SMCs. The state variables modelling a single SMC and EC are: free/unbound cytosolic calcium concentration \(c\), SR/ER calcium concentration \(s\), plasma membrane potential \(v\), open state probability of calcium-activated potassium channels \(\omega\) (for SMC only) and cytosolic IP$_3$ concentration \(I\). Where those without ‘tilde’ represent variables associated with SMCs and those with ‘tilde’ are associated with ECs. The equations governing the concentrations of Ca$^{2+}$ and IP$_3$ are simply a conservation of fluxes into or out of the cell, while the equation for the membrane voltage of either EC or SMC essentially corresponds to Kirchoff’s Law. We write fluxes as \(J\) or \(\tilde{J}\) corresponding to the SMC or EC, respectively. Additionally, we denote the reaction of an agonist on the surface of the endothelium as a flux \(J_{PLC_{agonist}}\) as it models the flux of IP$_3$ into the cell following the “reorientation” of the G protein in the cell membrane (see item (1) activating the G protein-coupled receptor (GPCR) in figure 1).

Time-dependent cytosolic Ca$^{2+}$ dynamics for an SMC and an EC were modelled by ODEs as shown in equations (2.1) and (2.2), respectively.

\[
\frac{dc}{dt} = \tilde{J}_{IP_3} - J_{SRuptake} + J_{CICR} - J_{\text{leak}} - J_{\text{VOC}} + J_{\text{Na}/\text{Ca}} 
\tag{2.1}
\]

and

\[
\frac{ds}{dt} = \tilde{J}_{IP_3} - J_{\text{ERuptake}} + \tilde{J}_{\text{CICR}} - J_{\text{leak}} - J_{\text{cation}} + J_0. 
\tag{2.2}
\]

\(\tilde{J}_{IP_3}\) and \(\tilde{J}_{IP_3}\) are the IP$_3$-induced Ca$^{2+}$ release from SR and ER, respectively. \(J_{CICR}\) models the CICR from either the SR or the ER, whereas \(J_{\text{leak}}\) accounts for a constant efflux of Ca$^{2+}$ from the SR or the ER independent of IP$_3$ activation. \(J_{\text{VOC}}\) models the influx of Ca$^{2+}$ from the extracellular space into the cytosol and is a function of the membrane potential. It is included only in the case of SMC, as the role of the VOCCs (considered to be mostly of T-type with low conductance) in ECs is deemed insignificant [10,29]. Instead, \(J_{\text{cation}}\) models the opening of non-selective cation channels. With a permeability ratio of 0.2–0.6 for Ca$^{2+}$ to monovalent cations, such as Na$^+$ and K$^+$ these non-selective cation channels allow Ca$^{2+}$ entry controlled by both membrane potential and cytosolic Ca$^{2+}$ concentration [29]. These terms together are responsible for bringing Ca$^{2+}$ into the cytosol. Ca$^{2+}$ is in constant efflux from the cytosol via the plasma membrane CaATPase pump modelled by \(J_{\text{leak}}\). In addition to this, the Na/Ca exchanger removes a single Ca$^{2+}$ ion from the cytosol by bringing in three Na$^+$ ions [30]. \(J_{SRuptake}\) and \(J_{\text{ERuptake}}\) model the filling of cytosolic IP$_3$ into the SR or the ER via an SERCA pump, respectively.

Calcium dynamics in SR/ER in an SMC or EC are modelled a shown in equations (2.3) and (2.4), respectively.

\[
\frac{ds}{dt} = J_{SRuptake} - J_{CICR} - J_{\text{leak}} 
\tag{2.3}
\]

and

\[
\frac{ds}{dt} = J_{ERuptake} - J_{CICR} - J_{\text{leak}}. 
\tag{2.4}
\]

Equations (2.5) and (2.6) model the membrane potential of the SMC and EC, respectively.

\[
\frac{dv}{dt} = \gamma(-J_{\text{Na}/K} - J_{\text{Cl}} - 2J_{\text{VOC}} - J_{\text{Na}/\text{Ca}} - J_{K}). 
\tag{2.5}
\]

\[
\frac{dv}{dt} = -\frac{1}{C_m}(\tilde{I}_K + I_{\text{residual}}) 
\tag{2.6}
\]

and

\[
\frac{d\omega}{dt} = \lambda(K_{\text{activation}} - \omega). 
\tag{2.7}
\]

To simulate the potassium efflux through calcium-activated potassium channels (K$_{Ca}$), EC is modelled here with \(\tilde{I}_K\), which is the sum of currents through large conductance (BK$_{Ca}$) and small conductance (SK$_{Ca}$) channels. Opening of BK$_{Ca}$ channels is dependent on the cell membrane depolarization and elevated cytosolic calcium, whereas the activation of SK$_{Ca}$ is only calcium-dependent. \(I_{\text{residual}}\) in equation (2.6) accounts for residual currents owing to the trans-membrane transport of monovalent cations and anions. \(C_m\) in equation (2.6) is the cell membrane capacitance of an EC. However, SMCs express only BK$_{Ca}$ channels [11]. \(J_{K}\) in equation (2.5) models the K$^+$ efflux in the SMC, which takes into account the open state probability of BK$_{Ca}$ channels modelled by equation (2.7). J$_{\text{Na}/\text{K}}, J_{\text{Cl}}$ and J$_{\text{Na}/\text{Ca}}$ in equation (2.5) account for the contribution of the plasma membrane Na/K pump, chloride current and Na/Ca exchanger, respectively towards establishing the membrane potential of an SMC.

Intracellular IP$_3$ dynamics in both SMCs and ECs is modelled by equations (2.8) and (2.9), respectively. Since we are studying the effects of a spatial variation in an agonist which is being advected in peripheral blood flow, only ECs are stimulated by it. We thus vary the parameter \(J_{PLC_{agonist}}\) that simulates the rate of agonist-induced intracellular IP$_3$ generation via PLC pathway and is added to equation (2.9) for an EC. The metabolism of IP$_3$ is accounted for by \(J_{\text{degrade}}\) and is assumed to be a linear function of cytosolic IP$_3$ concentration.

\[
\frac{dI}{dt} = -J_{\text{degrade}} 
\tag{2.8}
\]

and

\[
\frac{dI}{dt} = J_{PLC_{agonist}} - J_{\text{degrade}}. \tag{2.9}
\]

For the full expression of all the fluxes included here, see appendix A and also the study of Koenigsberger et al. [28]. Parameter values used in equations (2.1)–(2.9) are listed in tables 1 and 2.

