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A theoretical model of cytosolic calcium elevation following wounding in urothelial cell monolayers

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Abstract. Scratch wounding of a urothelial cell monolayer triggers a number of events including the release of soluble, diffusible signalling factors and mechanical stimulation of cells at the wound edge. These events cause a sustained elevation in cytosolic calcium concentration in the cells surrounding the wound and a transient rise in those further away. The precise form of this calcium transient is believed to play a central role in determining the subsequent response of individual cells and ultimately leads to a co-ordinated, population-level response that rapidly closes the wound. Here we present a framework for modelling the initial phases of this process. We combine a PDE model of diffusion in the extracellular medium and an ODE model of calcium signalling that has been tailored to represent urothelial cells. The ODE model is capable of generating a wide range of calcium transients, including spikes, bursts, oscillations and sustained elevations in the cytosolic calcium concentration. In multi-cell simulations of scratch wounding in a perfusion flow we find that the spatial position of the cells relative to the wound site leads to distinct classes of calcium response, with cells proximal to the wound exhibiting a sustained elevation and cells distal to the wound exhibiting a more transient elevation. We compare these results to existing experimental data and generate a number of novel predictions that could be used to test the model experimentally.

1. Introduction
Calcium signalling is known to play a central role in a wide variety of cellular processes. A key mechanism is the release of calcium from the endoplasmic reticulum. Typically, a small elevation in cytosolic calcium concentration ([Ca\textsuperscript{++}]) triggers further release of calcium via ryanodine- and IP\textsubscript{3}-receptor activated calcium channels, leading to a rapid and much larger elevation in [Ca\textsuperscript{++}]. This is followed by a rapid falling phase, so the overall pattern is that of a sharp calcium spike. A number of modelling studies have examined the mechanisms underlying calcium transients (for review, see [1]). These models are capable of reproducing a variety of experimentally-observed calcium transients [2, 3, 4, 5, 6]. In our own work we have integrated and extended these models to produce a theoretical model of calcium signalling in epithelial cells. Our hypothesis is that the calcium transient, defined as the precise temporal profile of the variation in [Ca\textsuperscript{++}], directs subsequent cell behaviour [7]. Our model is capable of reproducing calcium transients observed in normal human urothelial cells grown in monolayer cell culture [8].
Figure 1. (A) Diagram of the extended calcium signalling model. Leak currents are represented by straight arrows, pumped currents by arrows overlayed with circles, and channel currents by arrows overlayed with two ellipses. (B) An example of a cultured normal human urothelial cell monolayer. Cells have been loaded with fura red-AM and 5µM fluo4-AM (which produces the green fluorescence) and imaged prior to wounding using a spinning disk confocal microscope. Scratch wounding is performed by removing a strip of cells using a sharpened glass pipette (white dashed box).

Here we extend our model to describe calcium transients in a simulated urothelial cell monolayer following scratch wounding.

2. Model

We build upon the one-pool model of ER-dependent calcium signalling described in [8]. This model relates the cytosolic, extracellular and ER calcium concentrations via a system of ODEs and contains five key components: leak currents, SERCA pumping, plasma membrane Ca++ ATPase (PMCA) pumping, and the ionic and P2Y signalling pathways. Writing the cytosolic calcium concentration as \([\text{Ca}^{++}]_i\), the ER calcium concentration as \([\text{Ca}^{++}]_{\text{ER}}\), and the extracellular calcium concentration as \([\text{Ca}^{++}]_{\text{EX}}\), and denoting the various calcium currents as \(J_x\), the governing equations of the model are

\[ \frac{d[\text{Ca}^{++}]_{\text{EX}}}{dt} = 0, \]

\[ \frac{d[\text{Ca}^{++}]_{\text{ER}}}{dt} = -J_{\text{leak,ER}} + J_{\text{SERCA}} - J_{\text{IP3R}}, \]

\[ \frac{d[\text{Ca}^{++}]_i}{dt} = J_{\text{leak,ER}} - J_{\text{pump}} - J_{\text{SERCA}} + J_{\text{ionic}} + J_{\text{IP3R}}, \]

where all concentrations are defined relative to the total cell volume. The full mathematical details of this model and its implementation are given in [8] where we show that, given an appropriate choice of parameters, it can reproduce calcium transients observed in cultured urothelial cell monolayers that have been stimulated by application of the purinergic agonist ATP. Here we consider a virtual wounding experiment where cells are removed from a cell layer by scratch wounding. During scratch wounding soluble diffusible signalling factors such as ATP are released from the wound site and cells near the wound undergo mechanical deformation [9].

