Bioelectricity

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Lecture - 08

Welcome back to the NP-TEL lecture series on Bioelectricity. So, we are into the eighth lecture, and previously we have talked about the action potentials and the minus 90 millivolt potential difference across the membranes. And we briefly talked about the different ion channels and I promised to you that I will be coming back to the ion channels once I kind of talk about the different techniques which are been used. So, today what we will do, I will introduce you to the basic techniques which are been used for recording the electrical properties of the excitable cells, and from there we will move on to the properties of the ion channels which have been discovered in the whole process.

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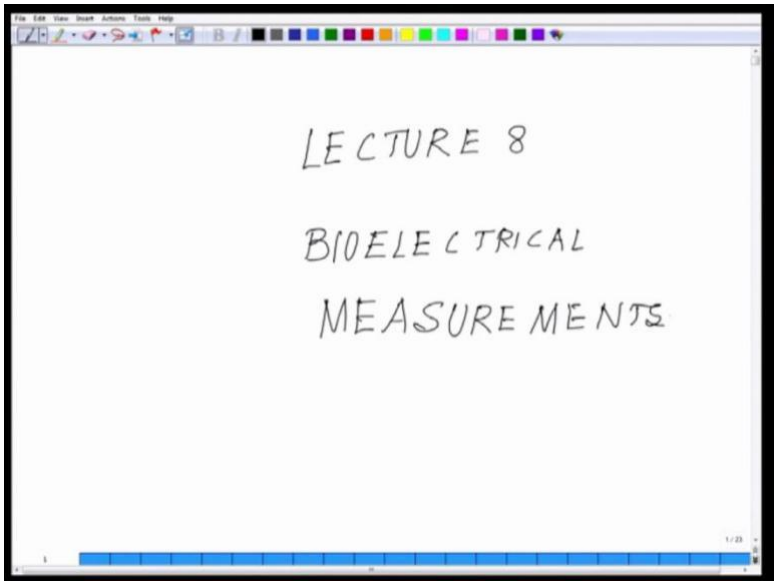

Let us start with the historical perspective, how the electrical measurements of the excitable cells have taken place. So, the full kind of move into the electrical measurements, so one of the fundamental things the way bioelectricity person treats a cell is that it treats a cell just like an equivalent circuit model. So, essentially it is like this. So, for example, this is our lecture eight - Bioelectrical Measurements.

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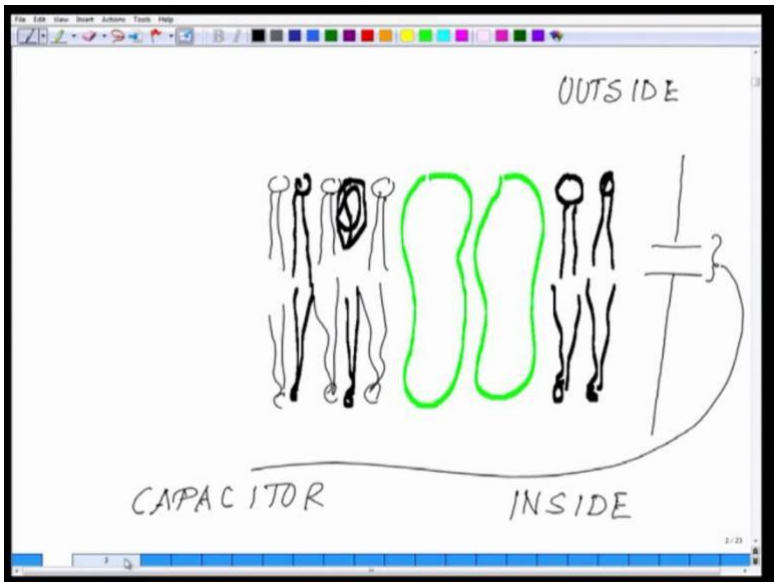

The way a cell is been treated is something like this. So, I have already talked early that a cell is a bi-lipid membrane like this, and then in between, you have the series of membrane proteins sitting like this, channels membrane proteins likewise, and then you have something like this. This is a kind of a and you have cholesterol molecules and all those things which are sitting here. So, essentially this barrier across, so this is indicating the outside the cell, and this is inside the cell. And we have already discussed the different molar concentrations and molar concentrations of the different ions. You can treat this membrane first of all like a capacitor, so the symbol of a capacitor.

So, essentially the way to treat it as a capacitor arises from the point. So, if you look at the membrane-like this, and if this is part of the membrane either look it bilayer and this is inside shown by I and this is outside shown by O. So inside it is negatively charged with respect to outside. So, these are the negative charges, and outside it is positively charged. So, it is almost similar to a parallel plate capacitor. So, if I connect the wire-like this out here, and I connect the wire-like this out here, so this is one of the ways how you can treat the cell. Similarly, you can treat the cell another way. You can consider this as a battery. So, essentially what you see is across this, if you call this positive and you call this negative terminal. So, if I show you blue this is the negative terminal and right here. This is another

way to treat the cell. Apart from it, the motion across something like from here, things are moving like this is basically things move like this or things moves

like this and also. So, the movement of the ions across this space some form of resistance, you can put as a resistance component.

So now you do, you add all of them together, and there you can call this as plate like this, parallel plates like this. So, essentially what you did, and call it outside or extracellular and this is your intracellular. So, essentially what we did actually, we treated the cell as an electrical circuit, it has all the three basic components. It has a capacitance across its membrane just like when we treat it as a parallel plate capacitor. It has a resistance that ensures or creates an obstruction on occlusion to the flow of ions across this membrane and it functions as the battery. So, if we translate this whole thing into a single diagram it will be something like this.

