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Osteo Intraorganelle Nanoporation under Electrical Stimuli

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Abstract:- Nanoporation is a highly effective method to increase permeability of intraorganelle membrane by using series of pico electric pulses. Using this technique, we can introduce specific drugs into the intraorganelle of regid cell like osreoblast and it has various application in medical science. The effect of phospholipids in respond to external pico electric fields, behaviour of water dipoles in the complex electric field landscape of the membrane interface and reorganization of water dipoles in pore formation process have been proposed in these studies. Pore characteristics such as life time, ion selectivity, size, kinetics of formation as well as number of pores are significant factors in the present study.

Keywords:- Pico seconds, Pulsed Electric Field (psPEF), intrigrated bio Micro chip, Dense Osteoblast cell, Intra- organelle, nanoporation

I. INTRODUCTION

The main cellular component of bone is osteoblast cell and its deficiency causes various bone diseases like osteoporosis which co-relates with Osteochondrodysplasia[1].Clinically osteoporosis can effectively be reduced by delivering drugs into the intraorganelle of osteoblast cell[2].It is only possible if the nano pores can be formed into the intraorganelle of that specific cell. To achieve this purpose, the complete electrical property of osteoblast cell should be investigated. But due to non uniform distribution of ions and rigid morphological structure of the osteoblast cell, it is more complex than other biological cell [3].This structure gives a challenge to the researchers for its nanoporation and its intra organelle characterizations. The nature of characterization was addressed by applying sufficient electric field to develop the pores within the membranes of the intraorganelle. So the electric field has a great influence on nanoporation of the intraorganelle [4-6].It is reported that the temporary permeabilization of the plasma membrane allows genes or drugs entering into the cell cytoplasm. This technique is very attractive in various applications such as electro chemotherapy, [7-9] tumor nodule treatments [10-12], the gene electrotransfection by nano second pulse electric field (nsPEF) exposure [13] and permeable to Propidium Iodide (PI) [14]. Beyond their potential effects on the cell membrane, the pico second pulse electric field (psPEF) shows a great interest because they also offer the possibility to disturb the intra cellular structures and functions [15]. However, the mechanisms implied in the effects of nsPEF as well as pico pulse under the non uniform micro fluidic system within the intra-organelle of living cells remain still unclear [16].In this context, a numerical model of a hybrid micro bio chip for psPEF exposure of intra organelle nanoporation of multilayer dense osteoblast cell and the equivalent electrical circuit of bi layer osteoblast which is placed within the proposed micro floudics system is proposed. [17].

In recent developments of micro technologies and micro fluidics techniques permit consideration of the design and fabrication of new innovative tools for biology. The main benefits of these technologies consist in their miniaturization and parallelization capabilities, as well as real-time observation in the case where transparent materials are used for the device fabrication .The use of micro fluidic devices in nanoporation is a remarkable exploration in the field of drug delivery system. Although the nanoporation process itself in sophisticated devices with full control over the process as well as cell debris separation and subsequent intracellular cell content analysis have not been reported yet.

In this research paper we explore the mathematical ,analytical and simulation model of intraorganelle nanoporation of bi-layer osteoblast cell.In the first part we design a 3D hybrid nanoporative device which is activated under the influences of pico electric field and the remaining part focuses the characterization of intraorganele nanoporation of the osteoblast cell.

II. THEORY

2.1 Intra organelle voltages

Saeid Movahed et al gave the following theoretical equations of outer and inner membrane potential of a biological cell[18].The outer membrane potential ($V_{org\,(o)}(t)$) and inner membrane potential $V_{org\,(i)}(t)$ are as follows

$$V_{org\,(o)}(t) = 1.5\ Rc\ E\ (t)\left[-e^{\frac{t}{\tau cell}} - 1(t-\tau) + e^{\frac{t-\tau}{\tau cell}}\cdot 1(t-\tau)\right]\cos\theta \text{---(1)}$$

$V_{org\,(i)}(t) = \frac{1.5\ \tau cell\ \ Rnuc\ \ E\ (t)}{\tau cell\ -\tau nuc}\left[\left(e^{\frac{t}{\tau cell}} - e^{\frac{t}{\tau nuc}}\right) - \left(e^{\frac{t-\tau}{\tau cell}} - e^{\frac{t-\tau}{\tau nuc}}\right).1.\ (t-\tau)\right]\ \cos\theta$ --(2)

As we know that E (t) = v/d, where v=applied voltage & d= distances in between two electrode.

