Advanced Neural Science for Engineers

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Lecture 44
Flexible biodegradable MEAs

Hi, welcome to this lecture, we in this lecture, we are going to further understanding on microelectrode arrays. And since we are going to acquire the signal from the brain, we say that it is an implantable microelectrode arrays. Now in the last lecture, we have seen that how the device that is flexible in nature that can we can fabricate using the micro fabrication technology. And that device can be implanted in the rat's brain. And then we can create an epilepsy by using convulsant like bicuculline followed by using AEDs antiepileptic drugs to understand whether the drug is effective or not.

And in this class, we want to go for one different set of a class of biomaterials that are not only biocompatible and your bio materials should not be biocompatible, but it should not have toxicity. But, we will be talking about bioresorbable that means, that the material that can be absorbed when implanted into the human's body or animal's body per se, to do that, we will be looking at how can we use that bioresorbable material is a substrate and what kind of metal layers on the bioresorbable material can be used that are biocompatible.

And, again, we keep our focus on epilepsy so, that because we have seen that last lecture, so, we do not really go into some other directions suddenly. So, keeping the epilepsy as a focus that means that we implant the device onto the brain of the rat, we acquire the signal, create an epileptic episodes and then measure those epileptic episode from 32 channels, use AED and see whether the baseline is recovered or not.

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Flexible Biodegradable Microelectrode Array for Recording ECoG Signals

Background

- Though the traditional ECoG electrode arrays can help us to study activities from cortical surface after implantation, it requires a secondary retrieval surgery, which can create an additional trauma to the subject.
- The arrays fabricated using transient materials can be used to fabricate the biodegradable microelectrode arrays. These arrays will be degraded and dissolved in body fluids depending on temperature and pH.
- The baseline LFPs, LFPs during an induced seizure and after recovery needs to be studied to understand the evoked response.

Research Goals

- To induce epileptic activities by peripheral (forepaw) electrical stimulation in rat
- To record baseline electrical activities with induced ictal and interictal discharges from a depth of the brain in an animal model (rats)
- Spatiotemporal analysis to determine the changes in electrical activities in different neurological conditions and distribution of signals across the depth

Objectives

- Validation of a protocol to induce epileptic activities by peripheral stimulation
- Design and fabrication of a microneedle electrode array (MEA) to record LFPs (normal with ictal and inter-ictal signals).
- Implantation of the array in animal model (rats).
- Recording and analysis of LFP signals in response to convulsant and antiepileptic drugs for studying the epileptic activity.

Novelty

- Validation of a forepaw stimulation protocol to induce epilepsy under ketamine anesthesia in rat
- The fabricated biodegradable array will be used to study the electrical signatures in normal, epileptic and recovered conditions and will be allowed to degrade *in vivo* conditions to avoid retrieval surgery.
- The OpenBCI Cyton Daisy Biosensing Board was used for *in vivo* recording from rat's brain.

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So, if you see the slide, we are talking about flexible biodegradable microelectrode array that is MEA for recording ECoG signals, ECoG stands for electrocorticography. So, what is the background? What are the research goals? And what are the objectives? And, what is the novelty? Always I told you that I am not just teaching you some lectures from some traditional books, but what I am teaching you here is the application of what we understand and this some of the things may not be available for in the textbooks.

But this is a part of the cutting edge technology in many labs, including my lab here at ISC. So, if you do not get the exact same kind of content in the books, do not worry about it, but you will find a lot of terms for example, ECoG, LFPs, local field potentials, biodegradable temperature, pH, body fluids, electrical stimulation, animal models, spatiotemporal analysis, and many more. So, more, a lot of things you will find it but how to apply it is what we want we are interested in what kind of research problems we can generate and all other existing problems and how we can generate these solutions.

So, in the background, what we look at is through the traditional ECoG electrode arrays, so though the existing electrodes which are the ECoG electrodes, that can be used to study the activities from cortical surface, it requires. What requires? Traditional ECoG electrodes requires a secondary retrieval surgery, which can create an additional trauma to the subject.

So that is the first thing that we understood that, we can use the traditional one, but it requires a retrieval surgery because when you implant the device, you need to retrieve it as well before the surgeon can start operating the patient. So, do understand from this perspective, that if you implant a device now, I told you about epilepsy and studying the drugs. But the other area of the epilepsy is intractable epilepsy. In intractable epilepsy. The device has to be implanted onto the subject's brain and then the bone is fused, or whatever you want to say.

And then we need, the doctor needs to wait now, why am using some basic word, non-technical word is that because that is not what we need to understand we need to understand that once the device is there, what is the difficulty with the existing devices. So, the existing devices once placed the brain once we understand that where is the misfiring of signals or where exactly the source of localization is there, we can take the device out when I say we as clinicians can take the device out and can resect that part of the tissue which is causing the problem. Now, to do that, like I said, you implant it, wait till the episode occurs, you resect it.

So, you have to go for the retrieval surgery, second is the array is fabricated using transient materials can be used to fabricate the biodegradable material microelectrode arrays. Third one is these arrays will be degraded and dissolved in body fluids depending on temperature and pH. Depending on the change in the temperature and the pH the material that we are using will get absorbed in the body. So, these are called biodegradable.

Now, what I was saying earlier, right now what we have seen is epilepsy, so, we implant the device in rat's brain, create epilepsy apply AED 1, anti-epileptic drug 1 and see where the baseline is required or not that much be understood. But in intractable epilepsy, just administrating drug would not be enough. And that case, the brain has the skull has to be open the device has to be placed and these are traditional devices. And then it is kept back and then you have to wait till the episode occurs, when the episode occurs, you again open it, take out the device and then you respect that area which was misfiring.

Now, you do secondary retrieving, retrieve means you take it out, but if the material itself that you are using for implantation, it will dissolve after 10 days, then you do not have to do retrieval surgery. So, you can directly operate the patient by just putting that the area that is causing the episodes of epilepsy. So, to do this kind of stuff, we have to use the materials which are biodegradable in nature and not just biodegradable, but bioresorbable. So, these arrays will be degraded and dissolved. Now, why it, how it dissolves? Dissolves in the temperature depending on temperature and pH. The baseline LFPs, LFPs stands for local field potentials and LFPs during an induced disease that means when that is an episode and after recovery needs to be studied to understand the evoked response. So, these are background, now let us see research goals.

So, first suppose is we need to fabricate the device then to induce epileptic activities by peripheral forepaw electrical stimulation in rat. Then next is to record the baseline electrical activities with induce ictal and interictal discharges from a depth of a brain in an animal model. So, do understand now, again, let us repeat and understand, instead of using the bicuculline drug in this case, we will be applying electrical stimulation to create an epilepsy to create a seizure.

The baseline needs to be recorded from the depth of the brain in animal models, the spatial temporal analysis to determine the changes in electrical activities in different neurological conditions and distributions of signals across the depth that is another important research

goal. And how we will validate we can observe, what are the objectives, to validate a protocol to induce epileptic activities by using peripheral stimulation.

The next is to fabricate MEA, to record LFPs. The next objective is implantation of the array in animal models that is here in this case we have read as an animal model. The last one is once you implanted you to record and analyze the LFP signals in response to a convulsant or by using the electrical stimulation to cause the epilepsy, convulsant also has the role to create an epilepsy and electrical stimulation also can create epilepsy if you apply electrical stimulation in the forepaw of the rat. And, then do the same thing which is study the efficacy of the AEDs, efficacy of the antiepileptic drugs for to understand the efficacy.

The last one which is novelty. What is the novelty in this? One is a validation of forepaw stimulation to induce under ketamine anesthesia in rat. So, can you create an epilepsy in the ketamine anesthesia, when the rat is under ketamine anesthesia. Can you create an epilepsy by applying electrical stimulation to the forepaw of the rat? The fabrication of biodegradable array will be used to study the electrical signatures in normal epileptic and recovered conditions. Finally, the OpenBCI system, Daisy Biosensing Board was used or will be used for in vivo reading or recording from the rat's brain.

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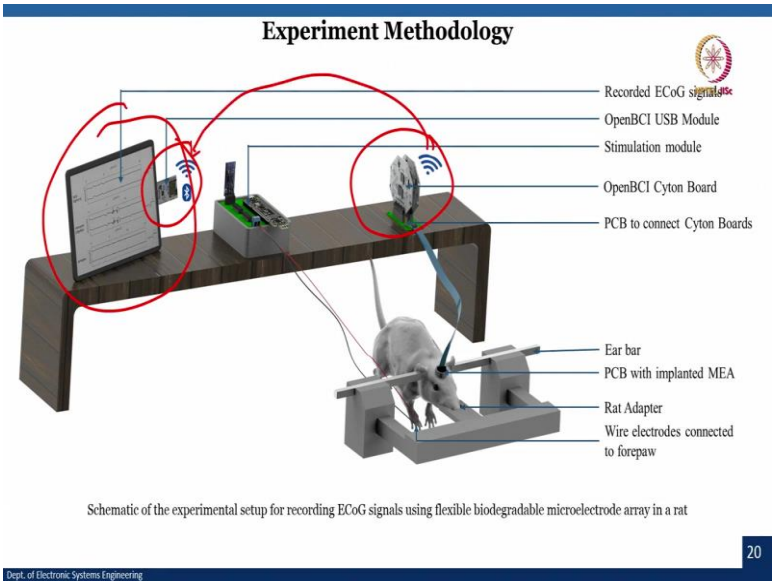

