Nanobio Technology Enabled Point-of-Care Devices

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Lecture - 24

Impact of Surface Roughness of PCB and PCB for Glucose Sensors

So, students today I will teach you one more very important topic for PCB's biosensor development. As you know I taught you already that PCB can be a useful addition for the biosensor development right. Because, why PCB is very important? You know that without PCB you cannot you will you cannot see any electronic instrument and we in our India even in your local place, you can see that easily we can get the PCB and we have very good PCB application technology.

Let us use them for biosensor development and important thing is that we can easily coat a good layer on the PCB substrate and that is substrate that is gold coated plate, we can use for biosensor development. So, that topic very deeply I will teach you today like how the PCB can be used for biosensor development and how we can clean the surface for PCB that I already started I will continue today and then their impact of surface for biosensor development. This is very important things that today I am going to cover.

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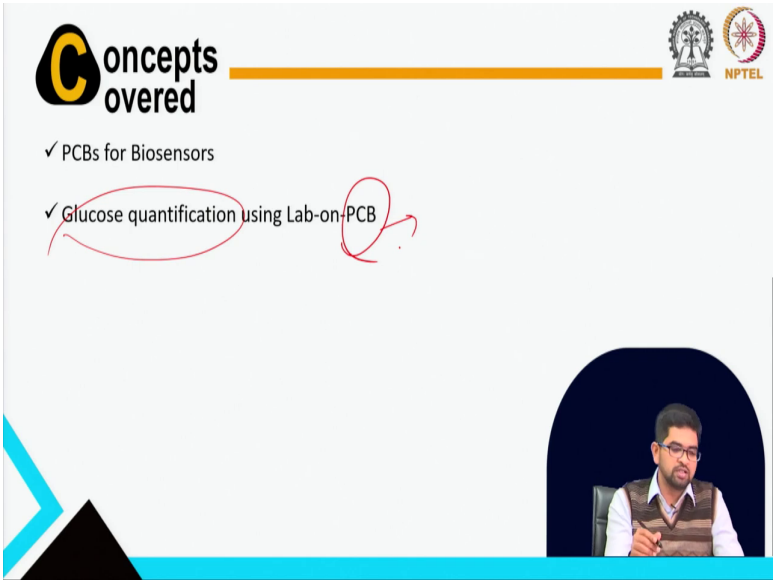

So, mainly that is why I will cover today first the PCB based biosensors and the let us take one example like glucose quantifications ok. So, glucose quantification that is I will teach you on PCB. As I told the lab on a chip technology you can remember, what is the lab on a chip technology?

Means, all the laboratory experiments that you are doing like sample purifications, sample then amplifications, then detections everything can be done on a single platform right, that is the lab on a chip technology. And, here why I said lab on a PCB because that chip will develop on printed circuit board. So, PCB means Printed Circuit Board, that PCB I will teach you very basic things today.

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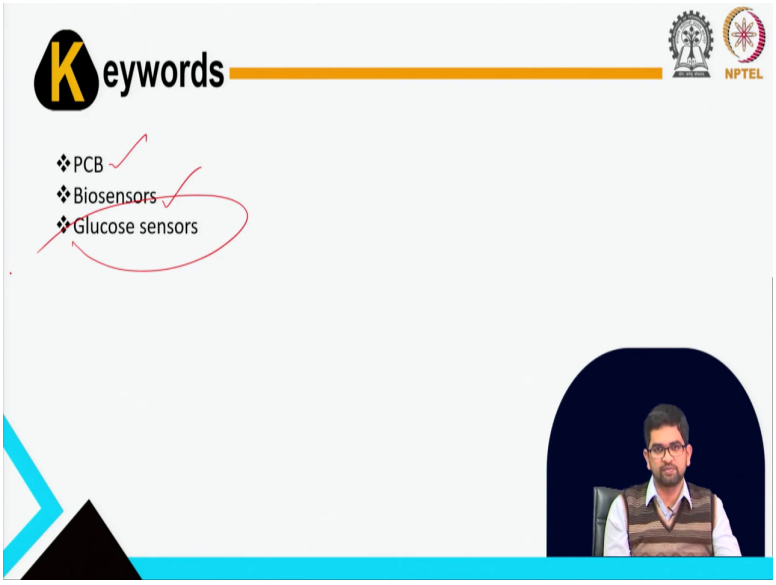

So, let us find out the basic keywords, if you want to study more in details, please search that PCB that is related for the biosensor development. And for example, today I will cover one example that is the glucose sensor ok.

