

Introduction to Biomaterials

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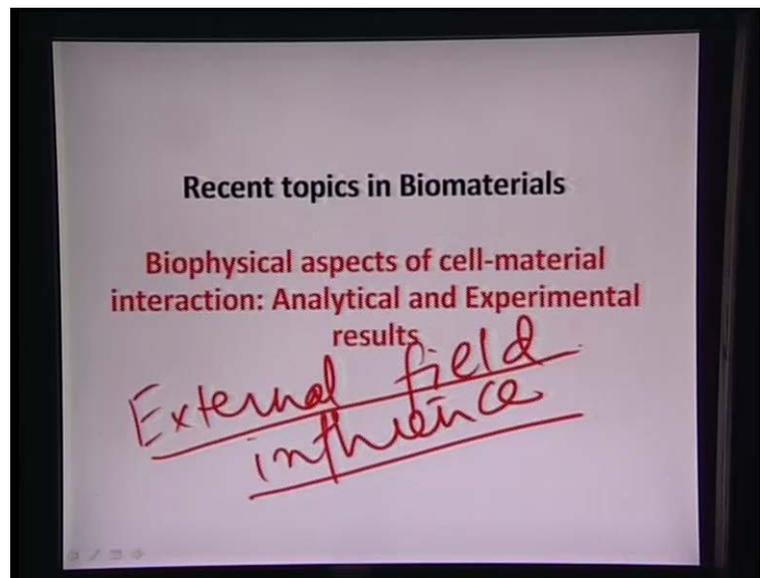
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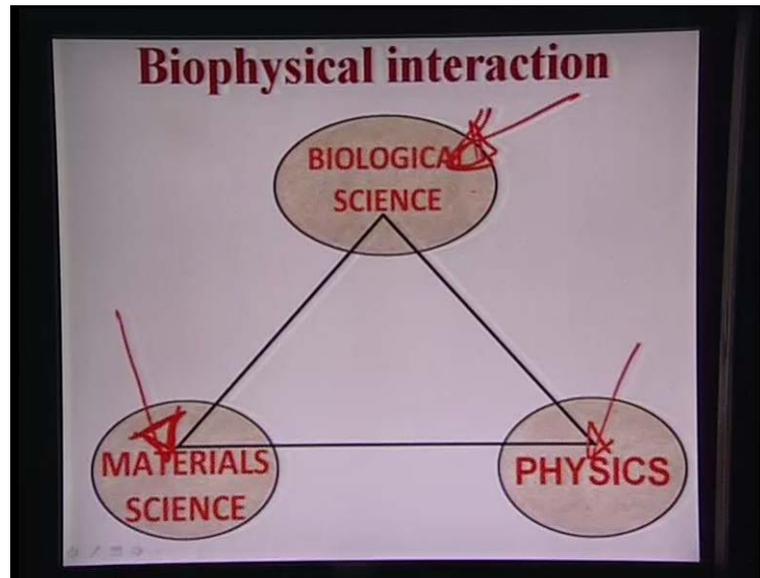
External field and cell – Material Interaction

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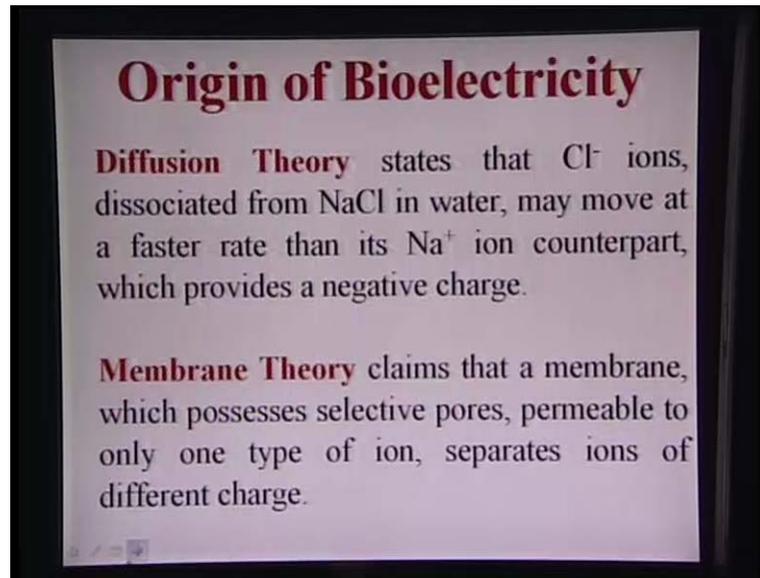
In today's lecture, as part of the recent topics in Biomaterials, I will be discussing the biophysical aspects of cell material interaction - analytical and experimental results. So, essentially, I will be dealing with that influence of external field on the cell material interaction and first few, external field influence. So, in first few slides, I will be mostly dealing with, how the electric field will essentially influence the cell material interaction, and then, it will be followed by series of experimental results, where I will be showing that, how the external magnetic field can potentially induce antibacterial, or antimicrobial property in materials.

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So, this biophysical interaction is essentially, it is at the crossroad of biological science, material science and physics. So, certain physical aspects, like electricity and magnetism, those kind of aspects is important to understand this biophysical interaction, and we will be also dealing with the material science aspects. Essentially, material science aspect means, like all the materials, they will not exhibit significant influence in the presence of electric field, unless they have some physio-electric properties, or because, if they are perfectly insulated, it may be possible that, they do not show, or they do not exhibit good biophysical interaction. And of course, biological science is involved in this kind of biophysical interaction.

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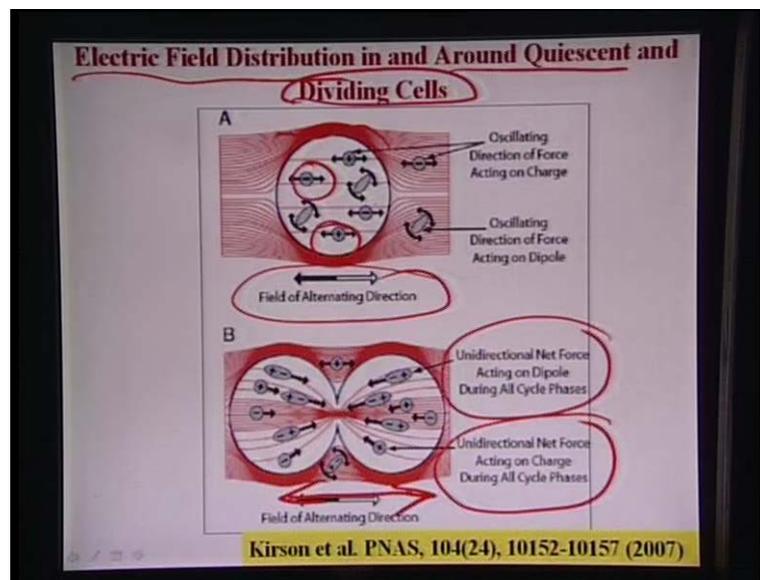
Now, origin of bioelectricity, essentially, if there are two school of theories; one school of theory is the diffusion theory, which states that, chlorine ions dissociated from Sodium chloride in water, may move at a faster rate than its sodium plus counterpart and therefore, it provides a negative charge. What it means that, in the chlorine ions, which is also available in large quantity in blood and other body plasma, as well as sodium plus ions; so, this diffusion theory states that, if chlorine ions, they move faster than sodium plus ions and in other words, if anions moves faster than cations, then essentially, that will lead to a net negative charge and that is what the concept of this diffusion theory; and, that is kind of one school of theory, which supports bioelectricity. The other school of theory is that membrane theory, which claims that a membrane, which possesses, which possesses a selective pores, permeable to one type of ions, separate ion of different charge. Now, here, one must remember that, cell as such, it is enclosed by a lipid bylayer and then, each organelle, for example, mitochondria, or even the nucleus, they will also enclosed by a bylayer.