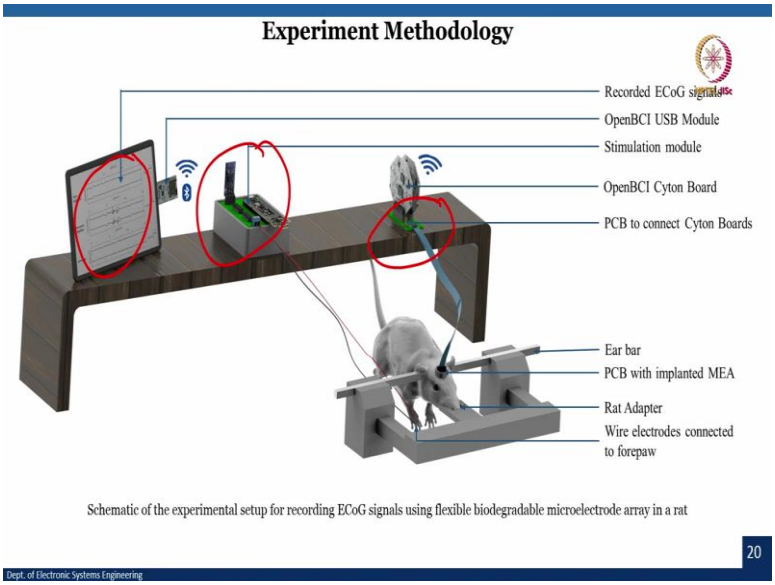

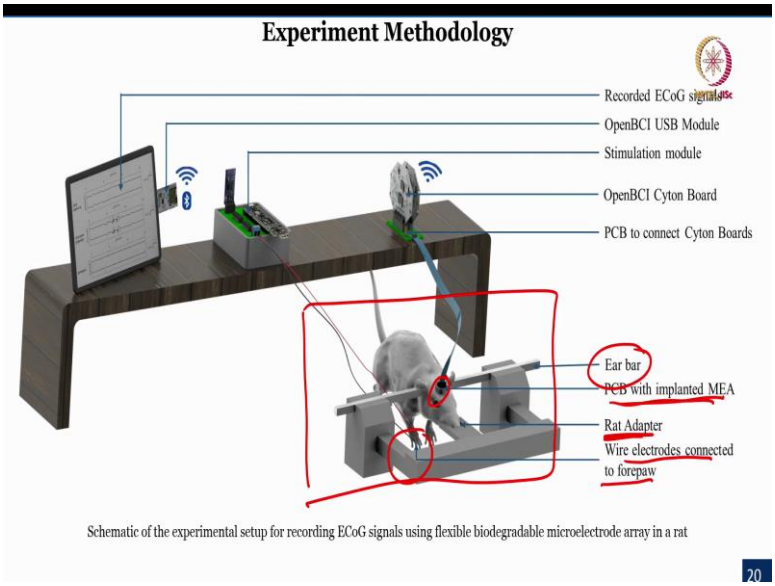

So, now, let us understand the experimental methodology. What are the experimental methodology? First what you see here, there is a tablet, this is a Dongle for Bluetooth. This is the OpenBCI Cyton Board and then the data is transmitted through the Bluetooth to your system. Within the tab you will be able to see the recorded EEG or in this case we because we are taking the signals from the brain it is electrocardiography or ECoG. There is an electronic module to apply electrical stimulation. This is a PCB, printer circuit board connected to the Cyton Daisy board.

Now, let us concentrate on this area. There are ear bars, PCB with implanted MEA, there is a adapter to the rat here, wire electrodes connected to the forepaw of the rat. So, this is a schematic of the experimental setup for recording ECoG signals using flexible biodegradable

microelectrode array. So, this is the experimental methodology. Now, we are to perform the experiments in a similar manner. So, let us start looking into it.

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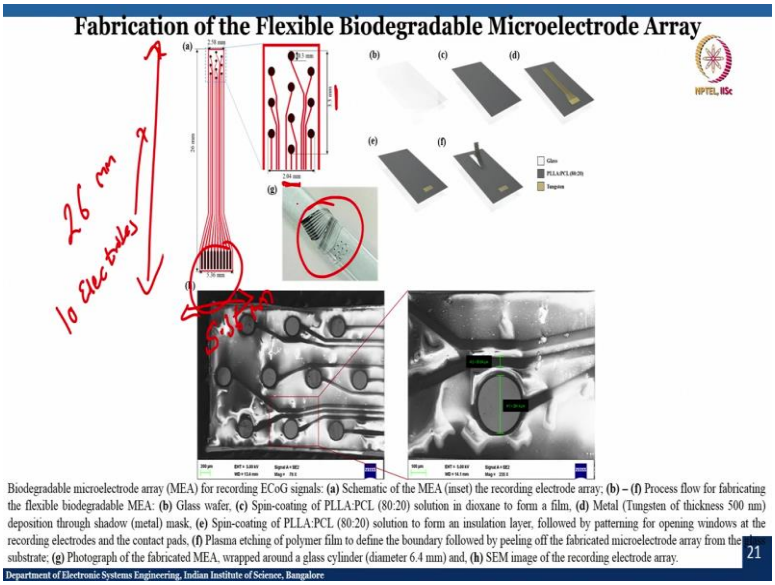

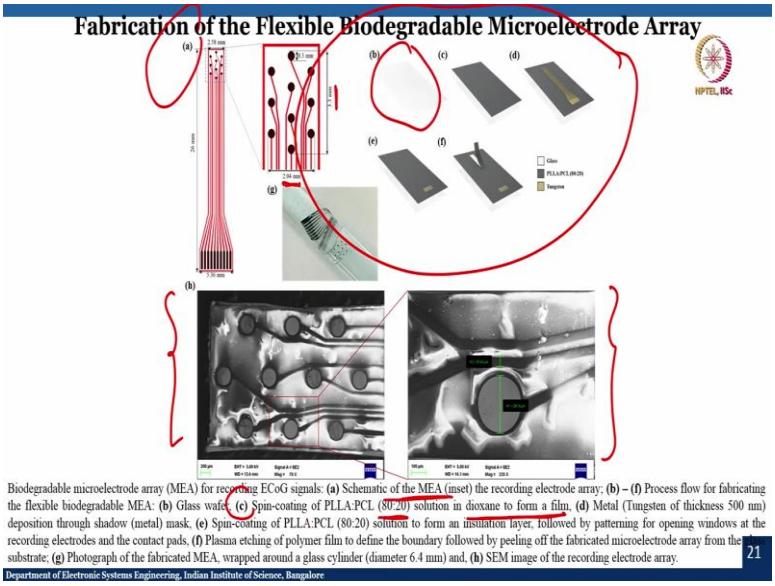

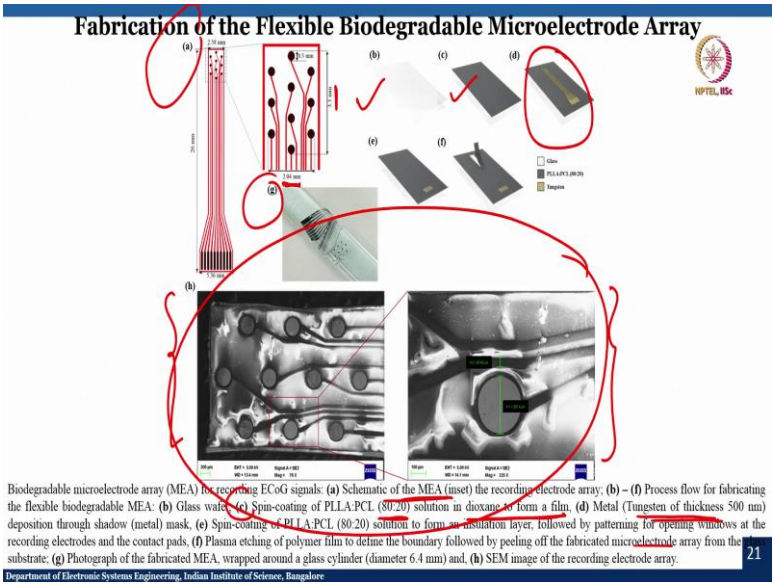

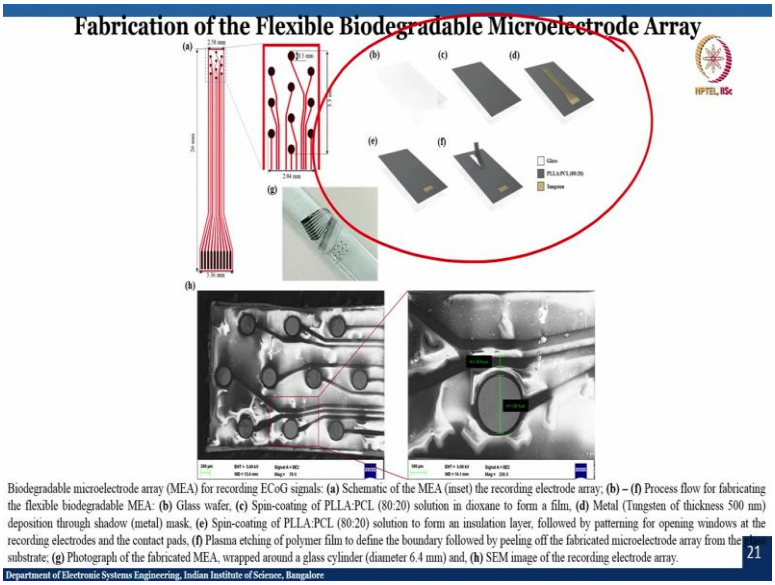

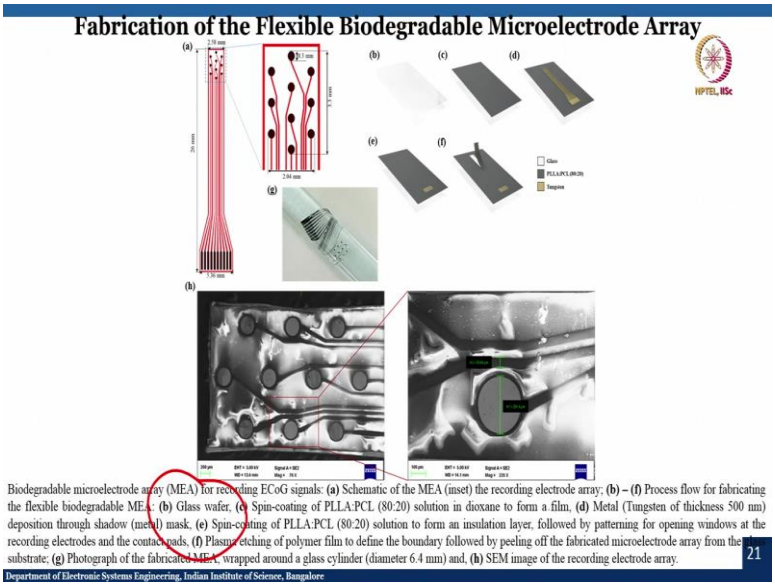

The first and foremost is we have to fabricate a flexible biodegradable microelectrode array. In this case what is the device? The device is this one. How many, what is the length? Length is 26 millimeter. What is the width? The width is 5.36 millimeter, this width and the length is anywhere here. When you zoom in the area of our interest where the recording electrodes are placed that area from the bottom of the bottom electrode to the top of the top electrode is the length is given. Length is how much? 3.3 millimeter by 2.04 millimeter that means that we can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 10 electrodes at max.