It is now well-known that for straight arteries, where the WSS is relatively constant, ECs line up with their long axis parallel to the major flow direction. To simulate the geometry of a straight segment of an artery, a cut is
Table 1. Table lists parameters used in describing single cell SMC model as described by Koenigsberger et al. [28].

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F$</td>
<td>maximal rate of activation-dependent calcium influx</td>
<td>0.23 $\mu$M s$^{-1}$</td>
</tr>
<tr>
<td>$K_a$</td>
<td>half saturation constant for agonist-dependent calcium entry</td>
<td>1 $\mu$M</td>
</tr>
<tr>
<td>$G_{Ca}$</td>
<td>whole cell conductance for VOCCs</td>
<td>0.00129 $\mu$M mV$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>$v_{Ca1}$</td>
<td>reversal potential for VOCCs</td>
<td>100 mV</td>
</tr>
<tr>
<td>$v_{Ca2}$</td>
<td>Half point of the VOCC activation sigmoidal</td>
<td>$-24$ mV</td>
</tr>
<tr>
<td>$R_{Ca}$</td>
<td>maximum slope of the VOCC activation sigmoidal</td>
<td>8.5 mV</td>
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<tr>
<td>$G_{Na/Ca}$</td>
<td>whole cell conductance for Na$^{+}$/Ca$^{2+}$ exchange</td>
<td>0.00316 $\mu$M mV$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>$v_{Na/Ca}$</td>
<td>half point for activation of Na$^{+}$/Ca$^{2+}$ exchange by Ca$^{2+}$</td>
<td>0.5 $\mu$M</td>
</tr>
<tr>
<td>$B$</td>
<td>SR uptake rate constant</td>
<td>2.025 $\mu$M s$^{-1}$</td>
</tr>
<tr>
<td>$c_p$</td>
<td>half point of the SERCA activation sigmoidal</td>
<td>1.0 $\mu$M</td>
</tr>
<tr>
<td>$C$</td>
<td>CICR rate constant</td>
<td>55 $\mu$M s$^{-1}$</td>
</tr>
<tr>
<td>$s_c$</td>
<td>half point of the CICR Ca$^{2+}$ efflux sigmoidal</td>
<td>2.0 $\mu$M</td>
</tr>
<tr>
<td>$c_e$</td>
<td>half point of the CICR activation sigmoidal</td>
<td>0.9 $\mu$M</td>
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<td>$D$</td>
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</tr>
<tr>
<td>$v_d$</td>
<td>intercept of voltage dependence of extrusion ATPase</td>
<td>$-100$ mV</td>
</tr>
<tr>
<td>$R_L$</td>
<td>slope of voltage dependence of extrusion ATPase</td>
<td>250 mV</td>
</tr>
<tr>
<td>$L$</td>
<td>leak from SR rate constant</td>
<td>0.025 s$^{-1}$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>scaling factor relating net movement of ion fluxes to the membrane potential (versus related to cell capacitance)</td>
<td>1970 mV$^{-1}$ $\mu$M</td>
</tr>
<tr>
<td>$F_{Na/K}$</td>
<td>net whole cell flux via the Na$^{+}$–K$^{+}$–ATPase</td>
<td>0.0432 $\mu$M s$^{-1}$</td>
</tr>
<tr>
<td>$G_{Cl}$</td>
<td>whole cell conductance for Cl$^{-}$ current</td>
<td>0.00134 $\mu$M mV$^{-1}$ s$^{-1}$</td>
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<td>$v_{Cl}$</td>
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<td>$G_K$</td>
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<td>$v_K$</td>
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<td>$l$</td>
<td>rate constant for net K$_{Ca}$ channel opening</td>
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<td>$c_w$</td>
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<tr>
<td>$b$</td>
<td>translation factor for membrane potential dependence of K$_{Ca}$ channel activation sigmoidal</td>
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<tr>
<td>$v_{Ca3}$</td>
<td>half point for the K$_{Ca}$ channel activation sigmoidal</td>
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<td>maximum slope of the K$_{Ca}$ activation sigmoidal</td>
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</tr>
<tr>
<td>$k$</td>
<td>rate constant of IP$_3$ degradation</td>
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Table 2. Table lists parameters used in describing single cell EC model as described by Koenigsberger et al. [28].

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</tr>
</tbody>
</table>

$J_0$ | constant calcium influx | 0.029 $\mu$M s$^{-1}$ |
| $C_m$ | membrane capacitance | 25.8 pF |
| $G_{tot}$ | total potassium channel conductivity | 6927 pS |
| $v_K$ | K$^+$ equilibrium potential | $-80$ mV |
| $x$ | | 53.3 $\mu$MmV |
| $z$ | | $-80.8$ mV |
| $\tilde{m}_{Na}$ | | $-0.4$ $\mu$M |
| $\tilde{m}_{K}$ | | $1.32 \times 10^3$ $\mu$M mV$^{-1}$ |
| $\tilde{m}_{I}$ | | $0.30$ $\mu$M mV$^{-1}$ |
| $\tilde{m}_{Ca}$ | | $-0.28$ $\mu$M |
| $\tilde{G}_{K}$ | | 0.389 $\mu$M |
| $\tilde{v}_{rest}$ | | 955 pS |
| $\tilde{v}_{rest}$ | | $-31.1$ mV |

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assumed along a line parallel to the axis of symmetry of the artery. By ‘opening out’ the artery such that the wall lies on a plane, large populations of ECs are mapped onto a rectilinear grid with periodic boundary conditions at the edge of the ‘cut’. In contrast, SMCs are mapped perpendicularly such that an integer number of SMCs corresponds to the circumference of the artery. Homocellular and heterocellular couplings simulate the physiological contacts between apposing cells made by gap junctions and are modelled by equations (2.10)–(2.19). Each cell has at least four nearest neighbours of the same type (homocellular), whereas for heterocellular coupling, an EC is coupled to at least 13 SMC neighbours while owing to size variations, an SMC has only five EC neighbours. Cells at the extreme ends of the axial boundary of the grids were conditionally set as to act as sinks, preventing spurious reflective waves to propagate back into the physical domain.

