Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
Course Instructor: Dr. Hardik J. Pandya
Department of Electronic Systems Engineering
Indian Institute of Science, Bangalore
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Hi, welcome to this lecture. In this lecture, we will look at the devices that can be used or implantable devices that can be used to acquire signals from a rodent's brain, but in this case, we will talk about bioresorbable devices. In the last lecture, we looked at a device that has a 32-electrode microelectrode array, and we referred to it as high-density because we are using it for a rat's brain. In this lecture, we will be looking at how to design a device that can be used for the same application, such as understanding the efficacy of anti-epileptic drugs, but at the same time, how it can be used, and what the process is to fabricate the device. Additionally, we will discuss how the materials used for this device fabrication will bio-absorb into the brain and the device will eventually dissolve.

Let us see the background. The background is that while traditional ECOG electrode arrays can help us study activities from the cortical surface after implantation, they require secondary retrieval surgery. Isn't that correct? We discussed that once a device is implanted, the surgeon has to wait for the activities to be recorded, and then the device has to be retrieved after the activity is noted so that the surgeon can perform the resection. This retrieval or secondary surgery, to remove the device from the human brain, would create additional trauma to the subject. Arrays fabricated using transient materials can be used to create biodegradable microelectrode arrays or, we can say, bioresorbable arrays.

Depending on the temperature and pH, these arrays will degrade and dissolve in body fluids. Now, if we have these kinds of devices, we can understand their performance by recording baseline activities, which are the local field potentials and the activities during seizures. When we administer a drug, we can check whether the baseline can be recovered. So, what can be the goals of this particular work? The goals are the following: First, to induce epileptic activities by peripheral or cortical stimulation in a rat. In the last lecture, we discussed that we can use convulsion to create a drug to induce seizures.

In this case, we are going to use electrical stimulation. Now, if you recall one of the TA classes, we were discussing the electrical stimulation circuit, how to design that kind of circuit, and how we give the pulses to the rats for power. The second goal is to record baseline activities with induced ictal and intra-ictal discharges from the depth of the brain in animal models. Finally, we want to understand the temporal analysis to determine changes in electrical activities in different neurological conditions and the distribution of

signals across the depth of the device or brain. To achieve these goals, the following objectives must be met: First, the validation of the protocol to induce epileptic activities by peripheral stimulation.

The second goal is to design and fabricate a microgrid array to record LFPs. The third goal is to implant this device in an animal model, specifically a rat model, focusing on the rat's brain in that model. Finally, the goal is to record and analyze LFP signals in response to convulsions and the drug for detecting activity. However, I do not think we are focusing on convulsions here; rather, we are looking at electrical stimulation to induce epilepsy. The novelty lies in the validation of a post-stimulation protocol to induce epilepsy under ketamine anaesthesia.

We need to understand that there are different kinds of studies: one is an acute model, and another is a chronic model. In the acute model, generally, the animal is sacrificed after the surgery or after the experiments are done. In the chronic model, the animal has to live for a certain period, ranging from 1 week to 10 days to 1 month to 3 months, depending on the type of application you are looking at and the experiments you are performing. Then, you have to euthanize the animal, but in this case, when you perform any surgery, the animal has to be under anaesthesia. This anaesthesia can be induced using ketamine.

The fabricated biodegradable array will be used to study the electrical signatures in a normal case, which is the baseline, as well as in the epileptic and recovery conditions. Finally, the Open BCI Cyton Daisy Biosensing board will be used for in vivo recording. So, now here you see that we have a stimulation module. This is an older version of what we were using. Now, we have the hardware, which is a part of the TA class where we taught you how to design that particular electronic module and how this little module can generate the pulses so that we can provide electrical stimulation to the rat paw. There are several different protocols: we can either use DC or AC, and we need to determine the appropriate voltage, current limitations, and other parameters.

