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Analysis of Dense Osteoblast Surface Tension in a Micro Chip

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Abstract:- Surface tension of a osteoblast cell in a micro chip is analysed. The effects of electrical pulse, electrode configuration ,micro channel dimension and specific property of suspension media on cell surface tension of osteoblast cell, were investigated. The effective surface tension of outer layer is exposed at micro scale pulse but for inner layer it is in peco scale pulse although pulse interval is micro scale in both cases. We find that the surface tension is non uniformly and sinusoidal distributed over the membrane In every respect position the minimum surface tension is obtained at pole $\theta = 90$ &$\theta = 270$. But their numerical values are varied. Minimum surface tension is exposed near the high density point of high permeable area of the osteoblast cell. we also consider the effect of neighbour cells on membrane surface tension to make the result more important and realistic.

Keywords:- Bi layer-membrane, micro channel, osteoblast cell, membrane surface tension, dense cell.

I. INTRODUCTION

In clinical investigation the dielectric property of osteoblast cell plays an important role. Like other biological cell the dielectric properties of osteoblast cells are very remarkable. They typically display extremely high dielectric constants at low frequencies, falling off in more or less distinct steps as the excitation frequency is increased. Their frequency dependence permits identification and investigation of a number of completely different underlying mechanisms, and hence, dielectric studies of osteo materials have long been important in electrophysiology and biomedical application. In drug delivery system the electroporation of the membrane plays an important role to introduced the drugs into the osteoblast cell. Previously some work have been done considering the single layer structure of osteoblast cell which gives the limiting information about the cell electroporation. We are focusing on that limitation and consider the original bi-layer structure of osteoblast cell.

From the theory of electroporation it is come to know that the sufficient external electric field is caused for the generation of pores on membrane and if the electric field is removed than pores starts to reseal after specific time. This occurrences depends on the membrane elasticity which is the function of surface tension. So the electric field has a great influences on membrane. A numbers of experimental study carried on at the out side of the micro or nano fluidic but all the reported studies on cell membrane surface tension considered the cells in an infinitely large medium. In micro fluidic devices, the cells are usually located in micro channels or micro chambers. The results of the current published studies may not reflect the boundary effects of micro fluid-based electroporative devices. It should be focus out that all the reported studies on cell membrane surface tension considered the cells contained single layer placed in an infinitely large medium and electrodes are placed at bottom or top layer of the micro fluidic devices. As a result the results are influenced by reflect the boundary effects of micro fluid-based electroporative devices & perfect optimization is to be needed. As a result the original and realistic information about the surface tension of outer and inner membrane is limited for multi layer or bi layer cell mainly in rigid cell like bone cell.

We mainly focus on the above limitations and in our study, we investigated the membrane surface tension of a bi-layer osteoblast cell located in a micro channel in between two side wall electrodes. We also find out the effect of neighbour cells on membrane surface tension. The pulse shape was chosen as a square wave, which is widely used in the micro fluidic devices. This electric pulse shape can be easily generated by contracting the cross-sectional area of the micro channels or using a high-voltage pulse generator.

In first part of the present study we developed a 3D micro fluidic model of the system of our current study and the simple electrical equivalent circuit of bi layer osteoblast which is placed in between two electrode at the micro fluidic channel. Cell as a primary model for osteo cell induced by an external rectangular electric pulse. Although we can also use the triangular or saw tooth electrical pulse but the results are not expected ,where as in micro-fluidic channel the rectangular pulse is more convenient to apply. This model is used to investigate the process of measuring the surface tension of that inner and outer layers of osteoblast cell,

including their related numerical analysis. The modeling results are compared to analytical data reported in the literature.

We also studied the effects of the specification of rectangular electric pulse (duration,intensity,interval), electrode geometry(width,gap,shape,material),dimension of micro fluidic channel as well as the nature of suspension media on inner and outer membrane surface tension of osteoblast cell at radio frequency (10GHz) in three different parts. As the cells are in contact together tightly so we also consider the effect of neighbour cells on membrane electroporation to make the result more important and realistic .In remaining part of our study we numerically find out the dependency of surface tension on electroporation of osteoblast cell in micro fluidic channel. The mathematical modelling and governing equations of cell membrane surface tension are introduced in numerical simulation section and Result and Discussion deals with graphical and numerical approach of our current study. All the information of our study gives the new aid to the osteo clinical diagnosis and bone cancer treatment.

II. THEORY

Model description:

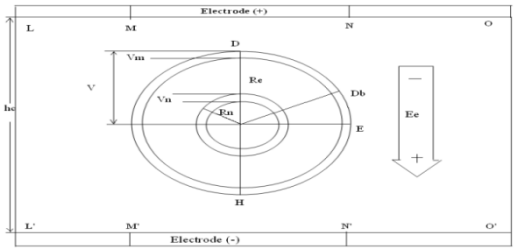


Fig. 1 Top view of the assumed 3D system of the current study. A cell of radius a is assumed in the micro channel of height hc. The micro channel is filled with the conductive medium. The required voltage of the electroporation is applied via the two electrodes of length d located on the wall of the micro channel.

Figure 1 shows a schematic diagram of the system of our current study. A double layer osteoblast cell having radius of $12\mu m$ and thickness of $9nm$ immersed in a micro channel of height $500\mu m$ is considered. The micro channel is filled with a conductive medium (an aqueous solution). The required electric pulse for electroporation is applied to the cell via the two electrodes placed on the side walls of the micro channel. The depolarized , hyperpolarized poles, equator line ,the border between the electroporated and the nonelectroporated regions on the membrane are indicated as usual. In the theory of cell membrane permeabilization, there are two kinds of pores in the cell membrane: hydrophobic and hydrophilic. But in our study, we investigated the change of surface tension to create the hydrophilic nanopores in the outer and inner membrane.

In our analytical and theoretical study, we applied bi-layer model to the system shown in Fig. 1 is to investigate the change of membrane surface tension by applying external electric field on bi-layer osteoblast cell in a micro channel at radio frequency. In this figure Rc & Rn denote the radiuses of the cell cytoplasm and nuclease respectively; $dm\ and\ dn$ denote the thicknesses of the cytoplasm and nucleus membranes respectively; $\gamma nc, \gamma nm, \gamma c, \gamma m\ and\ \gamma o$ describe the conductivities of nucleus membrane, cytoplasm, organelle membrane, cytoplasm, cell membrane and extracellular medium respectively, while $\varepsilon nc, \varepsilon nm, \varepsilon c, \varepsilon m, \varepsilon o$denote their permittivity, θ is the polar angle measured with respect to the direction of the field. For a given θ, the transmembrane potentials are $Vncos\theta$ and $Vmcos\theta$ *are the voltage of inner & outer membrane respectively.*

In our study, we applied this bi-layer model to the cell located in the micro channel to study the effects of various boundary conditions, including the pulse, micro electrode micro channel dimensions and basic property on cell membrane surface tension.