Number of electrodes are 10 electrodes and this will be on the flexible substrate as you can see here. So, now, let us understand how we can fabricate this device? But before we understand the fabrication of the device, it is also see the images that we obtained from the SEM. These are SEM images, scanning electron microscopy. So, biodegradable material

MEA for recording ECoG, Schematic of MEA which is in the inset process flow for fabricating the biodegradable material, which is this process flow here. So, the a which is this one is a process flow, like b to f is a process flow and a is a schematic of the MEA.

So, the b will be glass wafer. This is a glass wafer, spin coating of PLLA colon PCL in 80-20 ratio, in dioxane to form a film. So, that means that the glass wafer after glass wafer which is c spin coating of this particular material which is your PLLA is to PCL in the bracket 80 is to 20, I get over solution in dioxane to form a film.

The next one which is d so c, b is done and then you have c, c is also done. So, let us just write down here, yes, this is yes. Next one is d. In d what we do? In d, we have metal tungsten of thickness of 500 nanometer, deposition through shadow mask, metal mask is 5, because we do not have to do the lithography, if there is already tungsten that can be deposited on to a substrates like X, Y, Z like glass, like silicon anything.

So, the d is the metal deposition and then we followed by e with the spin coating of PLLA and PCL again to form an insulating layer followed by patterning of opening windows at the electrodes and the contact pads. Finally, plasma etching of polymer film to define the boundary followed by peeling of fabricator microelectrode array and the substrate. The photograph of the fabricator MEA wrapped around a glass cylinder and SEM image of the recording electrodes as shown in this particular figure. So, you can see that the g shows the actual photograph and h shows the SEM image, the electrodes which are not shiny are conductive. So, this is conductive.

Now, what we do is? Let us understand the process flow, and the process flow, really sorry. Let us see. Sometimes what happens is that we get too much into the thought process and we forget that what you are talking so, here we are looking at the microelectrode array and we need to understand the process flow. Now I have just told you what are these but do not worry about how to use it, we will now use it. So, the first one is glass wafer. You see glass wafer, so glass wafer followed by material try to form a film so, we will draw a film.

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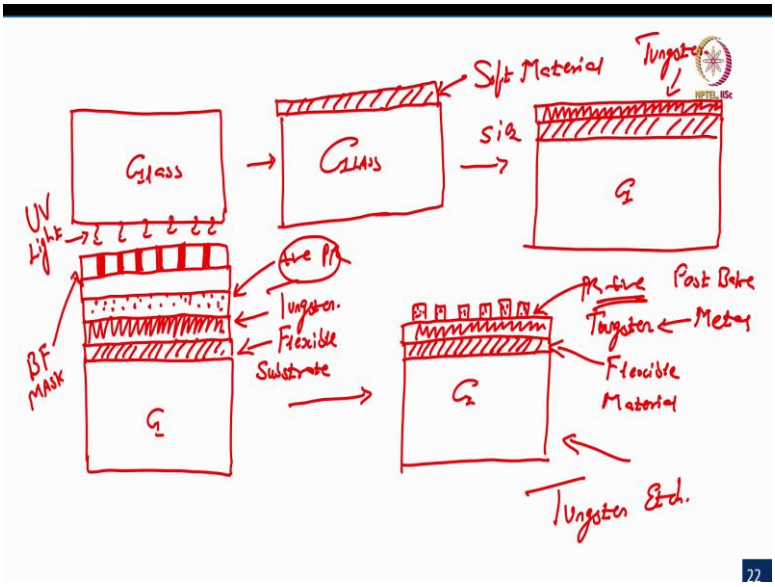

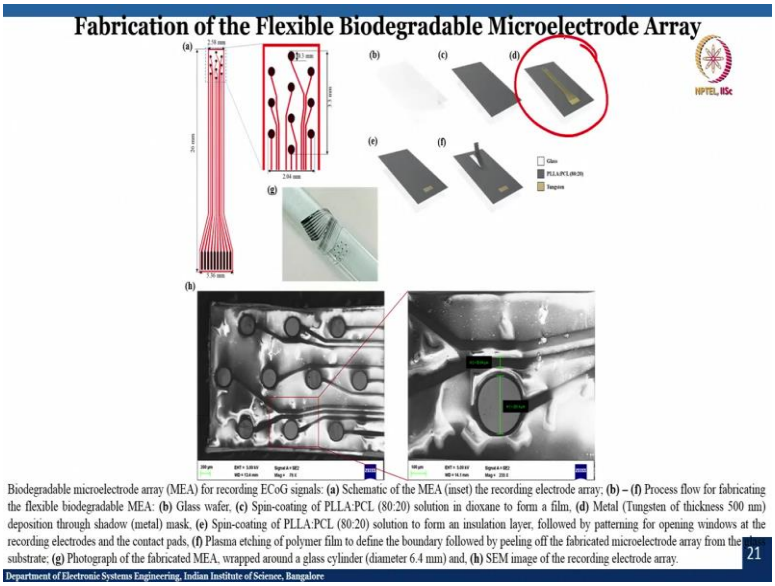

So, let us go to the fabrication process and it is very simple because we have to just design the electrodes. So, you take the glass wafer then on these glass wafer, we have spin coated the soft material, cure it whatever the process is. The next step in this one would be. Because you see here we have, in this case it is not gold. In this case, it is tungsten. Because tungsten is bioresorbable, is a tungsten. After this you need to pattern it. You see here, we had to pattern it.

So, for patterning, what we will do? We all know, what we need to do, but some of you may have forgotten, so I will draw for you. This is your flexible substrate. This is your titanium, sorry tungsten, on tungsten. So, in this case what happens is there are two ways, I will show it to you and let me draw the traditional way. Positive photoresist, we have coated the positive

photoresist and then soft bake it in 90 degrees centigrade for 1 minute on hot plate. Once you do that the next step is you all know.

What is the next step? Mask, and then mask you have to expose with UV light. So, what are the patterns on the mask? Patterns on the masks are following. So, what we have here is brightfield mask and this one is your UV light so, with the help of UV light you will expose the photoresist with UV light in it when there is a mask.

So, since it is positive photoresist the same pattern will transfer on to the photoresist or in another way we can say that the area which is not exposed. What are this? Photoresist, this is glass so, what we are doing here is that we have flexible material, you have metal which is tungsten, you have positive photoresist and positive photoresist is protected in the area or pattern such that the unexposed region became stronger in the mask and now we have also etch tungsten that is how it is now, see.

If I want to have one more step I can show it to you like this, this is how it is. So, if I did this wafer after patterning positive photoresist. What is a next step? Developing it and then post baking it. So, post bake, post baking at 110 degrees centigrade on hot plate for 1 minute. After this you dip this wafer in tungsten agent. And when you do that, what you get is?

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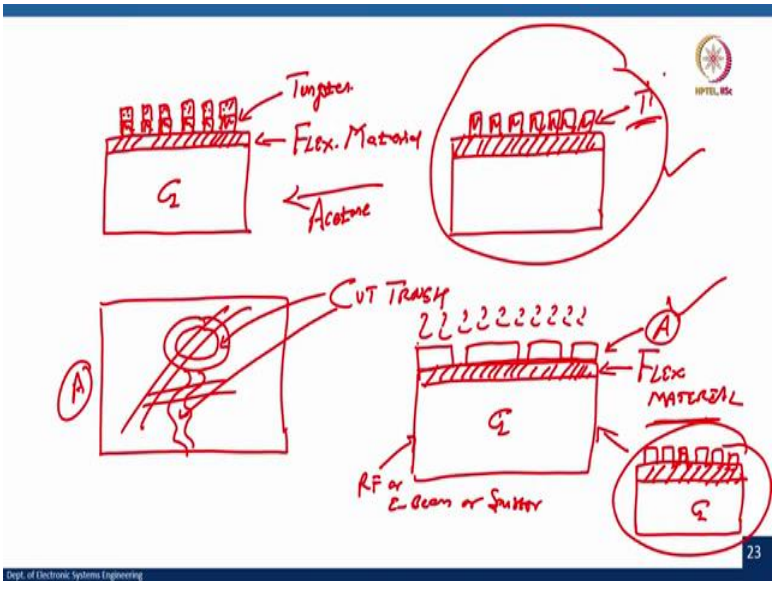
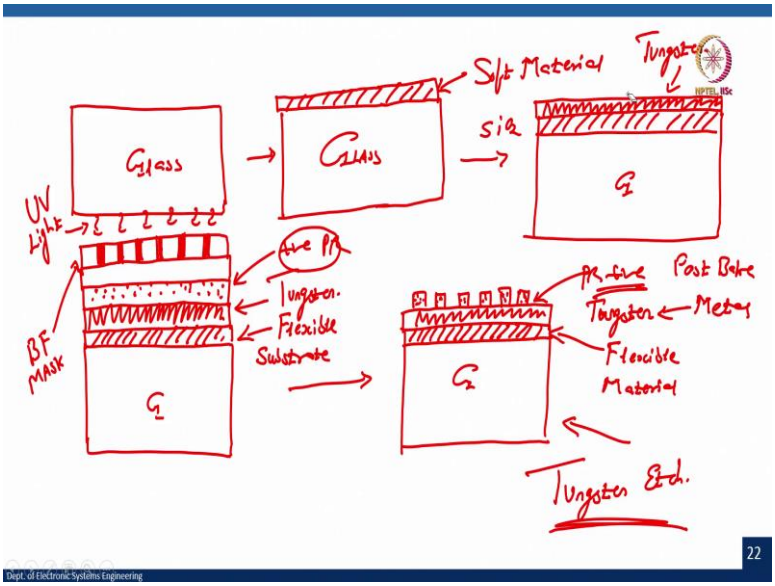
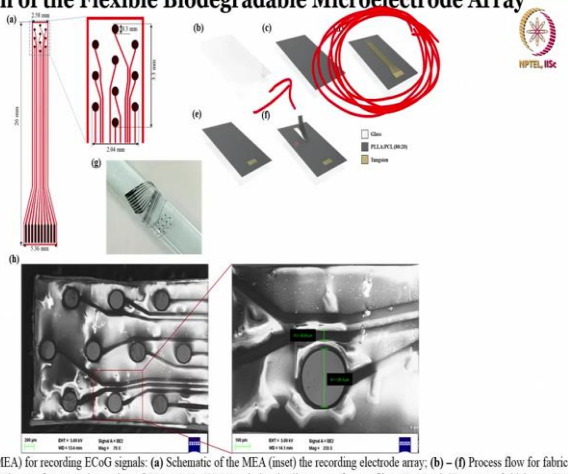