So, right now, let us not worry about those things, but understand that the electrical stimulation applied to the rat paw multiple times will induce epilepsy. What we are interested in is whether the device that we fabricate and implant in the rat's brain can capture those electrical signals, which are your ECOG signals. There is a PCB with an ear bar implanted MEA that acts as an adapter, while a wire electrode is connected to the focal area. There is a PCB that connects to the Creutzfeldt-Jakob boards, then we have the Open BCI system, an Open BCI USB module, and finally, the recorded ECOG signal that you can see on the display. This is our experimental methodology. If you look at the fabrication, it includes the following steps, and we need to understand the materials we are using to claim that it is biodegradable or bioresorbable. The materials we are using are PLLA, PCL, and tungsten.

Now, you do understand that these are all thin films, with a thickness of about 250 nanometers. For context, a human hair is between 50 to 80 micrometres thick. So, what are we talking about here? One micrometer is 1000 nanometers. So, when we say 250 nanometers, it is 0.25 micrometres. You do understand the dimensions: human hair is about 50 to 80 microns thick, so we are talking about 0.25 microns, which is close to 250 nanometers. We deposit this using sputtering or e-beam evaporation. But what is the base material? The base material is PLLA or PCL, but you have to coat this base material. We use glass as a holding substrate, and then on this holding substrate, we can deposit or spin-coat PLLA or PCL. After that, we cure the PLLA or PCL, followed by depositing tungsten. After depositing tungsten, we pattern it using photolithography techniques. As you know, the photolithography technique consists of the following steps: the first is spin coating.

After the metal is deposited—after tungsten deposition is done—what is the next step? You have to spin-coat photoresist. After spin-coating photoresist, the second step is soft bake. After soft baking, the next step is to load the mask and align the wafer. The next step is UV lithography, then you unload the mask and dip the wafer in the photoresist developer. The next step is hard bake. Now, since there is a metal involved, after hard baking and inspecting the wafer, the next step is metal etching. Finally, the last step is to dip the wafer in acetone to strip the photoresist, correct? After completing these steps, you will end up with this design. Once you have this design, you can then move on to the next steps. You will end up with this design, and then you can again coat PLLA or PCL. After this eighth step, we are here—this is the eighth step, right?

So, we are here, and after this, you again spin-coat PLLA or PCL, as it can also work as an insulator. After that step, you can open the contact pads and the recording electrodes. We remove PLLA or PCL from the recording electrodes. You can see here in the zoomed image that there are 10 electrodes, right? There are 10 electrodes. You see here, there are 10 electrodes. So, from these 10 electrodes, we will etch or remove the PLLA or PCL because these are supposed to conduct or acquire electrical signals. The same applies to the contact pads—if the insulating material is present on the contact pads, you cannot establish contact, isn't that correct? So, it is very easy to understand. Now, if you see this slide once again, in this particular image, the first step is glass. On the glass, you have PLLA or PCL, and once you do that, you cure the material, then deposit tungsten, and after depositing tungsten, you perform photolithography, as shown here.

After photolithography, you again spin-coat PLLA or PCL, followed by photolithography to open the contact pads and recording electrodes. In other areas, PLLA or PCL will still be present to avoid any short circuits. Then, you can release the device from the substrate holder. This is how we realize the device. Now, this is the device, which is shown to be 26 millimetres in length and about 2.58 millimetres—yes, millimetres.

36 millimeters 2.58 millimeters, correct. So, 2.58 millimetres in width, and then the contact pad is about 5.36 millimetres because we have to make proper contact with all 10 electrodes. Each electrode is about 0.3 millimetres, which is about 300 micrometres, right? 300 micrometres is the diameter of the electrode.

Each black circle that you see is about 300 micrometres. This black circle is tungsten, and from this electrode, we have removed the PLLA and PCL. In all other areas, PLLA and PCL are still present. Similarly, here the black areas at the bottom are the contact pads where PLLA and PCL have been removed. So, this particular device, which goes inside the rat's brain, is 3.3 millimetres by 2.58 millimetres, and then we can experiment.

