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Inefficiency calcium channels effects in calcium transients, action potential generation and propagation: A simulation Study on single cell and 1D model of human cardiac Purkinje fibre cells.

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Abstract

Calcium (Ca^{2+}) is essential in cardiac electrical activity and is the direct activator of the myofilaments, which cause contraction everywhere. Prolongation of action potential duration (APD), altered intracellular calcium transient $[Ca^{2+}]_i$ and propagation delay in cardiac myocytes are commonly observed heart failure (HF). This work focus on a simulation Study on abnormal Ca^{2+} channels effects on action potential generation, AP propagation and $[Ca^{2+}]_i$ in single and one dimensional (1D) purkinje fibre cell (PFC). In enhanced activity of sodium-calcium (Na^{+}-Ca^{2+}) exchanger, action potential generation cycle length reduces of 33% in HF versus normal condition, as well as great variation in maximum diastole potential (MDP). Systolic $[Ca^{2+}]_i$ is starts very early (~30ms) in HF. In 1D model which produces a more propagation delay (time taken to propagate action potential at a distance of 0.26cm (25 cells to 50 cells) is 0.150ms in HF, but in normal 0.0017ms) and reduces its conduction velocity. The failure sacroplasmic calcium channels AP changes its morphology. The simulation results shows an AP duration at 90% of repolarisation (APD_{90}) prolongation of 65% in heart failure versus normal conditions, as well as an 18% prolongation in APD_{50}. Peak systolic $[Ca^{2+}]_i$ and Diastolic $[Ca^{2+}]_i$ also have changes with that of the normal value. In this 1D model simulation, conduction velocity reduces. Results concluded that the $[Ca^{2+}]_i$ alternant might be responsible for the fluctuation in APD that produce T-wave alternans in a whole heart and this could be causal factor in the genius of ventricular fibrillation.

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Keywords: Excitability, Extracellular fluid, sacroplasmic recticulum, Conduction velocity, Action potential duration, Ion channels, Arrhythmias, Calcium transient, Na^{+}-Ca^{2+} ion exchanger.

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1. Introduction

Cardiac Arrhythmias and sudden cardiac death are among the most common causes of death in the industrialized world. Purkinje Fibre cell PFC is an important biological network of the human cardiac ventricular conduction system. It ensures the synchronized timing and sequencing of ventricular contraction [11]. One of the most important ailments that must be treated by the physician is cardiac failure ("Heart Failure" (HF)). Despite decades of research their causes are still poorly understood. First, the possibilities for doing experimental and clinical studies in human hearts are very limited. Second, animal hearts used for experimental studies may differ significantly from human hearts [heart size, heart rate, action potential (AP) shape, duration, and restitution, vulnerability to arrhythmias, etc [10]. Computer simulations of arrhythmias in the human heart can overcome some of these problems.

To perform simulation studies of different arrhythmias in human PFC, a mathematical model is needed that on the one hand reproduces detailed properties of single human PFC, such as the major ionic currents, calcium transients, and AP duration (APD), restitution (APDR), and important properties of wave propagation in human purkinje tissue, such as conduction velocity (CV) restitution (CVR). On the other hand, it should be computationally efficient enough to be applied in the large-scale spatial simulations needed to study different types of arrhythmias.

Mathematical model of the electrical action potential of purkinje fibre cell of human is given by Zhang in 2009. A description of I_{ion} for the human PF cell based on the model of the human endocardial cell by ten Tusscher et al. [10] and ten Tusscher & Panfilov [31]. This model, describing the properties of potassium currents in human PF cells based on the experimental data of Han et al. (2002) [6]. This model includes addition of two currents: a hyperpolarization-activated current (I_f) and sustained potassium current (I_{sus}). The descriptions for the inward rectifier current (I_{K1}) and the transient outward current (I_{to}) were reformulated, maximum conductance of the rapid (I_{Kr}) and slow (I_{Ks}) delayed rectifier potassium currents and the fast sodium current (I_{Na}) were altered because, these channels are distinctively different in channel kinetics and current densities between PF and ventricular cells [6].