Fabrication of the Flexible Biodegradable Microelectrode Array



Biodegradable microelectrode array (MEA) for recording ECoG signals: **(a)** Schematic of the MEA (inset) the recording electrode array; **(b) – (f)** Process flow for fabricating the flexible biodegradable MEA: **(b)** Glass wafer, **(c)** Spin-coating of PLLA:PCL (80:20) solution in dioxane to form a film, **(d)** Metal (Tungsten of thickness 500 nm) deposition through shadow (metal) mask, **(e)** Spin-coating of PLLA:PCL (80:20) solution to form an insulation layer, followed by patterning for opening windows at the recording electrodes and the contact pads, **(f)** Plasma etching of polymer film to define the boundary followed by peeling off the fabricated microelectrode array from the substrate; **(g)** Photograph of the fabricated MEA, wrapped around a glass cylinder (diameter 6.4 mm) and, **(h)** SEM image of the recording electrode array.

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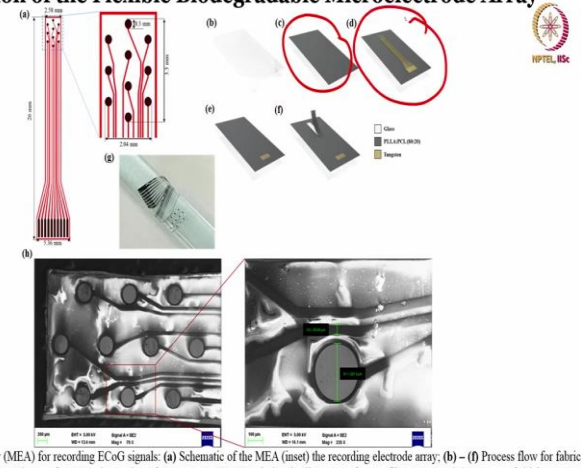

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This is what we will have after when you did the wafer in tungsten etch. What you will have is the tungsten will get etch in the area which was not protected by their photoresist and the area which are protected by photoresist, photoresist is still there. Next step after this dip the wafer in acetone if I dip wafer in acetone then what will I have, titanium. What will happen that when you dip the wafer in acetone, the photoresist will get stripped off and you will have your pattern, you see this pattern this one c from b to c these are the processes. Let me show you an alternative process or alternative trick.

So, if you want to pattern this gold as you can see here in the circle. What you need to do? Once you have this one, you can create a mask like this which is cut trough. What is cut trough I will just show it to you cut trough I will just give you an example. So, the white region this one is cut through. You got it. Now if I directly deposit material that means that if I take my flexible material is there and now what we need to do is, all this flex material you load the mask.

So, if I take the cross section of this mask then it will look like something like this and through this mask the next step would be, so, you can load this mask which is this one A after this you directly, this is a stencil mask you can directly use this in RF or E beam sputtering or RF or E beam evaporation or sputtering, so, what we have here is that we have a flex material on which there is a tungsten metal with fairly fine feature size and it can definitely stick on the flex material after this what you will have is that to this particular process if I remove the mask what I will have, so, you are again got the same thing that you got here, can you see this.

So, this is after that you just load the mask and deposit the wafer and you will have, I will just give an example there can be many, because the cross section can be like this, can be like this, it does not matter. So, this is an easy way to do it because it is a soft lithography. This is a conventional way of doing lithography both have a differences.

So, what I was thinking is that if you have a flexible material with tungsten as a next step that means that you have patterned tungsten as you can see here you can you have this PLLA and PCL mixture, 80 is to 20 on the glass and then you deposit directly patterned the gold using the stencil mask or trough mask after this step the next step is so, I will just remove it. So that we can quickly write it down for the next step.

The next step is to again coat the PLLA and PCL, why because PLLA, PCL is not just a substrate, but can also work as an insulating material, does not just work with a substrate but also acts as an insulating material. So, let us use spin coat PLLA and PLLA PCL.

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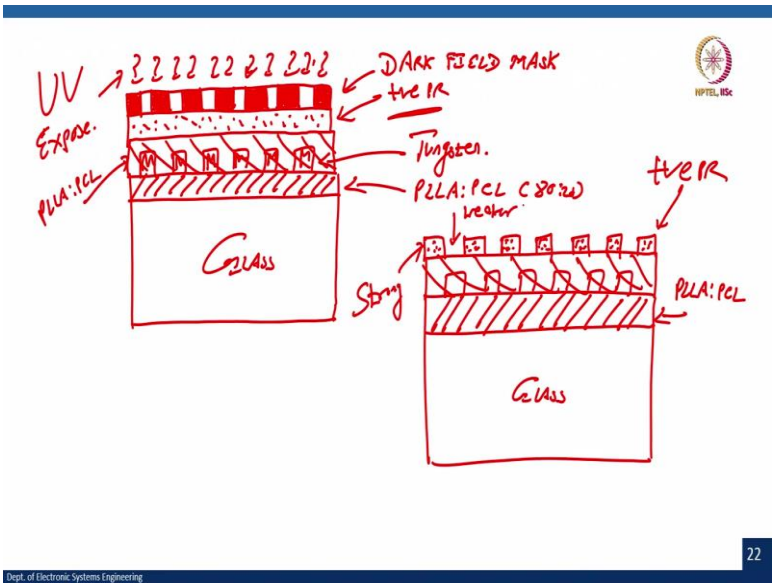

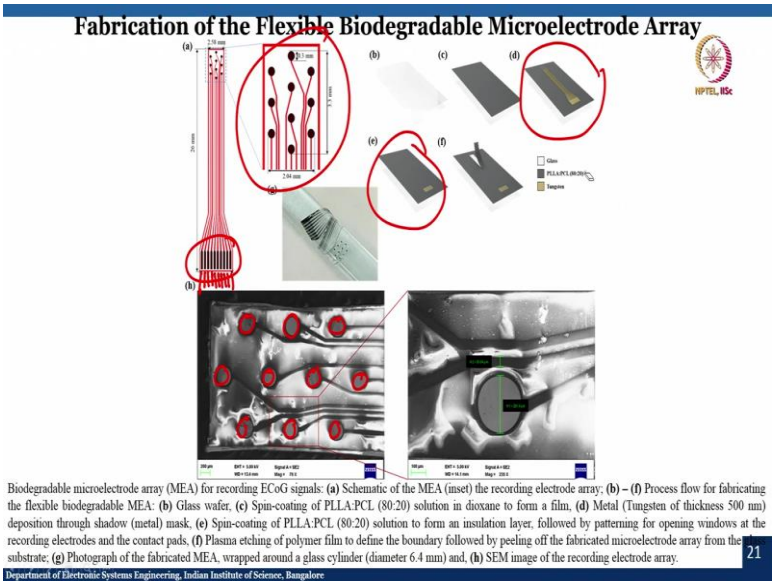

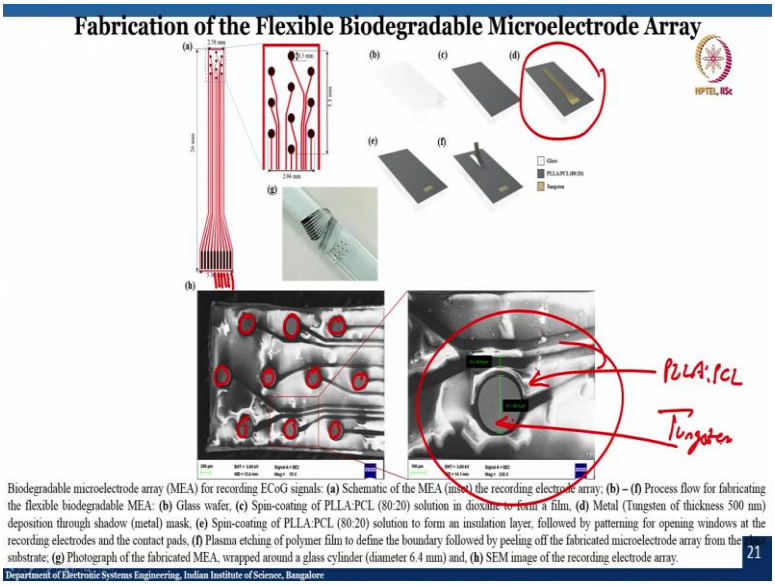

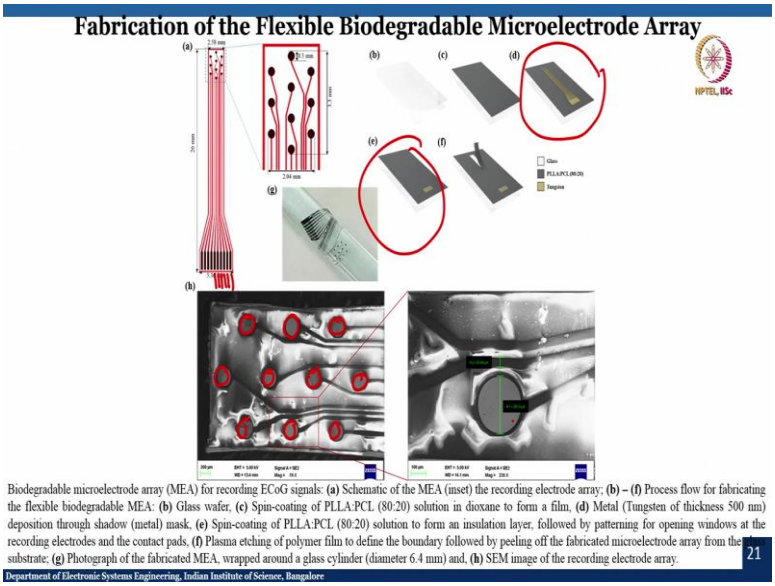

So, now what we have? We have fabricated our device on a glass substrate with this as PLLA, PCL, 80-20 ratio. On this we have our tungsten electrodes that everybody knows how you can create either liftoff technique or by conventional lithography, on this tungsten electrode I will again have PLLA and PCL. I am drawing like this, just to make it easy.

