Abstract

Fibroblasts are considered as a basic cell in wound repair. Regulation of cell proliferation and the cell productivity are important mechanisms involved in wound repair, which are still controversial. These mechanisms largely depend on the changes in cytosolic calcium concentration. Here an attempt has been made to study the variation of calcium concentration in the presence of excess buffer approximation. The model uses the partial differential equation with appropriate initial and boundary conditions which involve various biophysical parameters such as calcium diffusion coefficient, buffer binding affinity, total buffer concentration and source amplitude. The equations are solved by using finite difference method for a one dimensional unsteady state case. A computer programme has been developed in MATLAB 7.10 for the entire problem.

Keywords- Finite difference method, excess buffer approximation, diffusion coefficient, buffer binding affinity, source amplitude

1 Introduction

Many biological events such as embryogenesis, wound healing or the formation of primary solid tumors and metastases (secondary tumours) are still a challenge to understand completely. Because these events are closely related with other
mechanism in cells, particularly with the signals from local environment or extracellular medium and the mechanism within the cell like buffering, change in calcium concentration, influx and efflux mechanisms, etc. Calcium acts as messenger to perform various functions in cells.

Fibroblasts are a heterogeneous population of structural cells whose primary function is the production of extracellular matrix (ECM) for tissue maintenance and repair [17]. Thus, fibroblasts play a pivotal role not only in maintaining tissue integrity, but also in healing processes. They participate in fibrotic (scarring) disorder in lung, skin and other tissues. Fibroblasts cell has long been considered as a non-excitable cells [6]. There are some studies [7,9,10] which shows excitability of these cells in particular growth stages. The current hypothesis is that the excitability is important in calcium signaling in NRK fibroblasts since a strong interplay exists between the membrane potential and the internal calcium concentration [7, 13].

Experimental investigations [9, 13] with real NRK fibroblasts have reported that the buffer plays an important role in long action potential duration. Without buffering the AP-duration in NRK fibroblasts is very long and with very strong buffering a very short AP occurs [13]. These experiments are performed by using some non-natural buffer like EGTA and BAPTA. The presence of endogenous and exogenous buffer in cytosol is very important to maintain the calcium level below the toxic level. Theoretical investigation are reported in the literature [2,3,12,15,16] for the study of calcium diffusion in neuron cells, astrocytes [4,5], Myocytes [1], oocytes [14], hepatocytes [18] etc. But no attempt is reported in the literature for numerical study of calcium diffusion in fibroblasts cell.

In the view of the above, here, a one-dimensional model for calcium diffusion in a fibroblasts cell with excess buffer approximation has been developed. The model uses the partial differential equation with appropriate initial and boundary conditions, to describe the diffusion process with excess buffer in Normal Rat Kidney fibroblasts cell. The equations are solved by using crank Nicholson method for a one-dimensional unsteady state case. A computer programme has been developed in MATLAB 7.10 for the whole problem and executed on Intel(R) Core(TM) 2 Duo CPU, 4.00 GB RAM, 2.00GHz processor.

2 Mathematical Formulations

Calcium kinetics in fibroblasts are governed by a set of reaction-diffusion equations which can be framed assuming the following bimolecular reaction between calcium and buffer species [8,11]:
Numerical model to study calcium diffusion

\[
[Ca^{2+}] + [B_j] \xrightleftharpoons[k_+^-]{k} [CaB_j]
\]  

(1)

where \([B_j]\) and \([CaB_j]\) are free and bound buffer, respectively, and \(j\) is an index over the buffer species. Using Fick’s law of diffusion, we get the following differential equations [11,12]

\[
\frac{\partial [Ca^{2+}]}{\partial t} = D_{Ca} \nabla^2 [Ca^{2+}] + \sum_j R_j
\]

(2)

\[
\frac{\partial [B_j]}{\partial t} = D_{B_j} \nabla^2 [B_j] + \sum_j R_j
\]

(3)

\[
\frac{\partial [CaB_j]}{\partial t} = D_{CaB_j} \nabla^2 [CaB_j] - \sum_j R_j
\]

(4)

where

\[
R_j = -k_+ [B_j] [Ca^{2+}] + k_- [CaB] ;
\]

\(D_{Ca}, D_{B_j}, D_{CaB_j}\) are diffusion coefficient of free calcium, free buffer, and calcium bound buffer respectively; and \(k_+\) and \(k_-\) association and dissociation rate constants for buffer \(j\), respectively. For stationary, immobile buffers or fixed buffers [12] \(D_{B_j} = D_{CaB_j} = 0\).