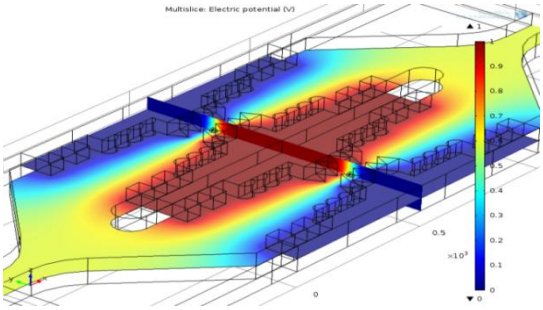


Fig 2 Complete electrical potential distribution within the 3D hybrid micro chip.

According to their electrical parameters, the extracellular medium, cytoplasm and organelle cytoplasm mainly exhibit conductive characteristic, so the capacitive component of them can be neglected . the cell membrane and organelle membrane mainly exhibit dielectric characteristic, so their conductive component can be neglected. Therefore, when studying the transmembrane potential across cell membrane, because inside the cell are mostly the conductive cytoplasm and organelle cytoplasm, the cell membrane can be regarded as a capacitance *C*m with the intra membranous organelles (cytoplasm, nucleolus, etc.) equivalent to a conductance *G*. Similarly, when studying the transmembrane potential across organelle membrane, the organelle membrane can be regarded as a capacitance Cn with organelle cytoplasm equivalent to a conductance *G*n. According to the above analysis, the equivalent complex domain *RC* circuit for studying cellular electroporation is presented and shown in Figure 3.With the help of complex domain RC model, we can explain the various effect of pulse, electrode ,micro fluidic channel & suspension media on bi-layer osteoblast cell membrane surface tension .All the theoretical part which is referred from different well known research papers are explanation as follows.

2.1 Effect of rectangular Pulse specification on outer & inner surface Tension:

C.Yao et al gave the following Schematic diagram of double -shelled spherical cell in suspension, which is used for theoretical explanation of outer and inner membrane potential of a biological cell**.**

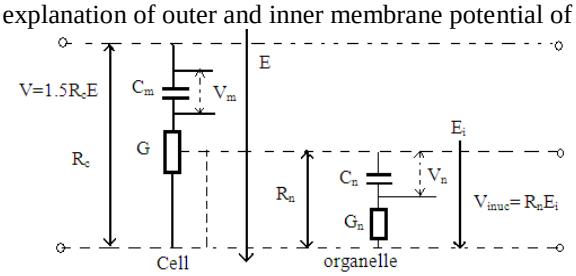


Fig: 3 Schematic of double -shelled spherical

According to the transfer functions defined by C.Yao the inner and outer membranes to a given rectangular pulse electric field *E*(*s*) can be obtained

$Vn(t) = L^{-1}[Hn(S).E\ (S)]$ ------ (1)

& $Vm(t) = L^{-1}[Hm(S).E\ (S)]$ (2)

Where,

$Hm(\ s\ ) = \frac{Vm\,(s)\cos\,\theta}{E\ (\ s\ )} = \frac{1.5Rc\cos\,\theta}{\tau cll\ S+1}$

& $Hn(S) = \frac{Vn(S)\cos\,\theta}{E(S)} = \frac{1.5\ \tau cell\ \ Rn\cos\,\theta}{(\tau nuc\ \ S+1)+(\tau cell\ \ S+1)}$

After simplification of equation (1)& (2) we get the outer membrane potential $(Vm(t))\ and$ inner membrane potential $Vn(t)$ are as follows

$Vm(t) = 1.5\ Rc\ E\ (t)\left[-e^{\frac{t}{\tau cell}} - 1(t-\tau) + e^{\frac{t-\tau}{\tau cell\ .}}\ 1(t-\tau)\right]\cos\theta - -$ (3)

$Vn(t) = \frac{1.5\ \tau cell\ \ Rnuc\ \ E\ (t)}{\tau cell\ -\tau nuc}\left[(e^{\frac{t}{\tau cell}} - e^{\frac{t}{\tau nuc}}) - (e^{\frac{t-\tau}{\tau cell}} - e^{\frac{t-\tau}{\tau nuc}}).1.\,(t-\tau)\right]\cos\theta$ ---------- (4)

As we know that$E(t) = \ v/d$, where v=applied voltage & d= distances in between two electrode. We replaces $E(t) = \ v/d$ in equation (3) & (4) and get outer membrane potential $(Vm(t))\ i$s

$Vm(t) = 1.5\ Rc\ \left(\frac{v}{d}\right)\left[-e^{\frac{t}{\tau cell}} - 1(t-\tau) + e^{\frac{t-\tau}{\tau cell\ .}}\ 1(t-\tau)\right]\cos\theta - -$ (5)

& inner membrane is

$Vn(t) = \frac{1.5\ \tau cell\ \ Rnuc\ \ (v/d)}{\tau cell\ -\tau nuc}\left[(e^{\frac{t}{\tau cell}} - e^{\frac{t}{\tau nuc}}) - (e^{\frac{t-\tau}{\tau cell}} - e^{\frac{t-\tau}{\tau nuc}}).1.\,(t-\tau)\right]\cos\theta$ ---- -(5)

According to the frequency response characteristic the inner and outer membrane potential are expressed as follows,

$Vn(t) = L^{-1}[Hn(S).E\ (S)]$ ---------- (6) &

$Vm(t) = L^{-1}[Hm(S).E\ (S)]$---------- (7)

After simplification of equation (6) & (7) we get the outer membrane potential $(Vm(t))\ and$ innermembrane potential $Vn(t)$ are as follows

$Vm(t) = 1.5\ Rc\ E\ (t)\left[-e^{\frac{t}{\tau cell}} - 1(t-\tau) + e^{\frac{t-\tau}{\tau cell\ .}}\ 1(t-\tau)\right]\cos\theta$---------- (8)

$Vn(t) = \frac{1.5\ \tau cell\ \ Rnuc\ \ E\ (t)}{\tau cell\ -\tau nuc}\left[(e^{\frac{t}{\tau cell}} - e^{\frac{t}{\tau nuc}}) - (e^{\frac{t-\tau}{\tau cell}} - e^{\frac{t-\tau}{\tau nuc}})(t-\tau)\right]\cos\theta$— ------- - (9)

As we know that E (t) = v/d, where v=applied voltage & d= distances in between two electrode. We replaces $E(t) = \ v/d$ in equation (14) & (15) and get outer membrane potential $(Vm(t))\ i$s

$Vm(t) = 1.5\ Rc\ (v/d)\left[-e^{\frac{t}{\tau cell}} - 1(t-\tau) + e^{\frac{t-\tau}{\tau cell\ .}}\ 1(t-\tau)\right]\cos\theta$----(10)

& inner membrane is

$$Vn(t) = \frac{1.5\ \tau cell\ \ Rnuc\ \ (v/d)}{\tau cell - \tau nuc}\left[\left(e^{\frac{t}{\tau cell}} - e^{\frac{t}{\tau nuc}}\right) - \left(e^{\frac{t-\tau}{\tau cell}} - e^{\frac{t-\tau}{\tau nuc}}\right).1.(t-\tau)\right]\cos\theta \text{------ (11)}$$