2.2 Calculation of the radius of nanopores

Based on the theory of membrane permeabilization,nano pores are initially created with a radius of r*. By increasing the applied electric field, nano pores start to develop in order to minimize the energy of the cell membrane. For the intra organelle with n nanopores, the rate of change of their radius of pore(r), can be determined by the following set of equations: [18]

$U(r, Vn, Ap) = \frac{D}{KT}\left\{4\beta\left(\frac{r^*}{r}\right)^4\frac{1}{r} - 2\pi\gamma + 2\pi\sigma r + \frac{[\Delta\varphi]^2 Fmax}{1+rh/(r+ri)}\right\}$---------- (3)

Where D is the diffusion co efficient, K= boltz man constant, T=absolute temp, $\varphi(r,\theta)$ =intra organelle potential. $\gamma = surface\ tention$.The constants of the above equations are defined in Table 1.

2.3 Calculation of the intra organelle pore density

The rate of creation of nanopores at intra organelle can be found as [18]

$\frac{dN(t)}{dt} = \alpha * e^{\left(\Delta\varphi/Vep\right)^2}\left(1 - \frac{N(t)}{Neq\,(Vn)}\right) - - - - - -$ (4)

Where $N(t)$ is the pore density.

2.4 Calculation of the intra organelle ion uptake:

It is known from the bio chemistry that the ion uptake can be calculated by following mathematical calculation [18],

$Iuptake = Kf[1 - \left(1 + Kp.te\left(1 + \frac{Kp.te}{2}\right)\right) * e^{-Kp.te}$] ------ (5)

$Where\ \ Kf = \frac{D.Sc}{Vc.d}$, $and\ \ Kp = e^x$,

$x = \left[\frac{9.\Delta R.Vp.a^2\varepsilon 0(\varepsilon w - \varepsilon c)}{8.Kb.T.d^2}.* Vn^2\right]$

D = Diffusion co efficient,

Vc = Area of the pore,

d = thickness. Sc = N. π. r^2,

$\Delta R.Vp = \pi.d\left(r1^2 - r^2\right)$,----- (6)

$T = T$ emp in kelvin.

Kb=Boltz man constant.

εw=permitivity of water,

εc= permitivity of cytoplasm.&

te= pulse duration.

$r1$= radius of pore ,

r=radius of initial pores,

$V_{org\,(i)}(t)$=intra organelle potential.

III. MATHEMATICAL MODELING OF NANOPORATION

The electroporation process, including the formation and expansion of the pores, are describedby the Smoluchowski partial differential equation . The Smoluchowski equation defines a pore density function, *n*(*r*, *t*), such that the number of pores with radius between *r* and *r* + *dr* at any given time, *t*, is *n*(*r*, *t*)*dr*. *n*(*r*, *t*) is described as

$\frac{\partial n}{\partial t} + D\frac{\partial}{\partial r}\left(-\frac{\partial n}{\partial r} - \frac{n}{kT}\frac{\partial W}{\partial r}\right) = S(r)$---------- (7)

Where *D* is the pore diffusion coefficient, *r* is the pore radius, *W* is the formation energy of a pore with radius *r*, and *S*(*r*) describes the transition of hydrophobic pores to hydrophilic ones, as

$S(r) = \frac{v_c}{kT}h\frac{\partial W_0}{\partial r}e^{W_0/kT} - v_d\,nH(r_* - r)$------ (8)

where *vc* is the pore creation rate, *h* is the membrane thickness, *Wo* is the formation energy of a hydrophobic pore, *vd* is the pore destruction rate, $r*$ is the radius at which hydrophobic and hydrophilic pores have the same energy, and *H*($r*-r$) is a step function at *r* = $r*$.Assuming that, *(i)* the expansion of the pores is negligible, and *(ii)* the temporal change of the minimum pore energy is negligible, a quasistatic asymptotic model of electroporation simplifies the PDE equation to an ordinary differential equation.The ODE defines the pore density, *N*(*t*), which is related to *n*(*r*, *t*) as

$N(t) = \int_{r=0}^{\infty} n(r,t)\, dr$---------- (9)

The quasistatic asymptotic equation for $N(t)$ is

$$\frac{dN(t)}{dt} = \propto e^{(V_m\ (t)/V_{ep})^2}\left(1 - \frac{N(t)}{N_0} e^{-q(V_m\ (t)/V_{ep})^2}\right)$$------- (10)

Where Vm is the transmembrane voltage, Vep is the characteristic voltage of electroporation, $N0$ is the equilibrium pore density at $Vm = 0$, and a and q are constants.