In cardiac muscle, depolarization of the sarcolemma initiates calcium (Ca^{2+}) release by activating sarcolemmal L-type Ca^{2+} channels, providing a local increase in cytoplasmic calcium concentration in the junctional space of the dyad [18]. This initiates Ca^{2+}-dependent activation of the sarcoplasmic reticulum (SR) Ca^{2+} release channels, which are ryanodine receptors (RyRs) [21, 22]. The subsequent release of Ca^{2+} from the SR further increases the Ca^{2+} in the junctional space and leads to regenerative RyR activation in a process known as calcium-induced calcium release (CICR) [23]. In such a regenerative process, the larger SR Ca^{2+} flux should prevent subsequent control by the surface membrane [8] and deep-SR Ca^{2+}-store depletion. However, the quantity of Ca^{2+} released from the SR has a graded dependence on the magnitude of the Ca^{2+} influx across the sarcolemma [8, 24], and the SR releases only ~50% of its content.

During the cardiac action potential (AP), Ca^{2+} influx via individual L-type Ca^{2+} channels activates a cluster of adjacent sarcoplasmic reticulum (SR) Ca^{2+} release channels RyRs, the consequent systolic Ca^{2+} transient is the spatial and temporal sum of such local Ca^{2+} releases [8,13,14]. It is now generally accepted that L-type Ca^{2+} current ($I_{Ca,L}$) is the major trigger for SR Ca^{2+} release [15,16], and that alternative pathways (Na^{+}-Ca^{2+} exchanger, T-type Ca^{2+} current) are weak triggers under physiological conditions [17, 19].

CICR leads to a 10-fold increase in cytosolic Ca^{2+} concentration, and hence, provides the necessary signal to initiate mechanical shortening of the cell's contractile machinery. The elucidation of the mechanisms that underlie the process of CICR has become possible in recent years with the development of experimental techniques for simultaneous measurement of LCC currents and Ca^{2+} transients [25-27, 8], and detection of local Ca^{2+} release events [28-29, 25]. These experimental observations gave rise to and later verified the local control theory of excitation-contraction (EC) coupling [25-26, 7, 18].

The variations of the concentration of intracellular Ca^{2+} are involved in the initiation of electric and mechanical phenomena. Increase in intracellular Ca^{2+} induces a hyper contracture of the cardiac muscles (7). The mechanism that links membrane potential changes to the release of calcium from internal stores to cause contraction of cardiac cells is unclear. In addition, sarcoplasmic reticulum calcium release may be partly regulated by membrane potential, since repolarization could terminate the rise in intracellular calcium. Thus, changes in the action potential will have immediate effects on the time course of the $[Ca^{2+}]_i$ (8).

The aim of this research work is to analyze the effects on AP generation, AP propagation and $[Ca^{2+}]_i$ by different types of abnormal condition such as enhanced activity of sodium-calcium (Na^{+}-Ca^{2+}) exchanger, hypertrophy and SR channel failure in human PFC. If any changes occurs in electrical activity of purkinje fibre cell,

which affects the excitation- contraction and synchronization between ventricular and purkinje fiber cell. This synchronization mismatch leads to life threatening ventricular arrhythmias.

2. METHODS AND MATERIALS

2.1. General

In this study, we use Zhang model of human PFC [6]. The electrophysiological behavior of a single PFC can hence be described with the following differential equation.

$$C_m\,(dV/dt) = -\,(I_{ion}) \tag{1}$$

Where,

C_m -Membrane Capacitance in (µF)
V -Membrane Potential in (mV)
t -Time in (s)
I_{ion} -is the sum of the transmembrane Ionic currents (pA/pF)

I_{ion} is given by summation of the number of ionic currents.

$$I_{ion}= I_{kr}+ I_{ks}+ I_{k1}+ I_{to}+ I_{Na}+ I_{b,Na}+ I_{Ca,L}+I_{b,Ca}+ I_{NaK}+ I_{NaCa}+I_{p,Ca}+I_{p,K}+ I_{sus}+ I_f \tag{2}$$

L-type Ca^{2+} ($I_{Ca,L}$), transient outward k^+ (I_{to}), rapid and slow delayed rectifier k^+ (I_{kr} & I_{ks}), Time-independent potassium current (I_{k1}), fast Na^+ (I_{Na}), Na^+-Ca^{2+} exchanger(I_{NaCa}), Na^+-k^+ pump (I_{NaK}), and background Na^+ ($I_{b,Na}$), sarcolemmal calcium pump ($I_{p,Ca}$), background Ca^{2+} ($I_{b,Ca}$), sustained current (I_{sus}), Hyperpolarization-activated current (I_f) and potassium pump ($I_{p,K}$).