So, essentially, this bylayer, they are not like a completely tight membrane. They also contains pores and this pores are of different sizes. So, essentially, membrane theory states that, if this porous membranes, or the pores in the membranes, if they selectively allow the movement of cations, for example, or movement of anions, for example, then what will happen? One side of the membrane, there will be net positive charge and other side of the membrane, there will be net negative charge. So, because of this charge

separation, that will also lead to the bioelectricity, net bioelectricity effect. So, there are two theories, one theory is, depends on the kinetic aspect of the movement of the cations and anions, and other theory is more related to the membrane theory, which is related to the transport of anions or cations through the membranes; and these membranes are, these basically, the cell membrane, or nuclear membrane, etcetera, etcetera.

Now, this, other things is that, now, there are some literature reports, which also states that, that, that, this electric field, they are distributed in and around quiescent and dividing cells; so, that means, when cells are undergoing mitosis, or cytokinesis, and mitosis, around that time, if, for example, if you look at here, now, if there is a field of alternating deductions, like AC current is flowing in this directions, then, what will happen? There will, in that AC current, there will be some positive ions and negative ions also will be moving, moving in particular direction. So, it shows that, unidirectional net force acting on the dipole and universal net force acting on the charges, like which are either positive charge, or negative charge.

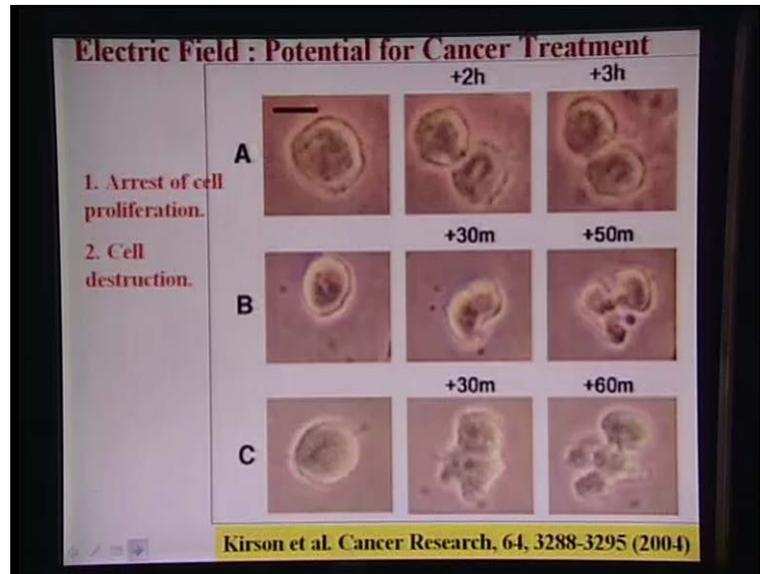
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So, if the situation will arise such that, that the net charge, positive charges, or net negative charges, will be higher at one side, compared to the dipoles, then, that will also lead to bioelectricity and in the top most part, it is shown that, you know that, if the positive or negative, cations or anions, if their movement is also influenced by the AC

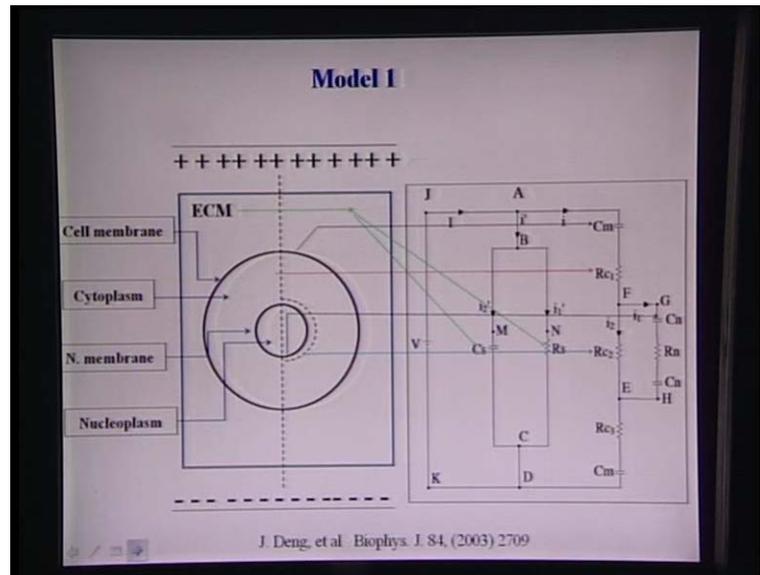
current directions, then, that will also lead to a non-uniform electric field distribution around the cells.

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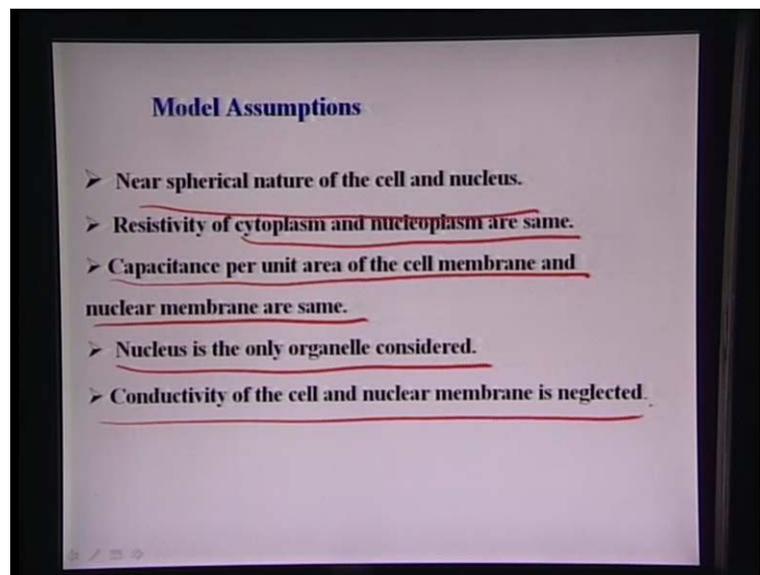
Now, this electric field influence also depends on the intensity, electric field intensity, as well as exposure time. Now, for the same type of cells, people have shown that, if you apply a certain magnitude of electric field, then, it will assist in faster cell division process, but if you apply the different magnitude of the electric field, for example, after 30 minutes and after 50 minutes, thereafter 30 minutes, up to 60 minutes, like different types of cells, same electric field magnitude, that can cause programmed cell death or cell apoptosis. So, what is the major message is that, that electric field influence on cell division, or electric field influence on cellular functionality is largely dependent on the cell type. So, it will have some influence on the particular cell type, for example, if it will aid a cell proliferation, or it will cause cell division; at the same time, that same electric field magnitude, or the same magnitude electric field can cause complete cell destruction, or cell apoptosis; and this kind of cell apoptosis will be very good for the cancer cell treatment, cancer cell treatment, because, cancer is essentially, uncontrolled way of cell division and by any chance that, you know, this cancerous cells that can be killed; that means, this uncontrolled cell growth can be reduced, and therefore, that will give benefit to the cancer stud, for the cancer patients.

