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Effects of spatially varying agonist concentration on calcium signalling in coupled vascular endothelial and smooth muscle cells

Shaikh, M.A [1], T. David [1] and D.J.N Wall [2]

[1] Centre for Bioengineering University of Canterbury, New Zealand
[2] Department of Mathematics and Statistics, University of Canterbury, New Zealand
Email: mas212@student.canterbury.ac.nz

Abstract: The arterial wall responds to local hemodynamic changes by altering vascular tone. Importantly it has been shown that atherosclerotic plaque formation occurs predominately in areas of low, oscillating wall shear stress. In addition due to convective transport these are particular areas where agonist and low density lipo-proteins accumulate. Vascular endothelial and smooth muscle cells mutually accomplish the change of vascular tone by detecting changes in hemodynamic variables such as wall shear stress and agonist concentration, and responding accordingly. Smooth muscle cells contract and relax and endothelial cells are now known to mediate this response. For an effective response, the smooth muscle cells synchronize their contractions through oscillations of cytosolic calcium and this is achieved by intercellular electrochemical coupling with neighbouring smooth muscle (homogenic) and endothelial (heterogenic) cells.

A system of such coupled populations of endothelial and smooth muscle cells, arranged in a 2D cylindrical grid (simulating the axi-symmetry of the arterial vessel) and modelled by a strongly coupled set of ODEs is used here to study the effects of a spatially varying agonist concentration on the calcium signalling in the cells.

Simulation results show different states of the coupled system associated with smooth muscle cells located at different spatial locations in an artery under the influence of various concentrations of agonist. The results show that increasing agonist concentration increases the oscillatory frequency of the smooth muscle cells and importantly this effect on one cell is propagated to the cells upstream and downstream via electrochemical coupling, affecting their response. Additionally there is a time-lag between the initial damped transient and the cell oscillating fully. This time-lag is a function of spatial position and provides evidence of the upstream wave propagation of both IP3 and Ca^{2+}. On the basis of this the results show that even relatively simple time-dependent and spatial varying agonist concentrations will provide a highly complex set of phenomena both locally and globally.

The results of this simulation, not seen before, show the important role of not only that intercellular communication plays in establishing, maintaining, or altering the extent of synchronization in smooth muscle cells but also the time-scales over which oscillating states begin to occur; providing a crucial insight into both the natural and pathological states of vascular/cellular systems.

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1 INTRODUCTION

Arterial wall is in constant contact with blood and the resultant mechanical and biochemical changes affect the vascular homeostasis by altering the tone locally. The physiological significance of alteration of vascular tone has long been discussed without notable consensus, but its importance is undisputed (Aalkjær and Nilsson, 2003). Understanding the basis of vasomotor homeostasis is important to explain pathogenesis of diseases such as atherosclerosis; their initiation and progression. Atherosclerosis is a major cardiovascular disease where the vessel is occluded by plaque formation. Lesions in the vascular wall characterize the inception of the disease and are localized predominantly in regions with low wall shear stress such as arterial bifurcations (Shaaban and Duerinckx, 2000).

The inner most layer of an artery, *tunica intima*, consists of endothelial cells which are laid parallel to the axis of the lumen and are the first direct interface between blood flow and vascular wall. The middle layer, *tunica media*, is made up of smooth muscle cells. This layer is thicker than tunica intima. Smooth muscle cells are circumferentially assembled and are excitable. Cells, via gap junctions, can communicate with neighboring cells of same type (homogenic) and different (heterogenic) (Bény, 1999). This coupled system of cells responds to hemodynamic variables.