As we know that E (t) = v/d, where v=applied voltage & d= distances in between two electrode. We replaces $E(t) = v/d$ in equation (14) & (15) and get outer membrane potential ($Vm(t)$) is

$$Vm(t) = 1.5\ Rc\ (v/d)\left[-e^{\frac{t}{\tau cell}} - 1(t-\tau) + e^{\frac{t-\tau}{\tau cell\ .}}\ 1(t-\tau)\right]\cos\theta \text{----(16)}$$

& inner membrane is

$$Vn(t) = \frac{1.5\ \tau cell\ \ Rnuc\ \ (v/d)}{\tau cell - \tau nuc}\left[\left(e^{\frac{t}{\tau cell}} - e^{\frac{t}{\tau nuc}}\right) - \left(e^{\frac{t-\tau}{\tau cell}} - e^{\frac{t-\tau}{\tau nuc}}\right).1.(t-\tau)\right]\cos\theta \text{------ (17)}$$

As we know when the electric field is applied on the biological cell its molecular & chemical property are changes which may cause the change outer or inner membrane potential and it controls the formation of pores on the membrane. If the external electric field is separated from the system the pores starts to reseal after some times. All this phenomenon is happened due to the change in elasticity as well as surface tension of the membrane.In this regards C. Chen , S.W. Smye, M.P. Robinson,J.A. Evans exposed in their review article that the alternative approach to the rupture of a bi layer membrane, but one which is still based on the notion of membrane instability but without reference to the creation of pores, considers the electro hydrodynamic instability of a planar layer of non-conducting liquid separated by two charged conducting liquids . This assumes that such a system is analogous to a bi layer membrane between two conducting fluids. The conditions for bi layer membrane stability are with respect to two kinds of perturbations of the two planar membrane interfaces: symmetric (the interfaces oscillate in phase with each other) and asymmetric (the interfaces oscillate in anti-phase). Since the BLM is moderately elastic, the symmetric wave, which is a squeezing mode, is permitted, whereas the asymmetric wave, which is a stretching mode, will be damped due to the large elasticity of membrane compression and the constant volume constraint that applies to the membrane.

According to this model, the membrane is stable with respect to long-wave perturbations if

$$U^2\ \varepsilon m = \frac{\Gamma h0}{2} \text{----------(18)}$$

Where, U is the membrane potential ,εm is the permittivity of the membrane, Γ = surface tension of the membrane and $h0$ is the thickness of the membrane.

The theory of electroporation reveals that if the electric field is applied on the membrane different membrane is developed at the outer and inner membrane.

So the surface tension also changes in both layers, which is mathematically expressed as

Surface tension of outer membrane= $\Gamma out = \frac{2*\varepsilon m*Vm(t)}{hm}$---------- (18A)

Where,$\varepsilon m, Vm(t), hm\ are$ permittivity, thickness, potential of the outer membrane ,If we put the value of $Vm(t)$From the equation (16) in equation (18A) we get,

$$\Gamma out = \frac{2*\varepsilon m*1.5\ Rc\ (v/d)\left[-e^{\frac{t}{\tau cell}} - 1(t-\tau) + e^{\frac{t-\tau}{\tau cell\ .}}\ 1(t-\tau)\right]\cos\theta}{hm} \text{--- (19)}$$

For inner membrane the surface tension is calculated as

$$\Gamma in = \frac{2*\varepsilon n*Vn(t)}{hn} \text{---------- (20)}$$

Where,$\varepsilon n, Vn(t), hn\ are$ permittivity, thickness, potential of the inner membrane, than if we put the value of $Vn(t)$from the equation (17) in equation (20) we get,

$$\Gamma in = \frac{2*\varepsilon n*\frac{1.5\ \tau cell\ \ Rnuc\ \ (v/d)}{\tau cell - \tau nuc}\left[\left(e^{\frac{t}{\tau cell}} - e^{\frac{t}{\tau nuc}}\right) - \left(e^{\frac{t-\tau}{\tau cell}} - e^{\frac{t-\tau}{\tau nuc}}\right).1.(t-\tau)\right]}{hn} \text{-------- (21)}$$

Finally the equations (19) and (21) are the main effective equation which shows the the numerical dependency of pulse duration , intensity & interval on outer and inner membrane surface tension.

2.2 Effect of electrode specification on outer & inner surface tension:

From the knowledge of di-electro spectroscope of cell membrane it is find out that

$$\frac{1}{2\gamma 0} + \frac{1}{\gamma c} = Ri * \emptyset * Gf * \left({}^{4}/_{9}\right) \text{----------(22)}$$

Where $Ri = resistances\ of\ cytoplasm, \emptyset = volume\ fraction,$

$$Gf = \left(\frac{widt\ of\ electrode\ * lengt\ of electrode}{inter\ electrode\ gap}\right)$$

Replaces $\frac{1}{2\gamma 0} + \frac{1}{\gamma c} = Ri * \emptyset * Gf * \left({}^{4}/_{9}\right)$ in equation (4) & (11) we obtained

$$\tau cell = Ri * \emptyset * Gf * \left({}^{4}/_{9}\right) * \frac{\varepsilon i\ \varepsilon m}{dm} Rc \text{---------- (23)}$$

& $\tau nuc = Ri * \emptyset * Gf * \left({}^{4}/_{9}\right) * \frac{\varepsilon i\ \varepsilon nm}{dn} Rn$ ---------- (24)

On the other hand we know from the theory of electrochemistry the value of produced voltage at metal electrode$(v) is$ expressed as

$v = -\left(\frac{K*T}{z*e}\right) log(Cmet)$---------- (25)

Where K= Boltz man constant, T= absolute temperature, z= valancy of electrode material & e=charge of an electrode,$Cmet$= ionic concentration of electrode material.

As we replaced $\tau cell = Ri * \emptyset * Gf * \left({}^{4}/_{9}\right) * \frac{\varepsilon i\ \varepsilon m}{dm} Rc$ and $v = -\left(\frac{K*T}{z*e}\right) log(Cmet)$ in equation (19) find out the dependency of electrode width, length ,material and geometry on outer membrane surface tension and if we put the numeric value of $\tau cell = Ri * \emptyset * Gf * \left({}^{4}/_{9}\right) * \frac{\varepsilon i\ \varepsilon m}{dm} Rc$ and $v = -\left(\frac{K*T}{z*e}\right) log(Cmet)$ along with $\tau nuc = Ri * \emptyset * Gf * \left({}^{4}/_{9}\right) * \frac{\varepsilon i\ \varepsilon nm}{dn} Rn$ in equation (21),then we trace the inner membrane surface tension can be controlled by the above electrode specifications.

2.3 Effect of micro fluidic channel specification on outer & inner surface tension:

As we know that if radius of cell is r and channel height H than relative resistances of cell placed in the micro channel is consider as $R = r/2H$ .If we replaces $R = r/2H$ in equation (19) & (21) then the effect of channel height on outer and inner membrane surface tension.