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Now, what we have tried to do recently, like, we tried to understand that, how an individual biological cell, if it is placed in an electric field, electric field means, between positive electrode and negative electrode, how it will respond to the electric field. Now, to solve this problem analytically, we have made some assumptions.

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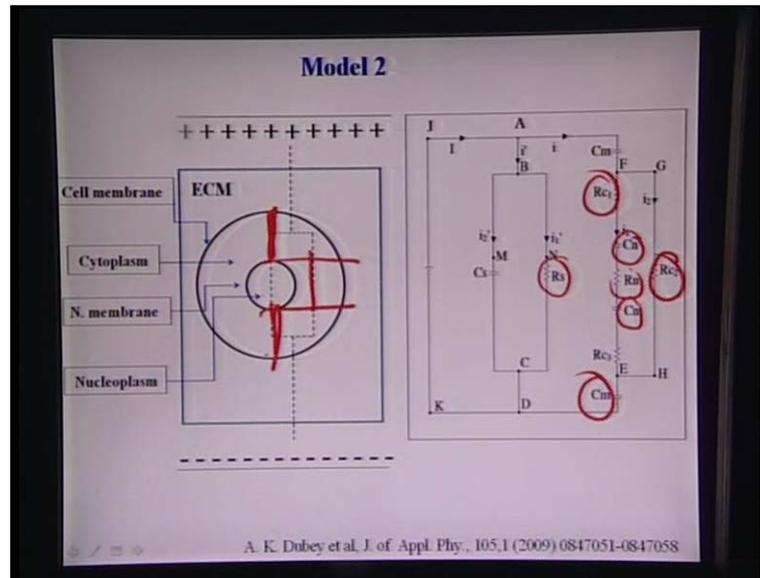
First assumption is that, this is a near spherical nature of the cell and the nucleus. And then, second one is that, resistivity of the cytoplasm and nucleoplasm are considered to be the same; so, there is no difference in the resistivity. Then, we have considered the

cytoplasm and nucleoplasm as a resistor; cell membrane and nuclear membrane as a capacitor. And then, third assumption is, the capacitance per unit area of cell membrane and nuclear membrane are the same. Then, fourth problem is that, that although it is widely known, that a living biological cell, they contains number of organelles, for example, golgi body, mitochondria, ribosome and so on, for the sake of simplicity of the model, we have only considered the presence of nucleus; not any other organelles.

Fifth one is, the conductivity of the cell and nuclear membrane is neglected. The last assumption that was made in this analytical problem is that, conductivity of the cell and nuclear membrane is neglected. So, based on this assumptions, we have attempted to solve this problem, to understand that, how a biological cell would respond to the electric field. Now, here in this **view** graph, what is shown that, this is a cell membrane and which is a capacitor; this is a cytoplasm, which is a resistor; this is a nuclear membrane, which is a capacitor; this is a nucleoplasm, which is a resistor. Now, when a current will pass through this dotted line, which is shown, this current can bypass the nucleoplasm and then, still it can go through the cytoplasm and go to the E C M. So, from one end of the cytoplasm, it can go to the other end of the cytoplasm. Finally, it will go to the E C M, exo-cellular matrix, or the current can pass directly through the cytoplasm and nucleoplasm, and go to the other part of the cytoplasm, and finally, get out of the cell, to go to the extra cellular matrix.

Now, whether the current will pass directly through the E C M, not entering the cells, that is case one. Case two, whether current will pass only through cytoplasm; case three, the current will pass through cytoplasm, and then, go to nucleoplasm, and go to **(())** in case three. For all this three cases, one can construct the resistance capacitance **(())** and then, one can apply the Kirchhoff's law, different nodes here and try to solve this, **this** equations, to find out that, what will be the time constant for this **(())** circle.

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Now, we have solved this analytical problem and here, you can see that, there are resistance and capacitance parameters. So, C_n is the capacitance of the nuclear membrane and this is the, R_n is the resistance of the nucleoplasm; R_{C2} is the resistance of the cytoplasm, your C_m is the resistance of the, capacitance of the cell membrane; R_{C1} and R_{C3} is the spatial value, with the spatial dependent of cytoplasm resistance. So, this R_{C1} , let us say, this is R_{C2} resistance here and this is the R_{C3} resistance.

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Calculated Time Constant

• **Model 1**

$$\tau_1, \tau_2 = \frac{-C_m C_n [R_1 + R_2 (R_3 + R_4 + R_5) - R_6^2]}{-R_2 (C_m + C_n) + R_1 C_m + (R_2 + R_3) C_n \pm \sqrt{[R_2 (C_m - C_n) + R_1 C_m - (R_2 + R_3) C_n]^2 + 4 R_2^2 C_m C_n}}$$

• **Model 2**

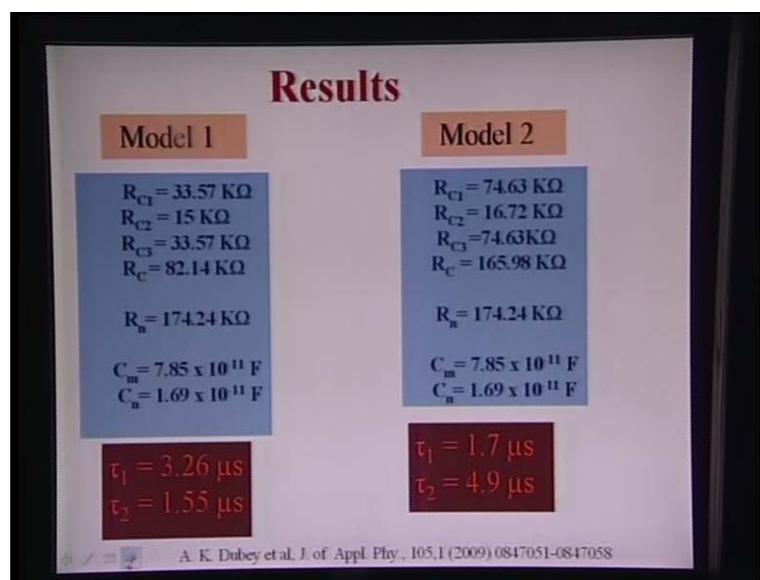
$$\tau_1, \tau_2 = \frac{-C_m C_n R_3 (R_4 + R_5 + R_6)}{-R_2 (2C_m + C_n) + (R_1 + R_3) C_m \pm \sqrt{[R_2 (2C_m - C_n) + (R_1 + R_3) C_m]^2 + 4 R_2^2 C_m C_n}}$$

• The values of τ_1 and τ_2 are always real and positive.

A. K. Dubey et al., J. of Appl. Phys., 105,1 (2009) 0847051-0847058

Now, if you solve that multiple equations, then, you finally, get the analytical equations, which has some form like $Ax^2 + Bx + C = 0$. So, Ax has 2 roots and this Ax is actually τ_1 and τ_2 ; that is the interaction constant, or the time constant value for this biological electrical (()) of the biological cell that is (()). And then, what you can see here that, if you know all the capacitance value and all the resistance values in this equation, then, you can essentially find out the value of the τ_1 and τ_2 , and depending on whether you get this (()) model, or own model, you can get this τ_1 and τ_2 value; these, these values are from the text book.