It is reported that,when the transmembrane voltage achieves the required voltage of electroporation some pores are formed in the membrane. The formation of the pores increases the membrane conductivity of the membrane and is electrically modeled as an additional current density, Jep, inside the membrane. Jep is written as

$J_{ep}\ (t) = N(t)\frac{\pi {r_p}^2}{h}\sigma_p V_m K$---------- (11)

where N is the density of the pores, rp is the pore radius, σp is the conductivity of the solution inside the pore, Vm is the transmembrane voltage, h is the thickness of the
Membrane, and K is

$$K = \frac{e^{v_m} - 1}{\frac{\omega_0\, e^{\omega 0 - n v_m - n v_m}}{\omega_0 - n v_m} e_m^v - \frac{\omega_0\, e^{\omega 0 + n v_m + n v_m}}{\omega_0 + n v_m}}$$----- (12)

Where $w0$ is the energy barrier inside the pore, n is the relative entrance length of the pore, and $vm = qe,kT\ Vm$ is the non-dimensional transmembrane voltage. Assuming that the electric field inside the membrane is uniform, the transmembrane voltage is written as

$V_m = E_* h$---------- (13)

Where E_* is normal electric field.

The pore current density inside the membrane can be translated as an increase in the membrane conductivity using the relation

$J_{cp} = \sigma_m E_*$---------- (14)

The conductivity of the membrane at the points that pores are formed is calculated as
$\sigma_m(t) = \sigma_{m0} + \mathrm{N(t)}\sigma_\mathrm{p}\pi {\mathrm{r_p}}^2\ \mathrm{K}$---------- (15)
where $\sigma m0$ is the conductivity of the membrane before electroporation. The nonlinearity of the equation 3.4 comes from N which was described as

$$\frac{dN(t)}{dt} = \propto e^{(V_m\ (t)/V_{ep})^2}\left(1 - \frac{N(t)}{N_0} e^{-q(V_m\ (t)/V_{ep})^2}\right) — (16)$$

Under equilibrium conditions (15) and (16) maintain the transmembrane voltage below the required voltage of electroporation, which is about 1V.Required voltage of nanoporation is the threshold at which notable increase in the density of the pores and membrane conductivity and consequently decrease in the transmembrane voltage occur. The increase in the transmembrane voltage leads to the increase of pore density which lowers the conductivity of the membrane and subsequently the transmembrane voltage.

IV. ANALYTICAL MODELING OF NANOPORATION.

We will initially focus on a simple analytical, passive, and linear model of the cell. However, it provides useful information on the threshold for the onset of nonlinear effects. After introducing the concept of electro-effects that depend on pulse duration, we will discuss advanced models that include changes in cell structures. Fig2.7 shows a cross-section of an osteoblast cell, with the only membrane-bound substructure shown being the nucleus. These are assumptions that limit the applicability of the model to a temporal range that is determined by the dielectric relaxation times of membrane and cytoplasm. Although we focus here only on the plasma membrane, it will be shown that the conclusions that are drawn from the discussion of this simple model can easily be extended to predict electrical effects on the inner cell structures.

The assumptions used in most models for membrane charging and electroporation as well as intracellular electro-manipulation are that the membranes are perfect insulators, and that the permittivity of the liquids in and outside the cell can always be neglected.

Starting from the resting voltage, V_r, (which is on the order of 70 mV for many cells), the voltage across the plasma membrane will increase with time until the end of the pulse at time τ, and reach a value of $V_n(\tau)$ at the poles given by

$$V_n(\tau) = fE\left({}^{D}/_{2}\right)\left[1 - exp^{\left({}^{-\tau}/_{\tau_c}\right)}\right] \text{--------- (17)}$$

Where f is a geometry factor (1.5 for spherical cells), and D is the diameter of the (spherical) cell. The charging time constant,τ_c, for the cell (plasma) membrane is given as:

$$\tau_c = \left[\left\{\left(1 + 2v_{org}\right)/\left(1 - v_{org}\right)\right\}\rho_1/_2 + \rho_2\right] C_m a \text{-------- (18)}$$

V is the volume concentration of the spherical cells, ρ_1 is the resistivity of the suspending medium, ρ_2 the resistivity of the cytoplasm, C_m the capacitance of the membrane per unit area, and a is the cell diameter. The electric field in the membrane at the poles of the cell can be estimated from the condition that the current density at the interface of cytoplasm and membrane is continuous.

$$\sigma_M = \sigma_c \text{ ------ (19)}$$

The condition that the total voltage across the intraorganelle of the cell,V_n, is the sum of the voltage across the cytoplasm and twice that of across the membrane. Assuming the electric field along the axis of a cell with diameter D, and membrane thickness, d, is constant in the cytoplasm and in the membrane, respectively, gives us:

$$E_c D + 2E_M d = V_n \text{------- (20)}$$

Combining the two equations allows us to calculate the electric field in the membrane, depending on the applied voltage,

$$E_M = V_c/((\sigma_M/\sigma_c)D + 2d). \text{-------- (21)}$$

Since the conductivity of the cytoplasm is generally on the order of 1 S/m, and the conductivity of the membrane is on the order of (10-5) ms/m^2 , the first term in the denominator is small compared to the second. Consequently:

$$E_M \approx V_c/2d. \text{------- (22)}$$

This means that it is reasonable to assume that the entire voltage across a cell in this case is applied across the membranes (at the poles of the cell). This can lead to a strong thermal loading of the cell membranes at these positions. The energy density (W) deposited in the membrane.