From the basic concept of electro kinetic theory of micro fluidic devices, the channel resistances Rch is numerically defined as $Rch = \frac{2*Kz}{\sigma m * d}$,where $Kz = \frac{2k(k^2)}{k(1-k^2)}$ and k=2 for biological ell,σm= conductivity of medium and $d = dn = dm$=$thickness\ of\ membrane$.After simplification it is turned as

$d = (2 * kz)/(Rch * \sigma m)$---------- (26)

On the other hand width of the channel is defined as

$Wc = [V0 * (Rch/Rch + 2 * Raqu)] * [\frac{1}{2*k} * k^2]$---------- (27)

Where,$V0$ =innetial velocity of fluid.&$Raqu_{=}$ resistances of aqua medium. By replacing value of equation (26) & (27) in equation (19) & (21) then we can explore complete numerical equation which shows the effect of channel width and resistances on outer and inner membrane surface tension.

2.4 Effect of suspension media specification on outer & inner surface tension & pore density:

As the medium conductivity is a function of membrane potential which is the main component of surface tension and. So medium conductivity has also a large influences on outer and inner surface tension of the bi-layer single and dense osteoblast cell.

2.5 Effect of neighbours cell in osteoblast electroporation:

Maxwell derived his mixture equation for the effective conductivity σ of a dilute suspension based on a simple example. According to Maxwell the effective conductivity is as given bellow

$$\frac{\sigma e - \sigma}{2\sigma e + \sigma} = f\ \frac{\sigma e - \sigma p}{2\sigma e + \sigma p} \quad - - - - - (27)$$

Where σe is medium conductivity and σp is individual cell conductivity.f is the volume fraction of cell. According to Bruggeman formula

$$\frac{\sigma - \sigma p}{\sigma e - \sigma p}\left(\frac{\sigma e}{\sigma}\right)^{1/3} = 1 - f \quad - - - - - (28)$$

The above equation however, is again an approximation that, only for certain conditions, represents a better approximation from Maxwell equation. Furthermore, for a special case of heterogeneous medium with spherical particles arranged in an SCC lattice, Rayleigh obtained the following result .

$$\sigma = \sigma e\left(1 + \frac{3f}{\frac{\sigma p + 2\sigma e}{\sigma p - \sigma e} - f - a\frac{\sigma p - \sigma e}{\sigma p + 0.75\sigma e} f^{10/3}}\right) - (29)$$

Where a is a numerical factor which, according to Rayleigh, is 1.65. Later, Tobias and Meredith, following the same procedure, obtained the same formula with the corrected value of the numerical factor a being 0.523 instead of 1.65 .The effective conductivity σ placed in equation (4) and (11) in the places of γc and γnc respectively.This numeric changes also implemented in equations (19) and (21) at $\tau cell\ and\ \tau nuc$,which gives the relationship of surface tension for dense cell.

III. NUMERICAL SIMULATION

In this study, above equations are solved numerically to find the electric potential developed in outer and inner membrane to investigate the creation of nano-pores on the cell membrane. The MATLAB-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. All the numerical values used in simulation are taken from Table-I.

IV. USED PARAMETER

Table I Values for constants and parameters used in the simulations(constant parameters are collected from different ref).

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 μm
	Cell membrane thickness(d)	0.006µm
	Nuclear radius(rn)	6 μm
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	$0.31 * 10^{-9}$

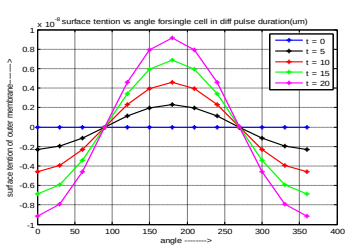
V. RESULTS AND DISCUSSION:

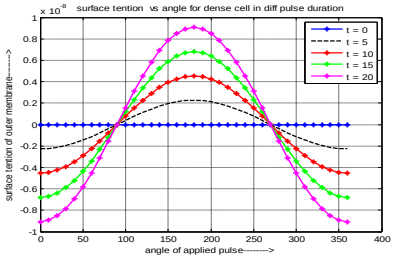
The quantitative information used in the simulations is provided in Table 1. A cell of radius a (12 μm) is considered in the microchannel of height (500μm). The necessary electric field (1v) was applied by the two electrodes of width (50 μm) located on the walls of the micro channel. The electric pulse span was on the order of microseconds to peco second operated at radio frequency (10 GHz).

5.1 Evaluation of pulse:

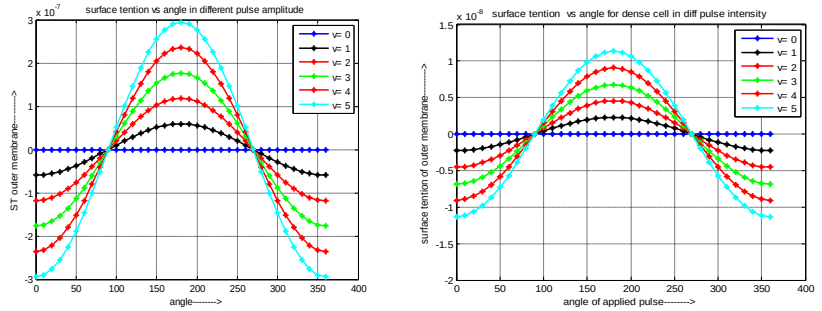
5.1.1 Outer membrane:

(a)





(b)



(c)

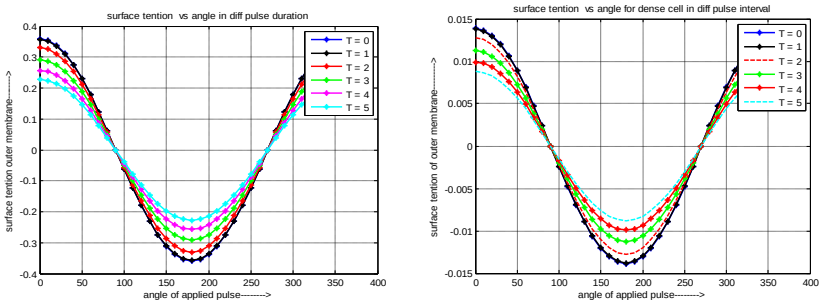
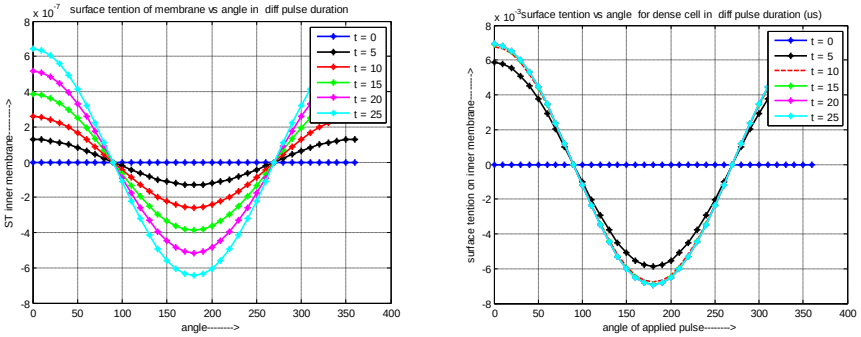


Fig. 4 Pulse evaluation of the outer membrane surface tension of a single and dense osteoblast cell located in the micro channel of height (500µm), cell of radius a (12 µm), electrodes of width (50µm). a Variation of pulse duration. b . Variation of pulse intensity , c. Variation of pulse interval**.**