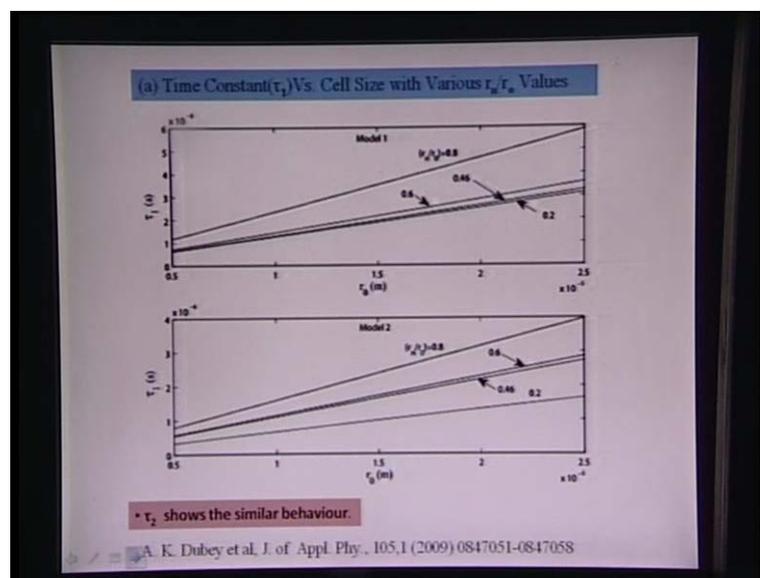
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Now, from a standard textbook, we have taken this value for a biological cell and we have found out the resistance of the cytoplasm, and you can find out that R is equal to $\rho \cdot l / a$; and from that, you can find out the, what is the resistance R_{C1} , R_{C2} , R_{C3} . It is of the order of kilo ohm and then, resistance of the nucleoplasm is 174, that has been also taken from a standard text book. Now, capacitance of the membrane and capacitance of nucleoplasm, nuclear membrane; that is also 10^{-11} Farad and from that, what we have found out that, this τ_1 and τ_2 values is around 1.53, 3.26 micro seconds; so, that means, it is very low fraction of seconds. And here, what you can see that, same τ_1 and τ_2 values, is 1.7, 4.9 microsecond. So, what it essentially tells you that, this τ value is less than 10 micro seconds. So, it is roughly, τ is somewhere at around 1 to 5 microsecond; so, that means, that if a individual biological cell, if it is placed in an electric field, then it will respond to that electric field

within less than 10 micro seconds, which is a very small time scale. And, however, such scenario will be different, if multiple such cells are placed in the E C M and then, they are considered between this positive and negative electrode and then, similar way, if you consider that current will flow through all this individual cells, then, that case, this time constant value may not be less than 10 micro second. We have not solved this kind of problem, but there are opportunities, that one can trying to solve this multiples cells in the E C M and trying to adopt the same kind of strategy to find, or similar strategy to find out that, what will be the tau value for the capital n number of cells, when they are placed in an extra cellular matrix.

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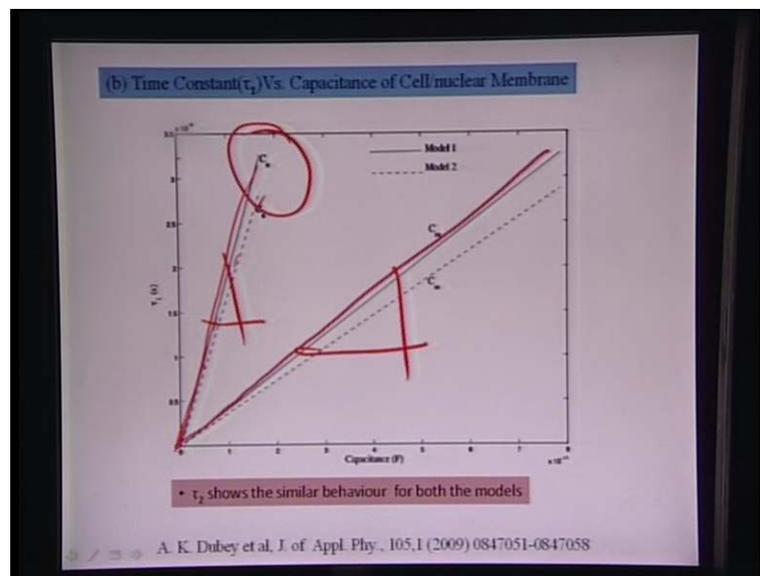


Now, second approach, what we have done, that is, that how this tau value changes with the cell size. Now, how tau value changes with the cell size, that means, that R value. Now, if you remember that, I have already taught you earlier that, there are four cellular adaptation processes. Cellular adaptation means, when a cell is placed, or when a cell experiences their particular external damage kind of scenario, coming to that influence of the cell size on the interaction time constant. Now, if you remember it correctly that, there are several ways that a biological cell can adopt through a changing external environment, what is known as cellular adaptation process. Now, there are four types of cellular adaptation process. One is atrophy; one is hypertrophy. So, depending on whether cells will decrease its size, or cells will increase its size, essentially, that term, the biological term that is used to describe such situation is known as atrophy and

hypertrophy. Now, and the cells can do it, because, cells have very much elastic-like properties and therefore, cells can increase its size, or decrease in size. So, therefore, the changing cell size, should in principle, have an influence on the time interaction time constant, what we have evaluated following, following this typical electrical analog of single biological cell.

Now, this slide tells you that, if there are τ_1 and τ_2 , this two values that we can get from this equations, earlier equations, and then, if you vary the R_n by R_0 value, R_n is the radius of the nucleus and R_0 is the radius of the cell. Now, if you vary this ratio of the radius of the nucleus and radius of the cell with, let us say, from 0.2 to 0.8, now, depending on the value of this, or independent of this value of this ratio R_n by R_0 , that time constant will monotonically increase with increase in the R_0 value. And, what you have seen here, that is in the 10 to the power minus 5 meter, so, close to micro meter range, and in this range of the increasing in the size of the cells, and depending on the variable, that is R_n by R_0 ratio, the time constant essentially increases, with increase in the overall cell size.

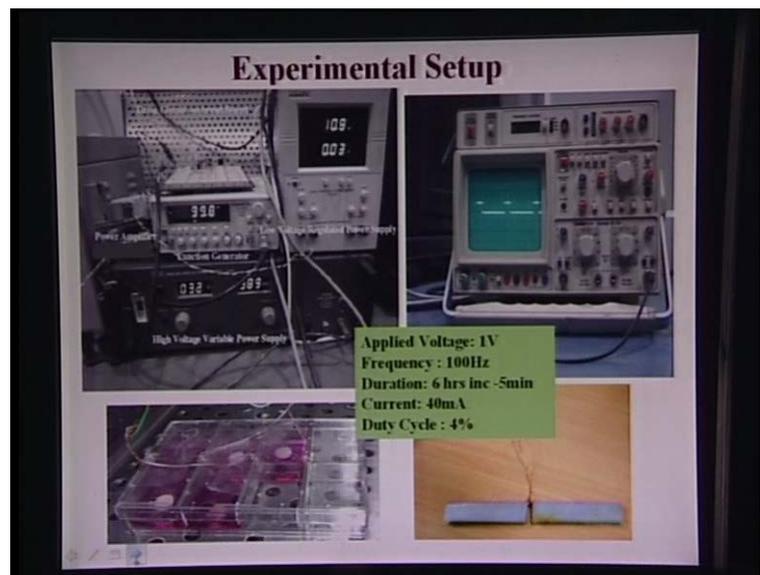
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The other important variable in this analysis, is the capacitance. Now, if you change the capacitance of the nuclear, sorry, nuclear membrane, or if you change the capacitance of the cell membrane, in both the cases, or in model one, let us say, what you see, there is a steep increase in the time constant with the capacitance values. So, whether the C_n

increases, or the C_m increases, τ is bound to increase; but what you see here that, influence of C_n on the increase in τ is much more drastic, or much more rapid, because, the slope is much higher compared to the slope of that τ versus C_n . So, accordingly, what you can say here that, C_n , the capacitance of the nuclear membrane will have much more marked effect on the time constants of this biological cell and what we believe that, that nuclear membrane traditionally is a bilayer structure and there is a called nuclear pore complex and through this nuclear pore complex will assist much more flow of the ions, or according to the membrane theory leads to the more by electricity. Now, increase in the capacitance of the nuclear membrane essentially means, you are thinking of that spacing between the two membranes, that which constitute that nuclear membrane and the structure and we believe that, possibly that causes the change in the interaction time constant.

