Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Week - 06
Lecture - 21

Welcome to this lecture. In this lecture, we are going to talk about a flexible macular electrode array. If you recall from the last lecture, I have shown you how the fabrication of such kinds of devices can be done using our photolithography process. So, now let us see once a device is ready, how can you interface this device with an electronic interfacing board and then how can you collect the data? Now, I am sure that in the TA class, you were shown that once the data is there, right? How to process the data, is similar to the EEG data that you acquire and then we use the Saturn board and then we process this using MATLAB. We have some digital filters. In reality, everything can be done or everything should be done on the hardware, right? With software that can be your form where can be there, the software should be used mostly for UI purposes. So, we wanted to understand how the device performs and what kind of neural signals we can acquire. Once we understand how these neural signals are acquired, which are called local field potentials, since it is a signal coming from multiple or more than 5-10 neurons, we do not, we cannot say this is an x n potential. And once you have these signals, we want to see whether we can understand the efficacy of an appropriate drug, right?
So, this starting was the ECoG device, which is an electro-cartography device that can be used during surgery to understand. That can be used invasively, right, through the surgery process where craniotomy is done, the device is implanted, the skull is stitched back, and then the data is acquired to understand which area in the brain is misfiring. Again, you do the craniotomy, remove the device, and reset that part of the brain. So, to improve the resolution, we started fabricating a 32 or high-density microelectrode. Now, 32 numbers look very small, but as I said earlier, in the rat's brain, this number is big. So, even 32 channels become a high-density microelectrode. And if you see the slide, the material that we used as a substrate is polyimide. The polyimide was coated on a silicon wafer, but finally, the device is made out of a polyimide substrate and on the polyimide substrate, we have titanium and gold. Once you pattern titanium and gold in this particular fashion, which is here, we can again spin core polyimide as an insulating material and then perform photolithography to etch polyimide from the recording electrodes and the contact electrodes, right? So, once you do that, you can realize the device and the device looks something like here, which is figure number g.

We can also look at the SEM images and from SEM images, we were able to see what the electrode size is. Now, this is already what we have discussed. Now, once you have the

device, then you can use the EIB, which is an electronic interfacing board. This interfacing PCB is designed to interface with EIB to signal acquisition, which means, that one is for interfacing with the device, and the second device is to use or to connect with the signal acquisition system. Now, that is one thing that we can connect the device to the EIB, but how this device will stay on the rat's head, right? It will not fall. So, we need to fabricate an EIB holder. Now, you may have seen or if it is not still there in the lecture which is covered now, we have a 3D printing laboratory experiment.

Now this 3D printing laboratory experiment will show you how to design 3D printing casing and how to print it. So, with the 3D printing, we were able to design an EIB holder which is an electronic interfacing board holder for housing the EIB and microelectrode ok. So, you can see here the EIB and the microelectrode are housed within this EIB holder and now once you do that this device you can see this area here that goes into the rat's brain and the remaining things with this contact pad which is right over here right this one comes out which can be connected to the EIB holder. So, now this entire thing will fit well on the rat's brain. So, there is a hole for this screw multiple holes right.

So, that the EIB holder stays form on the rat's brain or rat's head ok. Now corresponding engineering drawings are shown in Figure A, top view side view isometric view and front view. Where b shows the explorable view of the 3D-printed EIB holder. Again like I said if you have already seen the 3D printing lab recording then you already know, if you are not seen then you will see in subsequent lectures how the 3D printing can be done when you see the recorded lab videos. Now once you have this you need to also understand the impedance value of my different devices. That means that you have to take the contact from 32 channels which is why you can see this whole wire on the right cables.

You can then see or measure the impedance by doing a frequency sweep from 0 to 10000 which is your 10 kilohertz and what we found is that the frequency or the average impedance of the electrodes is close to 29-kilo ohm at 1 kilohertz which is similar to what we observe in the literature. Now since it is a flexible device. So, when you flex the device and when you make it again straight, is there a change in the impedance because of this flexing? So, to do that we have done the banding cycle test and about 250 times we have banded the device and measured the impedance. When we did that we found that the impedance values are not significantly changing when we normalize the impedance values.