Figure 3 shows the computational layout of the cellular grids and the coupling established between them. Here, ECs are homocellularly coupled with one another via Ca$^{2+}$ and membrane potential, whereas SMCs are coupled with other SMCs via IP$_3$ or Ca$^{2+}$ or both, in addition to the membrane potential [31,32]. The coupling coefficients are treated as free parameters that allow a variation of numerical experiments to be carried out.

2.1. Homocellular coupling

The equations given below model homocellular coupling for both SMC and EC. It should be noted that the coupling is deemed to be linear in form and proportional to the difference in either concentration or potential between cells. The coupling functions are defined as a flux in a similar fashion for ion transfer and can therefore be simply added to the summation of fluxes in the conservation equations for any of the concentrations (Ca$^{2+}$ and/or IP$_3$) or membrane voltage. Here, $J_{\text{SMC}}^{\text{SMC}}$ and $V_{\text{SMC}}^{\text{SMC}}$, for example, are coupling fluxes for concentration and voltage, respectively.

$$J_{\text{Ca}}^{\text{SMC}} = -p_{\text{Ca}}(c - c_i), \quad (2.10)$$

$$V_{\text{SMC}}^{\text{SMC}} = -g(v - v_i), \quad (2.11)$$

and

$$J_{\text{IP}}^{\text{SMC}} = -p_{\text{IP}}(I - I_i). \quad (2.12)$$

Equations (2.10)–(2.12) are added to equations (2.1), (2.5) and (2.8), respectively.

Equations (2.13)–(2.15) are added to equations (2.2), (2.6) and (2.9), respectively. Subscripts $k$ and $l$ represent the state variables of nearest homocellular neighbours of each EC or SMC at each time step.

2.2. Heterocellular coupling

Here the coupling equations are for heterocellular conditions and are similar in form to the homocellular coupling functions.

$$J_{\text{Ca}}^{\text{EC}} = -P_{\text{EC}}(c - c_m), \quad (2.16)$$

$$V_{\text{EC}}^{\text{EC}} = -G(v - v_m), \quad (2.17)$$

and

$$J_{\text{IP}}^{\text{EC}} = -P_{\text{IP}}(I - I_m). \quad (2.18)$$

Equations (2.16)–(2.18) are added to equations (2.1), (2.5) and (2.8), respectively.

$$J_{\text{Ca}}^{\text{SMC}} = -\tilde{P}_{\text{SMC}}(c - c_m), \quad (2.19)$$

$$V_{\text{SMC}}^{\text{SMC}} = -\tilde{G}(v - v_m), \quad (2.20)$$

and

$$J_{\text{IP}}^{\text{SMC}} = -\tilde{P}_{\text{IP}}(I - I_m). \quad (2.21)$$

Equations (2.19)–(2.21) are added to equations (2.2), (2.6) and (2.9), respectively. Indices $m$ and $n$ refer to nearest heterocellular SMC or EC neighbours, respectively, to each cell. $g$, $G$, $p_{\text{Ca}}$, $P_{\text{EC}}$ and $p_{\text{IP}}$, $P_{\text{IP}}$ in equations (2.10)–(2.21) are electrical (membrane potential), Ca$^{2+}$ and IP$_3$ coupling coefficients of homocellular and heterocellular junctions, respectively. The ‘tilde’ sign represents the association of a variable or coefficient with an EC.

As a default configuration, two cases (cases 1 and 2) of a healthy vessel are considered here. Ca$^{2+}$ and IP$_3$ coupling and Ca$^{2+}$, IP$_3$ and membrane potential coupling across MEJs are implemented in these two scenarios, respectively. To simulate the pathological state, two further cases are developed; firstly IP$_3$ coupling, in addition to the default EC homocellular coupling, is implemented to simulate upregulation of Cx43 in lesion-prone areas [32,33]. Table 3 lists the modes of coupling considered in the four cases taken into account in this study. In progressive atherogenesis, (e.g. early atheroma, Cx37 and Cx40 expressions are downregulated thus making Cx43 the dominant intercellular communication in ECs [31,32]). To simulate this scenario, homocellular and heterocellular membrane potential and Ca$^{2+}$ coupling are disabled between ECs and this is treated as case 4. Table 4 lists the parameter values to establish each case.

2.3. Numerical algorithm

The system of coupled sets of nonlinear ODEs was solved using an explicit Runge Kutta(4,5) scheme.
implemented in a robust open source software called RKSUITE written by Brankin et al. [34]. An arterial segment of an axial length of approximately 5 cm with radius of 50 μm was simulated by mapping 82,944 cells (59,904 SMCs and 23,040 ECs) around a circular domain as shown in figure 3. As it was not viable to execute such a large problem on a serial platform because of computational expense, the algorithm was written to run on the massively parallel IBM Blue Gene/L architecture available at Canterbury (www.bluefern.canterbury.ac.nz). Coupling of the cells was enabled by using the Message Passing Interface library for interprocessor communication. Each node of the Blue Gene contained a specified number of both EC- and SMC-coupled cells. We have used a number of nodes of Blue Gene to accommodate the total number of cells and this corresponds to 40–648 cells per node. Figure 4 shows a unit comprising 13 SMCs and five ECs mapped on to a single node of Blue Gene/L compute node. For the particular results presented here, the parallelization consisted simply of a one-dimensional connection of nodes, which when coupled together forms an axial segment of a vessel. This is easily used using the toroidal topology of the Blue Gene architecture.