And then, I will open the windows such that I can access the contact area, what I am doing here, you see this particular image in this electrode, these electrodes are patterned then you have this PLLA PCL coated on those electrodes and then we will open the contact only in this region you will see and in this region 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 this black color contact pads. Only from black color contact pads and the electrode that is recording electrode, we remove the PLLA, PCL.

How can we do that? We have to open the contract you can see here very clearly it is these are this is the, this one is PLLA, PCL and this one is tungsten. So, from d, we have reached to e and now we had to open the contact pads and recording electrode pads. So, we had to perform the lithography and for that we can spin coat the positive for the resist, perform soft bake, followed by loading the mask and this mask should open the area from the contact pad and from the recording electrodes. So, in a way, we can if the positive photoresist is there we can use a dark field mask.

See, what kind of mass is this? This is a dark field mask and then you have UV exposure this is your glass, when you do this is a positive photoresist So, whatever the pattern is that same pattern will come and what will I have or what we can have, we can have a beautiful PLLA as a PCL as a substrate, this is supporting substrate is glass on this will have tungsten electrodes and then this second PLLA: PCL on that we have photoresist and let us show the photoresist by dots.

This is my positive photoresist after exposure when you develop the photoresist the area which is not exposed become stronger because it is positive photoresist or the same pattern that is in the mask will appear on the wafer that you can see very clearly that the area which was not exposed got stronger, the area which is exposed got weaker after this we will go for a post bake 110 centigrade one minute on hot plate, this is easy, everyone good.

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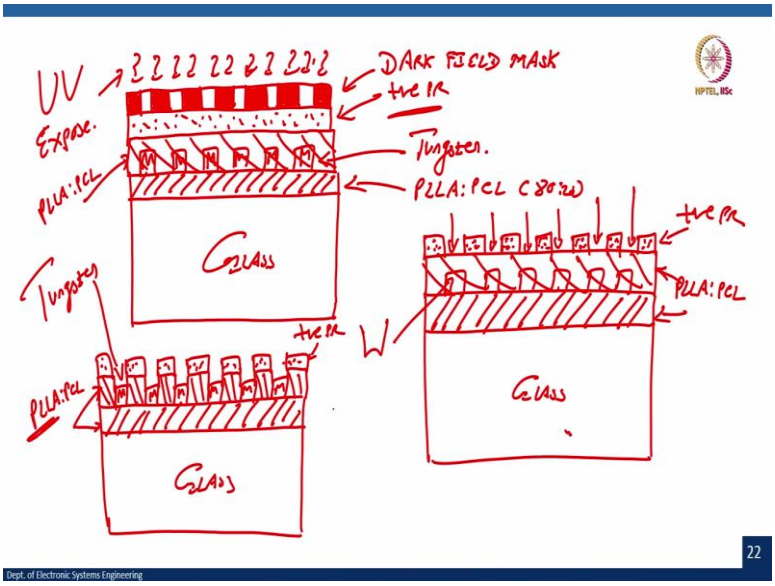

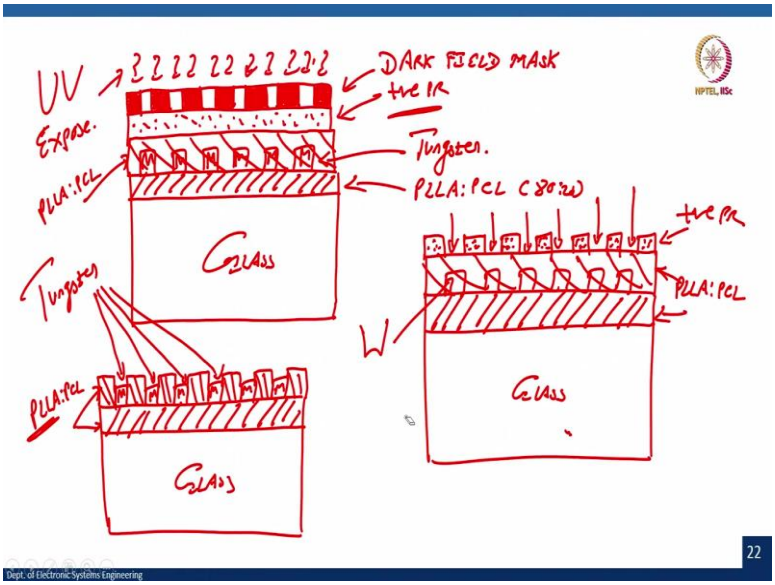

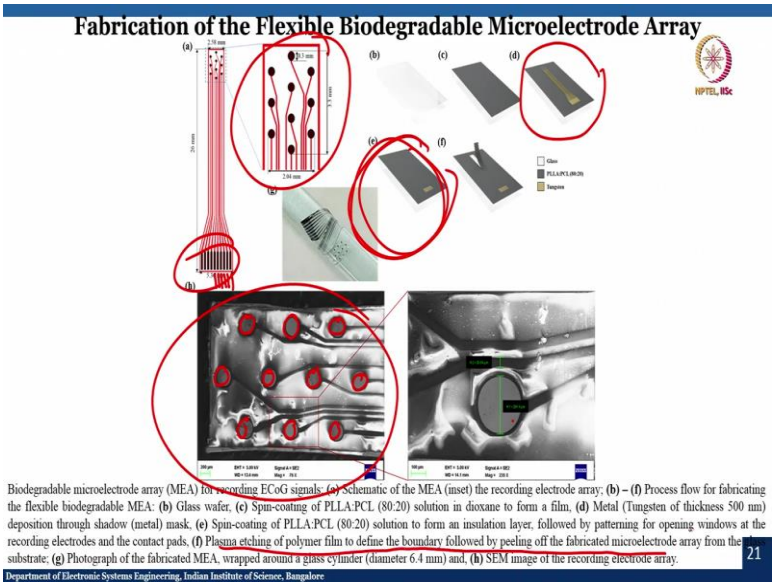

But, after this what is the step, after this step is that we have to etch the PLLA: PCL from these regions and this is my positive photoresist and these are tungsten. So, if I etch PLLA PCL from the area which is not protected using etching technique then what will I have? I would have tungsten PLLA: PCL and glass, easy because only the region which is protected by the photoresist like this one the PLLA: PCL will stay the region which was not protected by photoresist this will get etched as you can see here because it is a positive photoresist because that area which is not protected of photoresist will get etched in the PLLA: PCL etching.

And now, after this once it is done we can dip the wafer in acetone. If I dip the wafer in the acetone, what will happen? My photoresist will get etched. So, now I have my tungsten everywhere like this. So, where are we? We have reached here where the this one where the

PLLA: PCL is etched from the contact electrodes which is these electrodes and from the recording electrodes which are these electrodes remaining everywhere there is an insulating material, then last step is that you go for the plasma etching, f is plasma etching a polymer film to defend boundary by peeling off the fabricated microelectrode array. So, you can peel off the electrode array to be utilized for the experiments for the implantation.

So, what we have seen is? We have seen how can we fabricate this microelectrode array for implantation and once you have microelectrode array, now, the next step is that how we will implant it in the rat's brain. So, the these are the steps that we had to understand how to use it.

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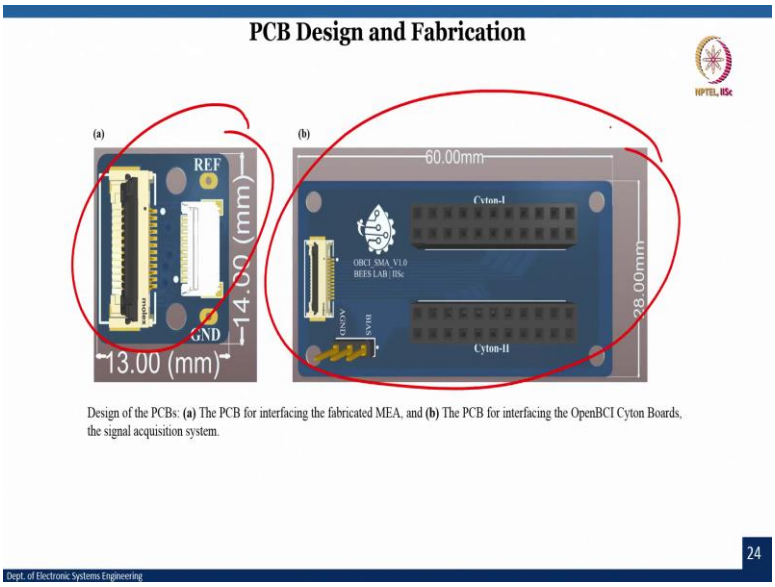

To attach the microelectrode array we have a PCB for interfacing the fabricated arrays which is here and then PCB for fabricating OpenBCI Cyton Boards which is this one b, a signal acquisition system. So, these are the PCBs that we have designed developed here.