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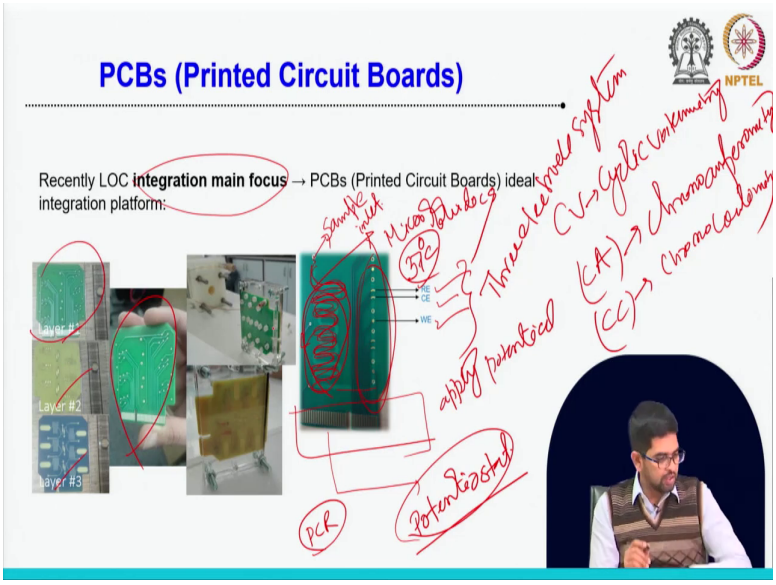

I think few things I already cover last class that printed circuit board is really important, because of mainly the integration technology that we can easily integrate many things right. You can remember last class I showed you just I am summarizing that we can easily integrate this you can see micro fluidics right, the you can see this channel, serpentine this is the micro fluidics channel. So, there may be a sample right, sample inlet.

So, what is this, where you can easily drop the sample and then this sample will flow through this micro fluidics right and then it will come to your detection area. So, this is the your detection area. So, one is your working electrode, one is your counter electrode, one is your reference electrode and this is your adapter where you can connect with your potentiostat, potentiostat right. So, through this potentiostat maybe you can apply some potential which potential you want to apply.

So, you have to select a electrochemical technique. So, if you want to apply the potential maybe you can select chronoamperometry CA right, chronoamperometry you can remember right or you can apply chronocoulometry right coulometry that is CC. Even you can apply cyclic voltammetry CV, cyclic voltammetry. Just you can play around the software here and based on this three electrode system right.

So, we need three electrode system, why I already taught you last class. This is the three electrode system right, that three electrode system will easily integrate on PCB platform. See there is a different different shape you can see, like different different PCB we can design and you can see the different different layer. It means on the top of the PCB biosensor that we fabricated already, here also we can integrate different layer based on the micro fluidics, based on your requirement.

Even you can see this serpentine that is the micro fluidics, here sample flow right. Sometime you may need to increase your temperature of the solutions, but maybe 37 degree Celsius your reactions is the best. So, what you can do on the back of this micro fluidics, you can use some heater localized you can heat the in this area local area.

And, you can increase the temperature of the solution that is the concept of you know PCR, that Polymerize Chain Reactions. Means, sometime you may need increase the temperature, sometime you may decrease the temperature. I mean you can heat cool, cooling and heating also can be done in the I mean this solutions. You can use, you can play like which temperature is required for your reactions right; that is things also you can try with the printed circuit board.

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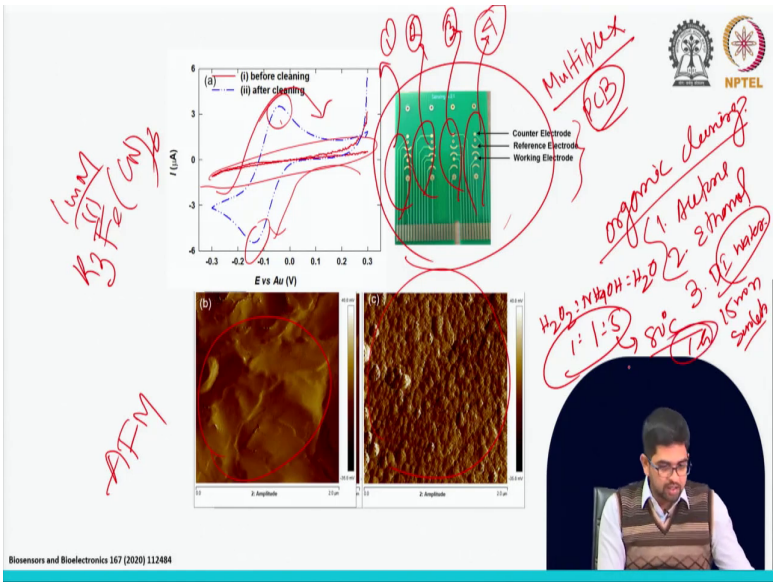