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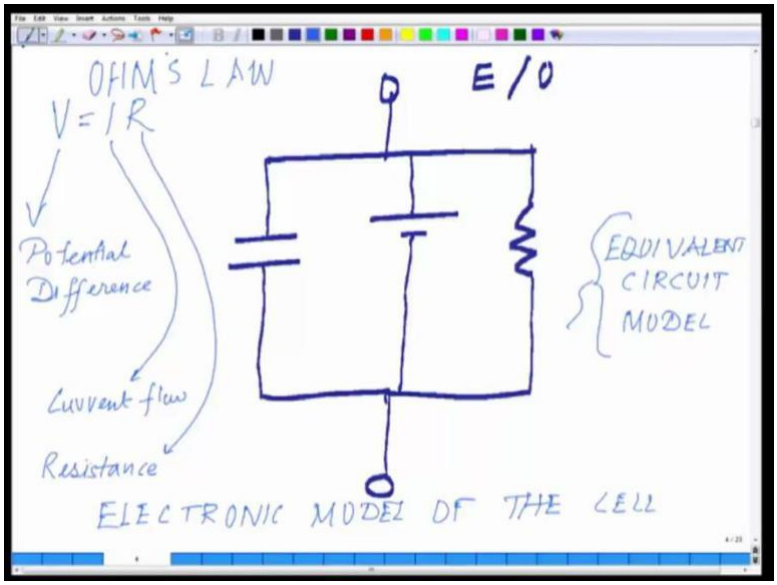

I draw the side, this is extracellular side – E or outside the side, you can you have the capacitors you have the resistance and you have the voltage across it. So, this is called an electronic model of the cell or electrical model of the cell. This is also called the equivalent circuit model. Now this equivalent circuit model, as it is in front of you now you can treat the cell accordingly. You can measure capacitance, you can measure resistance you can measure the voltage and of course the current flow across it.

And the thumb rule in this game is V is equal to I R, where V is equal to ohm's law, where V is equal to potential difference, I is your current flow, and R is your resistance. So, now, just the same way, we draw the equivalent circuit model, now what I will do, I will draw a cell

and I will show how the different kind of measurements which are been followed and the challenges and how far we are by twenty thirteen. So, before I start this let me tell you that these kinds of measurements treating the cell as an equivalent circuit likewise, even much before all these circuit components were discovered, the discovery of evidence of bioelectricity was pretty much there. We have mentioned earlier also from the time of Volta, Galvani all these things were there.

It was just it was never formalized, it took a while it was during the last century that everything kind of got formalized because by the time there is a formal field of electrical engineering which was there. So, everything was kind of structured. So, what we see during last century apart from the development in terms of semiconductor, high-end amplifiers, miniaturization of electrical devices, and very sophisticated good measurement techniques, apart from all these things what you see, the whole field of bioelectricity is slowly getting formalized, because it is kind of discreet even all over the place. And there is no standard wherein one textbook where you can cover all the whole spectrum of bioelectrical phenomena. So, over the period of time and different centuries and a different time, people have a different kinds of experiments, now slowly we trying to understand that these are very very fundamental events which are regulating our day to day events of our life.

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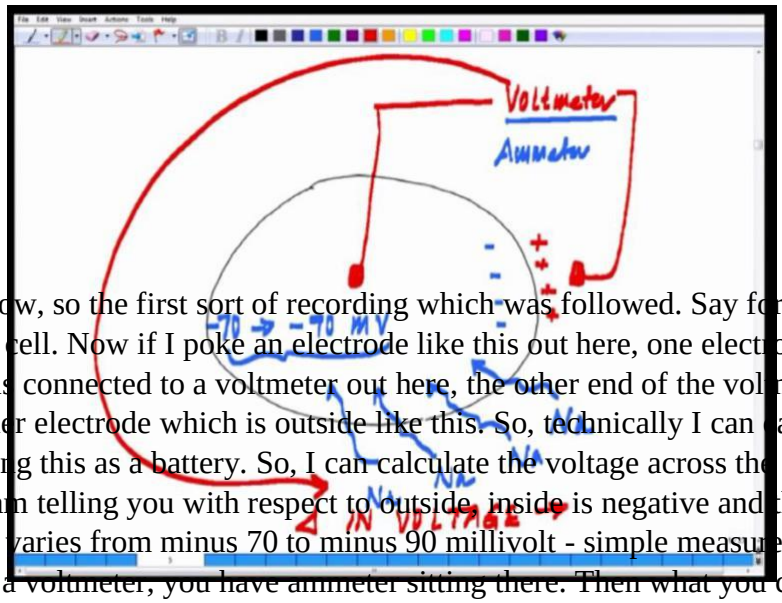

So, coming back now, so the first sort of recording which was followed. Say for example, I constraint this as a cell. Now if I poke an electrode like this out here, one electrode like this, and this electrode is connected to a voltmeter out here, the other end of the voltmeter is connected to another electrode which is outside like this. So, technically I can calculate because I am treating this as a battery. So, I can calculate the voltage across the cell, so that is what repeatedly I am telling you with respect to outside, inside is negative and this is minus 90 millivolt, and it varies from minus 70 to minus 90 millivolt - simple measurement. Second thing, if instead of a voltmeter, you have ammeter sitting there. Then what you can measure is there is a movement of sodium likewise, it should be able to measure the current across it.

And indirectly what you actually measure is you measure, because of this, you measure the change in voltage that is what you exactly measure.