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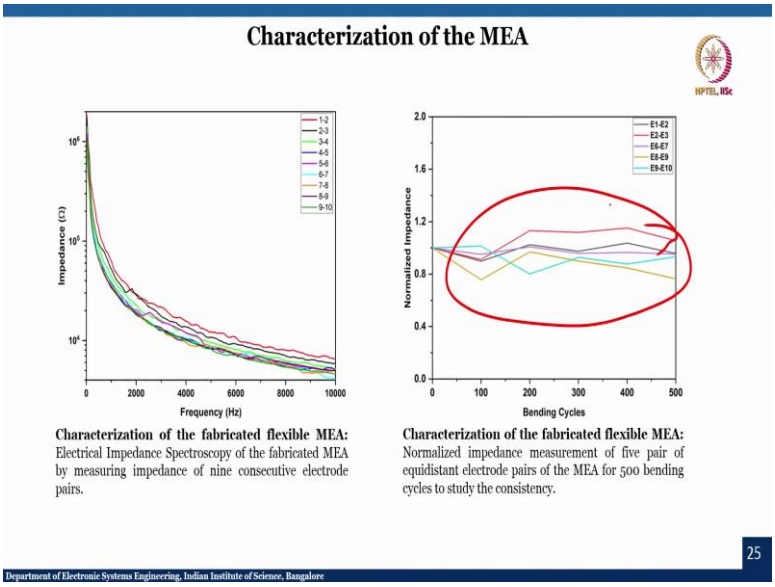

We had to characterize this microelectrode arrays. So, you perform this impedance measurement from 0 to 1 megahertz and what you will find that that the 9 consecutive electrode pairs we need to understand how the impedance is varying across different frequency. However, the normalized impedance should not change much when we bend the electrodes or bend the device many times, it is a flexible device. So, the impedance of the electrode should not change too often when you are going for higher number of bending or until it is 400, 500 times you can use this device.

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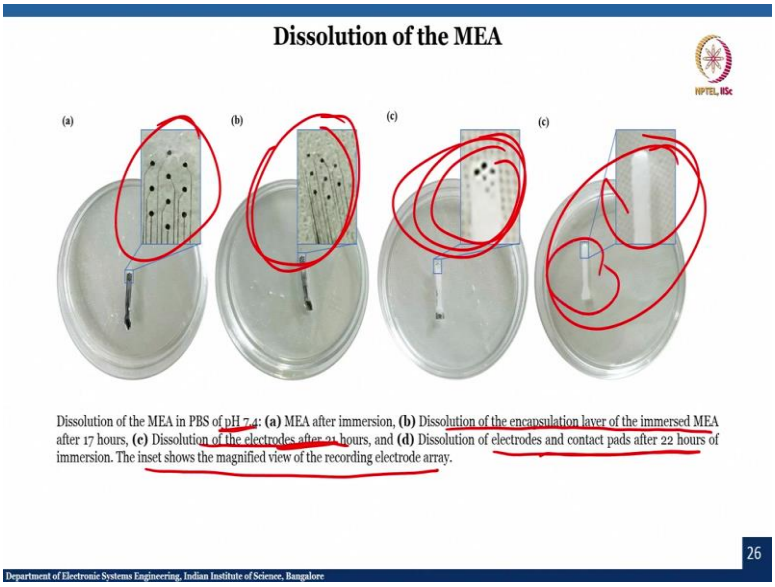

Now, what did I say? I have said that this is a bioresorbable device that means that if I have the device in pH of 7.4 the a one here is a complete device intact after immersion after you

immerse it in pH 7.4. Second is dissolution of the encapsulant layer of the immersed MEA after 17 hours. You can see here the dissolution of the encapsulating layer. Dissolution of the electrodes after 21 hours you see electrodes are dissolving.

These are electrodes in contact after 22 hours, everything is dissolved. This inset shows the magnified view of the recording electrode array here. Here is a recording array, here is the recording array, here is the recording array, here is the recording array from immersion to 17 to 22 you can see that the electrodes are gone. And if you keep this device for longer time, this device will be completely dissolved or absorbed, desorbed. It is bioresorbable, it will dissolved in the PH 7.4.

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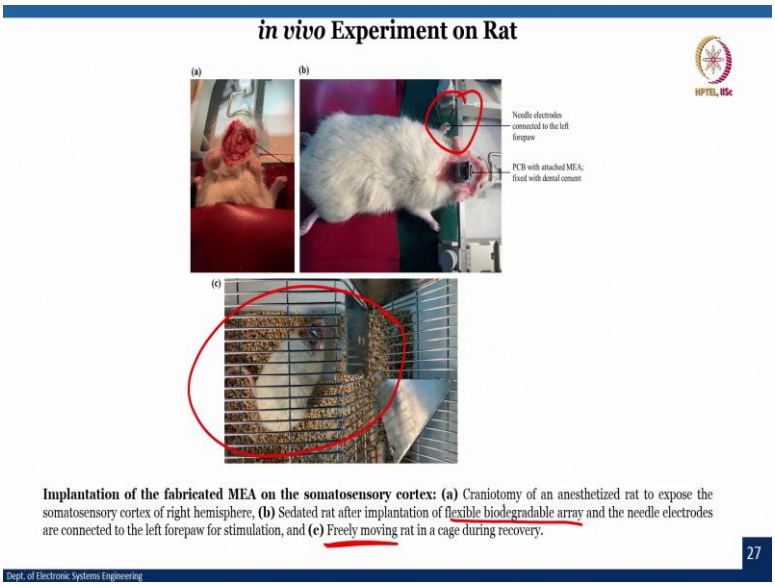

So, let us see that how we are going to perform the rat experiments. You can see here the rat brain is open and then implanted device onto the rat's brain and stricture it back. Now you have PCB attached to the microelectrode array which you can see here. That microelectrode array has been implanted in the rat's brain.

Once you do that, you let the rat recover. And then once the rat is recovered, it can freely move and then you start the experiment part. So, the a is craniotomy of anesthetized rat here to expose the somatosensory cortex is the region where we implant the device on the right hemisphere. The next one is the sedated rat after implantation of flexible biodegradable array and the needle electrodes are connected to left forepaw, you can see that the needle electrodes are connected here.

And finally, the free moving rat is here. So, we will see how to utilize each one during this anesthesia. Are we going to measure anything or once the rat is recovered completely how we are going to measure the signals?

So, what we will do is I will show you the way the surgery is done in the next class. And we will also see that once you implant the device, how can you create the epilepsy and when you create the epilepsy how the signals will look like. So, I think it is a lot of things that we have been learning in this particular class. So, let us do the final leg of this particular lecture. And then we will stop. So, since you have implanted a device, and you have stricture it back and you have basically it is fixed with dental cement and rat is happy then what the signals looks like?

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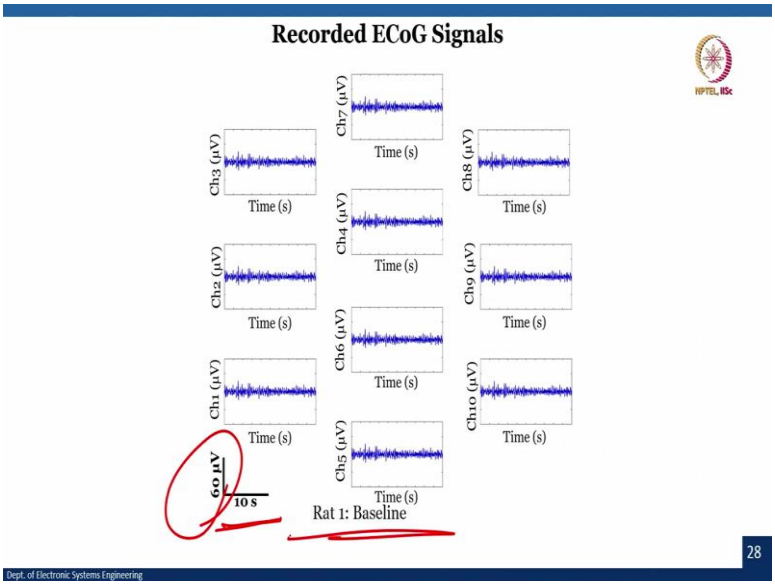
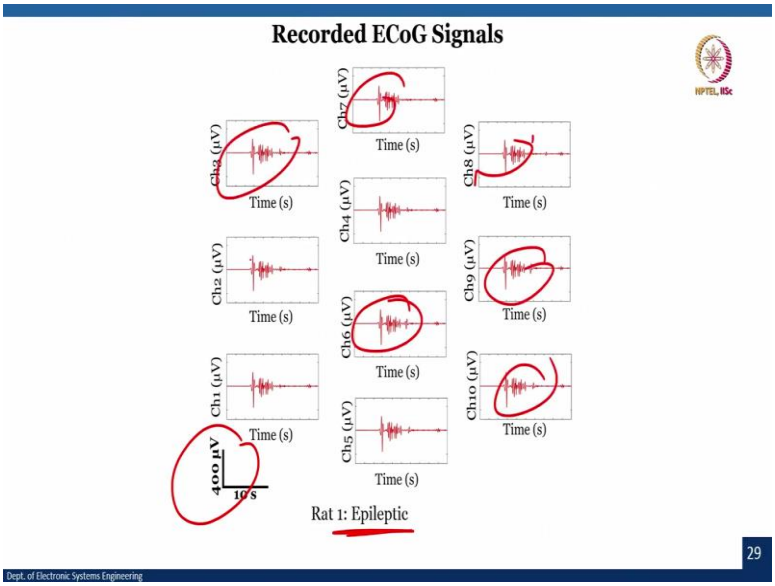
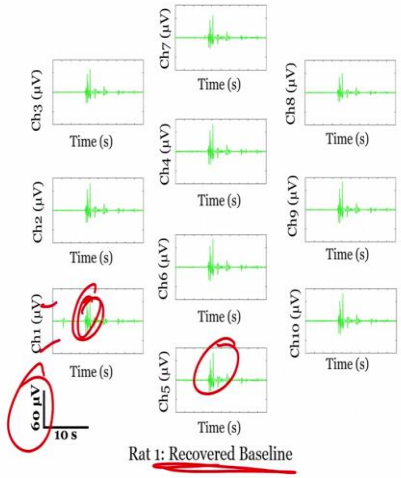


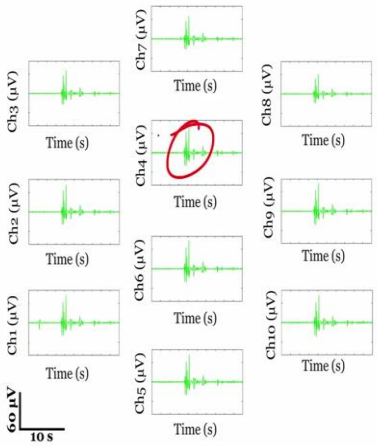


Recorded ECoG Signals



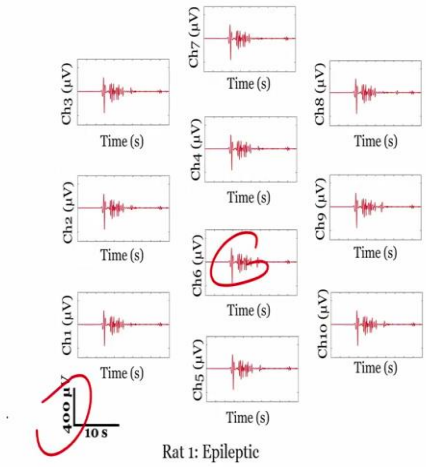
Rat 1: Recovered Baseline

Recorded ECoG Signals



Rat 1: Recovered Baseline

Recorded ECoG Signals



Rat 1: Epileptic

So, these are the baseline signals as you can see that 60 micro volts is the y axis 10 seconds data is a baseline signal, which are your ECoG signals. Now, you can see that from all 10 channels, we are able to find out the ECoG or acquire the ECoG signals, that means all 10 channels should be touching the brain surface. In this case, what you see is that we are looking at that recorded ECoG electrode, recorded electrocorticography signals. And for this one, we have created an epilepsy as you can see that the signals amplitude is now 400 milliseconds, sorry 400 micro volts.