Let us show you this all the technology like PCB, this is the another design of the PCB that I showed you last class, that PCB I already used like three electrodes. Here, this is a multiplex PCB right, multiplex PCB. Why I am saying this is the multiplex? Because, you can see 1, 2, 3, 4, 4 see unit there.

So, here may be you can try 1 detection, here 1, here 1, here 1. So, 4 detection can be done simultaneously, that is multiplex printed circuit board and last class I already taught you let us summarize that this PCB when we received from factory, there is lots of contamination. We need to go for some pre-treatment, we need to go for some cleaning method. You can see like before cleaning, you can see almost there is no peak, this is just like a if you see 1 millimolar K3 Fe CN6.

So, here iron is a 3 and oxidation stage. See, here if you see like first reduction because we started from 3 Fe3, then first reduction and then oxidation. See, at the beginning before the cleaning there is no peak, but after the cleaning we are getting very good peak so, cleaning necessary. And, I showed you right already the AFM images, see this is the before cleaning the lots of organic stuffs there and after the cleaning the surface is very clean and you will get very good cyclic voltammetry.

Now, today why I showed you again this figure because I wanted to start one again important topic today. Means, after the cleaning the surface, you have to be very much careful that your surface is properly clean or not.

Although, you are following like your best cleaning method, I taught last few classes you can remember first the organic cleaning, organic cleaning. What is the organic cleaning? Like one first acetone, then ethanol, this and then third after the organic cleaning, these two acetone ethanol for 15 minute 15 minute sonication, path sonication.

Then, you can go in the DI water, DI water you can take and you can go for again 15 minutes sonications right. So, these sonications will help to remove some organic contaminant, but still some contaminant may be there. So, you have to remove very clearly so, you have to use like hydrogen peroxide, ammonium hydroxide and water mixture is 1 is to 1 is to 5 and let us sonicate it again for 30 minute or you can boil, not boil, this is just heat at 80 degree Celsius for 1 hour.

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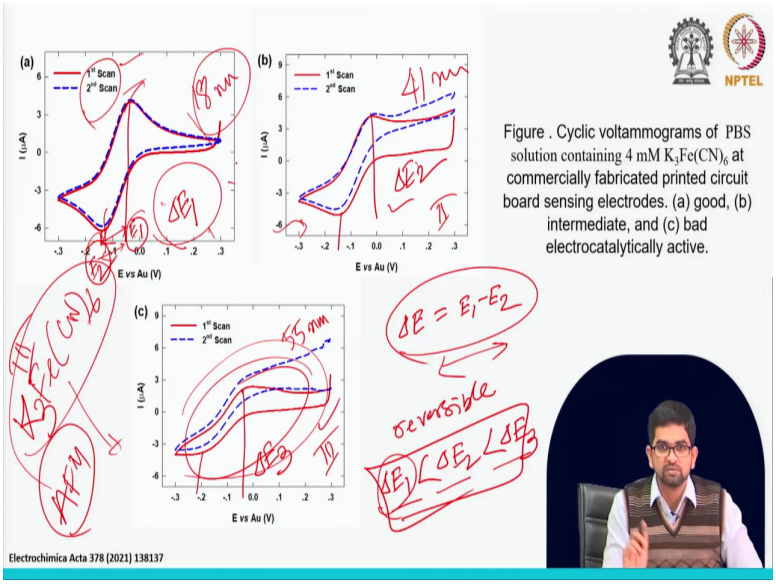

So, you may get very good surface. But, sometime after the cleaning you may not achieve your required cyclic voltammetry what you are expecting, that cyclic voltammetry you may not achieve.