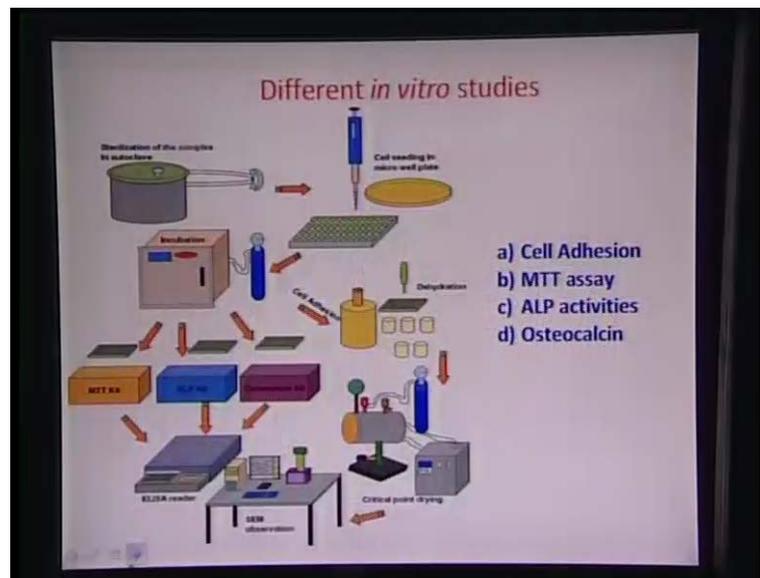
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Now, what we have done, we have designed and fabricated a pass electric field generator and with which, one can apply different magnitude of the voltage and in the first case, we have applied that 1 voltage and then, we have, where we have frequency, we have fixed at 100 Hertz. Now, duration, what we have done that, we have first incubated the cells in contact with materials for 12 hours; then, we give the shock for 5 minutes and then, we let the cells incubate for another 6 hours, then, give the shock for 5 minutes, electrical shock and then we let the cells to incubate or proliferate on the materials has for another 6 hours. So, total 24 hour cycle and in between, there are 10 minutes of the

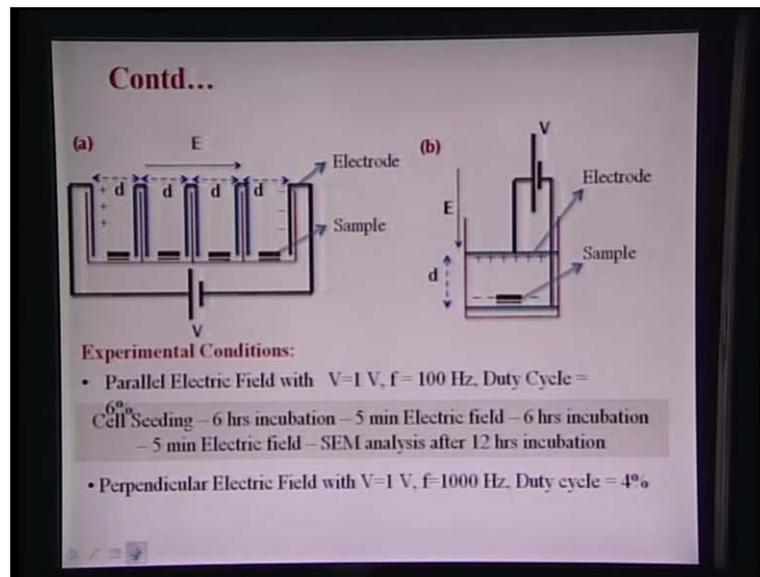
electrical field treatment. Now, this is like 8-12 plate and you can see, this is the cell culture solution and this white stuff are samples. Now, two type of samples we have used; one is the pure hydroxyapatite, because hydroxyapatite is considered as bioactive and biocompatible material and the another set of sample that we have used, HAP Barium titanate. So, we have added the 40 percent Barium titanate because, thinking that, the addition of Barium titanate will increase the, or will induce the physio-electric properties in the HAP, which is otherwise a perfect insulator. Now, with the addition of the physio-electric HAP barium titanate phase, we can increase the physio-electric strength coefficient, so on, so, thereby we can study the material aspect of this electric field influence experiments.

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Now, we have used that standard protocol for this, incubating of the **L929** cells and then, what we have done that, we have constructed two types of conditions, one is the parallel electric field and one is the perpendicular electric field.

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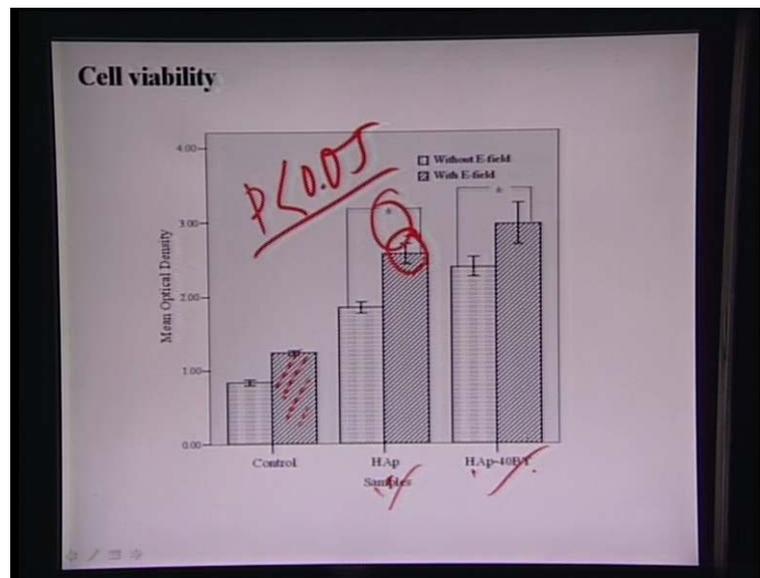
So, parallel electric field, what we have seen that, in case of the parallel electric field, we apply the one volt field and 100 Hertz and duty cycle is 6 percent. So, here, the electrodes are parallel to that well plate wall and then, we have put the samples here. So, one end is positive, one end is negative and then, current is, field is applied externally. And, in the particle perpendicular electric field, what we have done, that is, top is the positive electrode and the bottom of the cell wall is a negative one; and then, we have connected, we applied the field and then, again the similar parameters were used.

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Now, what we see here that, in case of the without electric field, pure hydroxyapatite and HAP 40 percent Barium titanate; so, these are the L 9 2 9 cells, that is mouse fibroblast cells, which are seeded on these 2 samples. So, this is without electric field, 24 hour culture; this is with electric field, 24 hour, after 24 hour culture, but with 10, with 10 minutes of electric shock. Now, from a visual inspection of these 2 surfaces, for individual material, HAP and Barium titanate, where HAP, without electric field, with electric field and HAP Barium titanate with electric, without electric, with electric field, one can clearly see that, more number of cells are proliferating in the presence of the electric field. Therefore, the field effect is pronounced, irrespective of whether it is a hydroxyapatite, or hydroxy Barium titanate composite.