On further simplification we get [1]

\[
\frac{\partial [Ca^{2+}]}{\partial t} = D_{Ca} \nabla^2 [Ca^{2+}] - k_+ [B]_\infty ([Ca^{2+}] - [Ca^{2+}]_\infty )
\]

(5)

where \([Ca^{2+}]_\infty\) is the background \([Ca^{2+}]\) concentration taken to be 0.1 µM, \([B]_\infty\) denotes the total buffer concentration and \(\nabla\) the Laplacian operator i.e.

\[
\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}
\]
The point source of calcium is assumed at $x=0$ and as we move away from the source, the calcium concentration achieves its background value i.e. $0.1 \, \mu M$. Thus, the boundary conditions for the above problem are: [11,12]

$$\lim_{x \to 0} \left( -D_{Ca} \frac{\partial Ca^{2+}}{\partial x} \right) = \sigma$$  \hspace{1cm} (6)

$$[Ca^{2+}]_{t=0} = 0.1 \, \mu M$$  \hspace{1cm} (7)

$$\lim_{x \to \infty} [Ca^{2+}] = [Ca^{2+}]_{\infty}$$  \hspace{1cm} (8)

The model equations (5-8) are solved numerically using Crank Nicholson method. The grid is shown in fig (1) for one dimensional unsteady state case. The length of cell is assumed to be $10 \, \mu m$ [6].

Using Crank Nicholson Method the above equation (5) takes the following form:

$$\frac{u_{i+1}^j - u_i^j}{k} = D_{Ca} \left( \frac{u_{i+1}^{j+1} - 2u_i^{j+1} + u_{i-1}^{j+1}}{2h^2} + \frac{u_i^{j} - 2u_i^{j+1} + u_{i+1}^{j}}{2h^2} \right) - k_f \left[ B \right]_{\infty} \left( u_i^j - u_{\infty} \right)$$  \hspace{1cm} (9)
where ‘u’ denotes the calcium concentration, which is a function of \((x, t)\) and ‘h’ represents spatial step and ‘k’ represents time step, ‘i’ and ‘j’ represents the index of space and time respectively. Since, the above equation is not valid at centre \((i=0)\); therefore the approximation at the center is given by:

\[
(1 + \alpha) u_0^{j+1} - \alpha u_1^{j+1} = \alpha u_0^j + (1 - \alpha - \lambda k) u_0^j + \alpha p + \lambda k u_\infty
\]

(10)

where \(\alpha = \frac{D_{Ca} k}{h^2}\); \(\lambda = k_j^+ [B]_e\) and \(p = \frac{2 h \sigma}{D_{Ca}}\)

Approximation for the rest of the nodes is given by,

\[
-\alpha u_{i-1}^{j+1} + (2 + 2\alpha) u_i^{j+1} - \alpha u_{i+1}^{j+1} = \alpha u_{i-1}^j + (2 - 2\alpha - 2\lambda k) u_i^j + \alpha u_{i+1}^j + 2\lambda k u_\infty
\]

(11)

The resulting system provides simultaneous algebraic equations for unknown node concentrations \(u_i\). Gaussian elimination method has been employed to solve the resulting equations for each time step.

The numerical results are computed using a program developed in MATLAB using 7.10 on a Intel(R) Core(TM) 2 Duo CPU, 4.00 GB RAM, 2.00GHz processor.

3 Numerical Results and Discussion

This section shows the numerical results in the form of figures for calcium profile against different biophysical parameters. The biophysical parameters used in proposed model are stated in Table (1). The proposed model illustrates the variation of cytosolic calcium concentration for the exogenous buffers.
The one well known exogenous buffer is EGTA. The purpose of using this buffer is to delay the time required to achieve steady state. So that the calcium diffusion kinetics can be studied in fibroblasts cell. Total buffer concentration decreases the calcium concentration in cytosol to such an extent that IP3 receptor will not be activated and calcium oscillations will not occur. Here all the investigation were done assuming that calcium is buffered using 20µM EGTA.