So, you may ask why you said that ammonium hydroxide, hydrogen peroxide water mixture 1 is to 1 is to 5 ratio its getting very good. But, in the PCB case when we are getting the printed circuit board from the industry, then we are going for the this very vigorous cleaning, but still, you may get some bad data.

Its depend batch to batch, variation during their fabrications. Some organic stuff that may cover the active side of PCB surface is very strongly, it may not remove. Also, it can also if you try like very vigorous cleaning, also it can damage also the surface. So, you can guide your PCB factory, I means there they have their own R and D sections, you can guide them.

We need this kind of surface, we need this kind of surface clean less, this kind of surface roughness and that is good for my biosensor and they can fabricate for you ok.

This this information I will share with you now, that you can tell and they can fabricate for you. They can take care their fabrication accordingly and they can give you those surface. See, here I will show you 3 figures of cyclic voltammograms that we receive 1, 2, 3 right. These 3 cyclic voltammograms we receive after cleaning, but problem is that, what cleaning means just same cleaning that I told like organic cleaning, ammonia hydroxide, hydrogen peroxide and water is all the cleaning going to followed.

But some cases you may get very good, very good cyclic voltammetry in the (Refer Time: 11:39) K3 Fe CN 6, this mixture. You may get very good cyclic voltammetry. It means this surface is really good and you can use for biosensor development, without proper cleaning do not use them for biosensor development because you may not get the best signal. There may be reproducibility issue. See, in the 2nd and 3rd case, you can measure the delta E, delta E value delta E.

So, say this is the I am just repeating again, please try to remember now. So, in this cyclic voltammogram, just try to remember. So, this is the say oxidation peak potential right. So, this is I can say E1 and this is the reduction peak potential right. So, this potential the reductions happen. So, I say this is the E2. So, I can say delta E equals to E1 minus E2 right. So, E1 and E2 if you if you calculate this value, this delta E value we can use a very important parameter.

How? Because, this will give you the surface behavior, if your surface properly clean or not. See, in the first case this surface it looks like very clean, that is why E1 and E2 they are very close right. I also taught you; you can just try to remember; if right these are very close, it means your reactions on the surface is very means reversible or semi reversible right, reversible. But, if delta E delta 1 is not very close see in this case, oxidation reductions peak they are not very close.

So, in this case so, if I say this is the delta E1 and in this case this one delta E2 and this case delta E3, see delta E value actually increasing right. From this value, you can predict the surface. It means this is the best surface and then slowly your sensor surface is actually not much good, because this surface showing slowly irreversible phenomena. This oxidation reduction is not happening in the very means not reversible way.

So, this surface is not so good for the biosensor development this surface is not so good after the cleaning. Still, some organic stuffs and contaminant there, that is the conclusion from this cyclic voltammetry. So, 1, 2 and 3, this cyclic voltammetry if you compare. So, here actually we compare 1st and 2nd scan means we can try the many scan. If the there is a variability, there is a change of the current or potential during the scan.

So, in the first case is so stable the surface, if your surface is not stable during the different different scan you may get different different value. See, the current changes, their potential can be change because your surface is not that much stable; that is another information you may get from this cyclic voltammetry ok, clear. So, this delta E1, delta E2, delta E3, this value you have to calculate and then you have to take the decision. This is the summary of this slide.

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Let us come the very important images of this 3 cyclic voltammogram that I have showed you in the last. These 3 figures, let us take this AFM image; Atomic Force Micrograph image, I wanted to take and then I wanted to see that surface how it looks like right. See, if this surface is very clean and you are getting very good cyclic voltammogram, in this case you see the surfaces looks like this and Root Mean Square, mean RMS value.

So, this is RMS root mean square value, in this case 18 nanometer, second case see this is the 41 nanometer RMS, 41 nanometer and third case RMS value is 55 nanometer. So, let us make a conclusions here. When you will use the printed circuit board, let us try to use one cleaning method. After the cleaning method, you have to collect your cyclic voltammetry on that surface ok. And, that cyclic voltammetry guide you if the surface is properly clean or not, means delta E value. This delta E value.

And, you can check like their AFM images and collect their RMS value. If your RMS value is near like 18 nanometer or less than or nearby 10 nanometer, in that case you may think yes your surface is really clean. But, if your root mean square is very high, like see if you go more and more RMS value if see if you see the compare. So, in this case the 18, 41 and 55; see here 18, here 41, here 55 nanometer. It means if the RMS value increase, your surface actually [FL] irreversible surface.