$$W = \sigma_M \mathrm{E_M}^2 \tau \text{-------- (23)}$$

The time-dependent intraorganelle voltage $V_n(t)$ produces electro pores with a distribution of radii. Essentially, the Smoluchowski equation (SE) governs the growth and decay of pores and their evolution in radial-space. The continuum Smoluchowski theory yields the following equation for the pore density distribution function $n(r,t)$, with r being the pore radius and t the time variable .

$$\partial n(r,t)/\partial t - \frac{\left\{D/(K_B T)\right\}\delta\left[n(r,t)\left(\delta E/\delta r\right)\right]}{\delta r} - \frac{D\delta^2 n(r,t)}{\delta r^2} = S(r) - -(24)$$

Where $S(r)$ is the source (or pore formation) term, while D is a pore diffusion constant, and E(r) is the pore-formation energy. The pore formation "S" term depends on the trans-membrane potential and has a strong exponential on its magnitude. The equation no 3.15, 3.16 and 3.22 shows that the pore energy is completely influenced by radius of nanopore which are generated into the intraorganelle.

V. NUMERICAL SIMULATION

5.1 Simulation tool:

In this study, above Equations are solved numerically to find the each and every parameters of nanoporation to investigate the creation of nano-pores on the cell membrane. The Mat lab 7.2 & Comsol-4.3a, commercial package was used in the numerical simulations. In order to discrete the solution domain, unstructured meshes were applied. The solution domain was broken into small meshes to allow meshes to fully cover the solution domain without overlapping.

5.2 Used parameter

Table 1 parameters used in the simulations [18].

parameter	Cell parameters	value	
conductivity (S/m)	Extracellular medium (σe)	10×10^{-3}	
	Cell membrane(σm)	1.2×10^{-7}	
	Cell cytoplasm(σc)	0.039s	
	Nuclear membrane(σn)	10×10^{-1}	
	Nuclear cytoplasm(σn)	0.08s	
relative permittivity	Extracellular medium(εe)	80	
	Cell membrane(εm)	22	
	Cell cytoplasm(εc)	93	
	Nuclear membrane(εn)	22	
	Nuclear cytoplasm(εn)	93	
Geometry parameter (μm)	Cell radius(rc)	$12\ \mu m$	
	Cell membrane thickness(d)	0.006µm	
	Nuclear radius(rn)	$6\ \mu m$	
Constant parameters	$N0$	$1 * 10^{9}$	
	D	$5 * 10^{-14}$	
	K	$1.38065 * 10^{-23}$	
	T	300	
	β	$1.4 * 10^{-19}$	
	γ	$1.8 * 10^{-11}$	
	$Fmax$	$0.7 * 10^{-9}$	
	σ	$1 * 10^{-6}$	
	rh	$0.97 * 10^{-9}$	
	ri	$0.31 * 10^{-9}$	
	r	$0.7 * 10^{-9}$	
	r 1	$1.2 * 10^{-9}$	

5.3 Simulation result and discussion

5.3.1. Pico electric field simulation

As it is revels that the pico pulse signal has a an important role for nanopore formation within the intraorganelle or nucleous membrane so author gives an extra concentration for the design of pico pulse generator.To obtain the perfect pico signal the author smart control FPGA technology and the following schematic diagram is used for simulation of pico pulse ,which is applied to the COMSOL simulated 3D hybride micro chip ,that provide the best option for intraorganelle nanoporation of rigid osteoblast cell.The simulating pico pulse electric field exposer system is describe bellow