This impedance measurement was called electrical impedance spectroscopy which is EIS; this was performed after immersing the recording electrodes into a PBS of 7.4 pH. Now 7.4 pH because it is close to the CSF pH. CSF stands for cerebrospinal fluid.

Now once you have everything ready you need to start performing the experiments on the rat. So, there are 3 terms, one term is and I think I have discussed this thing. Let us revisit this term, in vivo within the body, ex vivo tissue is taken out of the body and studied in vitro when the cells are grown in the laboratory. In vivo within the body, ex vivo when the tissue is taken out or the blood is taken out and you study that particular biosample are ex vivo studies. In vitro is when you take a few cells and grow the cells in the lab to make a different kind of structures using the cells right or you can say that for example, people study spheroids right.

If you further understand the role of spheroids can be to study the effect of the immunotherapy drug or chemotherapy drug in the in vitro environment which is the environment that we can have in the laboratory. The spheroids are formed using the cells and cells are loaded into the U-tube plate. Then the spheroids, the cells accumulate together to form some kind of structure like this, what we call a spheroid. These spheroids are studied further for several applications my question or the the course is not to understand how the cell culture is there how we can use the spheroids what chemotherapy or what immunotherapy what are the T cells, what the helper cells or killer cells or regulatory cells the question is about in vitro. So, when I say in vitro it is growing the cells in the laboratory or performing experiments in the laboratory with the samples that are made in the laboratory. This is the easiest way that I am using to explain to most of these students who come from different backgrounds.

So, do not hold me accountable for every small definition that I am using and am trying my best to make it as easy as possible. So, that you all understand what the terms mean. In- within the body, ex- take out the tissue and understand our cells and that is that. In our case we are looking at tissues so, is tissue and understand and then when say in vitro becomes something in the laboratory. So, that is what it is now if you see this particular image. What you understand is that when the device is implanted into the rat's brain the recording electrodes right the recording electrodes are in contact with the brain. Once you do that once you place it well you can put a dura back and multiple screws are used, one is for reference, one is for the ground followed by stuttering the brain back or the skull back or placing the part of that bone which was taken out during craniotomy back on the brain of the rat and followed by the dental cement which is right over here.

You already have the device coming out through the remainder of the device coming out from the brain which is connected to the EIB holder. Through the EIB interfacing board the connections are taken out. So, this is the whole process and you will appreciate the complexity, but at the end of the day, the rat is alive for the time that we want it to be alive. There are certain protocols that we need to follow, so that the animal is not

uncomfortable after the surgery and we have a vet with us in the institute who takes care of all the protocols that we have written through which we get the ethics. So, we have the surgeon to do the surgery, we have the vets to understand the protocol is followed, we have a proper animal facility. I have shown you what kind of operation room we have for rodent surgery with everything that you require for performing the rodent experiment.

Since we are talking about rodent experiments we will stay there we also have a non-human primate and we will not go to that section in this particular course, but maybe in the next course sometime in future. Now once you have recorded the ECoG signals which are your electrocardiography signals you can see that the signals from 32 channels are acquired baseline these are called baseline signals because these are initial signals that you obtain from the brain without creating or inducing epilepsy using convulsants or using the electrical stimulation on the red spot because both will create epilepsy. So, here you can see that all the channels are showing data and the y-axis is about 200 microvolts, this is for the 10-second recording that we are showing. So, that you understand and can see how channel 1 to channel 32 how the channels are behaving. Some channels are flat for example, chanel number 9 in this case shows kind of a flat line channel number 22 is a flat line. So, that happens and certain channels start recording certain channels will start kind of not showing good data because the channel is because the electrode is not touching the brain properly.

So, it requires a proper protocol and optimising the surgery and surgical protocol as well to make sure that all the things start showing some kind of reading. Nevertheless, we could induce epilepsy and when we induce epilepsy then you can see that the seizures are there because you can see how the y-axis is at 3000 microvolts and suddenly you see a lot of activity happening in a lot of electrodes among the 32 electrodes that are shown here. The question is whether we can get back to the baseline which means, can cure this epilepsy or can test the efficacy of the anti-epileptic drug AED. So, if you now administer once you create these seizures which you can see on this particular slide and now if you want to understand how the anti-epileptic drug is effective or whether AED is effective or not then you can administer this AED and once you administer this AED you will see that the ECOG signals from all 32 channels they are close to the baseline. Now we are back at 100 microvolts right from 3000 microvolts.