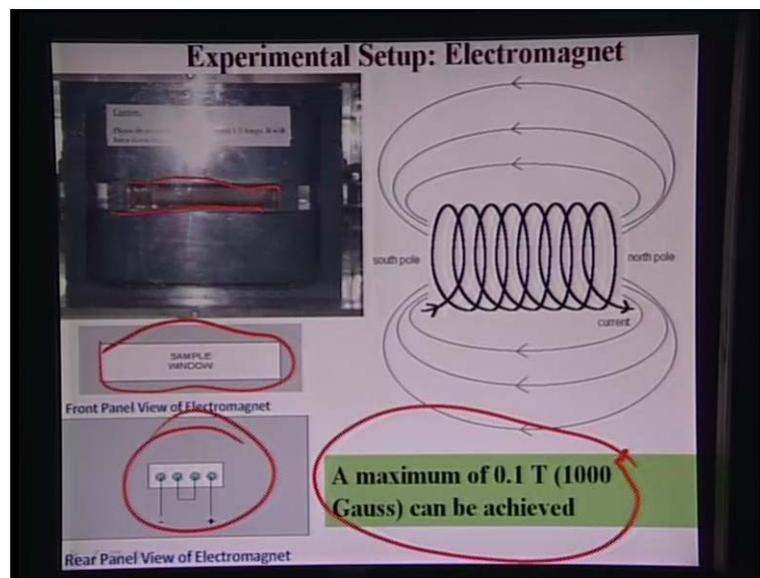
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Now, we have done some MTT analysis, trying to see that, how this influence, it is quantitatively. Now, this is for the control sample; the hashed one is that with electric field; un-hashed is the without electric field. Now, what you see here that, this dot is actually P less than 0.05, and then, what you see here, post, after post (()), we find that, for both HAP and HAP Barium titanate, that mean O D values, after applying the electric field, is statistically significant, compared to the absence of the electric field. What it makes us to believe that, that if you apply the electric field, then, one can essentially enhance the cell proliferation, particularly L 9 2 9 cells, on these two materials HAP and HAP 40 percent Barium titanate. Now, further experiments are in progress, trying to see that, whether you can optimize the electric field, or you can optimize the electric field

application sequence; in what way you can enhance the more cellular functionality on these electrically treated surfaces. And, the final aim is that, if we, if we are able to optimize, or if you are able to enhance significant the cellular proliferation on these two electric field treated surface, then ex vivo tissue formation is possible. And, such ex vivo tissue formation, prior to the implantation in the human, or animal, would be helpful for Osseo integration of these materials, inside the in vivo environment. So, that was the ultimate goal.

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Now, in next few minutes, what I will show you, I will show that, you know, we also designed and developed a magnetometer electromagnet, and that electromagnet, that is based on the simple physics principle and we have put a slot here; and through this slot, one can put the 6 well or 8 well plates, inside the magnetic field. So, this is called the sample window and this is your front panel view of this electromagnet. Now, by changing the turn, or you can, by passing the different field, one can create up to 1000 Gauss or 0.1 Tesla field; 1 Tesla, 0.1 Tesla magnetic field, one can achieve with this electromagnet.

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Summary of major effects of magnetic fields on various cells			
Magnetic field	Cell type	exposure	Effects
Static, 10T	Human fetal Lung fibroblasts	—	No effects on proliferation
Static, 7T	Human Tumor cell lines	prolonged	Inhibit cell growth
Static, 5T	E. Coli WP2 urvA	—	No mutagenic effect
Static, 7mT	Lymphocyte	—	Cell no. remains same
4.75 T Static + 0.7mT pulsed at 500 MHz	Human lymphocyte	1 h	Increased Ca ²⁺ influx without proliferation
Static, 0.2T	Human skin fibroblast	1 h	Cell morphology modified; Cell viability unaffected
Gradient magnetic field of 6T with 60T/m	He La Cells	—	Reversibly change the direction of conventional flow
Static, 10T	Human glioblastoma A172 a. imbedded in collagen gels b. Cultured in absence of collagen	7days	a. Cells oriented perpendicular to the direction of magnetic field b. No any specific orientation pattern
Static, 7T	Melanoma, HTP63, Lymphoma cell	64 h	Reduction in viable cell number

Now, there are different effects. This is the kind of table; it shows the summary of the major effects of magnetic fields on different cell types. Now, what it says is that, if you apply static 10 Tesla magnetic field on the human, **human** fetal lung fibroblast cells, there is no effects on proliferation. Now, if you apply the 5 Tesla field on the e-coli bacteria, no mutagenic effect. If you apply 7 Tesla field for the human tumor cells, inhibit cell growth and if it inhibit cell growth, that is quite good. So, what you see from the, major message from this slide is that, depending on the magnetic field strength, as well as the cell type, the influence, the effect is also variable, like, either you have no effect on the cell proliferation, or it can inhibit the cell proliferation, or it can orient the cells also. For example, when we apply the static cell field of 10 Tesla, cells are oriented perpendicular to the direction of the magnetic field. However, no such, not such any specific orientation pattern is found, when it is cultured in absence of collagen. So, what it says that, human A172 cell lines, when it is embedded in collagen gels, then, cells are oriented; but, when it is embedded without any collagen, then, there is no specific orientation. So, it is not clear, whether that magnetic field can, or magnetic field has the potential to orient the cells in a particular direction. It also depends on the microenvironment, or environment just outside the cell. So, if you spread the collagen on the surface, then, there is a potential that, this collagen containing on the material surface, the cells can have some orientation.

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Static, 14T	Muscle cell assemblies	---	a. Change in morphology b. Cell colonies are extended along direction of mag. Field
Static, 0.4T	MG63, Osteoblast like cells	a. 6hr. b. 24hr.	a. Increases ALP activity, ECM release b. reduction in proliferation of growth factor
Sinusoidal Magnetic field 1mT, 50Hz	a. Cancerous Leucocytes(CD4Ly) b. Leucocytes(CD4Ly)	1hr.	a. Increased adherence b. Only small effect of adherence
Intensity: (2.3-0.56) μ T (15Hz-KHz)	Human Fibroblasts	---	Enhance DNA synthesis

Now, these are like, you know, other literature reports, where it says that, when you apply the static 14 Tesla field and on the muscle cell assemblies, there is changes in morphologies, and cell colonies are extended along the direction of the magnetic field. So, when you do similar type of test in MG63, that is, osteo-sarcoma cell lines, it increases the ALP activity, that is, alkaline phosphate activity and also E C M, that extracellular matrix release and at the same time, if you apply 24 hours, there is a reduction in proliferation, or growth factor. So, again, I repeat, depending on the magnetic field strength, depending on the cell type, as well as the exposure time, which is, typically varies from 1 hour to 7 days, that effect is also very different.