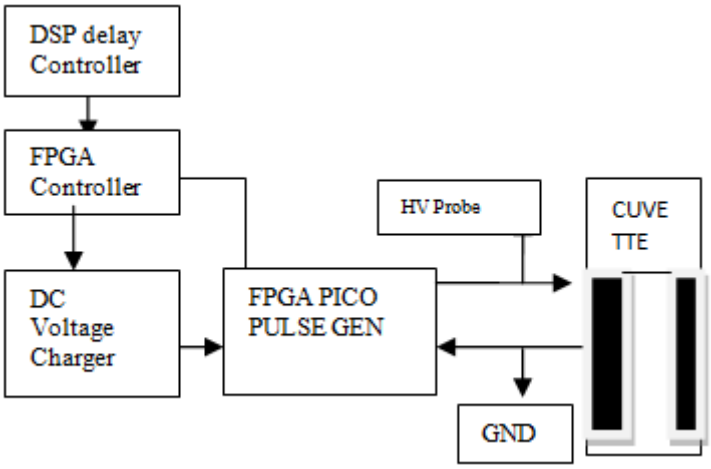

Fig.1: Pico second pulse electric field exposure

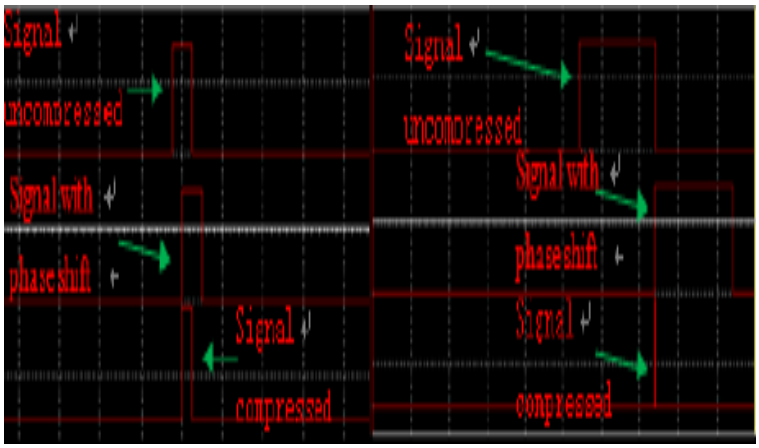

Fig: 2 Simulated output for FPGA control pico pulse generator[19].

The Fig.2 and Fig 3 shows the pico pulse electric field exposure system. The exposure set-up is composed of Pico second pulse generator unit, high voltage probe, DSP delay controller, FPGA controller, and FPGA based pico pulse generator which allow delivering the pulses to the biological medium. Pico pulse is generated by embedded FPGA programmable pulse generator which is shown in figure 1 and it is monitored by FPGA controller and. The pulsing sequence was controlled by a DSP delay controller. The voltage waveform was monitored using a voltage probe. The voltage in the final pulse was slightly reduced due to medium temperature rise. The medium temperature during pulsing was monitored using a fast radiation thermometer (RT) with a response time of 10 ms, sufficiently fast to monitor the overall temperature change during the repetitive pulsing. The applied voltage across the biochip electrodes is measured by a HV probe .The probe has a large frequency bandwidth (6 GHz) and is designed to have the output terminated into the system with a voltage ratio of 1:10. For the measurements, the two conductor pins of the probe are placed in direct contact with the input or output of the gold bismuth electrodes.

5.3.2. Devices Simulation device

It is reported that the effective nanoporation in intraorganelle membrane or nucleous membrane need specific type of pulse, microfloudic chip and suspension media. The author explores in the section 3.5.1. that the optimum value of the applied pulse having the duration of 5 peco second , pulse intensity of 1 volts is

suitable and effective intraorganelle nanoporation of osteoblast cell. it also exposed that intraorganelle nanoporation can easily obtained if the shape of the electrode is triangular or saw tooth which are made by gold bismuth alloy in specified micro channel having the height and width of micro -fluidic channel should be moderate and same & it would be taken as 200 micrometer for successful and effective intraorganelle nanoporation. The resistances of micro channel should be as high as possible for above purpose.With the lelp of above discussion the author optimised the system and design a simulating micro floudic chip on the bases of following specification with in which the osteoblast cell is placed ,to explore the numerical and analytical characterization of intraorganelle nanoporation. The simulation tool is composed of a 100μm thick SU8 micro fluidic channel including thick bi metallic electrode (Bi and Au) electrodes with a typical thickness of 50μm, in which cells suspended in a biological medium are injected. Bi-metallic is chosen as material for the electrodes because of its excellent electrical properties and bio compatibility. The biochip is designed in such a way that the pulsed electric field is absorbed and dissipated mainly in the biological medium placed between the electrodes within which cells to be treated are flowed.

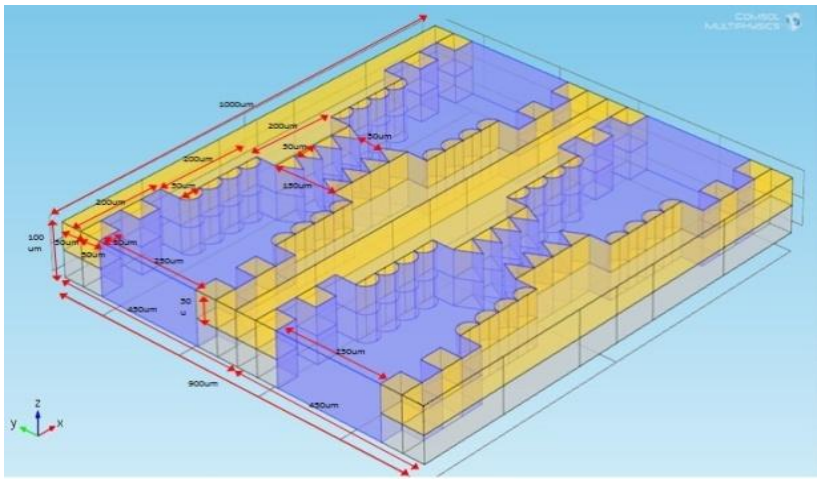

Fig 3: Dimension and design of microelectrode placed within the 3D hybrid micro chip[20].