Table-1

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Constant</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_j^+$</td>
<td>Association rate</td>
<td>320 (mMsec)$^{-1}$</td>
</tr>
<tr>
<td>$k_j^-$</td>
<td>Dissociation rate</td>
<td>0.06 (sec)$^{-1}$</td>
</tr>
<tr>
<td>$[B]_\infty$</td>
<td>Total buffer concentration</td>
<td>20 µM</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Source amplitude</td>
<td>1 pA</td>
</tr>
<tr>
<td>$D_{Ca}$</td>
<td>Diffusion coefficient</td>
<td>220 µm$^2$/sec</td>
</tr>
</tbody>
</table>

Figure [2] Calcium concentration profile with respect to distance where $D_{Ca}=220$ µm$^2$/sec $[B]_\infty=20$ µM and $\sigma=1$pA.
Figure (2) shows the changes in calcium concentration with respect to distance from the source at various time intervals. Here it is assumed that source is situated at \( x=0 \). It is observed from the figure that cytosolic calcium concentration decreases rapidly till \( x=2\mu m \) and after that it decreases slowly up to \( x=5\mu m \) and between \( x=5\mu m \) to \( x=10\mu m \) calcium concentration in cytosol falls gradually approaching towards the background concentration \( i.e. 0.1\mu M \). As time increases the calcium concentration near the source increases and attains its maximum concentration at \( t=0.1 \) sec.

Figure [3] Variation of calcium concentration with respect to time where \( D_{Ca}=220 \mu m^2/sec \) \( [B]_\infty =20 \mu M \) and \( \sigma =1pA \).

Figure (3) shows the calcium concentration profile at various positions with respect to time. As time increases, initially the calcium concentration increases rapidly and after some time it reaches steady state. As distance increases calcium concentration decreases and takes less time to reach steady state. Here the experimental values foe EGTA buffer (with 20 \( \mu M \) in cytosol) has been used to find the effect of buffer on calcium diffusion in fibroblasts cell.
Figure [4] Calcium concentration against distance for different source amplitude when $t=0.02$ sec, $D_{Ca}=220$ $\mu$m$^2$/sec and $[B]_\infty=20$ $\mu$M.

It is observed from the figure (4) that as source amplitude increases the cytosolic calcium concentration near the source increases almost proportionally. The fall in calcium concentration is sharp from $x=0$ $\mu$m to $x=2.5$ $\mu$m and thereafter this fall becomes gradual and it reaches background concentration at $x=10$ $\mu$m.

Figure [5] Calcium concentrations with respect to time for different source amplitude when $x=0$ $\mu$m, $D_{Ca}=220$ $\mu$m$^2$/sec and $[B]_\infty=20$ $\mu$M.
Figure (5) shows the temporal variations of calcium concentration in normal rat kidney fibroblasts. The calcium concentration increases sharply between $t=0$ to $t=0.02$ sec. and thereafter increases gradually and reaches steady state in 0.1 sec. It is observed from the above figure that calcium concentration is higher for higher rates of $\sigma$ (source amplitude).

![Figure showing calcium concentration changes](image)

Figure: [6] Calcium concentration in the presence of different buffer concentration with respect to distance, when $t=0.06$ sec, $D_{Ca}=220 \, \mu m^2/sec$ and $\sigma=1pA$.

It is observed from the figure (6) that when the total buffer concentration is very low (4 $\mu$M) the more free calcium ions are present in cytosol, which makes the concentration very high. When we change the total buffer concentration in cytosol at (40 $\mu$M), cytosolic buffer quickly bind with calcium ion and makes the buffered calcium. Thereby, the calcium concentrations in cytosol near the source suddenly, decreasing as compared to low buffer concentration. Such models can be developed to understand calcium dynamics in fibroblasts cell under different conditions.

References

[6] De AD Ross, PH Willems, PH Peters, EJ VanZoelen and AP Theuvenet, Synchronized Calcium Spiking resulting from Spontaneous Calcium Action Potentials in Monolayers of NRK Fibroblasts, Cell Calcium, vol (22) (1997b), 195-200