Cytosolic calcium concentration is an important entity which modulates the tone of an excitable cell such as in a smooth muscle cell and proportionates the force of contraction (Gonzalez-Fernandez and Ermentrout, 1994). In the resting state the calcium concentration is lower intracellularly than extracellularly. Various active and passive processes work towards maintaining this resting state by either extruding calcium out of the cell or refilling the intracellular calcium stores through Sarcoplasmic Reticulum Ca-ATPase (SERCA) pump. Agonists such as adenosine triphosphate and noradrenalin (NA) released from perivascular nerves can bind to the extracellular side of G protein coupled receptor, which activates a membrane bound phospholipid phospholipase C (PLC). PLC hydrolyzes membrane bound phospholipid phosphatidylinositol 4,5 biphosphate (PIP_2) to form inositol 1,4,5-triphosphate (IP_3) (Lamont et al, 2003). IP_3 binds to its specific receptors on sacroplasmic reticulum and calcium from these stores is released in cytosol. Increase in cytosolic calcium can induce further calcium release from SR stores via ryanodine receptors on SR surface, a process called calcium induced calcium release (CICR) (Berridge 2007). Increase in cytosolic calcium concentration depolarizes the membrane and allows influx of extracellular calcium through voltage operated calcium channels (VOCC), further increasing the calcium inside the cell. In addition, the Na/Ca exchanger also brings in calcium from outside. Increased calcium ions binds to calcium specific binding sites on cytoplasmic side of the calcium activated potassium channels (K_{Ca}) in the plasma membrane, resulting in activation of outward positive current to restore the resting membrane potential (Ledoux et al, 2005). VOCC also close due to repolarize membrane potential thus decreasing the rate of influx of extracellular calcium. Simultaneously Ca^{2+} is continuously extruded by Ca-ATPase in plasma membrane and pumped back in the SR by the Ca^{2+} dependent low affinity SERCA pump.

1 METHOD:

A system of nine nonlinear ordinary differential equations has been used to simulate calcium dynamics in an endothelial and a smooth muscle cell modeled by Koenigsberger et al (for details of the mathematical model refer to Koenigsberger et.al, 2005). Five of these equations model an single smooth muscle cell (SMC) with following equations:

$$\frac{dc}{dt} = J_{IP3} - J_{SRuptake} + J_{CICR} - J_{extrusion} + J_{leak} - J_{VOCC} + J_{Na/Ca} \quad (1)$$

$$\frac{ds}{dt} = J_{SRuptake} + J_{CICR} - J_{leak} \quad (2)$$

$$\frac{dv}{dt} = \gamma(-J_{Na/K} + J_{Cl} - 2J_{VOCC} - J_{Na/Ca} - J_K) \quad (3)$$

$$\frac{dw}{dt} = \lambda(K_{activation} - w) \quad (4)$$

$$\frac{dI}{dt} = J_{PLC_{agonist}} - J_{\text{degrade}} \quad (5)$$

where *c* is calcium concentration in cytosol, s is calcium concentration in sarcoplasmic reticulum, *v* is membrane potential, *w* is open state probability of calcium activate potassium channels (K_{Ca}) of an single SMC. Here $J_{PLC\,agonist}$ is a parameter. Calcium dynamics in single SMC and propagation of its effects to the near neighborhood are basis of vasomotion. It is widely agreed upon that a cytosolic oscillator model in conjunction with a membrane oscillator can model the transient oscillatory behavior of cytosolic calcium concentration, which is sensitive to agonist stimulation or availability of a second messenger such as IP_3 (Aalkjær and Nilsson, 2005). Equations (1) and (2) here, model the cytosolic oscillator and equation (3) models the membrane oscillator. Since cytosolic IP_3 concentration mainly drives the oscillatory response, equation (5) models the intercellular IP_3 concentration. The efflux of potassium necessary to repolarize membrane plasma membrane is dependent on the open state of the delayed rectifying channels, simulated here by equation (4). The system is numerically solved using Runge Kutta (4^{th}, 5^{th}) order method. Following equations simulate the calcium dynamics for a single endothelial cell:

$$\frac{d\tilde{c}}{dt} = \tilde{J}_{IP3} - \tilde{J}_{ERuptake} + \tilde{J}_{CICR} - \tilde{J}_{extrusion} + \tilde{J}_{leak} - \tilde{J}_{cation} + \tilde{J}_{0} \quad (6)$$

$$\frac{d\tilde{s}}{dt} = \tilde{J}_{ERuptake} + \tilde{J}_{CICR} - \tilde{J}_{leak} \quad (7)$$

$$\frac{d\tilde{v}}{dt} = {}^{1}\!/\!_{C_m}\,(I_K + I_R) \quad (8)$$

$$\frac{d\tilde{I}}{dt} = \tilde{J}_{PLC_{agonist}} - \tilde{J}_{\text{degrade}} \quad (9)$$

Here, $\tilde{c}$ is cytosolic calcium concentration, $\tilde{s}$ is calcium concentration in endoplasmic reticulum, $\tilde{v}$ is plasma membrane potential and $\tilde{I}$ is cytosolic IP_3 concentration in a single endothelial cell (EC). Similar to SMC, equations (6) and (7) model the cytosolic oscillator for a single EC. Equation (8) models membrane oscillator, but in this case membrane potential is depended on the sum of potassium efflux through large and small conductance channels BK_{Ca} & SK_{Ca} and residual current constituting sodium and chlorine currents (details in Koenigsberger et.al, 2005). Equation (9) models cytosolic IP3 dynamics. $\tilde{J}_{PLC\,agonist}$ is again a parameter, which is triggered by attachment of the agonist present in the blood.