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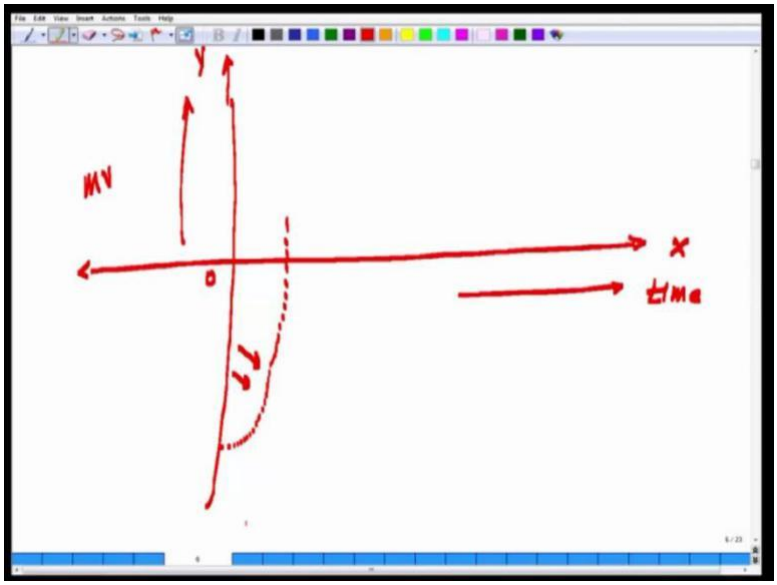

So, whenever we talk about an action potential, what you are measuring here is the on the scale and this is zero and y-axis. So, the x is the time, and this the volt in millivolt. So, what you essentially measure is this, this is where the electrode is measuring the influx of sodium likewise. If you compare this, this is what we are measuring. Now this kind of measurement that has been done falls under…

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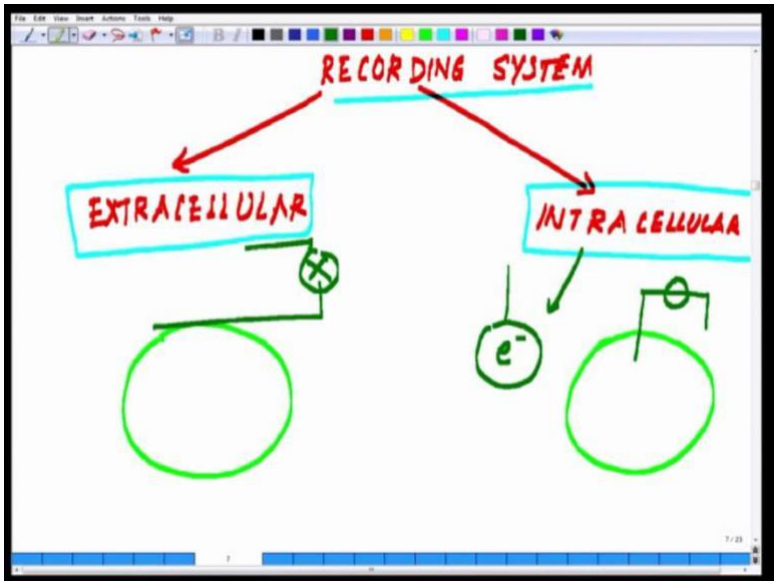

So measurement techniques could be now classified into two groups. The first group is called extracellular recording; the second group is called intracellular recording. So, these are the configuration of the recording system of recording electrodes. Let us first distinguish what is the difference between extracellular recording and what is the difference between intracellular recording. So, the word itself indicates when the electrode is outside the cell that is called extracellular recording; when the electrode is inside the cell, it is called intracellular recording. So, here electrode is if I differentiate the electrode by E like this. So, in this situation, this is the cell, this is the cell and the electrode is something in this position with respect to also here you have the ((Refer Time: 14:08)). So, it is inside the cell. But the second configuration is out here like this, and the electrode is outside the cell with respect to your measuring device you have and there is an electrode at a distance, this is the other configuration.

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So, if I go back on to the previous one, what you see here this kind of action potential pieces and falling down likewise, what you see here is coming from intracellular electrodes, intracellular recording of AP- action potential.

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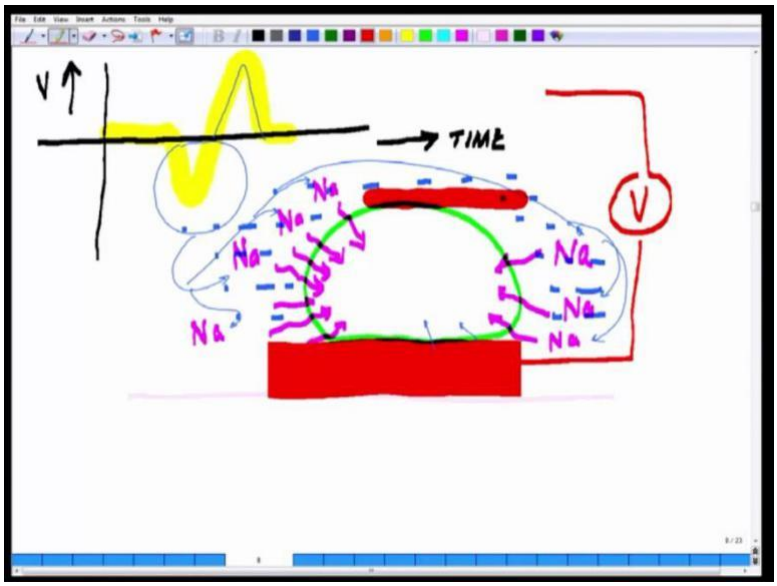

So, went to the previous slide show, this is a classic intracellular recording, but then what will happen in an extracellular recording, coming back. So, now let us see this situation in the next slide what I will do, I will highlight this one in the next slide. So, let us put the cell like this. So, this is the base; on this here, you have a cell sitting on like this. And

now you have two options, imagine an electrode is sitting either sitting underneath like this on the surface or you can have ultra diagrammatic configuration; and another electrode which is an electrode at an if this is an electrode sitting and on top of that this is the cell and this electrode is connected to the voltmeter. And other led off the voltmeter is out at a distance and this cell is in an extracellular field like this it is waved kinda field like this.