This is a device fabricated in our lab. When you perform or go for characterization, you can see the SEM image of the electrodes. You can see this one; some electrodes are covered with some material. Now, the electrode area is empty, which is like tungsten. Empty means there is no PLLA or PCL, but all the other regions appear to have some material, don't they? This material is nothing but PLLA and PCL. So, on these recording electrodes, the PLLA and PCL are etched out.

If you look at the statements here, right in the figure caption: "Biodegradable microelectrode array for recording ECOG signals." A is a schematic of the MEA (microelectrode array). B to F is the process flow where you have glass wafers, and spin coating of PLLA and PCL (80:20 ratio in dioxane) to form a film. Then there is curing, followed by the deposition of tungsten with a thickness of 500 nanometers.

I mentioned 250 nanometers earlier, but in this device, we have used 500 nanometers. You can change the thickness, but in this case, it is 500 nanometers, not 250. The example of 250 still holds; I wanted to show you the dimension difference between human hair and a single human hair. One human hair has a diameter of about 50 to 80 micrometers, and 250 nanometers is 0.25 micrometers. So, 500 nanometers would be 0.5 micrometres. This is deposited using either a shadow mask or spin coating, PCL to form an insulating material.

See, again, there is a difference. One is that I mentioned we can deposit the metal and go for conventional lithography, but there is something called a lift-off technique and a shadow mask technique. So, let's focus on the shadow mask technique to avoid confusion.

We'll start with the metal mask. A metal mask has an open area, while all other areas are covered. This line shows that the mask is completely covered. This is a metal, a full metal. The area in question is a through-hole in the metal. Now, if I take a wafer and load this mask onto the wafer—wafer on the backside, mask on the front side—what happens

after deposition? After I unload the mask, the wafer will look like this. The tungsten material will get deposited through the through-hole present in the metal mask. This is your tungsten.

So now, no lithography is required, right? You just take a metal mask, load it onto the wafer, and you have your patterns ready. You can use this technique, known as a metal mask when the feature size is not too small. But what is "not too small"? That's difficult to define.

In this case, the diameter of the electrode is 300 micrometres, and the connecting wires, the lines that go through, are close to 100 micrometres. With a stencil mask, you can create through holes and connecting wires. For example, a metal mask might have a contact pad as a through-hole, and the same applies to all 10 electrodes. The entire design can be made with a metal mask.

After depositing tungsten, you spin-coat and cure PLLA and PCL, load the metal mask onto the substrate, deposit tungsten, and unload the mask to have the pattern directly. This process is done with a metal mask, which is also called a shadow mask because it shadows or protects the areas you don't want to deposit material on.

You can also use traditional lithography, as shown in steps 1 to 8. This is a more conventional approach. However, when the feature size is larger, like hundreds of micrometres, you can opt for a metal mask.

Now, let me explain the lift-off technique. Assume we have an oxidized silicon wafer—silicon and silicon dioxide. On this silicon dioxide, we have aluminium interdigitated electrodes arranged like this. Now, I want to deposit a sensing material on these electrodes. The sensing material could be for a gas sensor, for example. We want the sensing material to cover only the interdigitated electrodes, not the contact pads.

So, we have a photoresist, which we cure, spin-coat, soft-bake, expose, and load the mask, then develop the photoresist to create the desired pattern. After that, we deposit the sensing material as required.

Let us have a different colour for the sensing material, which is deposited everywhere, isn't it? Like this, let's say this is the sensing material, OK. Let's say it is indium oxide. This one is what? This one is our aluminium, and this one is SiO_2, SiO_2 and silicon, correct? So, you have the sensing material only in this region, OK, and this is a contact pad. So, contact pad 1, contact pad 2, and this is my indium tin oxide. These are aluminium interdigit electrodes, correct?

This is correct. If I want to—let's say A is our final design—if I want to go for A, the next step for this particular case is—this is step one.