Fig 3 shows the top view of nanoporative devices. This numerical model satisfy the all simulating and analytical values of intra organelle nanoporation. Length, Height & Width of the microchip are 2300 , 100, 900 μm respectvely.The inlet and outlet path are same i.e 10μm.Within the micro chip a non uniform sidewall having the mixed dimention micro-electrode is places which is made by bismuth and gold.The shape of this electrode is the combination of three different shaped electrodes. The central part consists of triangular, medial part consists semicircle and terminal part attached with square shaped electrode. The length, width and height of the micro electrode are 1000,900,100μm.as the electrode is hybrid in nature so inter electrode gap is non uniform throughout the whole micro channel. The inter electrode distances of central, medial and lateral part are 50,150,250μm respectively**.**

5.3.3. Electric field simulation

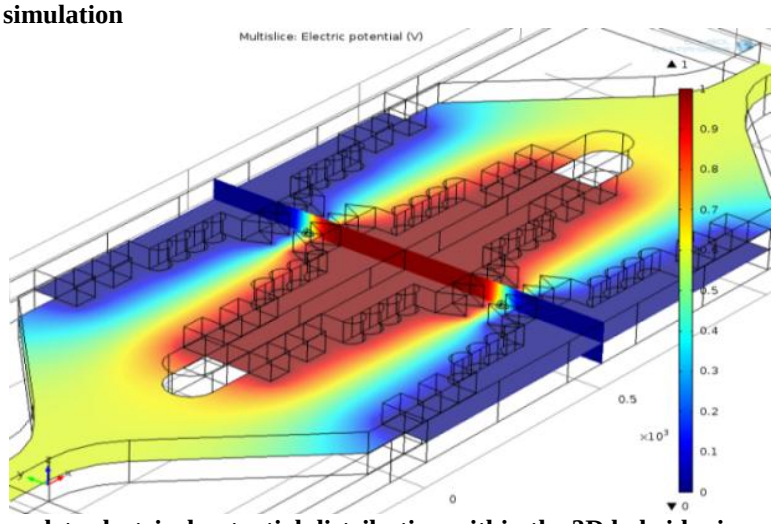

Fig 4: complete electrical potential distribution within the 3D hybrid micro chip[20]

Fig 4 depicts the potential distribution with the proposed 3D micro chip where we find the non uniform distribution of potential and intra cellular organelle also effect by this field .At pole $\theta = 90^{\circ}$ And $\theta = 270^{\circ}$ *the* maximum potential are exposed which is similar as our numerical result. It explore that the centre part (300-600) μm of the chip holds the maximum uniform potential where the nano pores are generated at the intra organelle.. All the observation of COMSOL simulation is as similar as numerical and analytical values of intra organelle nano poration of bi-layer osteoblast cell.

5.3.4. Characterization of nanoporation

5.3.4.1. Energy of pore

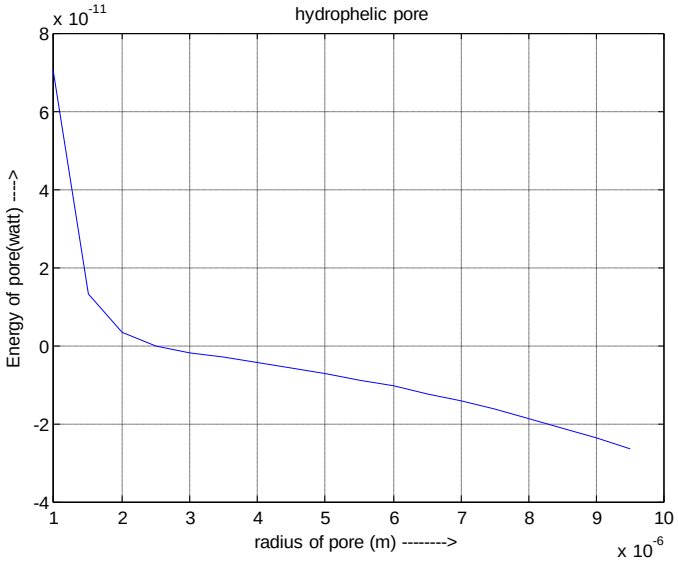

Fig5: Variation of pore energy with respect to azimuthal angle in different pulse intensity

In Fig 5 pore energy is gradually increase with the increase of pore radius. These pores expand and once the radius of them exceed a critical value a rearrangement in phospholipids molecules converts them to stable hydrophilic pores .These graphical representation focus on optimum condition for nanopore formation over the membranes of intraorganelle.