2.2. 1D Array of Purkinje fiber cell with gap junction

To avoid more complex cable equation 1D model, this work aims to formulate a computational theoretical multicellular fiber model for human PFC action potential that agree with recent experimental data Han et al. (2002). It is also efficient for large-scale spatial simulations of cardiac arrhythmias. The model includes a simple calcium dynamics that reproduces realistic calcium transients and a positive human contraction staircase and allows to realistically model calcium-dominated I_{CaL} inactivation, while at the same time maintaining a low computational load.

The computational model for 1D array of PFC with gap junction is developed and it composed of interconnected cell of zhang models with constant gap junction conductance. In that each single cell PFC dynamics are considered as a single potential node. The coupling current between two purkinje fiber cells is given by

$$I_{cup1}= (P_1-P_2)*G_1 \tag{3}$$

Where,

I_{cup1} - Coupling current between $cell_1$ and $cell_2$
P_1 - Membrane potential at $cell_1$
P_2 - Membrane potential at $cell_2$
G_1 - Conductance between $cell_1$ and $cell_2$

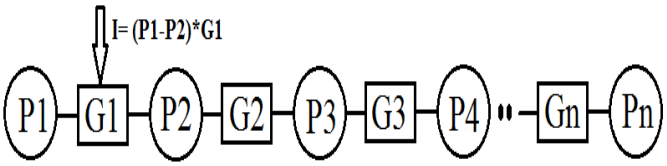

Fig. 1 1D Array of Purkinje fiber cell with gap junction

The gap junctions were distributed uniformly along the length of the fiber. The gap junctions were represented by channels having resistive properties that are constant (static model) with respect to voltage and time. With the static model, the channel conductance within the junction was fixed with respect to time and voltage.

2.3. Normal calcium Transient of single PFC (μM)

Calcium channels involve in $[Ca^{2+}]_i$ transient is shown in Fig.2. L-type calcium channels ($I_{Ca,L}$) release calcium in the diadic space or subspace (SS), where sarcolemmal membrane and membrane of the sarcoplasmic reticulum (SR) are in close proximity. Ryanodine receptors (I_{rel}) sense this elevation of calcium in the subspace and respond with a release of calcium from the SR. Through diffusion (I_{xfer}), the calcium released in the subspace travels to the cytoplasm (cyto). Sodium-calcium exchangers (I_{NaCa}) and the sarcolemmal calcium pump ($I_{p,Ca}$) pump calcium out of the cytoplasm to the exterior of the cell. Calcium pumps in the membrane of the SR (I_{up}) pump calcium out of the cytoplasm back into the SR. Small leakage current (I_{leak}) leaks calcium from the SR to the cytoplasm.

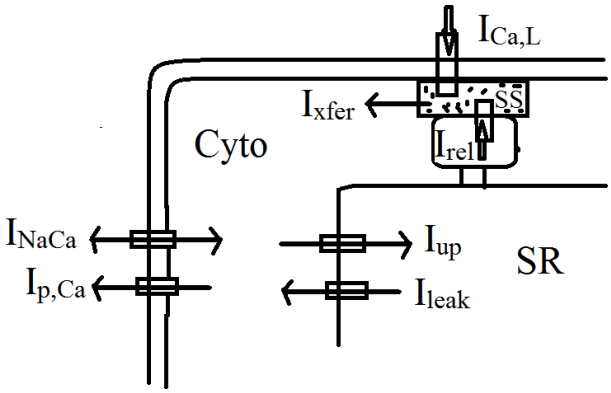

Fig.2 Calcium channels involve in $[Ca^{2+}]_i$ transient process.