The next step is, on one, we will first deposit the indium tin oxide and then we will have the photoresist spin-coated and protect the indium tin oxide from an area like this. So, this is my photoresist, and it is in colour so that it becomes easier. So, this is my photoresist. Now you said, how have you designed this photoresist? How is it patterned in this way? You know, right? Spin-coating photoresist everywhere on IN_2O_3, followed by soft bake, followed by loading a mask, and then exposing the photoresist through the mask, developing the photoresist, hard bake, and you will have this kind of pattern, right? Now, once you have this pattern, the next step is you dip this wafer in IN2O3HN, but the difficulty in this case is that the IN2O3HN, which is the etching material HN of X, affects what? Aluminium.

That means if I dip this wafer in IN2O3HN, my aluminium will also get etched. Why? Because IN2O3HN affects or etches aluminium. So, in this case, what can we do? You understood, right? The problem is if I go for this etching, you know what it will result in? Let me just draw it for you so that you have a better idea. If I go for this kind of etching, it will result in the following: you have IN, you have a silicon dioxide or oxidized silicon substrate—this is an easy way to say it—or you can say silicon dioxide on a silicon wafer. After that, what you have is the electrons, say aluminium electrons. But what will happen is that the aluminium electrodes, which are in the centre and protected by the photoresist—so what happened here? You see what happened when the indium tin oxide was getting etched in indium tin oxide etchant because indium tin oxide etchant also etches aluminium, the electrodes also got etched. You see here, there are no contact electrodes left. Why didn’t aluminium get etched from this place? Because it is already protected by the photoresist, isn't it? So, this is not useful because our contact pads are gone. These contact pads, do? For contacting or for connecting the interdigitated electrodes. So, if the contact pads are not there, our process fails. In this case, what can we do? Got it? In this case, what can we do? See, we need the contact pads.

So, let's see how lift-off will help us in this kind of case. How will the lift-off technique help us? So, for getting the indium tin oxide on aluminium, what we will do is we will take the wafer again—it is our oxidized silicon substrate. On that, what do we have? We have aluminium interdigit electrodes. Let me draw it like this, right? Now, what we will do is we will spin-coat photoresist. We will spin-coat photoresist. We have aluminium, SiO2, silicon, SiO2, thick photoresist, thick photoresist. After this, we will perform the lithography technique, and we will pattern the photoresist as shown in the schematic that I am drawing for you. See the beauty of lift-off, right? Amazing, amazing things to learn. So, now what we have is a photoresist, and this photoresist is patterned like this.

So, from this area, the photoresist is etched. See this area, this one? The photoresist is etched, right? This one, this one, this area. So, once the photoresist is etched or patterned, we will deposit indium tin oxide. We will deposit indium tin oxide, all right? Now, after depositing indium tin oxide, we will dip this wafer in acetone. When you dip this wafer in acetone, what will happen? You know the photoresist will strip off, and the indium tin oxide, which is on the photoresist, will be lifted off. So, what will you have? You will have an oxidized silicon substrate; the photoresist will get stripped off in the acetone because acetone is a stripping material for the photoresist.

So, what are we getting here? Why? Because from this area, from the electrode area, the photoresist will strip off. Now, when the photoresist strips off, the material on the photoresist will be lifted off. It will lift off because, while stripping, the material on it is lifting, right? So, it is lifting off. When you lift off, the area where the photoresist is not there, right? The area or the indium tin oxide, which is not coated on the photoresist, will remain intact, and that is why you have something that is our A star, and this A star is similar to A, as you can see from this drawing, OK. So, lift-off is a technique when your top material's etchant affects the bottom material. The top material's etchant affects the material at the bottom. OK, easy.So, the conventional lithography lift-off technique—now if you see the screen, this is what either you can go for, the metal mask, or we can go for the lift-off technique. After that, we have spin-coating this one to form an insulating layer. I told you that once you pattern it, you can again coat PLAPCL, and finally, plasma etching is used to remove the polymer from the area, which is your recording electrode and your contact electrode. The photograph of the fabricated MEA is shown in G, which is here, and you have an SEM image of the recording electrode array, which is shown in this case, OK. I'm sorry about covering the entire slide with thoughts of writing and drawings, but I thought that it is important for you to know that other than conventional lithography, we have a metal mask that can be used to pattern the material, or we have a lift-off technique when the material's etchant affects the material that is at the bottom. Similarly, as shown to you in the previous lecture, we require an electronic interfacing board.