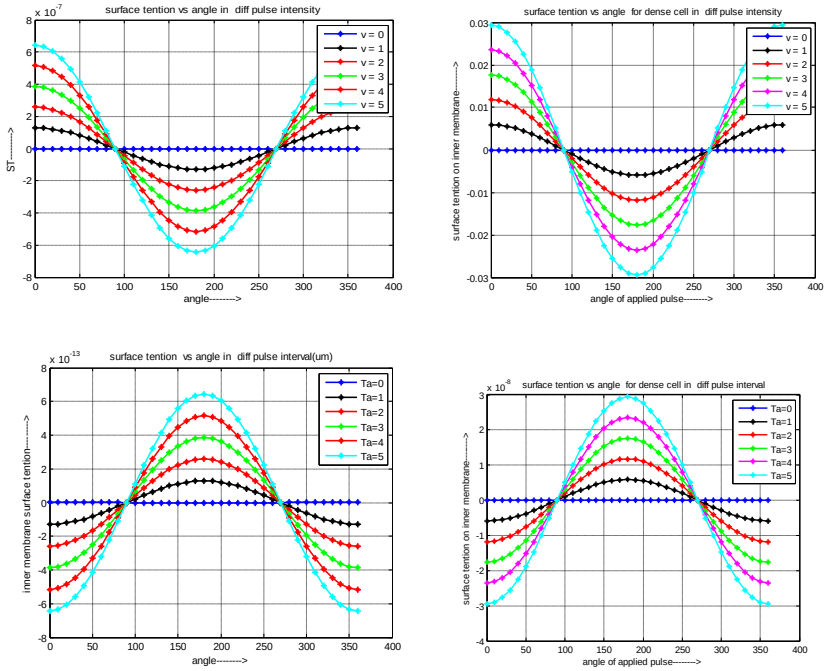
Figure 4 depicts the variation of outer membrane surface tension for single and dense osteoblast cell. In all cases the surface tension is varied in cosine form change in pole in between electric field and radius vector.In every conditions the zero surface tension is obtained at pole ($\theta = 90\ \&\ 270$) which assigned the minimum pressure and maximum pore radius at that pole, although value of surface tension is directly proportional with pulse duration and intensity but inversely proportional for pulse interval. As we know the critical voltage of a membrane potential is control by surface tension so if the surface tension is changed than the critical voltage is also non uniformly distributed over the membrane which assigned the different radius of the pore which are generated in the membrane for unique external electric field. Practically for efficient electroporation we need minimum surface tension which may cause the maximum pore density on the membrane. In 4.(a) this is for single and dense cell where positive surface tension exposed from pole $\theta = 91\ to\ 269$ and value is directly varied with pulse width and it should be minimum for efficient electroporations. We also find out the negative surface tension region in the remaining part of the membrane except$\theta = 0\ and\ 270$,which implies that such part of membrane will inherently have an inverse Kelvin vapor pressure effect, that resulting in increased water condensation. For single or dense cell the nature of the curve is same but in dense cell value of surface tension is lower as compare to single cell which indicates the rigidity of the previous cell. In 4.(b),(c) we vary the pulse intensity and duration, where nature of curves and effective pole positions are same but their numerical results are different which is explored as previous.

5.1.2 Inner membrane:

(a)



(b)



(c)

Fig. 5.Pulse evaluation of the inner membrane surface tension of a single and dense osteoblast cell located in the micro channel of height (500µm), cell of radius a (12 µm), electrodes of width (50µm). a Variation of pulse duration. b . Variation of pulse intensity , c. Variation of pulse interval.

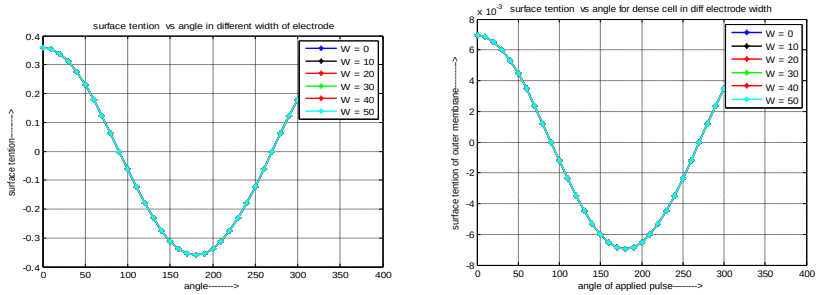
Figure 5 shows the variation of inner membrane surface tension for single and dense osteoblast cell. Here also in the cases the zero surface tension (ST)is obtained at pole ($\theta = 90\ \&\ 270$) which assigned the minimum pressure and maximum pore radius at that pole, although value of surface tension is same as outer membrane but nature of the curve is reverse which reflects the opposite di-electric property of membrane. In fig 5 (a) pulse duration is varied and it is found that no response in micro scale although only at peco scale we find some response but satisfied output is exposed at above that scale. This information supports the idea of window effect of the membrane. In this figure it is also revels that in inner membrane the surface tension is inversely proportional with pulse duration and negative surface tension is pointed out in between pole $\theta = 91\ to\ 269$ where increase the water condensation. The remaining part is under positive surface tension except $\theta = 90\ \&\ 270$ and it should be as low as possible for efficient electroporation. For dense cell characteristic curve is same as single cell but the value of ST is lower at same variation of parameters due to the rigidity of cell. In fig 5(b) pulse intensity is varied and it is observed that nature of the curve is as same as previous and ST is decreased when pulse intensity is increased .Numerical activity of dense cell in different pulse intensity gives the lower value of ST as same as previous due to Maxwell's effect. Fig 5(c) shows the change of surface tension of both osteoblast cell in different pulse interval and it gives the separate negative and positive surface tension region as compare to 5(a) and 5(b) although the surface tension is directly proportional with the change of pulse interval.

From the above discussion we can optimised the pulse (duration, intensity and interval) for efficient electroporation in micro-channel. Optimised value of the pulse duration, intensity and interval are 10 μs (outer membrane)and$10ps$(innermembrane),$1v, 10\mu s$ rspectively.

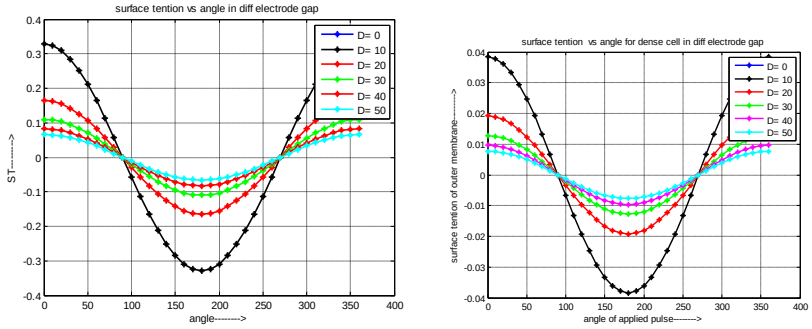
5.2 Electrode specification:

5.2.1 Outer membrane:

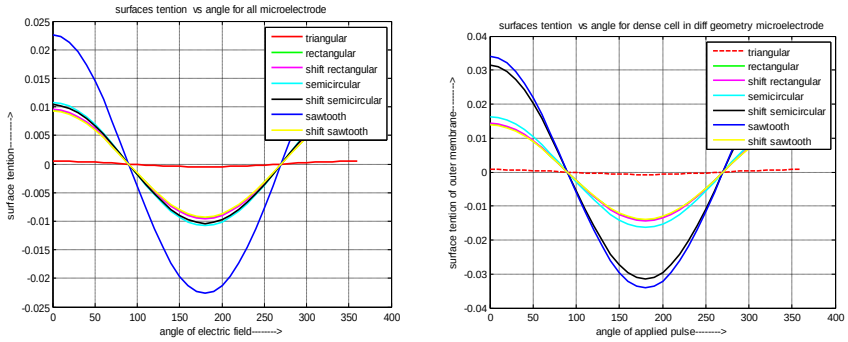
(a)



(b)



(c)



(d)

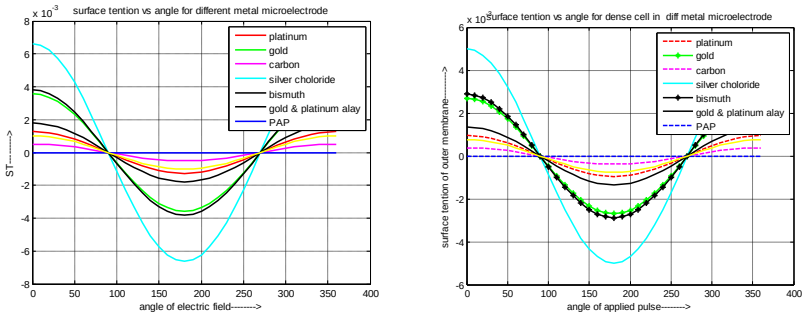


Fig. 6.Electrode evaluation of the outer membrane surface tension of a single and dense osteoblast cell located in the micro channel of height (500µm), cell of radius a (12 µm), electrodes of width (50µm). a Variation of electrode width. b . Inter electrode distances,(c) geometry of electrode , (d) Material of electrode.

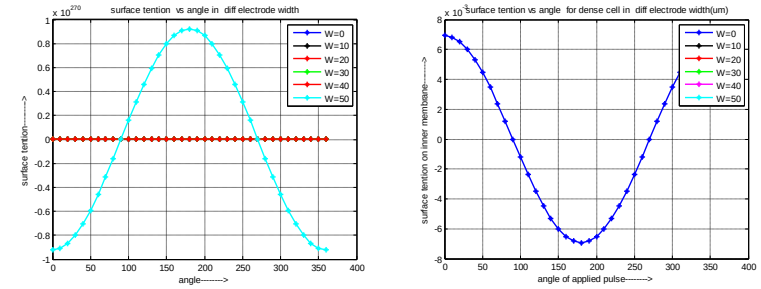
After optimization of pulse we are looking for optimal electrode for efficient electroporations of bi-layer osteoblast cell. To encourage this task we are taken different width, inter electrode gap, shape and material of electrode ,whose analytical and graphical representation are shown in fig(5) and (6). figure 6, explores the variation of outer membrane surface tension and pore density for single osteoblast cell. In fig 5(a) we change the width of electrode but we find that the variation of outer membrane surface tension because the electrodes are placed at the side wall of the micro channel and they have no effect on created electric field. But in fig 5(b),5(c),5(d) we varies inter electrode spaces, geometry & constrictive material of electrode respectively, where we have found the dependence of those electrode parameters on surface tension , although in all cases zero surface tension is obtained at the pole $\theta = 90$ & 270 which assigned maximum pore density region and location of biggest nanopores which is independent of microelectrode specifications for single or dense osteoblast cell in micro channel at radio frequency.

From fig 5(b) we find that the surface tension of outer membrane inversely proportional with inter electrode gap because this property of membrane is directly related to induced membrane potential which is effected by electrode gap which is justified by simple electrostatic equation but along with this we also observed the negative value of surface tension during the pole $\theta = 91\ to\ 260\ over$ the outer layer in all type of electrode geometry. Which is due to the residual pressure present in the outer membrane and it results increase the water condensation in the membrane. The remaining part is under positive surface tension except $\theta = 90$ & 270 and it should be as low as possible for efficient electroporation

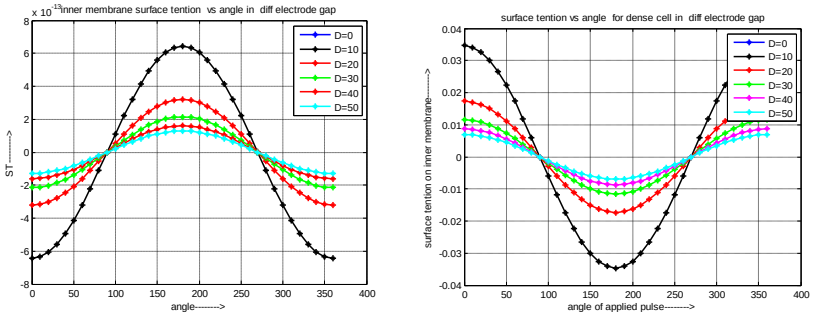
In fig 5(c) & 5(d) shows that the minimum surface tension is obtained if the geometry of the microelectrode is triangular which is made by carbon and bismuth and biggest density nano pore is also formed when microelectrode is triangular in shape which is made by carbon and bismuth . As melting point of bismuth is higher than carbon so practically bismuth is widely used electrode material for osteoblast cell electro-proration in micro channel at radio frequency. In every cases we observed lower value of surface tension is obtained for dense cell in similar type of electrode. Because when a number of cell are closely attached their effective conductivity and thickness is changed which effects the membrane potential as well as surface tension of the membrane.

5.2.2 Inner membrane:

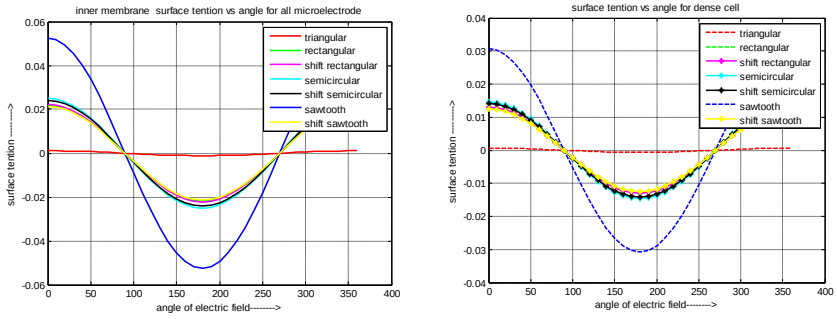
(a)



b)



(c)



(d)

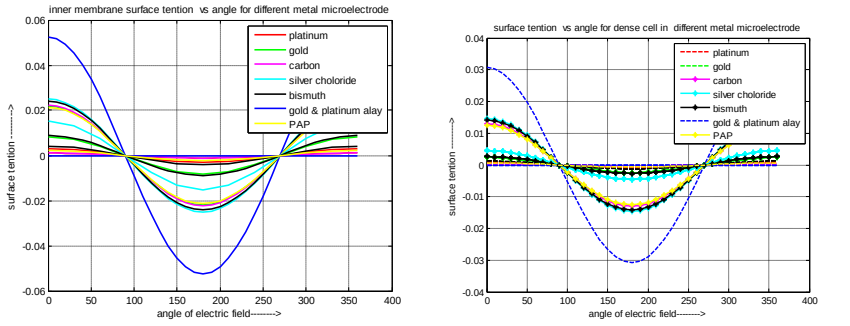


Fig6.Electrode evaluation of the inner membrane surface tension of a bi layer osteoblast cell located in the micro channel of height (500µm), cell of radius a (12 µm), electrodes of width (50µm). a Variation of electrode width. b . Inter electrode distances,(c) geometry of electrode , (d) Material of electrode.