2.4. Preparation of failure calcium channels

We modified the formulation of $I_{NaCa,}$ $I_{Ca,L,}$ $I_{NaK,}$ I_{up} and I_{leak} proposed by Zhang et al. (6) for human Purkinje fibre cells. The scaling factor of I_{NaCa} is modified to reproduce enhance activity of Na^{+}-Ca^{2+} exchanger (i.e, the maximal I_{NaCa}, (K_{NaCa}=1650 (pA/pF)) 65% increase from its normal level). The maximum current of I_{NaK} (P_{Nak}=0.75(pA/pF)) and $I_{Ca,L}$ current is modified to reproduce hypertrophy condition. In same way SR calcium pump I_{up} (V_{maxup}=0.0015(mM/ms) and SR calcium leak channel I_{leak} (V_{leak}=0.00017(mM/ms) activation time also modified to reproduces abnormal CICR process. All the data takes from human myocytes by Pribe and Beukelmann et al. (30).

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3. RESULTS AND DISCUSSIONS

Action potential generation of single PFC is achieved through simulation at normal physiological condition having maximum cycle length 1.2s, depolarization amplitude 29.3mV and its action potential duration (APD_{90}) 300ms is shown in *Fig*.3 panel A. The 1D model also predicts Action Potential (AP) propagation in multicellular fibre of length 0.5cm shows the characteristic like spike notch dome architecture, maximum diastolic potential (-75.65 mV), cycle length (1.2s), APD_{90} (300ms), conduction velocity (1.5m/s), and resting potential (-74.78mV) which is agreement with experimental data and Zhang model of single purkinje fibre cell. Action Potential of propagation in multicellular fibre of length 0.5cm with gap junction conductance (g_{con}) = 0.01 µS at control physiological condition is shown in Fig.3 panel B. The conduction velocity is more important in purkinje fibres. In this 1D model conduction velocity is 1.52m/s, (i.e) time taken to propagate action potential at a distance of 0.26cm (25 cells to 50 cells) is 0.0017ms at constant gap junction of 0.01 µS is shown in Fig.3 panel C. The main focus of this work is to analyse the calcium transient in single cell and AP generation and propagation effects in 1D model due to abnormal condition of different Ca^{2+} ion channels.

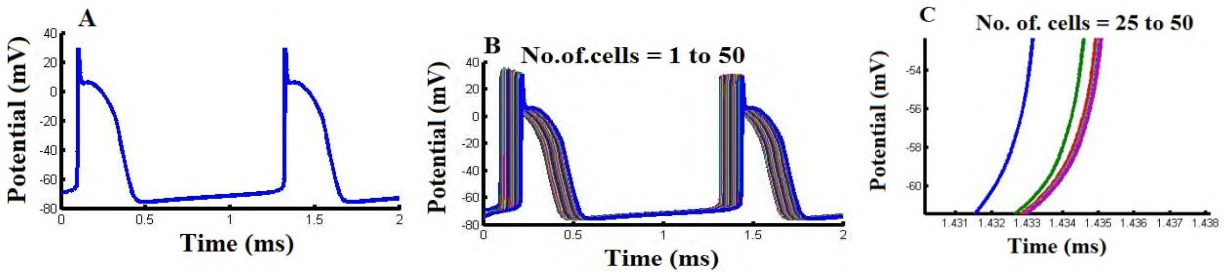

Fig 3.Panel A: Simulated action potential of single purkinje fiber cell at normal physiological condition. Panel B: Simulated action potential of 1D purkinje fiber cell of length 0.5cm at normal condition. Panel C: Action potential propagation time from 0.25cm to 0.5cm cells (26 cells).