This is not good for the biosensor development. It is not so clean. So, here you may get some bad cyclic voltammetry for the K 3 Fe CN whole 6 this mixture. So, this roughness value will guide you.

Accordingly, batch to batch variability of the PCB board that you can check. Means, when in the batch to batch means in the industry when they are fabricating printed circuit board, few batches may not good for like maybe there may be some fabrication issue, there may be some contamination issue, that can affect on your biosensor development.

So, for that you can just check cyclic voltammetry, check their AFM images and you just decide that you can use this one for biosensor development or not. This is very good informations, just try to remember.

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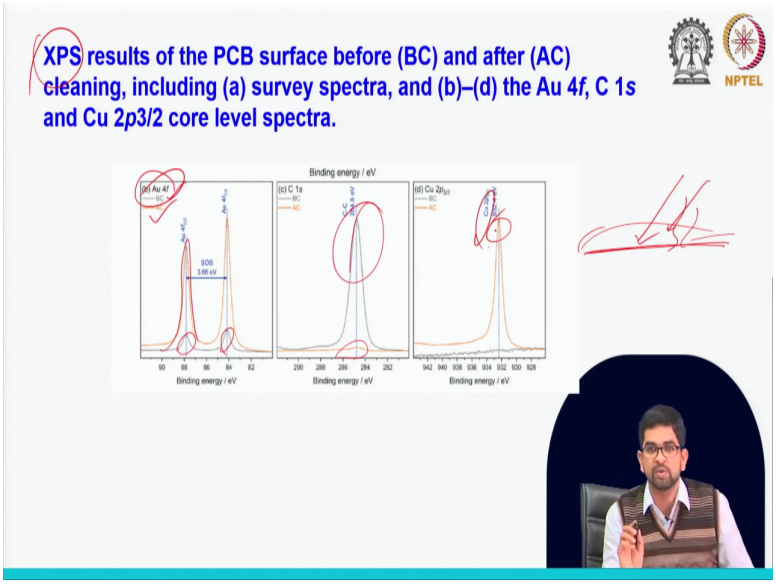

Then, after the AFM study if you wanted to check that, yes, your surface really clean or not, let us check the XPS, photo electron spectroscopy, X-ray Photoelectron Spectroscopy, that I showed all that in last class. Today, again I am summarizing here, you can see that the BC means just Before Cleaning, AC means After Cleaning. See, the gold peak, see this is the before cleaning is very less, but after cleaning it is very high.

It means you are getting the gold peak, its very high because the organic contaminant almost removed and carbon contaminant. See, the carbon contaminant in the case of after cleaning almost no, but before cleaning there is very high carbon contaminant there and also, we got the some copper. Because, as I told you last class also while PCB industry that fabricating gold on the PCB surface, there is a copper layer below and after they are using a gold layer.

So, during the cleaning process, some copper layer can be exposed or during the fabrications of the gold also, it may not fabricated very good way. So, you have to check there is some exposed copper layer or not, that you have to verify. So, if your cleaning is very very strong, if it can if it damage the gold layer then you may get some copper peak, that is if it is very highly intense copper peak, then you have to very much serious. You have to be very careful, that copper can affect on your biosensor. This kind of information you should have.

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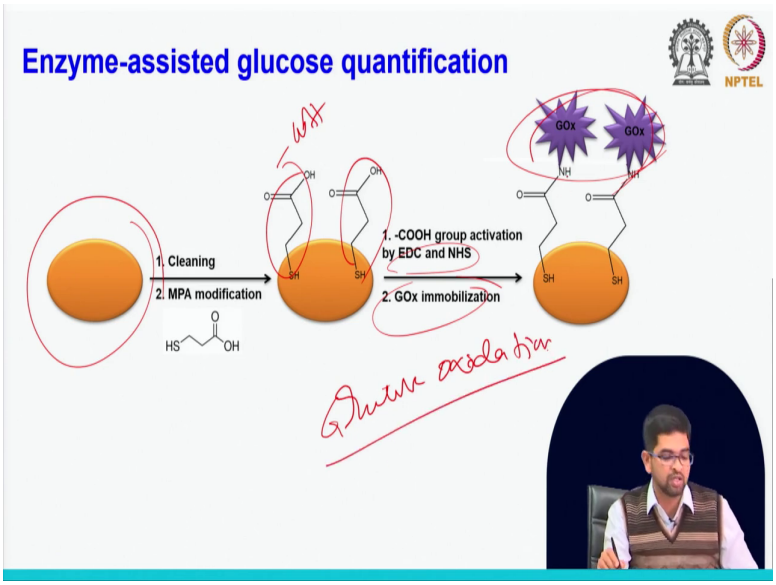

And, then this sensor can be useful for glucose detection. So, today I will show you the glucose detections. Now, means this PCB that you got all the informations after the cleaning, let us use them for glucose quantifications. So, I already taught you that how to modify the glucose oxidase on the PCB surface.