Figure 6, explores the variation of inner membrane surface tension and pore density for single osteoblast cell. Nature of the curves is reverse as compared to outer membrane which reflects the different dielectric property of the membrane. In fig 6(a) we change the width of electrode but we find that the variation of inner membrane surface tension is independent of it because the electrode is placed at the sidewall and it 's width does not have any effect on the variation on change in electric field. But in fig 5(b),5(c),5(d) we varies inter electrode spaces, geometry & constrictive material of electrode respectively, where we have found the dependence of those electrode parameters on surface tension , although in all cases zero surface tension is obtained at the pole $\theta = 90 \,\&\, 270$. Practically null surface tention is a virtual concept so we have make it as smsll as possible. Hence It signifies that the location of biggest nano-pores and highly dense nanopore region which is independent of microelectrode specifications for bi layer osteoblast cell.

From fig 5(b) we find that the surface tension of inner membrane inversely proportional with inter electrode gap because this property of membrane is directly related to induced membrane potential which is effected by electrode gap which is justified by simple electrostatic equation but along with this we also observed the negative value of surface tension during the pole $\theta = 0 \; to \; 89 \;\; \& \theta = 271 to \; 360 \; in$ the inner layer in all type of electrode geometry. Which is caused by the residual pressure present in the inner membrane and it indicates high water condensation area.

In fig 5(c) & 5(d) shows that the minimum surface tension is obtained if the geometry of the microelectrode is triangular which is made by carbon and bismuth and biggest density nano pore is also formed when microelectrode is triangular in shape which is made by carbon and bismuth . As melting point of bismuth is higher than carbon so practically bismuth is widely used electrode material for osteoblast cell electro-proration in micro channel.

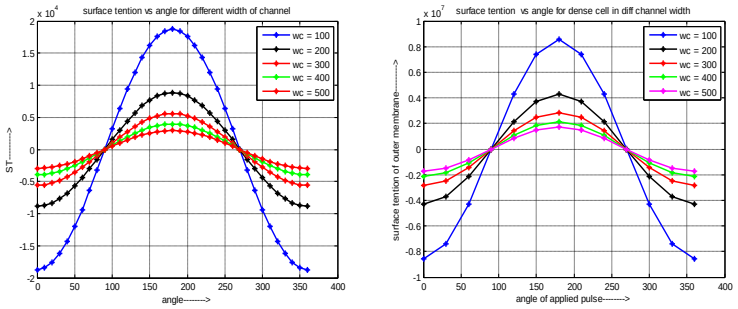
On the other hand when we study on inner membrane of a dense cell, it is found that the numerical values are lower but analytical graphs are as same as single cell which reflects rigidity of cell and the effect of neighbour cell on osteoblast cell in micro fluidic channel.

we can optimised the electrode width, gap, shape and material for efficient electroporation in micro-channel. Optimised value of the electrode width, gap are same i.e. 50 μm,and shape should be triangular in shape which is made by bismuth.

5.3 Channel specification :

5.3.1 Outer membrane:

(a)



(b)

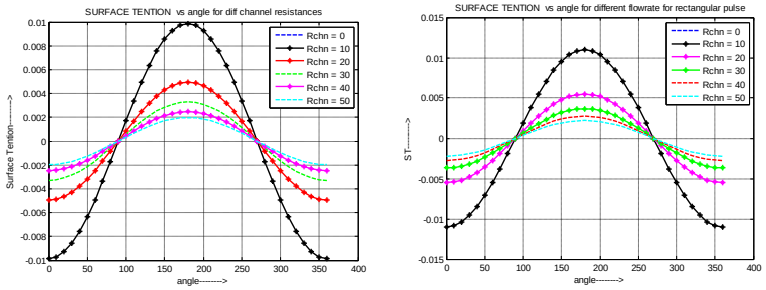
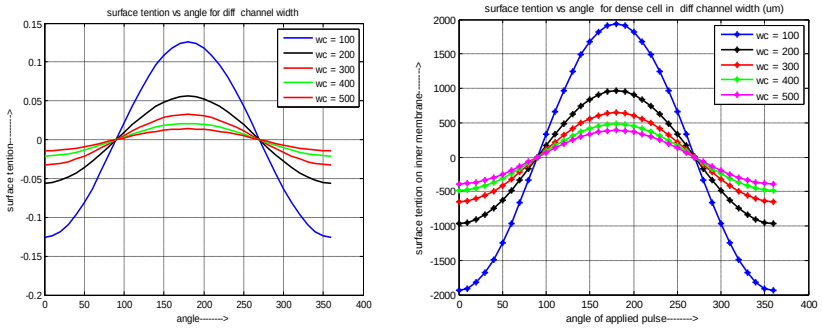


Fig7:Microfloudic channel evaluation of the outer membrane surface tension of a bi layer osteoblast cell applied pulse duration & intensity are 5µs & 1v respectly, cell of radius a (12 µm), electrodes of width (50µm) and inter electrode distances (50µm). (a). channel width,(b) channel resistances.

Figure 7 illustrate the effects of micro channel width and resistances on outer membrane surface tension which changes with the angle of applied electric field for bi-layer osteoblast cell. In all cases the zero surface tension is obtained at the pole $\theta = 90$ & $\theta = 270$,which is independent of micro fluidic channel specifications. Fig 7(a), 7(b), explore that the surface tension both are inversely proportional with channel width & resistances. With the variation of channel width and resistances the location of positive and negative surface tension region are fixed which display an important massage for drug delivery system.

5.3.2 **Inner membrane:**

(a)



(b)

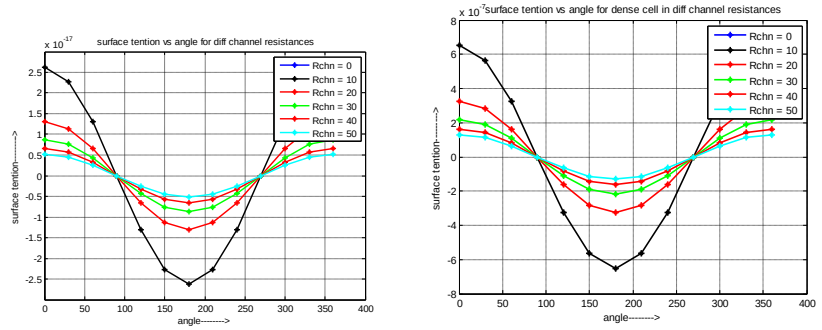


Fig 8: Micro floudic channel evaluation of the inner membrane surface tension of bi layer osteoblast cell applied pulse duration & intensity are 5μs & $1v\ respectly$, cell of radius a (12 μm), electrodes of width (50μm) and inter electrode distances (50μm).(a) channel width,(b) channel resistances.

