Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications

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Hi, welcome to this lecture. In the previous lectures, if you recall, we have seen an ECOG device with a 32-microelectrode array. We also examined a 10-microelectrode array bioresorbable or biodegradable device. Both devices were used to study epilepsy, allowing us to understand the signals we acquire from the brain. We observed how the efficacy of AEDs can be tested using this particular model. Now, let us proceed to understand how to create a single shank, or you could say a needle with multiple electrodes patterned on the needle using a silicon wafer.

When studying brain signals, researchers in the field of neuroscience often focus on understanding action potentials, i.e., signals coming from single neurons or, at most, multiple neurons. This is typically achieved by inserting a tungsten wire into the brain to capture signals from neurons. Given the extremely small thickness of the tungsten wire, only the tip of the tungsten wire can acquire the signal.

When talking about cortical arrays, the cortex is organized into columns, each consisting of six layers: layers 1, 2, 3, 4, 5, and 6. Layer number 4 is the input layer. For example, if you divide a pen into multiple layers, layer number 4 would be the input layer, layers 1, 2, and 3 would be the processing layers, and layers 5 and 6 would be the output layers. To capture signals from each layer, you would need an electrode or an electrode array on some material. The tungsten wire only conducts at the tip, so to capture signals from different stages and layers of neurons, an electrode array is necessary.

Today's lecture focuses on two parts: the first part addresses the single shank, and in the second part, we will explore multiple shanks with various designs. Finally, we will discuss some analysis from the tissue samples.

Let's examine the slide. We will discuss invasive neural system experiments where we use a single shank microelectrode array for depth neural activity analysis. Until now, we have focused on surface electrodes; now we have shifted to depth analysis. Planar ECOG electrode arrays can provide better resolution than EEG signals, but they offer a limited understanding of activities at brain depth. Local field potentials (LFPs) recorded by depth microelectrode arrays are used to study brain activities from cortical columns. We are

interested in understanding how electrical signals are generated in cortical columns and how these signals differ in various layers during an evoked response. For example, in previous lectures, when we touched the whiskers of a rat, we observed some evoked potentials. Similarly, electrical signals differ in different layers, particularly during an evoked response.

The baseline LFPs, LFPs during an induced seizure, and after recovery need to be studied to understand the evoked response and the effect of AEDs on LFPs at different depths. These are some of the interesting aspects we will explore.

Let's outline the research goals from this background. The research goals include recording electrical signals for baseline with induced ictal and interictal discharges from the brain's depth in an animal model. Additionally, we aim to perform spatiotemporal analysis to determine changes in electrical activities under various neurological conditions and the distribution of signals across the depth. The objectives are as follows: first, design and fabricate MEAs; second, implant the array in animal models; third, record and analyze LFPs in response to convulsions and anti-epileptic drugs to study epileptic activity. The electrical stimulation can also be used to create or induce epilepsy.

Let's review the schematic. In the schematic, you will observe that the structure of the MEA is different. We use a rat stereotactic apparatus, a micromanipulator (shown here), and a recorded LFP system. We also use an OpenBCI USB dongle and Cyton Daisy Board, similar to what we observed earlier. The new aspect here is the use of a micromanipulator to insert the needle into the brain. The micromanipulator has a step size of 1 or 2 microns, which allows for precise adjustment. The rat stereotactic apparatus helps hold the rat in place.

This study is conducted with the rat anaesthetized. We implant the electrode and measure signals. The MEA dimensions are 4.4 millimetres by 3.6 millimetres, but only the needle section goes into the rat's brain. To zoom in further, you can see there are 13 electrodes: one for reference and 12 distributed to capture signals from all channels.

The length of the shank that goes into the rat's brain is 3 millimetres. Let's understand the process for fabricating this microneedle or microneedle electrode array. We start with a silicon wafer (step number one), grow a thermal oxide layer (which can be done using wet or dry oxidation), deposit gold with a base layer of titanium, and then pattern the gold into the form of a microneedle. Next, we deposit an oxide layer using PECVD, followed by removing the oxide layer from the recording electrodes and the contact electrodes.