2.1 VASCULAR GEOMETRY

Results for simulation of a single uncoupled cell, an EC and SMC, are presented in section 3. A population of cells was also simulated. Anatomically, endothelial cells lie parallel to the vessel axis and smooth muscle cells are normal to the axis, connecting to one another, circumferentially. Typically, a smooth muscle cells is 50µm in length (around the circumference of the vessel) and approximately 5µm wide. An endothelial cell is almost 1.3 times as long as smooth muscle cell and approximately twice as wide (long axis of EC parallel to vessel axis. In contrast to Koenigsberger et.al (Koenigsberger et.al, 2005), where population was a lot smaller (of the order of only 30 ECs and 60 SMCs), we have simulated for 232 ECs and 3000 SMCs laid out as shown in figure 1.

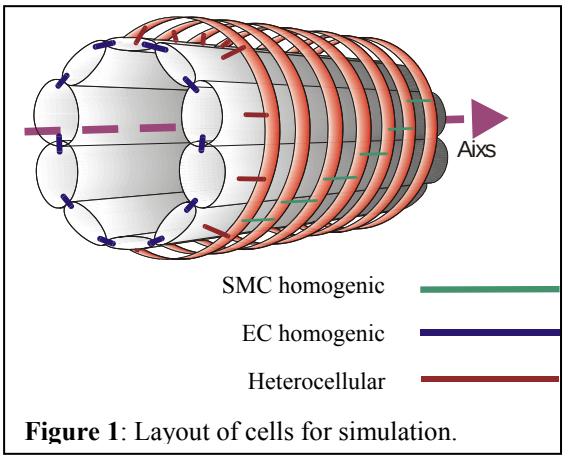

Figure 1: Layout of cells for simulation.

2.2 INTERCELLULAR COMMUNICATION

As shown in figure 1, each cell was two way coupled; homogenically and heterogenically. In homogenic coupling, an i^{th} SMC is coupled to four neighboring SMCs via its membrane potential (electrical coupling) and calcium concentration (calcium coupling). A j^{th} EC is coupled to four nearest neighboring via electrical coupling only. An EC is coupled to neighboring SMCs via electrical and IP3 coupling and vice versa.

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In contrast to Koenigsberger et.al (Koenigsberger et.al, 2005) where each SMC is coupled to 6 nearest neighboring SMCs, we homogenically couple a cell with 4 of its nearest neighbors to reduce the computational workload during simulation. Decreasing the number of neighboring cells did not cause a significant difference in the response of either an EC or SMC.

Equations modeling these coupling are:

Homogenic coupling between SMCs

$$V_{coupling} = -g\sum(v - v_k) \quad (10)$$

$$J_{Ca_{coupling}} = -p\sum(c - c_k) \quad (11)$$

Homogenic coupling between ECs

$$V_{coupling} = -\tilde{g}\sum(\tilde{v} - \tilde{v}_l) \quad (12)$$

Heterogenic coupling between an EC and neighboring SMCs

$$V_{coupling}^{EC\to SMC} = -\bar{g}\sum(\tilde{v} - v_k) \quad (13)$$

$$J_{IP3_{coupling}}^{EC\to SMC} = -\bar{g}\sum(\tilde{c} - c_k) \quad (14)$$

Heterogenic coupling between an SMC and neighboring ECs

$$V_{coupling}^{SMC\to EC} = -\bar{g}\sum(v - \tilde{v}_l) \quad (15)$$

$$J_{IP3_{coupling}}^{SMC\to EC} = -\bar{g}\sum(c - \tilde{c}_l) \quad (16)$$

where c_k and v_k are variables of neighboring SMCs and c_l and v_l are of neighboring ECs. The layout was programmed in C language and Matlab was used for visualization of results.

4 RESULTS AND DISSCUSION

A large population of cells was simulated, constituting an artery of approximately 15 mm in length and 32μm in diameter in contrast to Keonigsberger et al (Keonigsberger et al, 2005). This important difference in not only the number of cells but the arterial length has significant implications in simulating physiologically correct scenarios.