In fig.4 Panel A to D shows the Action potential generation, calcium transient, sacroplasmic recticulum and ryanodine channels effects due to normal (dark line) and enhance activity of Na^{+}-Ca^{2+} exchanger (dot line). At normal condition the PFC has peak amplitude of $[Ca^{2+}]_i$ is 0.7 µM. It starts its calcium transient after 100ms. During this initial 100ms, sodium depolarization takes place to activate L-type calcium channels. $[Ca^{2+}]_i$ plays vital role in generation of action potential automocity, contraction- excitation coupling and cardiac muscle relaxation. The enhance activity of Na^{+}-Ca^{2+} exchanger (i.e, the maximal I_{NaCa}, (K_{NaCa}=1650) increases 65% from its normal level) results shows in Panel A that action potential cycle length reduces of 33% in HF versus normal condition, as well as great variation in MDP. Simillarly $[Ca^{2+}]_i$ transients under HF and normal condition are shown in Panel B. systolic $[Ca^{2+}]_i$ is starts very early (~30ms) in HF. This quick activation of $[Ca^{2+}]_i$ happens because of enhanced activity of Na^{+}-Ca^{2+} exchanger reduced the reverse potential of calcium (E_{Ca}). So L-type calcium channel activate very early to increase SS and produce enough thresholds to open ryanodine receptors (~30ms). At normal condition, maximum time for Calcium concentration level back into SR from cytoplasm is 480ms. But in HF, maximum time for Calcium concentration level back into SR from cytoplasm is 250ms. SR load calcium from cytoplasm increase of 50% in HF versus normal condition is shown in Panel C. In normal PFC, ryanodine receptors open two times for CICR process in 2s. But in enhanced activity of Na^{+}-Ca^{2+} exchanger, ryanodine receptors open three times for CICR process in same time period is shown in fig.4 Panel D. In 1D model, consider 50 cells (length=0.5cm) in which first half (25 cell of length = 0.25cm) is set as enhanced activity of Na^{+}-Ca^{2+} exchanger and second half (25 cell of length = 0.25cm) is set as normal, its action potential generation and propagation is shown in Fig. 4 Panel E. Here action potential generation in first half peak amplitude (25 cells- HF) is slightly reduced; whereas second half peak amplitude (25 cells- N) is slightly increasedHF action potential generation is given as stimulation to normal cell,

which produces a more propagation delay and reduces its conduction velocity. In this 1D model conduction velocity is reduces, (i.e) time taken to propagate action potential at a distance of 0.26cm (25 cells to 50 cells) is 0.150ms at constant gap junction of 0.01 µS is shown in fig.4 panel F. From these results it is very clear enhanced activity disturb action potential propagation, action potential duration and also responsible for early depolarization. Enhance activity of Na^{+}-Ca^{2+} exchanger is mostly affects relaxation period and repolarization of $[Ca^{2+}]_i$ process and thus $[Ca^{2+}]_i$ alternant might be responsible for the fluctuation in APD that produce T-wave alternant in a whole heart and this could be causal factor in the genius of ventricular arrhythmias.