When this cell is sheeting an action potential what we essentially see is, there will be an influx of sodium ion from all over the place like this. Sodium is moving in from outside. So, this sodium movement essentially will for a very small fragment of time will meet the electrode which is sitting outside to field as if it is becoming negative because of this surrounding… So, show it like this, if I have a voltage plot sitting here, so y-axis is shown voltage and x-axis shown time. So, the baseline I am showing the baseline like this. So, this is the baseline. Now as soon as the action potential gets initiated, there will be a depth like this, because it will experience at that particular position and it will go up and down. So, this is the zone where the sodium is getting in, this is why the electrode will experience as if locally for a while all the positive charges have moved in.

And the electrode on the top of the electrode, you will see a slightly negative and then again ions from the other side will immediately rush in and shoot like this. And this is how you record the excess action potential. In this situation, the advantage and disadvantage, so the advantage is this you are not damaging the cell, your electrode is touching the surface. If you have electrode from the top as I was trying to tell you you could have electrode like this sitting on the top or you could have at the bottom.

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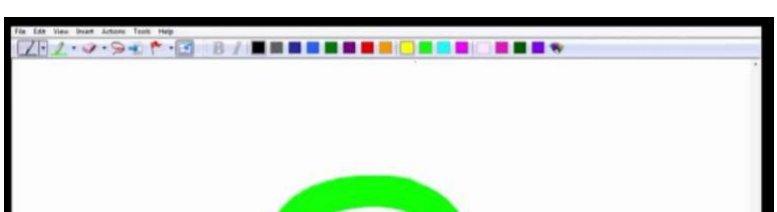

The disadvantage is something else, so the disadvantage is this, so once again draw the situation this is the electrode sitting and this is the cell that is sitting on top of your electrode like this. Now out here, across this gap, there is always a gap out there, we always assume that a cell and an electrode is almost sandwiched over each other, but if you look at the geometry of the cell essentially, so if I keep this cell takes you to a little bit of biology of the cell.

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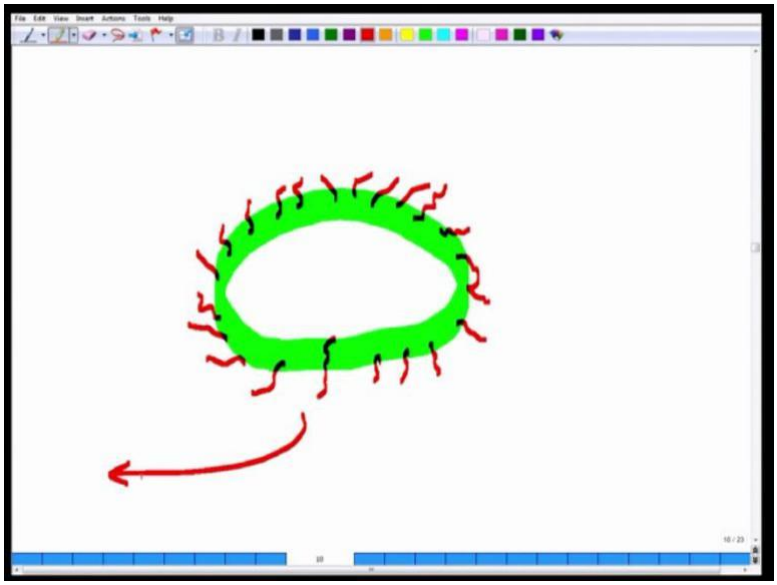

If you look at the cell is something like this is the cell on top of the cell surface you have a whole bunch of different kind of proteins, a whole series of proteins which are like this. It is decorated like this. Any cell is decorated like this. It is not a smooth surface as I am drawing.

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So, when this cell now goes back go back to the previous slide it is in the previous slide now if I add this component. So, I am using a different color just to mark the difference. Let me do something. Let me just change this color and let me introduce all the other components on top of the cell, all the different proteins which are present out there. Now, I introduce the electrode. Now if I put the electrode, now see the problem. The problem is, if you look at it very carefully, you will see a zone which is totally gap. So, that zone I am just putting into yellow now. Now this is the zone, see this gap.

This gap is the cause of a huge amount of leakage, whenever you are making a measurement. And this gap is called technically there is a name for this and this is called cell electrode interface. The cell electrode interface is one of the critical problems of a lot of leakage current which is taking place out here, because this connection or the sandwiching between the cell and the electrode is not perfect, and the cell is almost like a sponge structure. So, the options are either you make the surface of the electrode rough. So, it is something like this.

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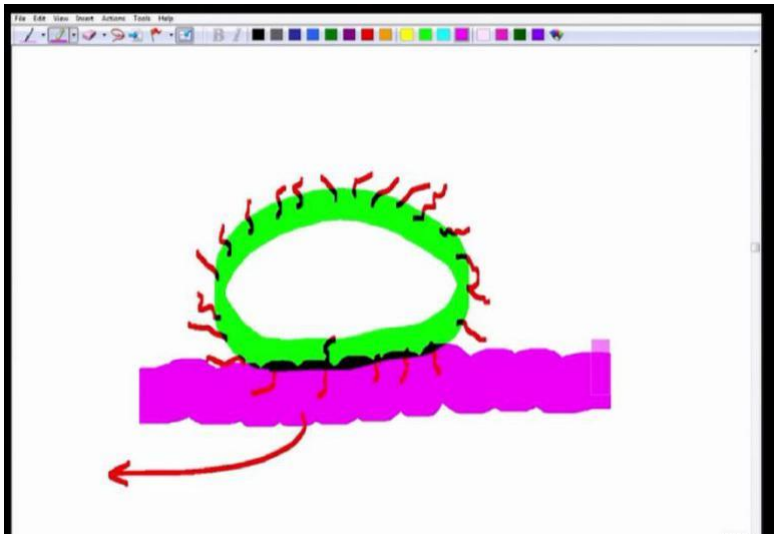

So, if this is the rough surface then you made the electrode surface, now let me presume the electrode surface with the pink color. I make the electrode surface something like this instead of making it perfect I make it, so that cell fix on that and kind of you know along with of the topographical feature and along with the ups and downs and bulges of the electrode the cell sets. This is one option by which you can reduce the loss of currents out there, loss of ions, loss of fidelity or improve the fidelity of the signal.