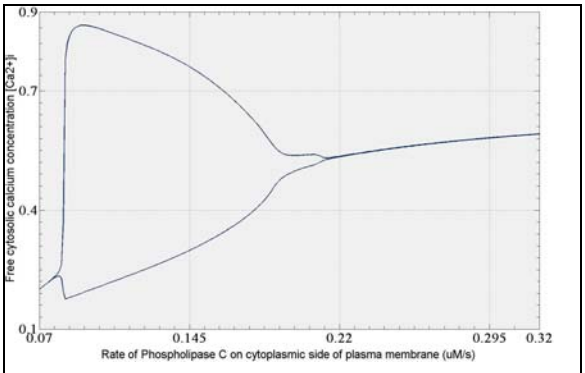

Figure 2 : Cytosolic calcium concentrations (in μM) of single smooth muscle cell over a range of agonist. stimulation

For our model we were able to simulate all the results of Koenigsberger et al (Keonigsberger et al, 2005). In case of single cell, the system simulating an uncoupled SMC oscillates when stimulated with a range of parameter values, shown in figure 2. The calcium concentration in SMC starts to oscillate when rate of PLC production reaches to 0.082μM/s and reaches to its maximum at 0.092μM/s. At values of $J_{PLC\,agonist}$ higher than this, the peak amplitude attained by oscillating cytosolic calcium concentration in a SMC decreases. The system again attains a steady state value when stimulated with 0.219μM/s. The range of

agonist over which the system oscillates is sensitive to intraluminal calcium concentration, thus increasing the extracellular availability of calcium would shift figure 2 to the left on x axis and the cytosolic calcium concentration in an SMC will oscillate at a lower value of $J_{PLC\,agonist}$.

Agonist concentrations used by Koenigsbereger et al were constant not only in time but in space. From a physiological perspective this is clearly a very simple assumption. In contrast for this present model the parameter $J_{PLCagonist}$ was constant in time but **spatially varied** along the axis of the vessel in increasing fashion shown in Figure 3, (there are, of course a large number of other possibilities modelling physiological conditions found in the arterial system, they are not included here but left for a future publication).

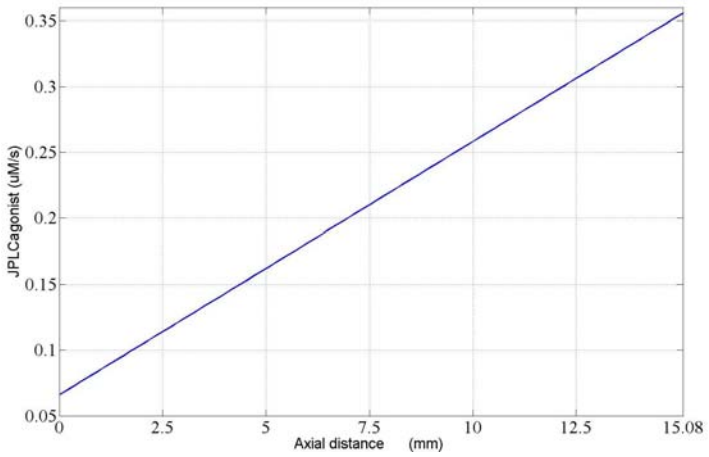

Figure 3 : Spatially varying agonist over an axial distance of 15mm

Both homogenic and heterogenic coupling (as detailed above in methods section) were then introduced and hence simulated a mixed population of coupled endothelial and smooth muscle cells in geometry depicted by Figure 1.

With all intercellular couplings active, the SMCs acted upon with low agonist concentration started to oscillate in contrast the single cell result. The initiation of an oscillation phase in time depended upon the location of an i^{th} SMC i.e. how far it was from a k^{th} SMC which was exposed to an agonist concentration high enough to cause oscillations in a single uncoupled cell.

To observe the effect of a spatially varying agonist concentration on cytosolic calcium concentrations of smooth muscle cells, a fast Fourier Transform (FFT) was taken utilising the entire time scale of 1200 seconds. The DC component was eliminated to gain clearer visualization.

In order to describe the complex relationships that exist between cells and the particular agonist concentration (which is a function of spatial position) we concentrate on three distinct smooth muscle cells. The choice of these spatial locations is driven by the what would seem to be "unexpected behaviour". These cells are positioned at 1.105, 1.365 and 2.145 mm along the axis of the artery designated as Cells 1,2 and 3 respectively. These experience rate of generation of PLC due to agonist concentrations of 0.085, 0.0925, 0.1075μM/s, respectively.