At regular intervals, state variable values were passed to the neighbouring (left and right) green-coloured node as shown in figure 4 (Blue Gene nodes). Numbers of computational nodes used in the presented scenarios ranged from 128 to 2048. The results for the simulation code showed perfect scaling, where an increase in both nodes and numbers of cells exhibited the same solution time. Tests showed that a small serial code produced the same results as a parallel simulation.

We have been able to simulate an arterial segment with a much larger radius (of the order of 2000 μm), where the computation comprised 251 SMCs and 1255 ECs surrounding the artery aligned along an axial length of 5 cm totalling 3.5 million cells (2,505,984 SMCs and 963,840 ECs) mapped on to 384 Blue Gene nodes (9036 cells per node). The results from this particular test provide phenomena similar to that seen with the 50 μm radius artery as noted below.

Table 3. The various coupling modes (V, membrane potential coupling; Ca$^{2+}$, Ca$^{2+}$ coupling; IP$_3$, IP$_3$ coupling).

<table>
<thead>
<tr>
<th>case</th>
<th>homocellular coupling</th>
<th>heterocellular coupling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SMC</td>
<td>EC</td>
</tr>
<tr>
<td>1</td>
<td>V, Ca$^{2+}$, IP$_3$</td>
<td>V, Ca$^{2+}$</td>
</tr>
<tr>
<td>2</td>
<td>V, Ca$^{2+}$, IP$_3$</td>
<td>V, Ca$^{2+}$</td>
</tr>
<tr>
<td>3</td>
<td>V, Ca$^{2+}$, IP$_3$</td>
<td>V, Ca$^{2+}$, IP$_3$</td>
</tr>
<tr>
<td>4</td>
<td>V, Ca$^{2+}$, IP$_3$</td>
<td>IP$_3$</td>
</tr>
</tbody>
</table>

Table 4. Table lists coupling coefficients in different intercellular communication configurations considered here; cases 1 and 2 simulate healthy, whereas cases 3 and 4 simulate pathological states, early and progressive atherosclerotic lesion, respectively. Tilde represents parameters for ECs.

<table>
<thead>
<tr>
<th>case</th>
<th>homocellular</th>
<th>heterocellular</th>
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<tr>
<td></td>
<td>g</td>
<td>g</td>
</tr>
<tr>
<td>1</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0.05</td>
</tr>
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Figure 4. Schematic of parallelization. Nodes are connected in one-dimensional space.
more complex scenario is present along the simple three domain state does not occur and a far large macro-scale-coupled simulations, this relatively decreasing function). It will be shown below that for (figure 5) on EC layer. The choice of this particular function of the agonist flux single coupled unit of an EC and an SMC as a function responds to areas of constant Ca\(^{2+}\) function of the agonist concentration (whose scale length is much larger separation and variation of agonists (i.e. atheroprone To simulate areas within the arterial segment where flow complex, it can be shown that the concentration bound-

ection reflected two important facts. Firstly, work by Comerford \cite{35} has shown that even in a time-dependent solution, the ATP concentration can be well-represented by a time-averaged profile in areas known to be prone to atherosclerosis. Secondly, that we wished to investigate areas of the arterial segment, where there existed both constant and 'linearly varying' ATP concentrations. Our reasoning behind this is that careful examination of the neighbourhood of fluid stagnation points (where lesions have been shown to exist as noted previously) shows a WSS of linear form followed in the work of Comerford \textit{et al.} \cite{35}. Indeed, the areas of constant ATP flux also helped in developing a pair of non-reflecting boundaries which allowed waves of Ca\(^{2+}\) concentration to properly exit the computational domain. Although in atheroprene regions, cells experience low WSS and the mass transfer characteristics are complex, it can be shown that the concentration boundary layer of mass transfer thickens in these areas and does not change much with pulsatile flow owing to the high Peclet number \cite{35}. Therefore, the simplification of a sigmoid agonist profile seems plausible.

Figure 2 shows the cytosolic Ca\(^{2+}\) concentration for a single coupled unit of an EC and an SMC as a function of the agonist flux \(J_{PLC_\text{agonist}}\). There exist three different areas as noted by Keonigsberger \textit{et al.} \cite{28}. These correspond to areas of constant Ca\(^{2+}\) concentration separated by a range where the Ca\(^{2+}\) undergoes oscillatory behaviour and whose amplitude is also a function of the \(J_{PLC_\text{agonist}}\) value (essentially a monotonic decreasing function). It will be shown below that for large macro-scale-coupled simulations, this relatively simple three domain state does not occur and a far more complex dynamic scenario is present along the arterial segment both in time and space.

We first present two (cases 1 and 2) prospective scenarios of intercellular communication corresponding to a healthy arterial segment within a region of disturbed flow and attempt to investigate the Ca\(^{2+}\) dynamics in the underlying SMCs.

To avoid confusion, we should note that there exist two oscillatory phenomena occurring during any one time. Firstly, that of the SMC itself (in the time domain) and secondly that of a spatial wave, where at some specified time, a concentration gradient exists between adjoining cells owing to a phase lag in oscillation of the adjoining cells thus forming a wave in space. For case 1, figure 6 shows the cytosolic Ca\(^{2+}\) concentration in SMCs laid out in the axial direction (left is upstream) for eight different times \((a–h)\) in an arteriole of radius 50 \(\mu m\). For the case of a spatially uniform agonist concentration (results not shown here), cells give a well-coordinated Ca\(^{2+}\) response, oscillatory or non-oscillatory, depending on the value of the agonist concentration or \(J_{PLC_\text{agonist}}\) value experienced by the ECs as expected when comparing with figure 2. In figure 6, however, in the presence of homocellular Ca\(^{2+}\), IP\(_3\) and membrane potential coupling between SMCs, Ca\(^{2+}\) and membrane potential coupling between ECs and heterocellular IP\(_3\) and membrane potential coupling across the MEJ, cells show spatially differential intracellular Ca\(^{2+}\) responses as a function of axial distance. Bands of varying Ca\(^{2+}\) concentration (a Ca\(^{2+}\) wave) are visible in the middle of the arterial segment in figure 6\(a, b\), where a steep gradient exists in the agonist concentration. Units of cells on either side of this steep gradient region respond in a non-oscillatory behaviour with different steady-state intracellular Ca\(^{2+}\) concentrations. However, the Ca\(^{2+}\) waves decrease their wavelength as time increases. Figure 6\(c–e\) shows the thinning of these oscillatory bands and by definition an increase in their wavenumber. In figure 6\(f\), we see this Ca\(^{2+}\) wave effect moving towards cells which were originally at low agonist concentration and in a non-oscillatory state. Noteworthy is the fact that an SMC from a single isolated EC/SMC pair would not normally oscillate at these concentrations as would be the case when comparing with figure 2. Moreover, thinning of the bands is a requisite to this propagatory response and is a consequence of the cells undergoing oscillatory desynchronization where cells show a phase lag between neighbours. Hence, at any time, there exists a concentration gradient capable of transferring Ca\(^{2+}\) across gap junctions. To test the relationship between the radius of the artery and the phenomenon noted above, a simulation was completed for an artery with 2000 \(\mu m\) radius, which allowed 3.5 million cells coupled together and mapped on to 384 Blue Gene nodes. Figure 7 shows Ca\(^{2+}\) concentrations for the 2000 \(\mu m\) radius artery. Direct comparisons can be made to figure 6 for the first 60 min. It is clear that there exists no difference in the concentration distribution. This is to be expected given the problem definition as we assume an axisymmetric condition.