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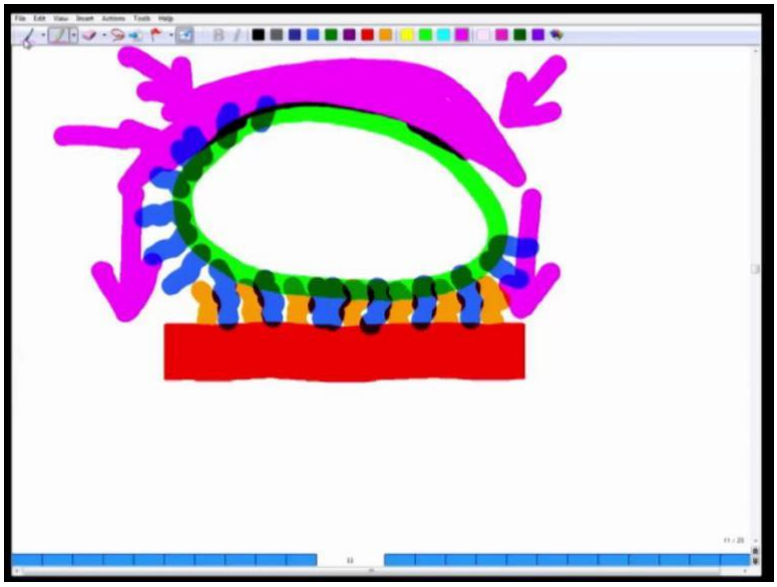

There is another way you can do which is the next technique is you have this electrode, electrode out here, you decorate the electrode with molecules. You decorate it with molecules which will bind to the cell, bind to those, these are the molecules you should decorate the cell and you have a cell out here at the top of it, and now cell has its own a proteins like that which I am showing in blue now. So that way we reduce the gap in the cell electrode interface, this is another way of doing it. So, there are several groups which are trying to develop different kind of antibody light molecules which will bind the cell, hold the cell much tighter on top of the electrode.

There is another technique. Another technique is that you push the cell on top of the electrode with some kind of gel, some kind of a hydrogel or some kind of cryo gel or some kind of a gel you push it down. So, it is something like this, what we are trying to do is let me pick up a fully different color, this is good. So, you are creating some kind of a gel out here which will

put sufficient pressure on the top. So, you are putting pressure like this, and since it is a gel-like structure, it is not going to damage the cell. So, essentially what you are doing is that you are creating a mechanical pressure, which is pushing the cell down. These are the different challenges what I showed you now of doing a recording using an extracellular electrode, but there are several advantages.

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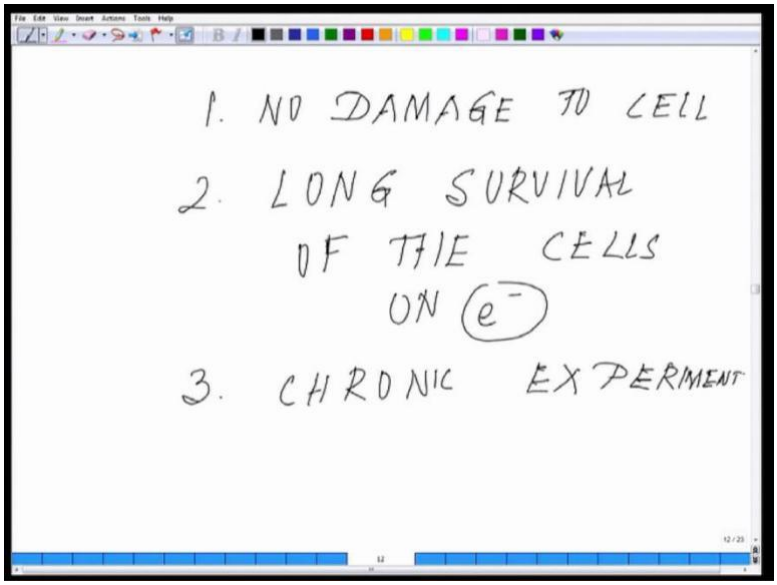

Now let us enumerate you have talked about the problem of cell electrode interface, talk about let us talk about the advantages. First - no damage to cell; second - long survival of

the cells on electrodes just I am showing thee. Then you can do all the chronic experiments or long term experiments related to drug discovery and all these things. This is the situation when I am talking to you about when you doing the recording outside the human body on a culture dish, but what will happen when you have to implant this extracellular electrode inside the body this complicates it a little further.

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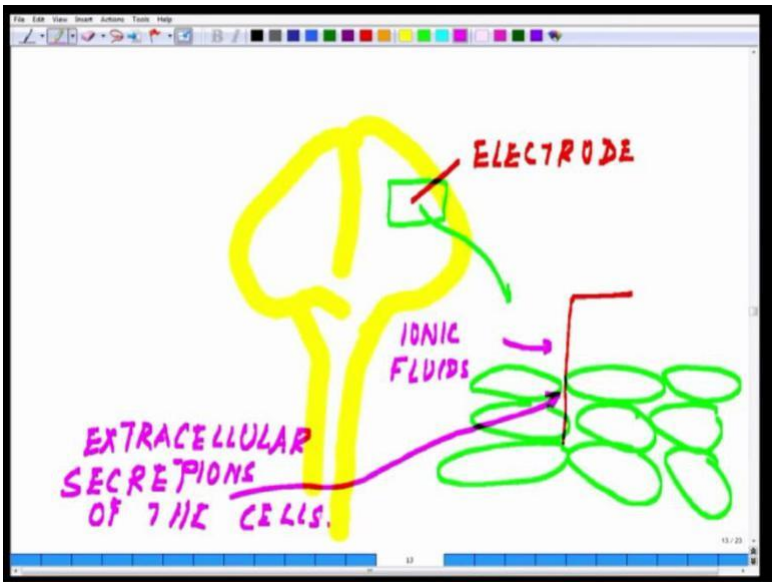