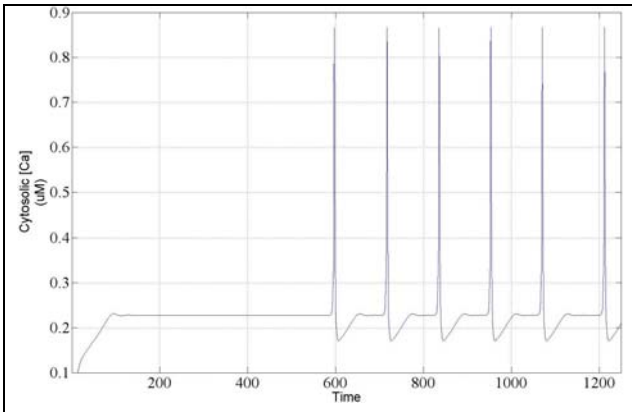

Figure 4: Cytosolic free calcium concentration in an SMC located at 1.105mm of axial distance, experiencing rate of generation of PLC due to agonist concentration = 0.085 μM/s.

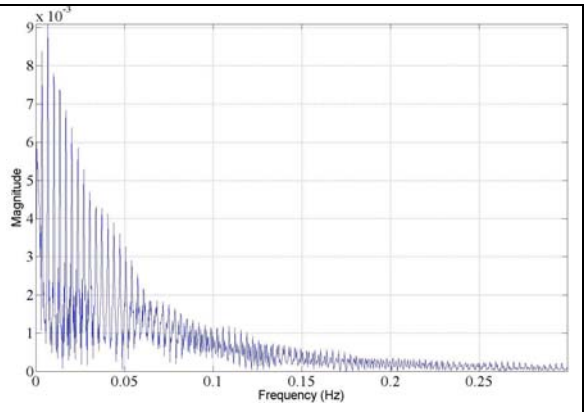

Figure 5: FFT Cytosolic free calcium concentration over an interval of 1200 seconds, of an SMC located at 1.105mm of axial distance, experiencing rate of generation of PLC due to agonist concentration = 0.085 μM/s.

3.4 Cell 1

Figure 4 shows the calcium concentration of cell 1. This cell lies at a spatial position where the agonist concentration is low and therefore we would not expect the cell to oscillate if it was uncoupled. It is shown that there exists a transient of approximately 30 seconds before it starts to oscillate. Our results indicate that the initiation of oscillation at time = 600 seconds is due to the process of IP3 diffusion upstream into the cell from those cells lying in a spatial position where their IP3 concentration (due to the higher agonist concentration) is larger. This is also true for the calcium concentration and thus also diffuses upstream.

Figure 5 shows the FFT for the whole time of 1200 seconds and indicates that the large majority of oscillation is at the low frequency range.

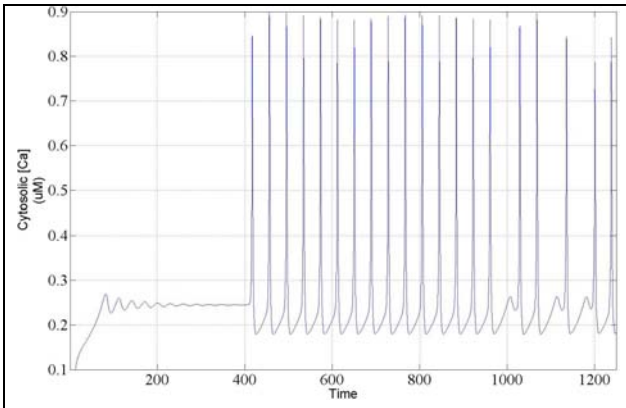

Figure 6: Cytosolic free calcium concentration in an SMC located at 1.365mm of axial distance, experiencing rate of generation of PLC due to agonist concentration = 0.0925 μM/s.

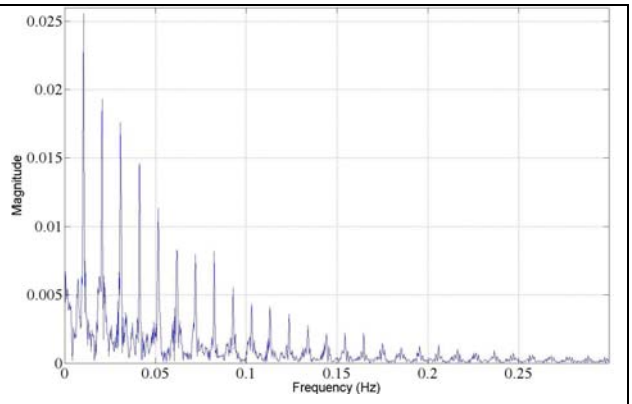

Figure 7: FFT Cytosolic free calcium concentration over an interval of 1200 seconds, of an SMC located at 1.365mm of axial distance, experiencing rate of generation of PLC due to agonist concentration = 0.0925 μM/s.