Figure 8 shows the time evolution of intracellular Ca\(^{2+}\) concentration in 12 selected SMCs co-located in groups of four at three upstream and downstream space domains (I, II and III), each at three different
time durations (each 50 s long). SMCs located in space domain I do not oscillate until the oscillations in the downstream SMCs desynchronize and Ca\textsuperscript{2+} is therefore transported differentially via gap junctions owing to the concentration gradient existing at any specific time. Also notable was the observation that absence of homo-cellular Ca\textsuperscript{2+} coupling between SMCs caused a cessation of the Ca\textsuperscript{2+} propagation to upstream cells. The proposed mechanism driving this behaviour is touched upon further in §4.

In the second case of intercellular communication in a healthy vessel, heterocellular Ca\textsuperscript{2+} coupling was enabled in addition to the previously described configuration of intercellular coupling while stimulated with the same agonist profile as before. Figure 9a–c shows the cytosolic Ca\textsuperscript{2+} concentration in the SMCs along the arterial segment at three different times. Compared with the Ca\textsuperscript{2+} dynamical response in case 1 where heterocellular Ca\textsuperscript{2+} coupling was disabled, the propagation of Ca\textsuperscript{2+} wave to the upstream SMCs is rapid in case 2. Similar to case 1, SMCs at axial locations corresponding to the steep spatial gradient in agonist concentration show bands of varying Ca\textsuperscript{2+} concentration. These bands in figure 9a, b, precede an increase of Ca\textsuperscript{2+} concentration in upstream cells in figure 9c. However, the extent of the thinning of the oscillatory bands (i.e. the degree of oscillatory desynchronization in adjoining cells) is not as severe as in case 1. Also, a comparison of figure 6f–h with figure 9c shows that the spatial wave in upstream cells is synchronized in case 2 when compared with case 1 despite the fact that the Ca\textsuperscript{2+} concentration is oscillating temporally in the SMCs of an upstream region in both the cases. More SMCs attain a steady-state Ca\textsuperscript{2+}
concentration on the downstream side than in case 1. Thus, the Ca\(^{2+}\) response in SMCs in the intercellular coupling configuration of case 2 is more definitive and rapid, compared with the case 1 where heterocellular Ca\(^{2+}\) coupling was disabled.

Inclusion of homocellular IP\(_3\) coupling between ECs, which simulates the first of the two pathological cases (case 3 in table 4) did not change the Ca\(^{2+}\) dynamical response in the SMCs and was not substantially different from the second healthy case as given in figure 9. Thus, upregulation of Cx43 in the presence of unaffected heterocellular Ca\(^{2+}\) coupling does not alter the spread of homogeneity among the SMCs axially.

Implementation of case 4 of table 4, however, had a different outcome when compared with other cases. Figure 10 shows the Ca\(^{2+}\) concentration in SMCs in the axial direction at time intervals of 2.5, 10 and 15 minutes.

![Figure 8](http://rsif.royalsocietypublishing.org/) Time evolution of intracellular Ca\(^{2+}\) concentration in adjoining SMCs at selected axial distances from three space domains I, II and III, of the arterial segment in oscillatory state. Domain I extends from 1.225 to 1.234 cm, II from 1.472 to 1.478 cm and III from 2.226 to 2.232 cm, axially. Four SMCs from each domain are colour-coded as black (solid line), grey (solid line), black (dashed line) and grey (dashed line)(cell with Ca\(^{2+}\) concentration in black (solid line) being most upstream in each domain). Ca\(^{2+}\) concentration in these SMCs is compared at three 50 s long time intervals, 2.08–2.92 min in (a),(d),(g); 19.6–20.416 min in (b),(e),(h) and 34.6–35.42 min in (c),(f),(i). These time intervals correspond to the state of the vessel segment shown in figure 6a,d,e. In the first time interval, cells either assume a steady state or oscillate in phase-locked loop, as shown in (a),(d),(g). In the latter time intervals, the Ca\(^{2+}\) oscillations in individual cells from domains II and III desynchronize, prior to the appearance of Ca\(^{2+}\) oscillations in SMCs located in domain I.