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Magnetic Field	Bacteria	Time	Results
Homogeneous: 7 Tesla Inhomogeneous: 5.2~6.1T and 3.2~6.7T	E-Coli	12 h Incubation period	1. Decline in the cell number 2. Effect of high magnetic field is very sensitive to changes in biological, mechanical or magnetic conditions
Clinical electromagnetic bone stimulator Bm=1.5 mT 2.7-10mT	E - Coli, Pseudomonas aeruginosa, bacillus magaterium, staphylococcus aureus	24h	No obvious differences were found between test and control with any of the four bacterial species
	E-Coli, Leclercia adecarboxylata and staphylococcus	T<30 min	1. Exponential decrease with mag field 2. Number of Cfu decreases with time of exposure

Now, this effect is also equally different, in case of the bacterial cells. So, earlier, all those two tables were shown, those were for memory and cell types. For bacteria cell type, most of the experiments are carried out in that e-coli bacteria, that is, the gram negative Escherichia coli bacteria. Some were also carried out on the pseudomonas species and this essentially, again this kind of a field, which is applied like 7 Tesla, or it varies sometimes up to 10 Tesla, the exposure time was 12 hours, 14 hour, or less than 30 minutes. What you, what you see, when you heat it for 12 hours, there is decline in the cell number and that is good, because, you want much less number of bacterial cells to proliferate, so, those infection, chances of infection will be less. Now, when you apply this 1.5 milli Tesla, that probably this magnetic field strength is quite low, even after application for 24 hours, there is no obvious difference were found between the test and control samples, for all the bacterial species. Now, when we apply for less than 30 minutes, there is a exponential decrease with magnetic field, and number of C F U increases; C F U means colony for mini unit, for the bacterial cells, will increase with time of exposure.

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Contd...			
F=50hz, B _m =2.7-10mT	Escherichia Coli	0-12min	The ability to form colonies decreased with increasing mag field and time of exposure
50Hz 10mT	E Coli P. Denitrificans	(1hr. each)	No change in bacterial morphology was observed in both type of bacteria
10T	Secondary structures of protein in E. coli and Staphylococcus Aureus	(5, 10, 20, 30, 40, 50 and 60) min.	The ultra strong static magnetic field has a great impact on the secondary structures of protein in E. coli and has little impact on those of protein in S. Aureus
30, 60 80, 100 mT (Magnetic fields were applied in aerobic and anaerobic conditions).	Streptococcus mutans, Staphylococcus Aureus and Escherichia coli	(24 hrs.)	Findings suggested that- Oxygen related to growth the cases of S. mutans, S. Aureus. No growth effects were detected on E. coli culture

So, similarly, if we apply the 50 Hertz and 2.7 to 10 milli Tesla for 0 to 12 minutes, that ability to form colonies decreased with increasing magnetic field. So, either that colony forming units decreased, or they, decreased to the increasing magnetic field, and time exposure, or there is no change in bacterial morphology; that is what has been observed for e-coli, that is 50 Hertz and 10 milli Tesla field.

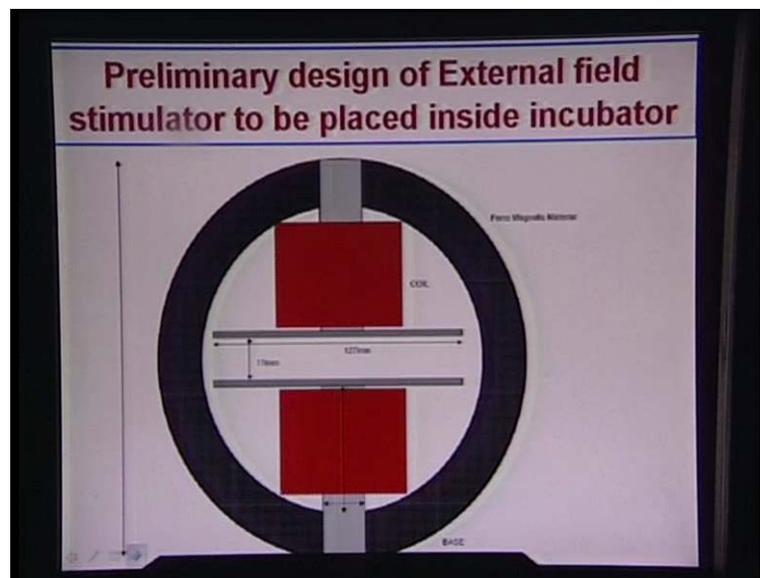
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Possible Mechanisms:	
•	Magnetic fields may induce active oxygen formation.
•	Radical reactions with these paramagnetic ion and oxygen are effected by magnetic fields.
•	Mechanism of effect of magnetic field may be different in gram positive and gram negative bacteria
•	Possible effect of electromagnetic field on the permeability of the ionic channels in the membrane or formation of free radicals due to magnetic field

Now, possible mechanisms is that, magnetic fields may induce active oxygen formation and this radical reactions with these paramagnetic ion and oxygen are affected by this

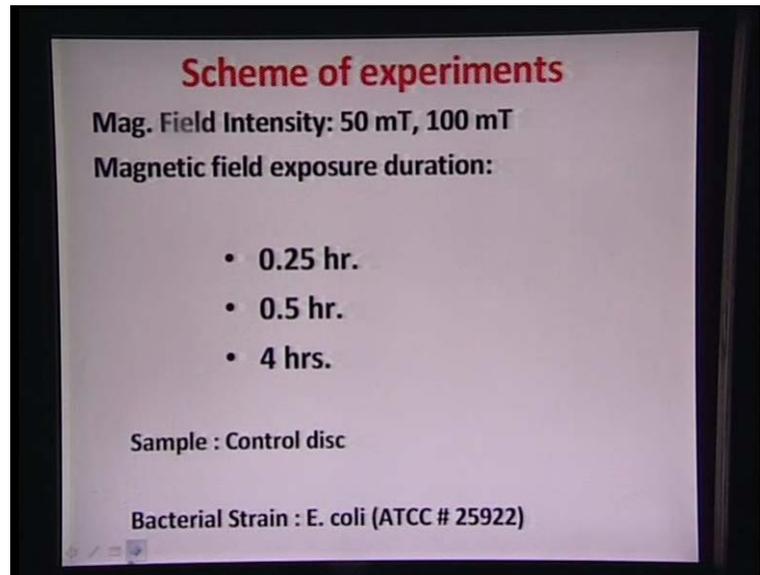
magnetic field and the mechanism of the effect the magnetic field may be different in gram positive and gram negative bacteria. So, that is the reason, when we have started investigating this particular aspect, we started with first, gram negative bacteria, that is e-coli bacteria, which is widely investigated and in the next stage, we are trying to find out similar influence on the gram positive bacteria, for example, staphylococcus epidermidis, or staphylococcus odious species. Now, there is also possible effect of the electromagnetic field on the permeability of the ionic channels in the membrane, or the formation of the free radicals due to the magnetic field. So, these are like possible mechanisms.

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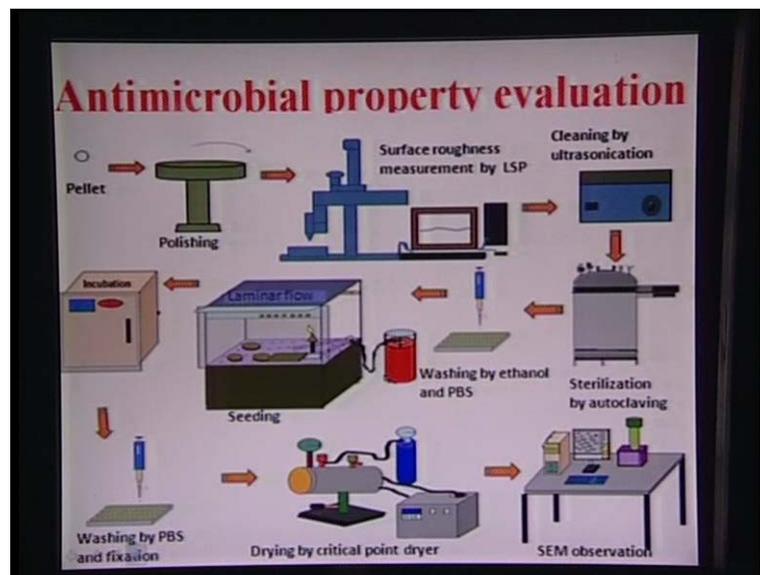
Now, these are the design of this magnetic field. You can see, that is the 17 millimeter, that is the distance through which this, the samples, that 8 well plates, or 12 well plates, they are introduced in this magnetic field. This is the, where the electrical wire coil things are there, depending on the (()), depending on the amount of current that you are passing through this coil, you can create magnetic field using, that is quite clear from the basic physic aspects.