Again, the readings are for 10 seconds, and this is the epileptic zone, because these are the burst of signals, as you can see, it almost kind of uniform adds in several channels. So, this is another thing of importance that we need to understand, we can create epilepsy by applying the electrical stimulation on the forepaw of the rat, that is why we have needed electrodes in the forepaw of the rat and then when you apply the stimulation that creates these burst of signals, which are the epileptic epileptograms, and then we induce or we administer anti-epileptic drug when we do that, what we can see is that now it is 60 microvolts.

So, we have kind of recovered the baseline. You can see this one, but still it is within 100 microvolts if there is a change only like within 60 I actually see from here to here is 60, it is just like 30 microvolts. So, we do not need to worry about this little bit of spikes of course, we need to understand if it is very high like this one, which is about 400 microvolts, why it is happening that means that the AED is not too effective.

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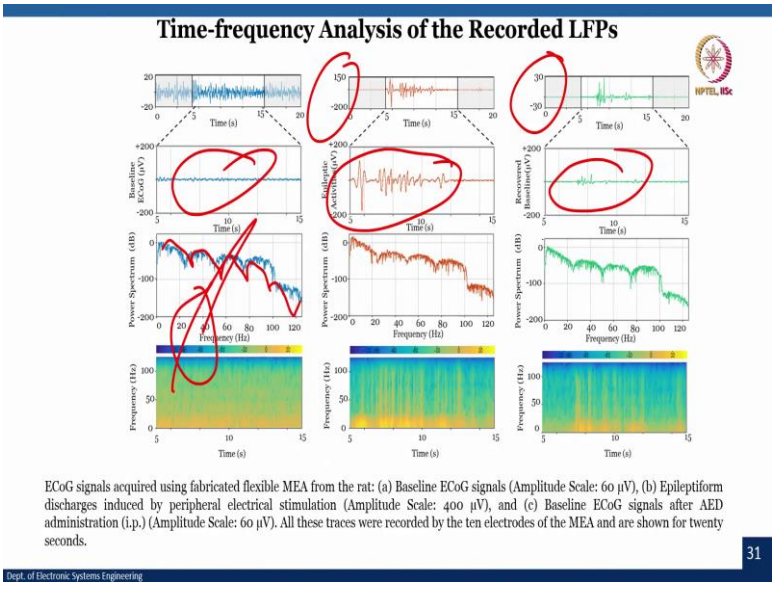

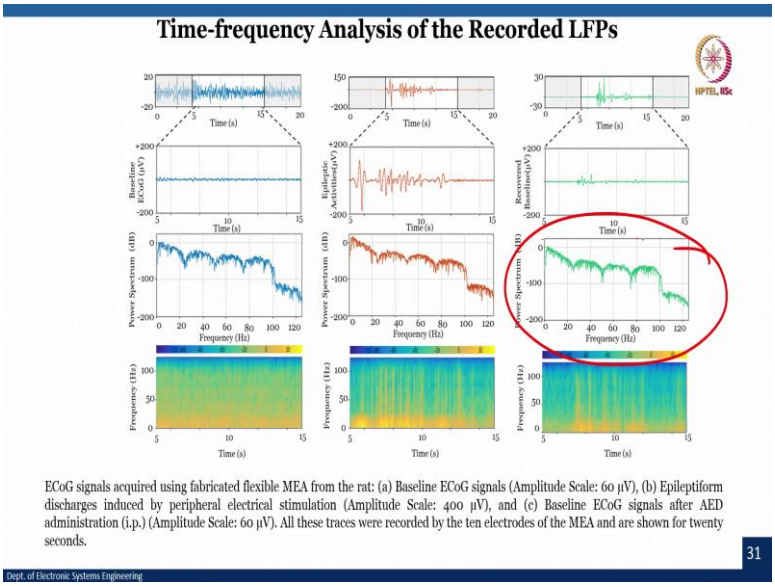

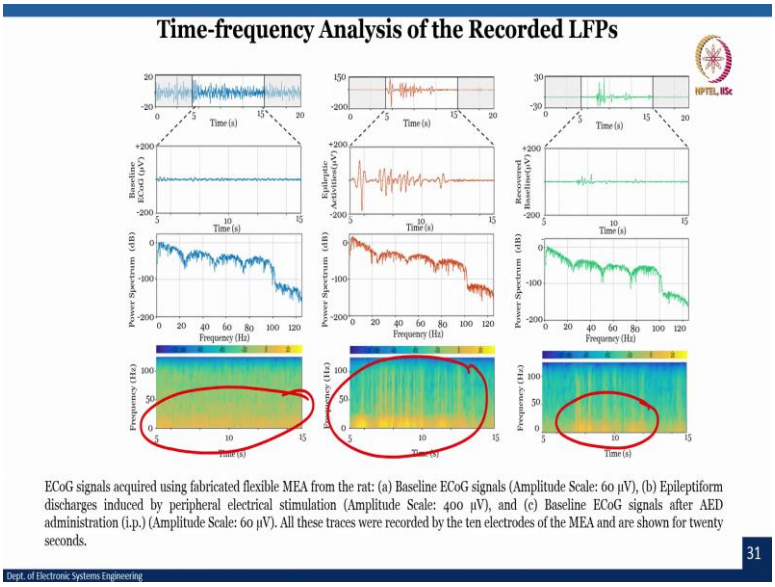

So, when you perform the time frequency analysis, the baseline here from minus 20 to 20. Here you can see that minus 200 to 150, here is minus 30 to 30 and for 10 seconds of data when we plot it from minus 200 to 200, you can very clearly see the epileptic episode here, it is not that here, it is not here, but when we plot the power spectrum analysis with respect to the frequency can very clearly see that the dip for everything is about in the dB is at least 20 dB for each frequency range from 20 to 40 you can see, 40 to 60 is kind of dipping like this and finally this.

Same thing goes here and same thing when we are administering the AED. So, from this frequency time analysis what we can see that in the initial stage you cannot see many yellow color spectrums, in this case you can very clearly see and here again it is kind of recovering back. So, one thing is that we have now bioresorbable microelectrode array, and we have we

have 10 electrodes for recording. All these electrodes along with the array is implanted into the rat's brain. We can apply electrical simulation to the forepaw of the rat sprain, create an epilepsy as you can see here and apply or administer anti-epileptic drug and you can see that the baseline is recovered. So, this is the slide.

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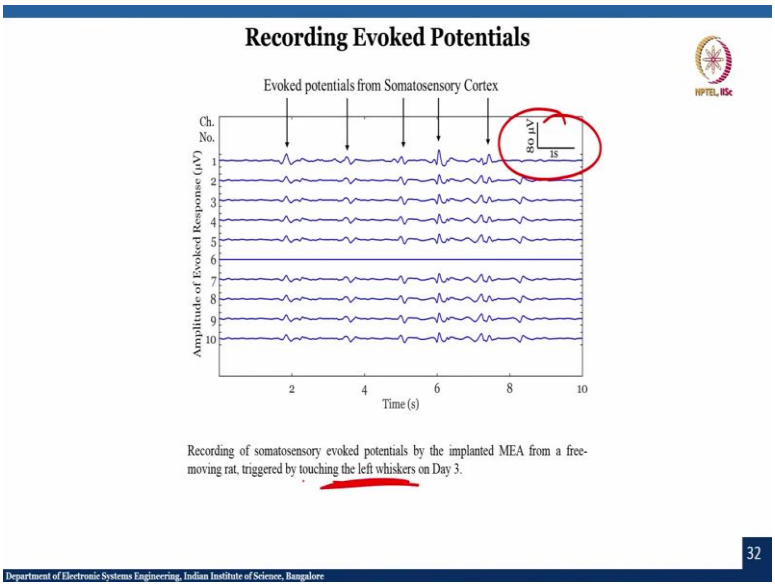

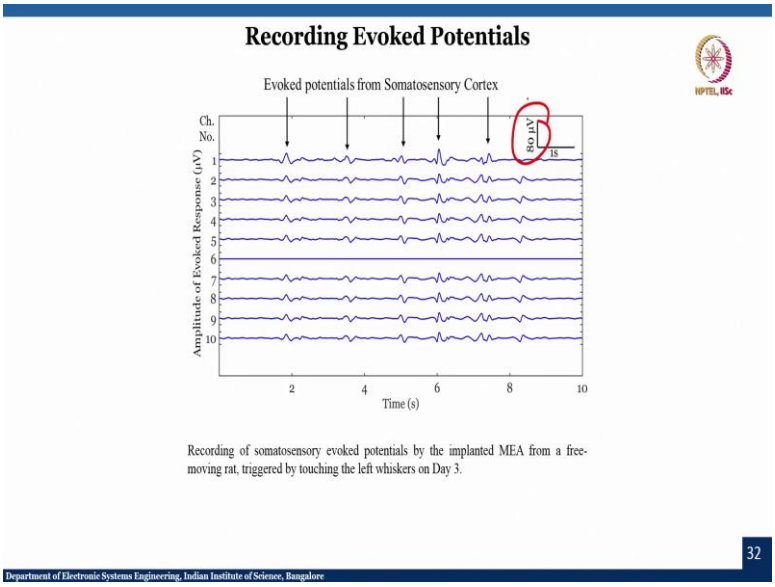

We can also record the evoked potentials. So, amplitude potentials at 80 microvolts 1 second, these are the signals. So, the recording of somatosensory evoked potentials by implanted MEA, for free moving, when the rat is free moving, then you can find out this somatosensory from the cortex by touching the left whiskers, when you touch the left whiskers immediately we will find out the change in the signals from the somatosensory cortex and these are called evoked response, these are in some microvolts range for each channel number and the

microvolts is about 80 microvolts, so y axis and it is for 0 to 10 seconds. So, for 10 seconds data we are showing it to you in this particular slide.