So, you can see a huge difference because we have administered the AED which is an anti-epileptic drug. So, the baseline after AED administration was recorded for 20 seconds. So, as you can see here this is 20 seconds this is 10 seconds just to show you the scale bar. So, I think I told you that we are showing for 10 seconds. This is close to 20 seconds. This is just a scale bar to understand.

So, it is close to 20 seconds of recording. Now, further to understand how the time-frequency analysis of the acquired ECG signals from the cortical surface in all 3 conditions is, we experiment by taking the baseline data. So, you can see A1 is a recorded baseline then we have these all for 40 seconds is here like 40 seconds and here are your microvolts 100 minus 100 to 100 is minus 10 to 10 here it is minus 1800 to plus 1800 and then you will see that what A1 shows is that A1 is a recorded baseline A2 is a power spectrum baseline recording for 20 seconds right from 10 to 30 microseconds. So, the total is 20 seconds then we have A3. A2 is the power spectrum and this power spectrum is also for 0 to 60-hertz frequency again that is because we are using a certain board and if you use it it is about 125. So, half becomes close to 60 hertz and then we have A3 which shows a spectrogram of the baseline duration of 20 seconds.

So, you can see that we were able to understand how the time frame analysis for the baseline is there then we performed the same experiment for the recorded signals during the epileptic episodes or seizures and you can see that B1 is a recorded epileptic activity after 10 minutes of bicuculline, it is a convulsant that will cause or it will help us to induce epilepsy and then we have B2 which is a power spectrum of epileptic activities which is right over here followed by spectrogram of the epileptic activities for 20 seconds which is B3 in this case then we go to C in C we can see that the effect of AED. C1 is a baseline which this one and then it was the baseline was recovered after administering the drug we recorded after 4 minutes of administration of the entrapped drug then we have C2 which is the power spectrum and you can see that for every 10-hertz frequency approximately 20 dB fall, you can see in the power spectrum and finally, the C3 is a spectrogram of the recovered baseline which you can see here. Further, if you want to understand the spatial analysis of the recorded epileptic activities then you can just take that episode where the seizures are there and from there we can understand which electrodes were firing more, which means that corresponds to the electrical activity of the brain in that region the epileptic is epileptic or seizures are more and from there what we can understand is that how this spatial electricity electrical traces with a black dotted line showing the bilateral spread of epileptic activities due to topical application of bicarbonate crystals this is this particular line correlation results with B somewhere in the centre it is more compared to on the sides you can see here. Of course, there is an episode where it is not like this there is nothing, no activity, but what we have done is we can just see how the electrical traces are there, where the maximum activity is there and then correlate that particular data there are a correlating the results with electrodes with dominant epileptic signatures which shows that correlation indicating seizure on the set zone is localized in the area affected by bicarbonate. So, the point is we drop the bicarbonate in the centre of the brain and then we suture the skull. So, the point is we were able to see that the maximum episodes were in the centre of the brain because the crystal diffuses from we put the crystal in the centre of the brain and then it starts melting

or diffusing at the point that I am going to make here or I am making here is that with this 32 electrodes that we can capture the data from the brain of the right.

Not only can we capture data, but we can also tell where the seizures are, seizure episodes or activities, and epileptic activities. If you further want to understand the source localization, then also you can use this kind of microelectrode array. So, let us understand what we have seen. I will just go through all the slides very quickly so that it helps. First, we are looking at the ECoG, so you can use this kind of application to understand which region is firing more. Then we saw the schematic of how the device is implanted into the rat's brain and how the data is acquired through the Cyton daisy biosensing board.

Then we saw how we fabricate the device which is a flexible microelectrode array. Then we moved to EIB which is the PCB design fabrication of the electrode interfacing board followed by the EIB holder that you can see in this particular slide. Once you see the EIB holder with the help of 3D printing we see how the electrical impedance spectroscopy can be performed and what are the impedance values for the electrodes. Then we show how we can implant this device into the rat's brains. Again the implantation process will be taught to you by the neurosurgeon.