5.3.4.2. Life time of nanopore

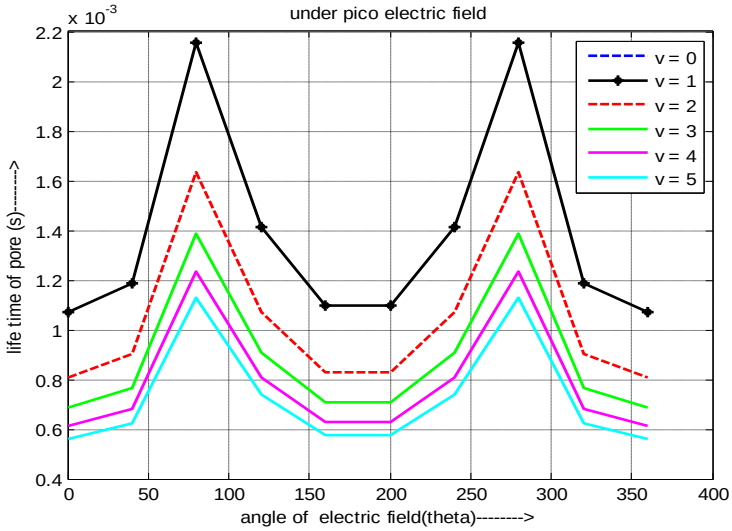

Fig6: Variation of life time of pore with respect to azimuthal angle in different pulse intensity

The Fig 6 explores the variation of intra organelle pore life time along with pole position of applied electric field for intraorganelle It clears that the life time of all the pores are not same. it is nonuniformly distributed for all the pores.It is also shown that pore life time is gradually increase as the angle of applied electric field is increase & maximum is obtain at an angle of $\theta = 90$ and $\theta = 270$ which is independent of

pulse, electrode, micro channel and suspension media specification due to different molecular structure of intraorganelle membranes.

5.3.4.3. Pore radius

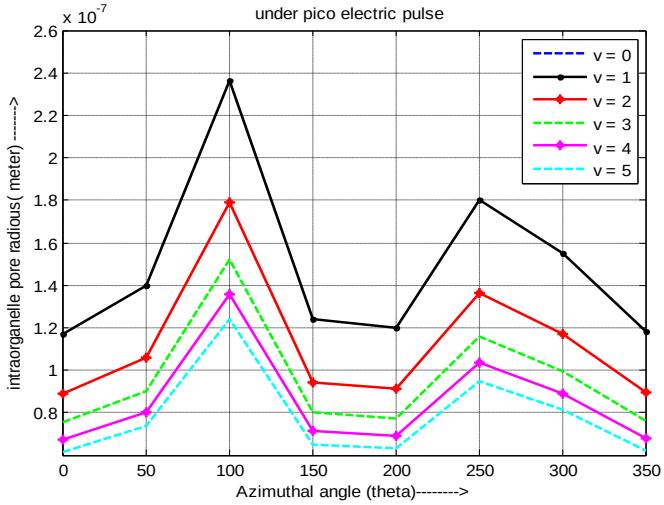

Fig7: Variation of intraorganelle pore radius with respect to azimuthal angle in different pulse intensity

The Fig 7 shows the variation of intra organelle pore radius along with pole position of applied electric field in different pulse intensity uder the influences of pico electric field. It clears that the radius of all the pores are not same it is sinusoidal distributed over the rmembrane.. As the nanopores are created, intra organelle potential increases and the biggest nanopores move just opposite to the equator (E). It is also shown that pore radius is gradually increase as the angle of applied electric field is increase & maximum pore radius is obtain at an angle of $\theta = 100$ which is independent of pulse, electrode, micro channel and suspension media specification due to the higher elasticity of layers.

5.3.4.4. Density of nanopore

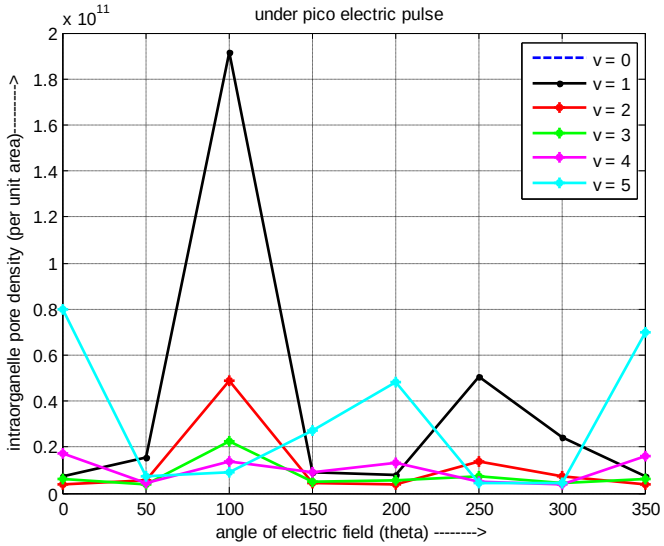

Fig8: Variation of pore density with respect to azimuthal angle in different pulse intensity

The Fig 8 depicts the variation of intra organelle pore density along with pole position of applied electric field.In case of pore density the pulse specifications have an acceptable effect although the maximum pore density locate at the pole ($\theta = 100$ & 250) which is independent of above variations. The effectiveness of pulse specification on inner membrane pore density is same .This information reflects the different permeability of the intraorganele membrane. For drug delivery system it is needed the maximum pore density region which is observed in this graphical analysis.