![Figure 9](http://rsif.royalsocietypublishing.org/) (a)–(c) Cytosolic Ca\(^{2+}\) concentration in SMCs at time steps 2.5, 4 and 6 min for an artery of radius 50 μm with heterocellular coupling enabled (case 2). The colour in each graph corresponds to the amplitude of cytosolic Ca\(^{2+}\) concentration in each SMC ranging from red (high Ca\(^{2+}\) concentration) to dark blue (low concentration), as depicted by the colour bar. Oscillations propagate to upstream cells in (c), whereas they were absent in (a,b).
30 min in the simulation. SMCs along the axial distance show moderately high Ca\(^{2+}\) concentration, either steady state in cells located at distance greater than 2.25 cm or oscillating with time in the rest of the vessel segment (upstream). Figure 10a when compared with figure 6a is notably different. Case 1 at time step 2.5 min (figure 6a) was piecewise homogeneous when compared with what is seen in figure 10a. We see Ca\(^{2+}\) concentration waves from the start of the simulation which leads to a global spatially oscillatory state in figure 10a,b, except for the part of the arterial segment where there exists coupling with ECs experiencing a relatively higher agonist concentration and thus attaining a steady-state Ca\(^{2+}\) concentration early in simulation. The homocellular coupling between ECs is limited to IP\(_3\) coupling only in this case and the heterocellular coupling is also restricted to IP\(_3\) transfer through MEJs (refer to case 4 in table 4). SMCs, however, remain homocellularly coupled via all three media. This simulates intercellular coupling in an advanced atheroma especially at the shoulder of the atheroma where expressions of Cx37 and Cx40 are severely downregulated and Cx43 is upregulated in ECs [31,32]. Thus, we see the propagation of a Ca\(^{2+}\) wave from SMCs provided with a relatively higher amount of agonist flux to those at positions where overlying ECs experience low agonist concentration. This propagation however was much faster when compared with case 1; notably figure 6f corresponds to 2.75 h. We discuss the mechanism behind this accelerated response in §4.

4. DISCUSSION

Atherosclerotic lesions preferentially occur at arterial bifurcations and inner walls of curvatures. Lesion formation is highly sensitive to the local blood haemodynamics resulting in intracellular processes that contribute to a global as well as a local response to haemodynamical changes. Intercellular communication plays a major role in eliciting these responses over multiple scales. Also, spatial gradients in mass transport dynamics of vasoactive substances to the endothelial surface occur in these regions owing to disturbed blood flow. We investigated four cases of intercellular communication with the EC layer experiencing an axial variation in ATP concentration simulated by a parameter \(J_{PLC_{app}}\) modelling a proportional change in the production of PLC, a precursor to IP\(_3\)-dependent increase in intracellular Ca\(^{2+}\) in ECs. In case 1, there existed homocellular and heterocellular coupling via Ca\(^{2+}\), IP\(_3\) and membrane potential (refer to table 4), downstream Ca\(^{2+}\) dynamics played a vital role in eliciting a response in upstream SMCs in a time-dependent manner.

The Ca\(^{2+}\) waves (figure 6a) observed in the first few seconds after the application of an agonist gradient (figure 5) in areas of steep spatial variation of stimulus was found to be solely gradient-dependent. Homocellular Ca\(^{2+}\) coupling between SMCs had a fundamental role in the increase of the wavenumber of these Ca\(^{2+}\) waves and the eventual propagation to upstream SMCs. These SMCs were coupled with ECs that were in an environment of low agonist concentration (i.e. would not necessarily oscillate if uncoupled). Low wavenumber of the Ca\(^{2+}\) oscillations in figure 6 is representative of synchronized Ca\(^{2+}\) oscillations in ‘bunches’ of SMCs experiencing a relatively uniform heterocellular IP\(_3\) transmission from ECs that are coupled to them. Thinning of these bands (higher wavenumbers) at later time steps suggests the desynchronization of these Ca\(^{2+}\) oscillations in neighbouring cells. In these SMCs, it is found that desynchronization is a requisite to the propagatory behaviour shown in figure 6.

We suggest that the mechanism behind such propagation behaviour involves, owing to the desynchronization of Ca\(^{2+}\) oscillations, at a specific moment in time, a difference in concentration between adjoining cells and thus a flux of extracellular Ca\(^{2+}\) through gap junctions. This is followed by the stimulation of the CICR-dependent cytosolic Ca\(^{2+}\) increase in SMCs. Figure 11 shows the temporal relationship between components of this process for one SMC located at an axial distance of 1.303 cm downstream. Note that Ca\(^{2+}\) flux through the gap junction precedes all other fluxes in time. The flux of CICR and SERCA activity continuously changes in time and correspondingly affects the cytosolic Ca\(^{2+}\) concentration in figure 11c. The influx of Ca\(^{2+}\) from an adjacent SMC enters the intracellular domain and is pumped back into the SR immediately via an SERCA pump. With an increase in the rate of change of Ca\(^{2+}\) via the SERCA pump, an increase in the rate of change of CICR and subsequent elevation of cytosolic Ca\(^{2+}\) can also be observed. Furthermore, disabling the CICR function caused a cessation of desynchronization and thus the propagatory effect. SMC VOCCs are not involved in this process because of the continual closure owing to membrane hyperpolarization induced by

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Figure 10. (a)–(c) Cytosolic Ca\(^{2+}\) concentration in SMCs at time steps 2.5, 10 and 30 min for case 4. The colour in each graph corresponds to the amplitude of cytosolic calcium concentration in each SMC ranging from red (high Ca\(^{2+}\) concentration) to dark blue (low concentration), as depicted by the colour bar.
neighbouring hyperpolarized ECs. Membrane coupling alone could not elicit the desynchronization process and thus no propagation was seen for this case. Homocellular IP$_3$ coupling between SMCs caused the thinning of bands (increase in the Ca$^{2+}$ wavenumber) but was unable to produce a propagatory effect as it could not activate the CICR mechanism in cells that were in a low agonist environment. The thinning was not via the CICR-dependent process stated above, but mainly owing to redistribution of IP$_3$ from enabled homocellular IP$_3$ coupling in this case.