Figure 8(a),(b), shows the effects of micro channel width and resistances on surface tension of inner membrane for osteoblast cell. The surface tension is widely effected by channel width and resistances. It is inversely related with channel width & resistances. This variation happened due to the placement of electrode at the sidewall of micro fluidic devices. We also find out the negative portion of surface tension because of residual pressure on membrane and it is the cause of maximum water condensation on the membrane.

5.4 Evaluation of suspension of media :

5.4.1 Outer membrane:

(a)

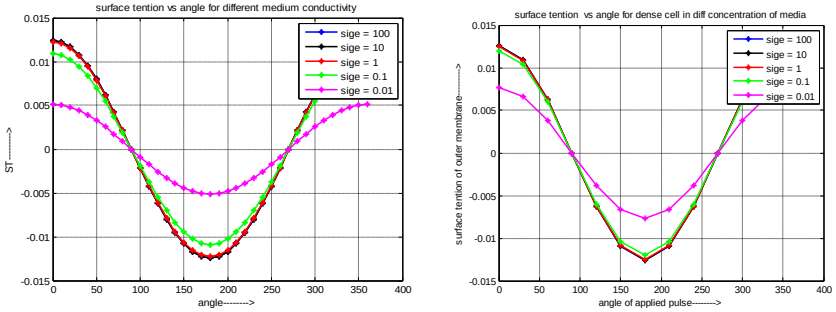


Fig9: Micro fluidic channel evaluation of the outer membrane surface tension of osteoblast cell applied pulse duration & intensity are 5μs & $1v\ respectly$, cell of radius a (12 μm), electrodes of width (50μm) and inter electrode distances (50μm) in different medium conductivity for single cell and dense cell.

Figures 9, illustrate the effects of conductivity of suspensions media on outer membrane surface tension for bi layer osteoblast cell. In all cases the zero surface tension is exposed at pole $\theta = 90$ & $\theta = 270$

which his not practically possible. But we make the surface tension as small as possible for efficient electroporation. We also find out negative surface tension region in between $\theta = 91$ & $\theta = 269$ which is due to residual pressure on membrane Although the value of surface tension is inversely related with the conductivity of media which reflects the important characterization of osteoblast cell. This value is independent of medium conductivity of the suspension media..

5.4.2 Inner membrane:

(a)

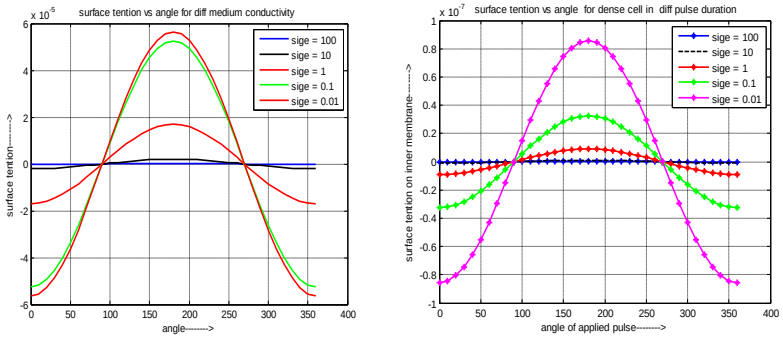


Fig 10: Micro fluidic channel evaluation of the inner membrane surface tension of a osteoblast cell applied pulse duration & intensity are 5μs & $1v\ respectly$, cell of radius a (12 μm), electrodes of width (50μm)and inter electrode distances(50μm) in different medium conductivity for single cell and dense cell.

Figures 9, illustrate the effects of conductivity of suspensions media on inner membrane surface tension for bi layer osteoblast cell. In all cases the zero surface tension is exposed at pole $\theta = 90$ & $\theta = 270$ which gives the idea of probable location biggest nanopores on the inner membrane and high pore density regions. We also find out negative surface tension region in before $\theta = 90$ & after $\theta = 270$ which is due to residual pressure on membrane and maximum water condensing in the membrane.. Although the value of surface tension is directly related with the conductivity of media which reflects the important characterization of osteoblast cell. The nature of the curves is opposite with compare to outer membrane which exposed the reverse dielectric property of the membrane. In case of dense cell the surface tension is low in compare to single cell in same variation of medium channel conductivity which is placed in the micro channel.

VI. CONCLUSION

In this study, membrane permeabilization of a bi-layer osteoblast cell in a micro channel was investigated. The previous studies on cell electroporation consider cells in an infinite domain, which does not reflect the finite boundary effects of micro channel walls on the membrane permeabilization process. In our study, we found that the pulse specification(duration .intensity, interval),electrodes specification (width, gap, geometry, material),height & width of micro channels and as well as the flow rate & conductivity of suspension media have a great influence on bi-layer single and dense osteo cell electroporation. In this regard we obtained the following conclusions.

In all cases the zero surface tension is exposed at pole $\theta = 90$ & $\theta = 270$ which is independent of pulse, electrode, micro fluidic devices and suspension media.

We also find out that a specific region of membrane holds positive surface tension which should be as low as possible for efficient electroporation and the remaining part of the membrane is evoke as the negative surface tension region in which implies that such part of membrane will inherently have an inverse Kelvin vapor pressure effect, that resulting in increased water condensation.

The numerical and analytical value of surface tension in outer and inner membrane are opposite to each another which implies the different dielectric property of the membrane.

In micro fluidic channel the membrane permeabilization can be performed with very low electrical pulse intensity (1-2v).To obtained the efficient electroporation in bi-layer osteoblast cell the optimum value of pulse duration and interval are same (5 micro meter) for outer membrane but different (5 peco meter & 5 micrometer) for inner membrane.

If the electric pulse intensity as well as the duration are constant than the electrode gap, geometry & material of electrode have great influenced on osteoblast cell membrane surface tension in micro-channel at radio frequency range.

From the above discussion we can optimised the pulse (duration, intensity and interval) for efficient electroporation in micro-channel. Optimised value of the pulse duration, intensity and interval are 10 μs (outer membrane)and$10ps$(innermembrane),$1v, 10\mu s$ rspectively.

After optimization of pulse we focus on the specification of electrode and for unique electroporation the value of the electrode width, gap should be same i,e 50 μm,and shape should be triangular in shape which is made by bismuth.

The surface tension is widely effected by channel width and resistances. It is inversely related with channel width & resistances. This variation happened due to the placement of electrode at the sidewall of micro fluidic devices.

The value of surface tension is directly related with the conductivity of media which reflects the important characterization of osteoblast cell.

In case of dense cell the surface tension is low in compare to single cell in same variation of pulse, electrode, micro fluidic channel and suspension media due their rigidity and change in effective permittivity of osteoblast cell in micro channel at radio frequency.

All these are related to the dielectric properties of the osteoblast cell which can also aid in understanding the basic physiological difference between normal and cancerous bone cells on a molecular level and finally all the information given in this article might provide a new light on drug delivery system and cancer treatment in bone cell. We are in process and more work has to be done to explore these possibilities.

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