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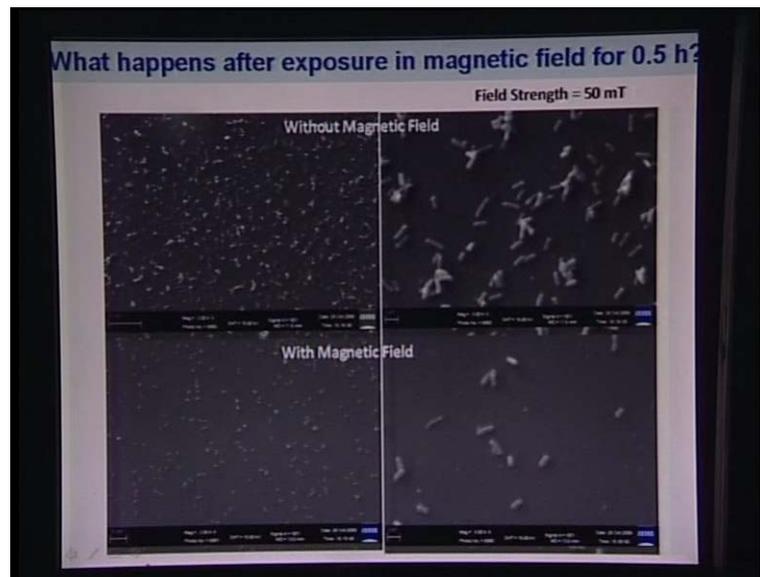
Now, initially, we have done this test up to 150 Tesla and then, we are increasing the field to 100 milli Tesla. Magnetic field exposure duration is varied from 0.25 hours to 4 hours, and we have done the, first used the sample control disc and then, we have also used the bacterial strain, that is e-coli strain from A T C C.

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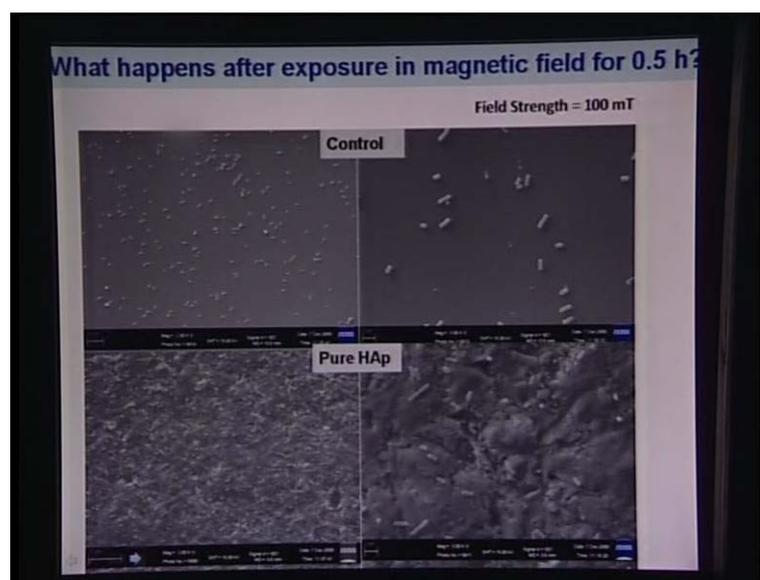
We have followed the standard protocol for the bacterial culture and after this standard protocol, we have, we used the scanning electron microscope to observe this bacterial cell morphology.

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Now, this is what happens, when you apply the 50 milli Tesla field and 30 minutes of the exposure. Without magnetic field, you can see that, so many, so many number of bacteria, they are adhering very happily. With magnetic field, like 0.5 Tesla, you can see, number of bacteria is reduced and also, both at high magnification, you can, you do not see that many number of bacteria, or that many number of colonies on the control disc.

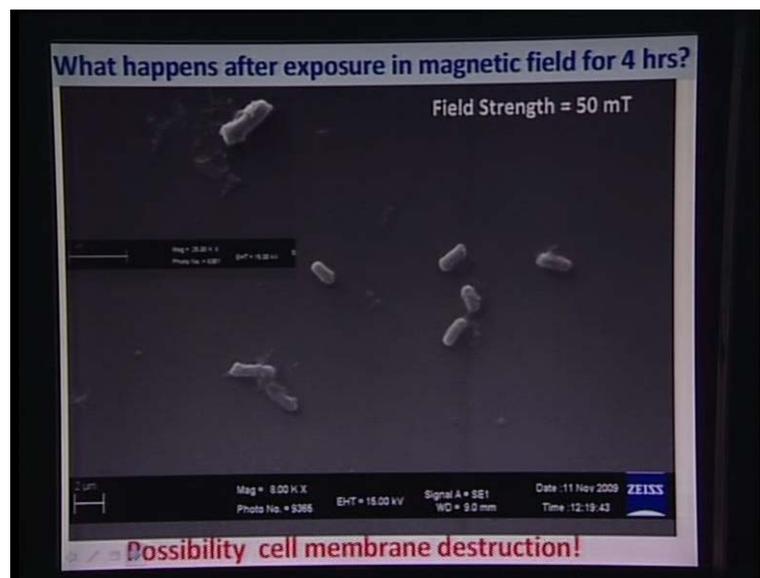
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Now, similar observation has been made, when you increase the, increase the magnetic field to 100 milli Tesla and exposure time is kept at 0.5 hours, that is 30 minutes. Now,

what we see here, in the control sample, again, it is, the number of bacteria is less; if you, if you see that, 50 milli Tesla and if you see that 100 milli Tesla, certainly, the increasing the magnetic field has a positive effect as far as the reduction in the number of bacterial cells are concerned. We have also done similar experiments on pure hydroxyapatite, because, if you remember correctly that, hydroxyapatite is one of the material which is widely regarded as excellent biocompatible, bioactive material; but, there are two issues of hydroxyapatite, and one of the issues is that, lack of bacteria seeder property. So, therefore, we tried to investigate, whether if you apply the magnetic field, whether you can induce antimicrobial property in the hydroxyapatite and we have demonstrated that, when you apply the 100 milli Tesla field, you can essentially reduce largely, or significantly, the number of bacteria those are seeded on this hydroxyapatite surface.

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This is like relatively high magnification SEM image, essentially showing the possibility of, there are possibility that, you know, there are cell membrane are destructed; intercellular elements are coming out and what we can see with that, what we can possibly see that, individual bacterial cells, they have the cell membranes are destructed; intercellular elements are coming out of the bacterial cells and therefore, it causes the death of the bacteria. So, however, we are still looking into the possible mechanism of the bacterial cell death. So, I think, I will stop here and then, we will start with a different topic in the next lecture.