In case 2, enabling the heterocellular Ca$^{2+}$ coupling had a rapid effect on intracellular Ca$^{2+}$ concentration in SMCs. The response showed trends similar to those seen in case 1 but the propagation of the Ca$^{2+}$ wave needed less oscillatory desynchronization among adjoining cells, as shown in figure 9. This is a much more definitive vasoconstrictive response. Elevated intracellular Ca$^{2+}$ in upstream SMCs, although oscillatory, increases actomyosin activity proportionally and hence mediates SMC contraction with a resultant reduction in arterial radius. Addition of Ca$^{2+}$ from ECs via heterocellular Ca$^{2+}$ coupling sensitizes the underlying SMCs to any further injection of Ca$^{2+}$ from an adjoining SMC. This aids the spatial wave originating in the cells under the steep agonist gradient to reach the upstream cells relatively quickly. Each upstream SMC now needs only a small homocellular Ca$^{2+}$ influx from an adjoining SMC to induce Ca$^{2+}$ oscillations in it (via the CICR pathway described above) thus producing a spatially synchronized Ca$^{2+}$ response. The heterocellular Ca$^{2+}$ coupling, therefore, abates the temporal desynchronization of intracellular Ca$^{2+}$ oscillations in the SMCs while achieving the propagation of a high agonist concentration signal to upstream cells. We thus suspect that heterocellular Ca$^{2+}$ coupling could be an integral part of the vascular apparatus eliciting a rapid global response to alterations in the local agonist concentration. This should help in preserving the structural integrity of the intima by reducing the effect of haemodynamically induced gradients acting on the endothelial surface by homogenizing the contractile activity of SMCs axially. It is yet to be investigated how circumferential gradients cause arterial responses in such cases.

Case 3 simulates a pathological scenario where the inclusion of homocellular IP$_3$ coupling between ECs simulates the upregulation of connexin Cx43 in atheroplane areas [32, 33]. No difference was seen in the response of the intercellular Ca$^{2+}$ for case 3 when compared with the response in case 2. The presence of heterocellular Ca$^{2+}$ coupling in both cases inhibits the gradient effects and resulting in a diffused spatial Ca$^{2+}$ wave and synchronized temporal oscillations of intracellular Ca$^{2+}$ concentrations in SMCs located upstream. This highlights the importance of heterocellular Ca$^{2+}$ coupling between SMCs and ECs in arterial segments.

Case 4, where EC homocellular coupling and EC/SMC heterocellular coupling were severely restricted, provided a different response to any of the above three cases. In cases 1–3, the presence of heterocellular membrane potential coupling suppressed the entry of Ca$^{2+}$ into the SMCs via their VOCCs owing to EC-mediated hyperpolarization of the SMC membrane potential. In this case, because of the inhibition of heterocellular membrane potential and the consequential disabled hyperpolarization, it allows the Ca$^{2+}$ influx via VOCCs into the SMCs following adequate IP$_3$ stimulation. This offsets the Ca$^{2+}$ concentration globally in the whole population of SMCs in the axial direction. Further exchange of Ca$^{2+}$ via homocellular gap junctions in SMCs, as we go forward in time, elicits the same response as seen in case 1. However, for an SMC, the time to reach an oscillatory behaviour in case 4 is much less than that observed in case 1. This observation points out the role and extent of coupling between two populations of cells. In case 4, where both cell types had weak heterocellular coupling and ECs were also weakly coupled homocellularly, the
response resembles a condition where the EC layer was non-existent and SMCs were experiencing the agonist directly onto their cell membrane. Relatively lower agonist concentrations stimulating ECs in this case would also produce a similar response suggesting that, unlike cases 1–3, the arterial segment with such intercellular coupling becomes hypersensitive and would contract even at low agonist concentrations.

5. CONCLUSION

In the coupling configuration cases representing a healthy arterial segment, the presence of homocellular Ca\(^{2+}\) coupling between SMCs ensures the propagation of a high agonist concentration signal to upstream cells at low agonist concentration. This is achieved rather slowly in the absence of heterocellular Ca\(^{2+}\) coupling, as in case 1 (figure 6) compared with case 2 (figure 9), where heterocellular Ca\(^{2+}\) coupling between ECs and SMCs ensures a rapid and spatially definitive response. In both the cases, the intracellular Ca\(^{2+}\) concentration in each SMC (situated axially at less than 2.5 cm) oscillates in a temporarily desynchronized fashion, the degree of which is much greater in case 1 than in case 2. This desynchronization is the main source of the propagation of the Ca\(^{2+}\) wave in both the cases in the upstream direction and is CICR-mediated between SMCs, as suggested by the results above. This behaviour ceases in the absence of homocellular Ca\(^{2+}\) coupling between SMCs.

When considering the cases of a pathological nature of intercellular coupling in the presence of an agonist gradient, case 3 was not different from case 2. Case 4 however highlights the inhibitory effect of hyperpolarized EC membrane potentials on SMC Ca\(^{2+}\) dynamics. In the absence of such a leash, the intracellular Ca\(^{2+}\) concentration oscillated in each SMC (situated at less than 2.5 cm) in similar fashion as observed in case 1 but the response was much more rapid. We suggest that because the cells become sensitive, even to the presence of low agonist concentrations, the vessel segment will be in a contracted state more frequently than in other cases. Thus the presence of heterocellular membrane potential coupling seems to be essential for imposing a range of agonist concentration where vasoconstrictive effects are seen.

Thus, heterocellular Ca\(^{2+}\) and membrane potential coupling could, on the basis of our results, have an atheroprone effect in an arterial segment stimulated by a time-averaged agonist gradient in coupled EC–SMC populations. Homocellular Ca\(^{2+}\) coupling between SMCs also has a compensatory effect and helps in homogenizing the response on a global scale in response to such gradients, the rapidness of which is heavily influenced by the presence or absence of appropriate heterocellular coupling.

Through the use of massively parallel simulations, our results have shown that there exist macro-scale phenomena which indicate the propagation of Ca\(^{2+}\) dynamics upstream within arterial segments. It is believed this is one of a number of possible explanations of how lesions may grow upstream in opposition to convecitive transport effects. The use of computational architectures such as Blue Gene in this project have demonstrated the ability to simulate highly complex cellular effects on a scale large enough to be seen with the naked eye. Further work will show the effects of complex tangential WSSs acting on the cytoskeleton of the cell and concentration patterns associated with complete arterial bifurcations.

The authors would like to gratefully acknowledge the National Heart Foundation of New Zealand who supported this work with a grant (no. E5693).

APPENDIX A

Values of the conductances, rate constants and coefficients used below are listed in tables 1 and 2, respectively.