5.3.4.5.Ion Selectivity of nanopores

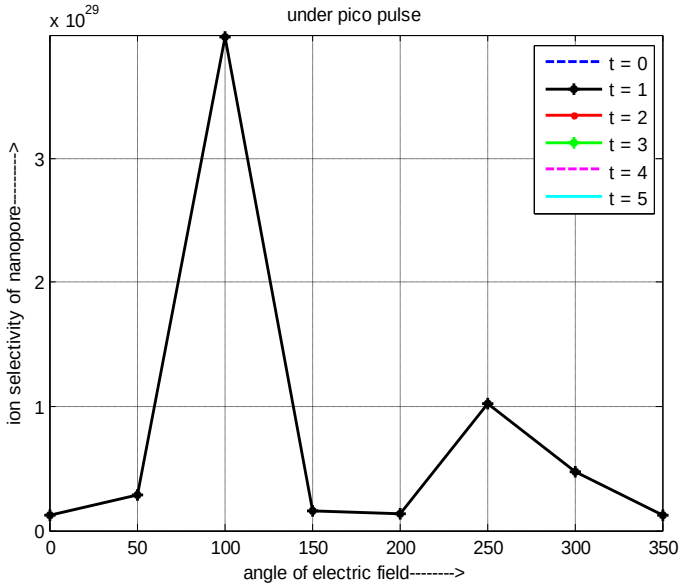

Fig9: Variation of ion selectivity with respect to azimuthal angle in different pulse intensity

The Fig 9 shows the variation of intra organelle ion uptake along with pole position of applied electric field for intraorganelle It is observed that the amount of ion which is uptake by the intra organelle is only occurred at pico scale pulse and its value is not same throughout the whole surface of the layer of the nucleus.. The maximum ion uptake occurs at pole ($\theta = 100 \,\&\, 250$) where the surface tension is minimum and it implies that such part of membrane will inherently have an inverse Kelvin vapor pressure effect, that resulting in increased water condensation.

VI. CONCLUSION

This paper mainly contributes the characterization of intraorganelle nanoporation with in a novel 3D hybrid micro bio device under the influences of pico electric field. The effect of important parameters specially intra cellular pore energy,radius,density, ion uptake and developed pressure on intra organelle is studied and explore the understanding of biological mechanisms, which can occur during nanoporation of bi-layer dense osteoblast cell. In this context we observe that when the micro pulse is applied the cell starts to response but it is unable to penetrate the cell intra organelle membrane where as the expected results will come when we apply the Pico pulse on the cell, a number of nano pores are generated on the intra organelle and chemicals are entered into the cell. On the other hand we also find out that ,in case of pore density the pulse specifications have an acceptable effect although the maximum pore density locate at the pole ($\theta = 100 \,\&\, 250$) which is independent of specification of pulse and micro chip. As we know the pore density of the organelle has great influences on ion uptake and in our study it is explored that the amount of ion which is uptake by the intra organelle is only occurred at Pico scale pulse and its value is not same throughout the whole surface of the layer of the nucleus. It is changed sinusoidal in nature over the surface. The maximum ion uptake occur at pole ($\theta = 100 \,\&\, 250$) where the surface tension is minimum.

We also report the numerical and analytical model of intra organelle nano poration of bi- layer osteoblast cell placed in a 3D hybrid micro biochip under the influences of FPGA based ultra sonic pico second pulse generator. The reported study encourage the specific micro chip multiple dimension microfloudic channel with irregular bi metallic (Bi and Au) side wall electrodes that are designed to deliver a maximum of energy to the biological medium containing multilayer osteoblast cell.The reported nanoporative device aided by a quantitative understanding of the interactions between cells and an external electric field. The micro device is effective in more advantageous conditions than conventionmal systems, small voltages and power consumptions, continuous flow, small sample volume, and negligible heating.

In summary, the present data provide evidence that psPEF introduce the ions with specified microchip possibly through the nucleus mediated pathway.The use of picoseconds pulses not only allows us to enter a new field of field-cell interactions, but it may open the door to a range of non invasive therapeutic applications. This research also explores the non-uniform natures of nanopores and electro mechanical property of outer and inner layer of intraorganelle .

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