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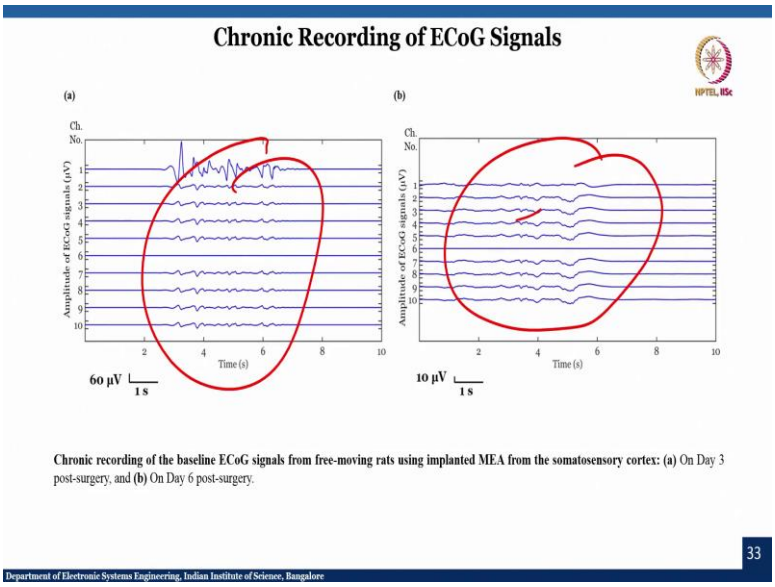

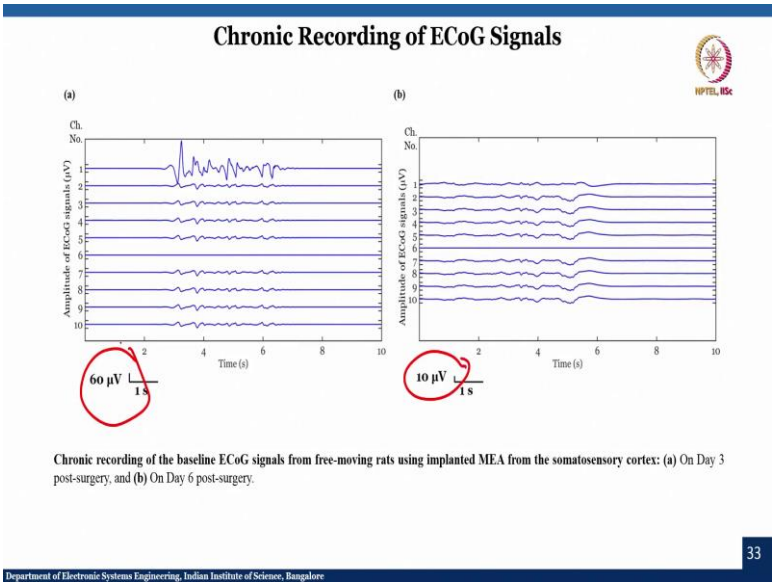

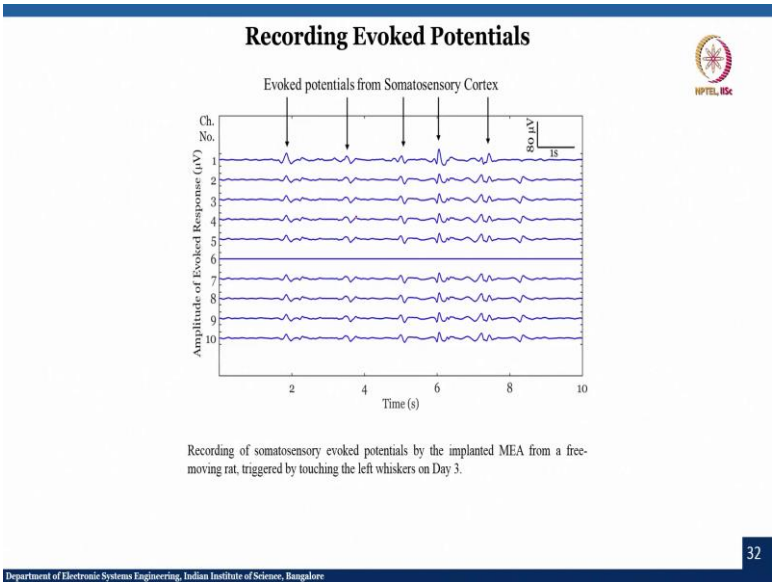

This is chronic recording that means that on day 3 post surgery on day 6 post surgery, you can see that slowly and gradually the device is not able to faithfully recover the signal the reason because the device is starting the electrodes are starting to be absorbed, bio resorbed on the rat brain. So, when that happens, the signal acquisition capacity of the electrodes will reduce and so you cannot see any ECoG signals, it is still 60 microvolts here you can very clearly see here also you can see, but at the 6 post-surgery the electrodes are dissolving.

Now, the one very fundamental question would be, why in the case of x the in vitro analysis only in 22 hours we were able to see the dissolving while in the brain it takes so long time? Because the pH the CSF is not in continuous touch with this electrodes. It is not continuously it is not dipped, it takes some time for bio reserving inside the brain.

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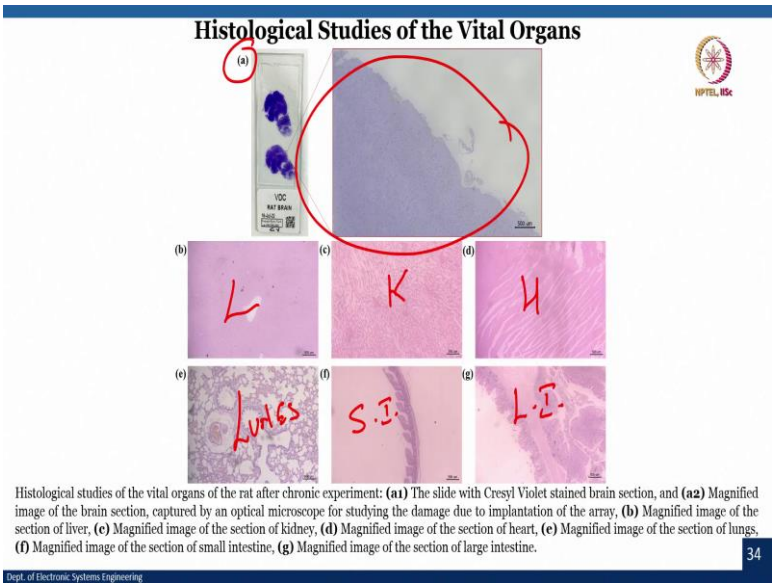

Now, these are the histology images from the rat's brain and some of the vital organs of the rat after chronic experiments. This slide with Cresyl Violet stained brain section is shown in a 1 which is your a here, but then magnified image of the brain section captured by optical microscope for studying the tissue damage is shown here then we have a magnified image of the section of liver. This is liver, then kidney, then heart, then lungs, small intestine, large intestine.

So, these are some of the organs, histology images from the organs. This is to understand whether the device that is absorbed in the body it is dissolved biodegradable, so, it is dissolved in the certain part of the body that is implanted, is it toxic? And is it creating any problem in other parts of the body or other vital organs are affected or not? So, we have to perform a histology study to understand whether these organs are affected or not. And this actual experiment should be for one to two to three months to actually understand how this the effect of these bio resorbed materials on to the different metal organs. So, let us stop it here what we have seen.

What we have seen is that if we have a microelectrode array and that too made up of the bio reserve material like PLLA: PCL, in 80-20 ratio and then we have materials like tungsten. Tungsten is also bioresorbable. Then you can have a biodegradable implantable MEA, microelectrode array that can be used for understanding the efficacy of AED. In next lecture, we will understand how the microelectrode or a needle electrode can be designed to be implanted in the rat's brain and get some signals out of it.

So, till then, if you have any questions, feel free to ask me through forum. We have updated all the questions asked by answering to them. But if you have more questions, feel free to send more questions. I hope you are understanding the next stage of the usage of the implantable devices in acquiring signal. Now we are just here to, until now what we have seen is just to acquire the signal. What about if we apply electrical stimulation and then acquire signal, so it is a advanced version. And that is what the course is all about.

So, we started very basic things, if you really understand and go back and see, we just started with silicone, our thermal oxidation techniques, lithography, PVD, CVD, micro machining and then we have seen that how flexible sensors are fabricated, then we deep dived and then understood bio potentials, then we further understood what are the different signals among that EEGs what we are interested in, how the EEG is generated, what is ECoG and then we have also further understand that, if you understand the EEG we can use to identify types of

epilepsy, then we further went, we went in or dive dipped in, dived in. And what we understood? We understood that now, if or that how the epileptic episodes are there, can we use this device to understand the efficacy of AEDs.

Then, we try to again do the retrieval surgery, what about we implant it and then reabsorbed it. So here we are talking about bioresorbable implantable MEA. Now going forward, we will look at the micro needle that can go in the cortical column, we have cortex cortical cortex is made of cortical arrays, and each array is a group of cortical columns. Each cortical column has 6 layers and how we can tap the signals from all these layers. So, we will look into this in the next class. Till then take care. Bye.