This is a situation, let us imagine say, for example, let us pick up some tissue. let us think we were talking about grain tissue like this. So, this is the now I want to implant an electrode here fine. So, if take the example, this is your electrode which you have implanted, but the problem arises when the electrode is out there is something a real-life problem. Here this electrode is interacting. So, first of all classify what all challenges this electrode are going to face. First of all, this electrode is in a dynamic ionic system, the dynamic ionic system first of all electrode material should be a souvenir that it does not gets corrugated, it does not get damaged because of the ionic material, because there are the whole bunch of sodium-potassium all over place chloride likewise.

The second thing a cell is a dynamic entity. So, a cell continuously secretes certain things in and around it. So, if I have to kind of amplify this image, it will be something like this, at the cellular level what is happening there are a lot of cells like this sitting here and likewise, these are the cells and have an electrode out here like this. So, now, this

the electrode which is sitting here is experiencing all the ionic fluids and the extracellular secretions of the cells.

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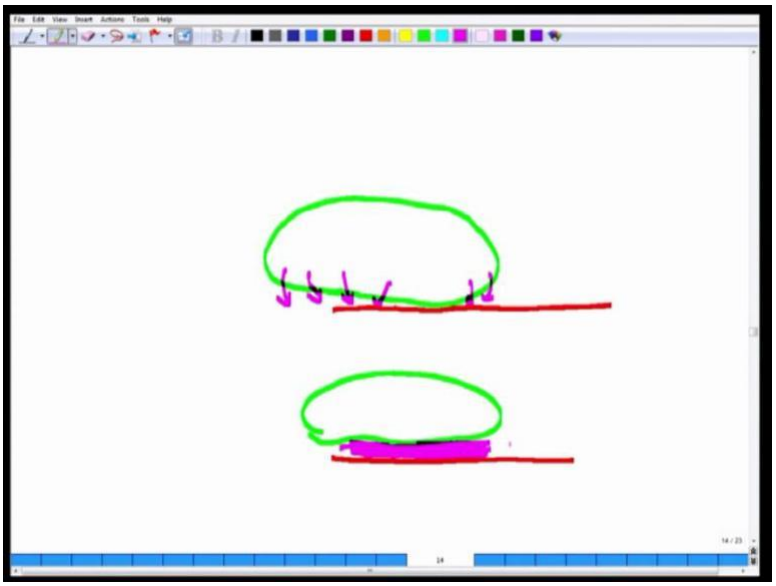

So, what do we really mean by extracellular secretion is something say for example, this is your cell and this is your electrode. Now, this cell is continuously secreting a different kinds of molecules. And these molecules essentially what they do is, this is your electrode and this is your cell, these molecules will eventually plug the connectivity between the electrode and the cell. And thereby you are losing upon the signal what the electrode is supposed to receive all the time. So, you are realizing that extracellular recording or implanting extracellular recording for a stimulation. So, these are multiple purposes in the liver situation, these are implanted into the brain for stimulation.

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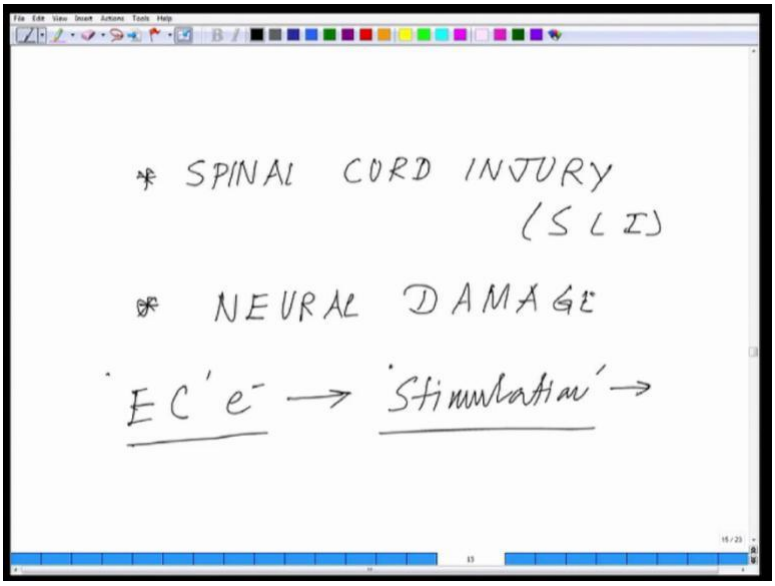

Say for example, for let us enumerate them for spinal cord injury or any kind of just short it is called SCI or any kind of other neural damage. So, these extracellular electrodes, I am just putting EC as extracellular electrodes are used for stimulation electrical stimulation. But they are the problem is that initially they are all fine, but over a period of time, because of all the different situations I told you narrated you their efficiency to transfer the signal reduces the fidelity of signal is been compromised.