The mechanical stimulus is believed to activate ionic channels in the cell membrane and we add a new term, \(J_{\text{mech}}(t)\), to Eq. 3 to represent this

\[ J_{\text{mech}}(t) = f_{\text{max}} [H(x_1, t) - H(x_2, t)] g_{\text{max}} [H(t_1, t) - H(t_2, t)] [\text{Ca}^{++}]_{\text{EX}}, \]

where \(x\) is spatial location of the cell, \(x_{1,2}\) determine the spatial extent of the mechanical deformation, \(t_{1,2}\) determine the duration of activation of the ionic channels and \(f_{\text{max}}\) and \(g_{\text{max}}\)
are scaling factors. A sketch of this model is shown in Fig. 1A. We also include a model of diffusion where the concentration of an extracellular signalling factor, $\phi$, evolves according to

$$\frac{\partial \phi}{\partial t} = D \nabla^2 \phi,$$

(5)

where $D$ is the diffusion coefficient and $\nabla^2$ the Laplace operator. We incorporate a uniform flow of the extracellular medium at a fixed speed $s = 5 \times 10^{-5}$ m/s. This corresponds to an experimental setup where the extracellular medium flows over the cell layer at a constant rate due to a perfusion flow. Solutions of the diffusion equation are calculated using standard techniques from the literature [10].

3. Results
We simulate a uniform layer of cells of total area 0.01 m$^2$, which represents a confluent layer of cultured urothelial cells similar to that illustrated in Fig. 1B. The cytosolic calcium concentration of each cell is governed by the ODE model described above using the default parameter set given in [8]. The parameters $J_{\text{ionic}}$, $J_{\text{mech}}$, and $J_{\text{IP3R}}$ contain dependencies on channel numbers and we expect them to vary from cell to cell. We therefore scale each of these parameters by a Gaussian probability distribution with a mean of 1 and standard deviation of 0.2. We set $x_1 = 0.001$, $t_1 = 1$ min and $t_2 = 10$ min. We assume the mechanical stimulation caused by wounding is symmetrical around the wound site, so that $x_1 = -x_2$.

Figure 2 shows the evolution of the extracellular ATP concentration and cytosolic calcium concentrations of the cell population during a virtual wounding experiment. Initially all of the cells have a resting cytosolic calcium concentration of 0.2 mM and no ATP is present in the extracellular medium. Following scratch wounding there is a brief elevation in ATP at the wound site, which is rapidly carried over the cell layer by the perfusion flow. Cells distal to the wound site undergo a transient elevation in cytosolic calcium concentration. Cells proximal to the wound exhibit a sustained increase in cytosolic calcium concentration due to the influence of the ionic current $J_{\text{mech}}(t)$.
of the plateau varies from cell to cell but the overall shape is preserved. Distal cells exhibit a characteristic single-spike response that lasts around ten seconds, with an onset time that is dependent on the spatial distance of the cell from the wound site.

4. Discussion

In this paper we have examined a theoretical model of cytosolic calcium transients in urothelial cells following scratch wounding. We observe two distinct classes of response within the cell population. Cells proximal to the wound site exhibit a sustained elevation in calcium, while cells distal to the wound site exhibit a more transient, single-spike behaviour lasting a few seconds. Both of these results compare favourably to existing experimental data [7]. A number of experimental tests of the model can be conceived. Direct mechanical stimulation of the cells should activate the ionic channels that mediate $j_{\text{mech}}$, leading to a sustained elevation in $[\text{Ca}^{++}]_i$ without the need for wounding. Alternatively, scratch wounding a monolayer in the presence of a P2Y antagonist should strongly attenuate the distal response but preserve the proximal response. Performing these experiments would be a direct test of the assumptions behind the model. Our model provides the framework for a detailed exploration of how calcium transients in individual cells direct subsequent behavioural responses.

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