So, you can see here in this case in D oxide is everywhere right, but in E you will see that the gold pads you can see the same thing gold dots are there on the needle right see here. That means, that the oxide is etched from the recording electrode and from the

contact electrodes all that place oxide is there. Then you perform front-to-back lithography and use DRI as we have seen which is called deep reactive ion etching. So, if you follow this process you will fabricate a microneedle and the size of the microneedle can be seen we are just showing so to understand the dimensions of the microneedle. And each electrode is this dot that you see on this shank is about 50 micrometres and the spacing centre to centre distance is also 50 micrometres.

So, this is the SEM image, right? Now from this once you have a needle what we do we integrate this needle with the EIB board. So, here the needle is ticked onto this pad and then the contact pads are there right? So, each and then electrode there ok. So, for each contact pad, we do wire bonding ok.

We perform wire bonding and then this one is connected to this one and this one is connected to your Cyton board. So, this is how the PCB design and fabrication are done, I am not going into detail because we have already taken or discussed this thing in detail in the TA class. You have to then perform the electrical impedance spectroscopy and from there we were able to understand the average impedance of the electrodes which is 581 kilo ohm at 1 kilo horse which is within the value reported in the literature. Now once you open the brain of the rat that means, you perform craniotomy and when you use the micromanipulator then the micromanipulator is and you can see here wire bonded you see all the wire bonds here very clearly right? And this is not in the in this is the needle and it is not right now implanted into the brain of the rat.

In this one, you can see the needle is now implanted in the rat's brain. This is the rat's brain, okay? The details are given here. You can see the acrylic arm connected to the micromanipulator, FFC cable, FPC connector, and the fabricated MEA wire bonded to the PCB. We are looking at implanting this particular microneedle into the somatosensory cortex, which is in the left hemisphere of the rat's brain. We are using the Cyton Daisy board to acquire the signal from the needle. The appearance of the signal is shown here. Panel A shows the number of channels, and here you can see the baseline versus time for different channels firing at different rates, particularly during an epileptic episode upon using the convulsant. The y-axis is 500 microvolts. Then, when we administered the appropriate drugs, we recovered the baseline.

So, once you acquire the data, the analysis remains almost the same. We performed a time-frequency analysis of recorded LFPs. Again, Panel A1, which is this one, shows the baseline LFP signal we recorded for 20 seconds. Panel A2 is the power spectrum analysis, and Panel A3 is the spectrogram of the baseline. Similarly, Panels B1 and C1 show recorded epileptic activities and recorded LFPs after 4 minutes of AED administration, respectively. Panel B1 displays the epilepsy data, and Panel C1 shows the

signals after 4 minutes of AED administration. Be careful about the y-axis—do not get confused by values like minus 10 to 10, minus 500 to 500, or 20 to 20, as these are all in microvolts. Even if the signals appear similar, the y-axis scale indicates differences. The same applies to Panels B2 and C2, which show power spectrum analysis, and Panels B3 and C3, which display the spectrogram of epileptic activities after 7 minutes of bicuculline administration. Bicuculline is a convulsant, and Panel C3 shows the spectrogram of the restored baseline after 4 minutes of AED drug administration.

You can very clearly see from this analysis that the activity is far greater during epileptic episodes. So now, if you can fabricate a single shank, can you fabricate a multi-shank? The advantage of a multi-shank is that you can take readings from three cortical columns simultaneously, right? So, instead of using one shank, if I use three shanks, you can cover a larger area and obtain data from multiple columns. There are various ways to achieve this, but the process remains the same. Instead of having one needle, we would have three needles. If you look at the slide, you can see a very nice three-shank electrode fabricated using microfabrication techniques, with each shank having a linear array. The spacing between the shanks is 200 micrometres, and the length of the needle is 3 millimetres—this is 3 millimetres. This particular image—this one here—shows just that.

The beauty of this particular design is that the electrode size is only 15 microns, and the spacing between electrodes is 25 microns, with a track width of 30 micrometres. Not only that, but you can also see the three masks used to fabricate this device—Masks 1, 2, and 3. The first mask is to pattern the shape of the microfabrication needle, the second one to pattern the electrodes, and the third to open the contact pads from the recording electrode and the contact pads themselves. The specifications are further detailed, showing the electrode dimensions, inter-electrode gap, and the number of electrodes on a single shank. Since each shank has 11 electrodes and there are three shanks, you get a total of 33 electrodes. The shank width is about 200 microns, the length is 3000 microns (about 3 millimetres), and the distance between the tip and the first electrode is 250 microns—that's this distance. The contact pad size is 300 by 300 microns, the tip dimension is 25 micrometres, and the tip angle is 30.25 degrees. The materials used in this design are silicon as the substrate, silicon dioxide as the insulating material, and gold for the electrodes and interconnects.