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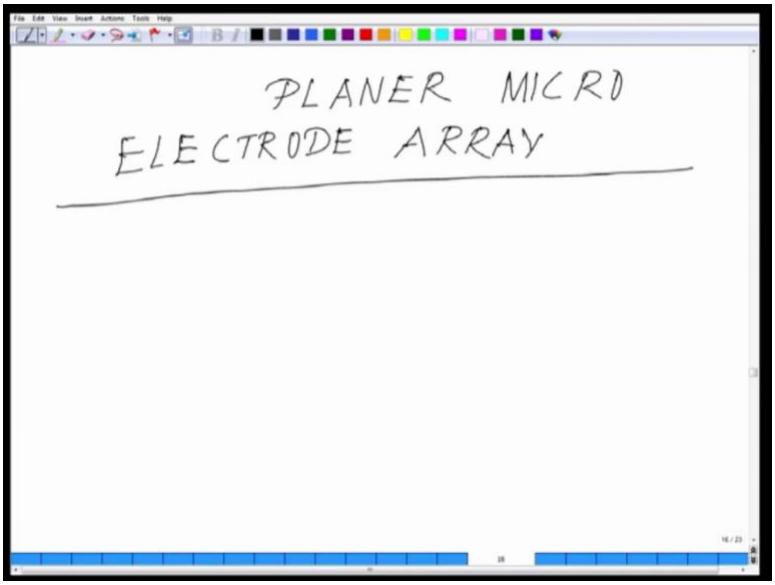

The same thing happens when you grow cells on top of electrodes and some of the devices, which are used by the (( )) industry are called perceptions are called planer microelectrode array. These planer microelectrode arrays are nothing but simply say for example, a backlight sheet on which you have a bunch of electrodes, which are on the surface. And there is a well where you can grow cells on top of the electrodes and you grow the cells. So, I told you there are two situations. So, you can use the (( )) for stimulation and for neuroprosthesis, and there is another situation where you can study the electrical properties of the cell in a culture dish. And it is something like this.

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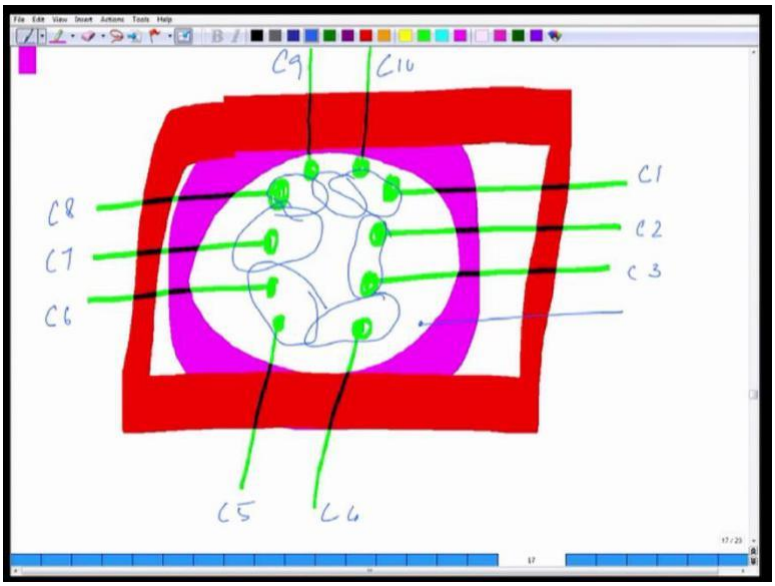

If you look at it the microelectrode arrays will look like this. It is kind of a sheet-like this on which you will see a series of the electrode which are embedded in it like this. And around this, there is a well it is something like this. Inside this well, we can actually grow the cells. So, you have let me look in the cell with blue. So, you really can grow the cells like this on top of this, any kind of cell and these planer and this electrode and there is ground electrode out here. So, each one of these is individual channels, the channel one, channel two, channel three, channel four, channel five, channel six, channel seven, channel eight, channel nine, channel ten. I am just showing it in channels, which I cannot really draw, but there are such 64 channels and 128 channel fantastic my queries which are available across the world.

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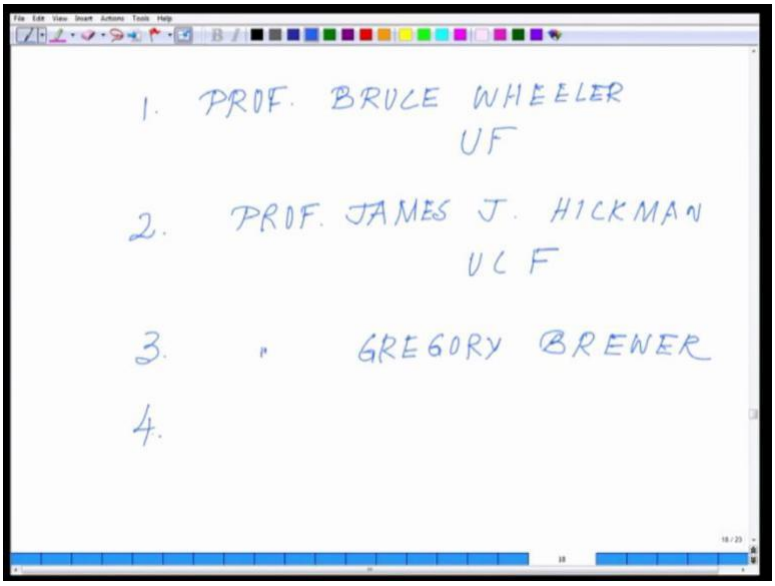

Here just before I proceed there is some individual's work which I want you guys to look online, I will be please to go through the work of these references, please go through Professor Bruce Wheller in the university of Florida James well. Then go through the work of Professor Bruce Wheller and Professor James J Hickman should go through some of his work on his microelectrode arrays and university of central Florida – UCF. Then you should go through the work of Professor Gregory Brener, and then there is one more group (( ))who's work I will in the next lecture, I will tell you there is another very fantastic group in Germany who are doing very nice work in this field. Please go through these some of their work they are really nice pieces of work which you guys will be really interested to look at how the world is moving in this direction.