3.4 Cell 2

Figure 6 shows, in a similar fashion to Figure 4 above, the calcium concentration. Here the transient time is smaller, approximately 15 seconds, and the cell starts to oscillate in the same way as that for cell 1. However this particular cell is in a position where it should oscillate immediately. We believe on the basis of our results that the transient occurs because it starts to immediately distribute its IP3 (and calcium) in an upstream direction, thereby lowering its IP3 concentration and thus unable to oscillate. IP3 (and calcium) from downstream eventually reaches this cell and it starts to oscillate as normal. Figure 7 shows the FFT of cell 2; the difference in scales for the frequency magnitude should be noted. Here the cell has a high magnitude low frequency oscillation, which should be expected given it's spatial position (and hence resulting IP3 concentrations corresponding to the rate of PLC generation due to agonist concentration).

3.4 Cell 3

Figures 8 and 9 show calcium concentration and FFT of cell 3 lying at a position relatively far downstream at a position of 2.145 mm from the start of the arterial tube. The cell lies at a position where the rate of PLC production is 0.1075μM/s. Here the cell has initial transient as it lies at a position where the PLC rate is high enough to provide both IP3 and calcium to other coupled cells without allowing the concentration to fall below a certain level at which it would not oscillate. FFT is this case shows multiple low frequencies with comparably high magnitude.

Cells further downstream oscillate in a similar fashion and distant cells which are in the position of highest agonist concentration do not oscillate at all. We should note here that the agonist concentration, although steady in time produces time–dependent behaviour over substantial periods of time. Indeed it is only after a very long period do the cells reach a stable equilibrium. However this is not a physiological condition since the wall shear stress and hence the agonist concentration will vary, typically over a cardiac cycle time and thus our results show that the cells will continue to oscillate in this fashion.

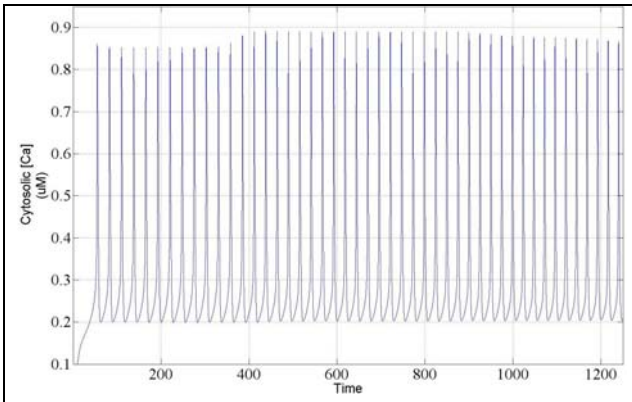

Figure 8: Cytosolic free calcium concentration in an SMC located at 2.145mm of axial distance, experiencing rate of generation of PLC due to agonist concentration = 0.1075 μM/s.

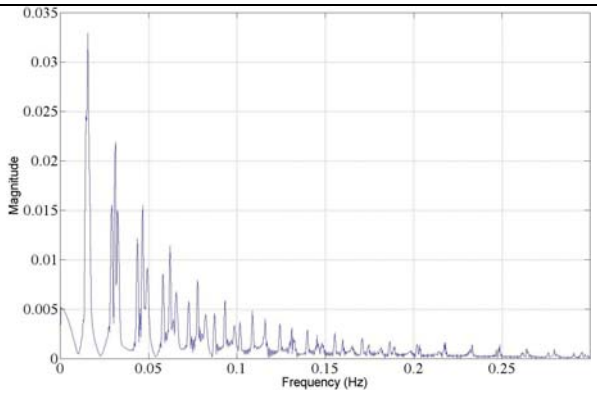

Figure 9: FFT Cytosolic free calcium concentration over an interval of 1200 seconds, of an SMC located at 2.145mm of axial distance, experiencing rate of generation of PLC due to agonist concentration = 0.1075 μM/s.

4 Conclusions

Conclusively, we suggest that the active intercellular communication plays an important role in propagating the effects of high agonist by recruiting the cells which are not stimulated to level where they can oscillate on their own. This can be a way of sending information to orchestrate an effective and collective response to a local hemodynamic change.

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