A.1. Smooth muscle cell intracellular Ca\(^{2+}\) dynamics

Following are the constituent currents for a single SMC, modelled by Koenigsberger et al. [28]. IP\(_{3}\)-induced Ca\(^{2+}\) release in SMC:

\[
J_{IP} = \frac{F}{K_t ^2 + F^2} \text{.} \tag{A1}
\]

Ca\(^{2+}\)-induced Ca\(^{2+}\) release from SR into cytosol:

\[
J_{CICR} = C \left( \frac{v}{s_v ^2 + s_v ^2 + c_i ^2 + c_i ^2} \right) \text{.} \tag{A2}
\]

Pumping of Ca\(^{2+}\) back into the SR via an actively operating Ca\(^{2+}\)-activated SERCA pump:

\[
J_{SRuptake} = B \frac{c^2}{c_b ^2 + c_i ^2} \text{.} \tag{A3}
\]

Ca\(^{2+}\) leak from SR:

\[
J_{lek} = Ls \text{.} \tag{A4}
\]

Influx of extracellular Ca\(^{2+}\) via VOCC:

\[
J_{VOCC} = G_{Ca} \frac{v - v_{Ca}}{1 + e^{-[(v - v_{Ca})/R_{Ca}]}} \text{.} \tag{A5}
\]

Efflux of Ca\(^{2+}\) through \(Na^+/Ca^{2+}\) exchanger:

\[
J_{Na/Ca} = G_{Na/Ca} \frac{c}{c + c_{Na/Ca}} (v - v_{Na/Ca}) \text{.} \tag{A6}
\]

Removal of intracellular Ca\(^{2+}\) through plasma membrane CaATPase

\[
J_{ATP} = DC \left( 1 + \frac{v - v_d}{R_d} \right) \text{.} \tag{A7}
\]

Dependence of activation of the Ca\(^{2+}\)-activated K\(^+\) channels on cytosolic Ca\(^{2+}\) concentration:

\[
K_{activation} = \frac{(c + c_o)^2}{(c + c_o)^2 + 2 \beta e^{-[(v - v_{Ca})/R_d]}} \text{.} \tag{A8}
\]
K$^+$ efflux through plasma membrane-bound $BK_{Ca}$ channels, where $\omega$ is the open channel probability of Ca$^{2+}$-activated K$^+$ channels in equation (2.7) expressed as a function of $K_{activation}$:

$$J_K = G_K \omega(v - v_K). \quad \text{(A 9)}$$

Influx of Cl$^-$ ions upon plasma membrane depolarization:

$$J_{Cl} = G_{Cl}(v - v_{Cl}). \quad \text{(A 10)}$$

A constant efflux of K$^+$ through Na$^+$/K$^+$ pump:

$$J_{Na/K} = F_{Na/K}. \quad \text{(A 11)}$$

**A.2. Endothelial cell intracellular Ca$^{2+}$ dynamics**

IP$_3$-induced Ca$^{2+}$ release in an EC:

$$\tilde{J}_{IP3} = F \frac{\tilde{I}^2}{K^2 + \tilde{I}^2}. \quad \text{(A 12)}$$

Ca$^{2+}$-induced Ca$^{2+}$ release from ER into cytosol:

$$\tilde{J}_{CICR} = \tilde{C} \frac{\tilde{C}^2}{\tilde{C}^2 + 3 \tilde{C}^2 + \tilde{C}^4}. \quad \text{(A 13)}$$

Pumping of Ca$^{2+}$ back into the ER via an actively operating Ca$^{2+}$-activated SERCA pump:

$$\tilde{J}_{ERuptake} = \tilde{B} \frac{\tilde{C}^2}{\tilde{C}^2 + \tilde{C}^2}. \quad \text{(A 14)}$$

Ca$^{2+}$ leak from ER:

$$\tilde{J}_{leak} = \tilde{L}. \quad \text{(A 15)}$$

Removal of intracellular Ca$^{2+}$ through plasma membrane CaATPase

$$\tilde{J}_{eff} = \tilde{D}\tilde{C}. \quad \text{(A 16)}$$

Ca$^{2+}$ influx through non-selective cation channels:

$$\tilde{J}_{cations} = \tilde{C}_{cat}(E_{Ca} - \tilde{v}) \times \frac{1}{2} \left(1 + \tanh\left(\frac{\log_{10} \frac{\tilde{C}}{\tilde{C}_{cat}} - \tilde{m}_{\text{cat}}}{\tilde{m}_{\text{cat}}}\right)\right). \quad \text{(A 17)}$$

Constituent currents modelling the K$^+$ efflux through large and small conductance Ca$^{2+}$-activated K$^+$ channels ($K_{Ca}$):

$$\tilde{I}_K = \tilde{G}_{cat}(\tilde{v} - \tilde{v}_K)(\tilde{I}_{BK_{Ca}} + \tilde{I}_{SK_{Ca}}). \quad \text{(A 18)}$$

where

$$\tilde{I}_{BK_{Ca}} = \frac{0.4}{2} \left(1 + \tanh\left(\frac{\log_{10} (\tilde{C} - z)(\tilde{v} - y) - x}{\tilde{m}_{\text{BK}}(\tilde{v} + x(\log_{10} (\tilde{C} - z) - y)^2 + \tilde{m}_{\text{BK}})}\right)\right). \quad \text{(A 19)}$$

and

$$\tilde{I}_{SK_{Ca}} = \frac{0.6}{2} \left(1 + \tanh\left(\frac{\log_{10} (\tilde{C} - \tilde{m}_{\text{sk}})}{\tilde{m}_{\text{sk}}})\right)\right). \quad \text{(A 20)}$$

The residual current comprising an inward Na$^+$ or K$^+$ current and an outward Cl$^-$ current:

$$\tilde{I}_{residual} = \tilde{G}_R(\tilde{v} - \tilde{v}_{\text{rest}}). \quad \text{(A 21)}$$

A constant term regrouping continuous Ca$^{2+}$ influx from other trivial pathways:

$$\tilde{J}_0. \quad \text{(A 22)}$$

**REFERENCES**