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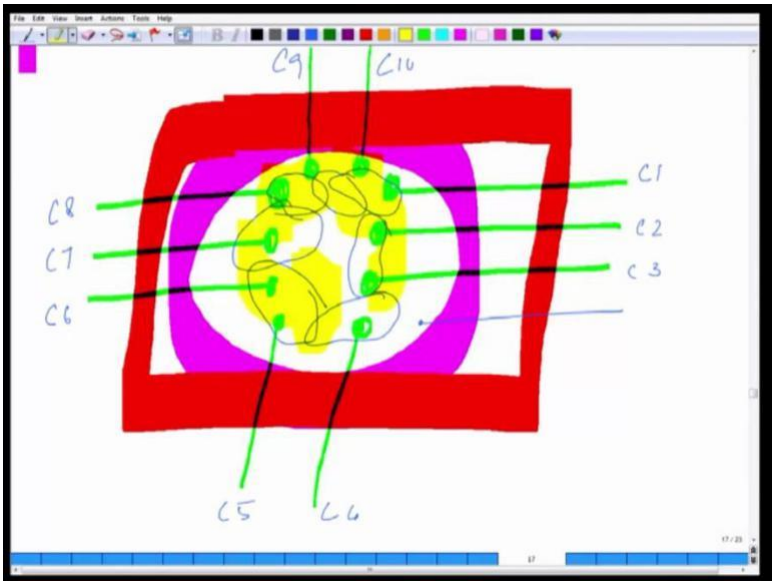

So, coming back, so you actually can on these kinds of planer microelectrode array and do one more exercise go to Google image and look for planer microelectrode arrays images of the planer microelectrode arrays, this will help you. So, pupils are developing a neural network on top of it. So, they are making something like you know like this. The network you are forming and actually, you can monitor the signal propagations within such networks. So, the whole field is really moving at a very interesting pace.

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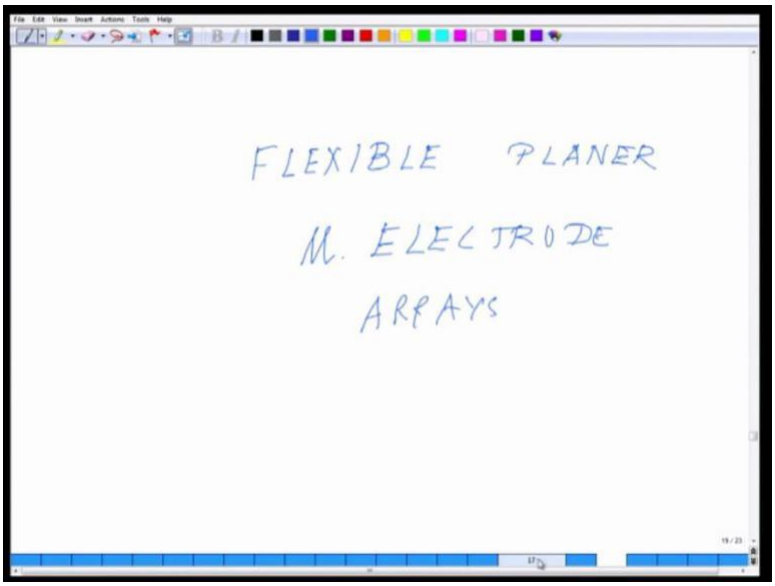

So, apart from it, there is the second generation modification that is taking place which is basically you are making these arrays which are flexible planer microelectrode arrays. What you are essentially doing the base what you are making out here, what I will show you you are making this base as a flexible base. So, they are much more easily, they are not a rigid structure, you can really do a lot of measurements on them. And out here all the recordings what you are getting are recordings what I explained out here from here this all the extracellular recording. Now correlate this structure what you have in front now which I showed in one of the previous slides with the structure that I showed you. So, this is one single electrode, and here what I showed you is ten different electrodes, and it could be sixty-four it could be one twenty-eight likewise and so on and so forth.

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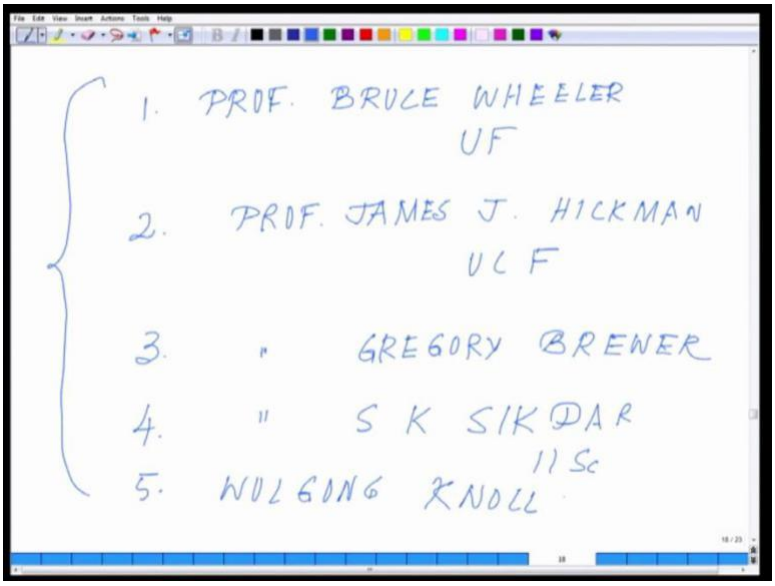

And those of you are in the Indian context, you may refer to the work or of Professor S K Sikdar who is working in the Indian Institute of Science in Bangalore. He also has a very active group out there. Then a few other people I will cross check for other people who are there in the field whose work I really will appreciate if you guys go through it, go online, give search and see the kind of things they are doing. So, what I will do now at this stage, I will be closing the lecture today, and we will continue again a little bit of a microelectrode array and then we will move onto the next technique which will be the intracellular techniques.

Thanks a lot.