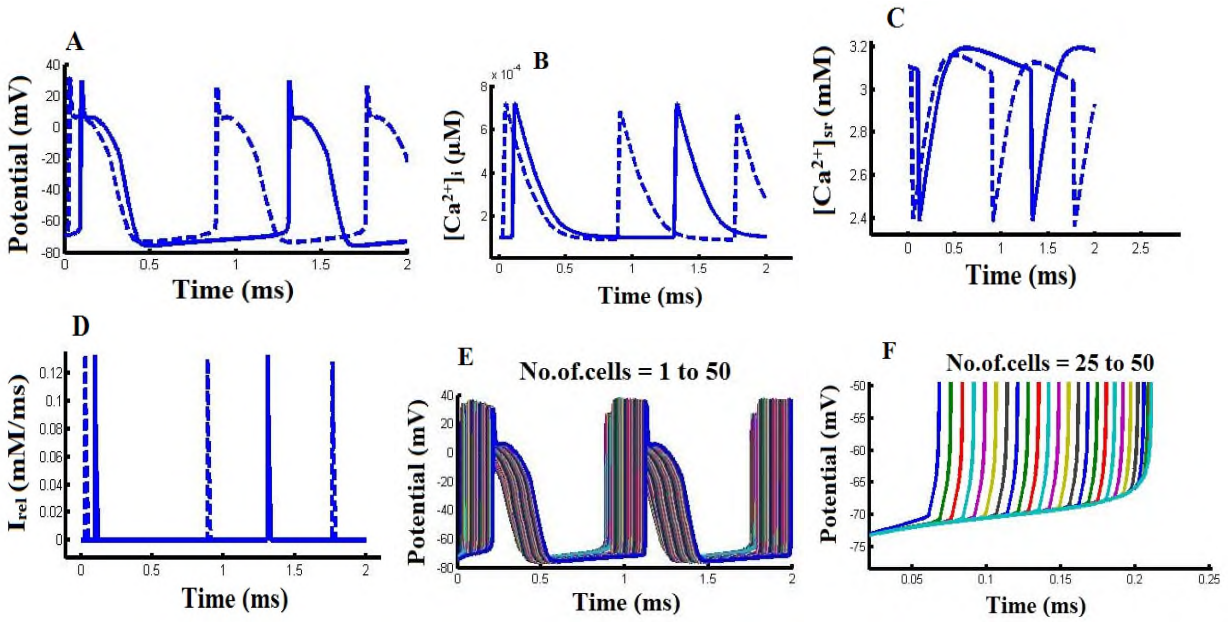

Fig 4.Panel A: Simulated APs of single purkinje fiber cell from normal physiological condition (dark line) and failing cell (dot line). Panel B: Simulated $[Ca^{2+}]_i$ transients. Panel C: Simulated $[Ca^{2+}]_{sr}$ concentration. Panel D: Simulated I_{rel} (ryanodine receptors) calcium release. Panel E: Simulated APs of 1D purkinje fiber cell of length 0.5cm, in that first 0.25cm fiber is failing myocytes and second 0.25cm is normal. Panel F:Action potential propagation time from 0.25cm to 0.5cm cells.

In Fig 5.A the AP shows the control and failure in sacroplasmic calcium uptake pump and sacroplasmic calcium leakage channels in single cell. Comparing with control sacroplasmic recticulum calcium uptake pump and sacroplasmic calcium leakage channels, the failure sacroplasmic calcium channels AP changes its morphology.

Simulation results of this work shows an AP duration at 90% of repolarisation (APD_{90}) prolongation of 65% in heart failure versus normal conditions, as well as a 18% prolongation in APD_{50}. Calcium transient under heart failure and normal conditions are shown in fig.4 panel B. Peak systolic $[Ca^{2+}]_i$ is slightly increases to 11%, where as Diastolic $[Ca^{2+}]_i$ increases upto 39% of the normal value. Ryanodine channel is a 4- states markov model of Shannon et al. and stern et al. [57]. Its activation rate (open) and inactivation rate (close) depends on sacroplasmic recticulum calcium load and subspace calcium levels are shown in fig.5 panel E and F. Peak systolic in open conducting state (k1) activates upto100ms in normal, where as in HF is activation period upto 200ms. In peak Diastolic is reduces upto 50% of the normal value. Inactivate closed state (k2) is activates after 100ms in normal, where as in HF is inactivation period upto 200ms. The peak Diastolic is increases upto 50% of the normal value. Peak systolic in sacroplasmic recticulum calcium concentration is slightly vary, where as Diastolic is reduces upto 75% of the normal value is shown in fig 5.Panel C. Calcium concentration in sacroplasmic recticulum is reduces during relaxation upto 17% of the normal value. In Fig 5.Panel G shows the AP of 1D purkinje fibre cell of length (0.5cm) .In that first half length (0.25 cm) purkinje fibre cell is consider as failure in sacroplasmic recticulum calcium uptake pump and sacroplasmic calcium leakage channels , then remaining half length is consider as normal purkinje fibre cell. The control fibre length (0.25 cm) with action potential duration (APD_{90}) increases to 15%,

maximum diastolic potential is less negative (-72 mV) and cycle length is increases upto 50% of the normal value. In this 1D model conduction velocity reduces, (i.e) time taken to propagate action potential at a distance of 0.26cm (25 cells to 50 cells) is 0.030ms at constant gap junction of 0.01 µS is shown in fig.5 panel H. From these results it is very clear that the Calcium channels in SR disturb action potential propagation, action potential duration and also responsible for early depolarization, which leads to arise synchronization problem in conduction and relaxation between ventricular and purkinje fibres.