So, you have the PCB for interfacing the fabricated MEA, which is shown here, and then we have the PCB for interfacing the open-side boards, which are the signal conditioning and acquisition systems. We perform the impedance measurement with frequencies from 0 to 10,000, using electrical impedance spectroscopy. We again test the bending for 500 bending cycles to see how the performance of the electrodes is from E1 to E10, and then we perform a very important experiment, which is the dissolution of the magnetoid array. So, with time, how the magnetoid array dissolve in the solution?

So, MEA in PBS of pH 7.4—you see here, right? So, 7.4, because it is similar to our CSF, which is cerebrospinal fluid. So, MEA after immersion in the solution shows the

dissolution of the encapsulation layer, as you can see here in this case, right? Then we have the dissolution of the electrodes, as you can see here. So, the dissolution of the electrodes and contact pads after 22 hours is shown right over here, and if we keep on increasing, then the whole material will get dissolved. Then we perform the surgery, implant the device, and the PCB with the attached MEA fixed with dental cement is shown here.

This rat recovers, and once the rat recovers, we take the data from the rat's brain. We take the data from the rat's brain, and you can see here the initial baselines for 3 different rats. We perform experiments on 3 different rats. You can very clearly see that there is an initial baseline, then when there are epileptic form discharges, what kind of activities are there? When there are recovered baselines, what kind of activities are there? Again, the scale bar is 1000 microvolts, 60 microvolts, and 100 microvolts.

So, you can see that these are all epileptic activities that are formed or caused due to the electrical stimulation on the rat's paw, right? It is not induced by drugs but by electrical stimulation. We perform the time-frequency analysis, as you can see on this slide. It is similar to our previous lecture where you can see the power spectrum for the baseline during the epileptic episodes the power spectrum for the recovered baseline, and so on, for 3 different rats. So, these are 3 different analyses. Then, what we are showing here is what happens after every 48 hours.

So, this is at 0—if you say time t equals 0 after the rat recovers—and then you wait for 48 hours. So, 48 hours t 0 plus 48 is 48 hours plus 48 hours, which is 96 hours, and in 96 hours, what we were able to see is that the recordings or the baseline were fading, and the same thing happens in the case of epilepsy and the AEDs. That means that with time, the electrodes are dissolving or bioresorbing into the brain of the rat. And now, we need to understand that once the material gets absorbed or degrades, what kind of experiments do you need to understand for the toxicity? So, we have performed experiments to understand whether the heart of the rat, lungs of the rat, liver of the rat, and intestine of the rat show any toxicity because of the device that we implanted in the rat's brain, OK. This particular plot shows the evoked response extraction from the somatosensory cortex.

So, the recording of somatosensory evoked potential by the implanted MEA for a free-moving rat triggered by touching the whiskers on day 3. So, when you touch the whiskers, these are the responses that we get from the brain of the rat from the somatosensory area. We see here how the signals are fading concerning time on day 1, on day 3, and on day 6. You can see that on day 6, the signals are fading, showing that the electrodes may have degraded into the brain of the rat. Now, why do we say it may have? So, this is the last slide for this particular lecture.

The point is that why we say that it may have degraded or it may have bio resorbed is because I am using MEA, right? It is dissolved because we need to cross-check not only from the signals that they are fading but also from the images that we can obtain if we have an animal MRI. If you have an animal MRI, we can see that the metals are fading on the electrodes, and that will confirm the data that we are obtaining. So then, we can say with certainty that it is because of the degradation of the metal, right? But here, the purpose is to fabricate the device, implant it into the rat's brain, and understand whether you can create epilepsy by applying electrical stimuli to the paw of the rat and whether we can recover the baseline. So, with this, I will stop here, and I will see you in the next class with a microneedle, and then we will stop there to cover the entire course structure.

Till then, I will see you in the next class. If you have any questions, feel free to ask us in the forum, and we will try our best to answer your questions. Thank you.