So you may have already seen that right how it is done. But once the device is there and you implant it. It is what I am more interested in showing to you. Once you implant the device then what we have seen we have acquired the signal which is a baseline. Once the baseline is acquired we induce epilepsy using bicuculline, a drug convulsant and then we can see the seizure activity. And then we administered an anti-epileptic drug to understand whether the baseline was recovered which you can see on this particular slide followed by time-frequency analysis experiments.

Here we were able to very clearly see the power spectrum analysis and the spectrogram of the baseline, the spectrogram of the epileptic activities and the spectrogram of the recovered baseline. Finally, we were able to do some spatial analysis and correlation analysis of the obtained epileptic activities. This particular work has already been published in this paper which is called a flexible implantable microarray for recording ECoG signals from rodents in biomedical micro devices. So, if you want to further understand in detail we have the paper for you. So, my way of teaching or conducting this particular lecture with this topic is to make you understand that not only you are learning how to fabricate a device, but you are also kind of getting some understanding about how to utilize the device and how to acquire the signals.

It is validated or appreciated and it is welcome by the research community such that we can publish it. So, the reviewers have accepted the protocol and the way we fabricated the

device and now it is in the public knowledge. So, this is what I wanted to show you when not everything that we are discussing is published, but most of the things that we are discussing are also published. The reason for pressing publication is that sometimes even whatever we think the reviewers help us to improve the quality of the work right. We may sometimes know how to represent our data correctly or the analysis that we are doing or to add some more analysis to give the end user more access or more understanding about the work that is performed. So, it is important for all the young researchers who are taking this course not just to learn fabrication, but to understand how the application of the device is there and then try to collect the data and try to publish it.

Now there are international conferences where publishers work, and there are journals where publishers work or submit your work of course, you cannot go to a journal right, but you can submit your work there and see what kind of feedback you get right and take the feedback optimistically. If you think that all the papers that we submit from IISc or even from IITs or NITs or something that you think is right is the top institutes in our country get accepted in one go then it is not correct it is not correct. Every researcher has to go and improve the work so that it becomes good for the journal to publish it. So, do not have some kind of what you call thought process without or perception right without knowing the truth. Truth always is every researcher works hard, every researcher tries to publish, every researcher gets some good feedback, major revision, rejection, minor revision and then it is accepted ok. So, this work even now is in theis accepted in general it took about 2 and half years for us to regressly work on this particular device.

The good thing is now we have a device that can teach you this particular application. Now, I want to switch gears and go to another device which we call biodegradable ECoG electrodes. Now, until now what we have seen is you have the ECoG device and you turn on the human brain right for intractable epilepsy. So, why intractable epilepsy because of that epilepsy the anti-epileptic drug fails. So, the only way to treat intractable epilepsy is by resetting the area that is causing these seizures or episodes.

To understand which area is causing the seizure we need to implant when we say we are a surgeon needs to implant the device which we call ECoG or ECoG. Now, once you implant a fuse, open, and take out, what about once you implant the device will dissolve or absorb or desorb? So, the bioresorbable device will get dissolved or absorbed in the brain itself ok? The question is where it goes, what materials we have to use, and what the toxicity effects right whether after dissolving is it toxic to another part of the rat's body because first we are doing it in the rat now. So, we have to check the heart of the rat, the liver of the rat, the kidneys and the small intestine and large intestine lungs to see that the toxicity effect is not there from the materials that we have used for fabricating this particular device.

So, we will take this as a separate lecture since I do not want to feed you a lot of things in the same lecture. So, let us wait for the next lecture and again I will cover the fabrication of this device and the novelty that we bring in. We are not material scientists. So, what we do is we get the literature to understand what materials I have been using and we fabricate these device sensors and then we do the experiments for certain applications right? This application also is still towards understanding the effect of elliptical drugs, but in this case, now we are using a bioresorbable platform.

So, we will see this thing in the next class till then you take care and if there are any questions feel free to ask us in the NPTEL forum. Thank you.