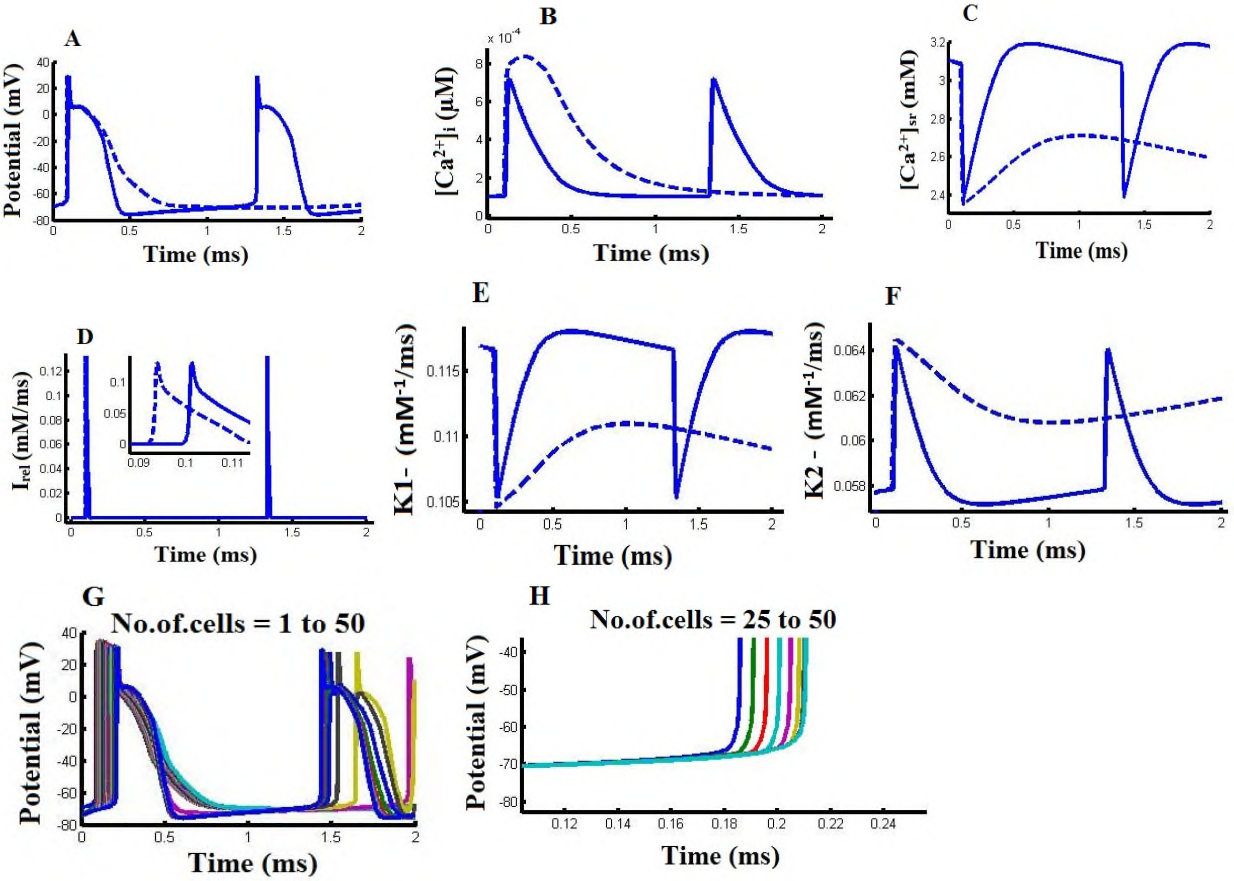

Fig 5. Panel A: Simulated APs of single purkinje fiber cell with normal physiological condition (dark line) and failing cell (dot line). Panel B: Simulated $[Ca^{2+}]_i$ transients. Panel C: Simulated $[Ca^{2+}]_{sr}$ concentration. Panel D: Simulated I_{rel} (ryanodine receptors) calcium release Panel E: Simulated K1 (ryanodine receptors active state) opening rate release. Panel F: Simulated K2 (ryanodine receptors inactive state) closing rate. Panel G: Simulated APs of 1D purkinje fiber cell of length 0.5cm, in that first 0.25cm fiber is failing myocytes and second 0.25cm is normal. Panel H: Action potential propagation time from 0.25cm to 0.5cm cells.