So, you can see the linear array here, the three shanks fabricated, and the dimensions as well. If you can fabricate a three-shank, how about a four-shank? Of course, you can do that. Sometimes, to get data from four different neurons, neuroscientists take four different tungsten wires and fuse them like this—1, 2, 3, 4. They are not touching, but they are fused so that data from all four neurons can be acquired simultaneously. These four electrodes are what we call a tetrode—four electrodes, tetrode. So, if a tetrode is good, what about having multiple tetrodes on the same shank?

So, yes 4 and 4 multiple tetrodes on the same shank right the distance between the electrodes like this is about 30 microns and also the length is 30 microns the distance between each shank is 200 microns the length of each shank is 3.1 millimetres and the distance from the first tetrode to the tip of the needle is close to 270 microns. Again we can change the dimensions and designs based on the application that we are looking at the contact pad area is 4.6 by 3.6 millimeters with each contact pad of 300 microns and the spacing between contact pads is 100 microns the distance between quadrupole electrodes is 100 microns edge to edge distance.

So, like I said we can change these dimensions based on the application that we are looking at and then we have already filed a pattern this is an actual fabricated device you can see 4 shank electrodes tetrodes on the 4 shank and then we are now planning to get some data from the rats spray. One thing I was kind of mentioning in the last to last lecture and even the last lecture is that once you implant the device right does it have any toxic effect it is not just only when you have a biodegradable material or bioresorbable material you will have this toxicity that you need to study, but even for the implants like micron needles or flexible devices or bioresorbable electrodes you need to understand for how much is the toxicity effect that is at how whether the device whether the material that is used in device is toxic or not. So, for that, we perform some histology studies histology is the study of tissues, cytology is the study of cells right histology of vital organs which are vital organs the vital organs of course, we have to look at the brain because we are using shank into the brain to see that how much is damage to the brain is an endotoxic effect in the brain also we need to see the liver which you can see here right we have to see the image of a kidney which is right over here then we have to see the section of the heart which is in here then we have to look at the lungs which are shown in e then we have to see small intestine which is shown in f and finally, we also look at the large intestine which is shown in g from this image analysis we have to identify whether there is any toxicity or not ok. So, this is where the importance of the fabrication comes into for the application now we talk about epilepsy, but we have a different application for Parkinson's, we have an application for understanding stroke right? So, how to acquire the signal how to stimulate the part of the brain what happens when you acquire and stimulate simultaneously can you look at the surface acquisition versus deep brain acquisition what happens what is the advantage of using this device or can we have devices that can acquire signal from the depth of the brain can we have devices acquire signal from the surface of the brain.

We will now see a little bit about the Parkinson model of what we are working on which is also a fabrication process we are looking at the surface electrodes to acquire the signal

and stimulate it and see the effect of stimulation on the Parkinson's right. Now there are many things that many different you know parameters through which one can see that the person is having Parkinson's, but one of the parameters like a gate right walking sometimes a turning and coming back becomes difficult. So, then there is an involuntary movement of the hands people cannot hold the pen not spoon right walking becomes difficult these are all the symptoms symptoms not the parameters symptoms of Parkinson's. And can you so so the right way to treat which we have subrecipitation he is a functional neurosurgeon so he he does surgery and treats a patient suffering from Parkinson's by using deep brain electrodes. So, electrodes go deeper into the brain the question that we are asking is whether surface electrical stimulation along with deep brain electrical stimulation would help in improving the life of a patient to cure or to cut down the tremors significantly.

So, we will see a little bit on the surface electrodes that are used for application in Parkinson right. But for now, this is enough that will be probably our last lecture on which we will look at the Parkinson model the device that we can fabricate using microfabrication and what can be the protocol to study the Parkinson model. With that I take a leave I will see you next class see you then bye-bye.