During hypertrophy condition, purkinje fibres cell calcium transient process initiated very quickly at 20 ms onwards. This type of irregular calcium transient process leads to early depolarization effects in action potential generation. Such an action potential generation process is shown in fig 6. Panel A. The Peak amplitude of AP is slightly reduce up to 26mV, MDP is also varies up to -72.3mV, calcium transient has no change from its normal level, but total cycle length (~680 ms) is very much reduces upto 47% of the normal value. In Fig 6.Panel B shows the AP of 1D purkinje fibre cell of length (0.5cm) .In that first half length (0.25 cm) purkinje fibre cell is consider as failure myocytes due to hypertrophy, then remaining half length is consider as normal purkinje fibre cell. The hypertrophy condition cell length (0.25 cm) action potential generation results as same as hypertrophy single cell, but this AP generation is affects the action potential generation and propagation in rest of normal purkinje fibre cell

length. The control fibre length (0.25 cm) action potential peak amplitude slightly increase (31mV), and cycle length is reduces upto 37% of the normal value. In this 1D model conduction velocity is reduces, (i.e) time taken to propagate action potential at a distance of 0.26cm (25 cells to 50 cells) is 0.145ms at constant gap junction of 0.01 µS is shown in fig.6 panel C.

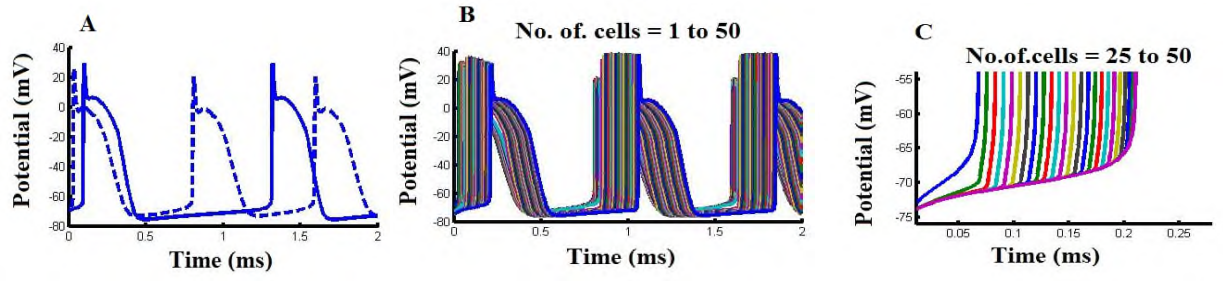

Fig 6. Panel A: shows the APs of control and HF (hypertrophy) in single PFC. Panel B: Simulated APs of 1D purkinje fiber cell of length 0.5cm, in that first 0.25cm fiber is failing myocytes (hypertrophy) and second 0.25cm is normal. Panel C: Action potential propagation time from 0.25cm to 0.5cm cells.

4. Conclusion

In summary , the parameters were identified and described in this work (Ion channels) that affect calcium transients, action potential generation and action potential propagation because the calcium transients alternans might be responsible for the fluctuation in APD that produce T-wave alternans in a whole heart and this could be causal factor in the genius of ventricular fibrillation. Arrhythmias condition due to the ion channels such as enhanced activity of sodium-calcium (Na^{+}-Ca^{2+}) exchanger, failure of sacroplasmic recticulum calcium channels and hypertrophy conditions are responsible for the following effects like AP cycle length decreases, prolongation of APD, and early depolarization. All these produce more propagation delay and less conduction velocity which may lead to worsening of the cardiac abnormality.

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