Here, just we use EDC NHS, then we activated it, when EDC NHS activations method we use, because surface already have your carboxylic group and that can be activated by EDC NHS. And, then we added glucose oxidase and then glucose oxidase already immobilized on the surface and then you tried glucose oxidation, glucose oxidations. And, this is a enzyme based glucose oxidation because already you have enzyme on the surface.

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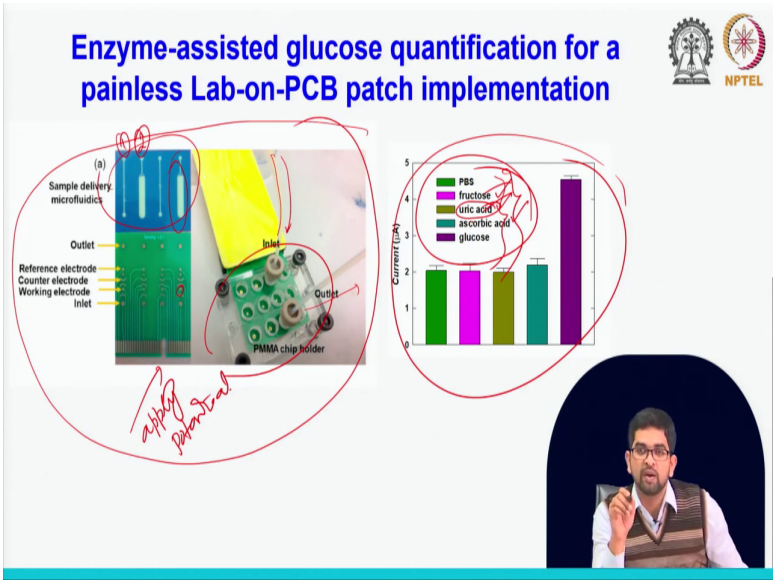

And, this is the actual structures that actually I wanted to show you, the structures of the PCB that we integrated some microfluidics. So, at the very first slides I showed you that microfluidics can be integrated some different different way. In this case, I am showing you that microfluidics can be just this another layer. So, here is two different type of the microfluidics we use 1 and 2, two pattern we use.

So, this is like just a simple channel and there is a different channel, looks like different that you can try different different microfluidics and their efficiency. So, this is the whole setup. So, one way you can I mean inject your solution like glucose solutions through the on inlet and through the outlet, all the excess solutions may be removed.

So, and then you can try the glucose oxidations means once glucose solutions come on yours on the glucose oxidase surface and then when you apply, you have to apply some potential right, you can remember. Suppose for example, just 0.3 volt, then this glucose will be oxidized you will get the amount of current based on the concentrations of the glucose and then you can make the calibrations curve.

And, here this is the very important figures that while you are measuring the glucose, then you have to try to find out the other interference species that may present in the real sample. What kind of interference species may present? Like fructose with the glucose right, uric acid, ascorbic acid.

Then, you try to find out their contributions on your background current. If their contribution on background very high, that it means your sensor surface is not so good for selective detections of the glucose. Because, I wanted to see the how much glucose present not the other.

So, because some (Refer Time: 23:08) they may have some different different concentration uric acid right, they may have uric acid. So, if uric acid also can oxidize in the presence of glucose, it can cause the background, it will give some false positive signal. You may think this signal you are getting because of glucose actually not because uric acid also can be oxidized.

But, one important things while you are using glucose oxidase, glucose oxidase is an enzyme and they are very very selective. So, its little bit safe side for you while you are using enzyme. But, this kind of interference species effect you have to be very much careful when you are using some non-enzymatic procedures, that I will teach you next class.

But just this is just for your understanding, while you are developing biosensor, definitely you should check all the interference species present in your real sample and check their contribution. Now, let us come.

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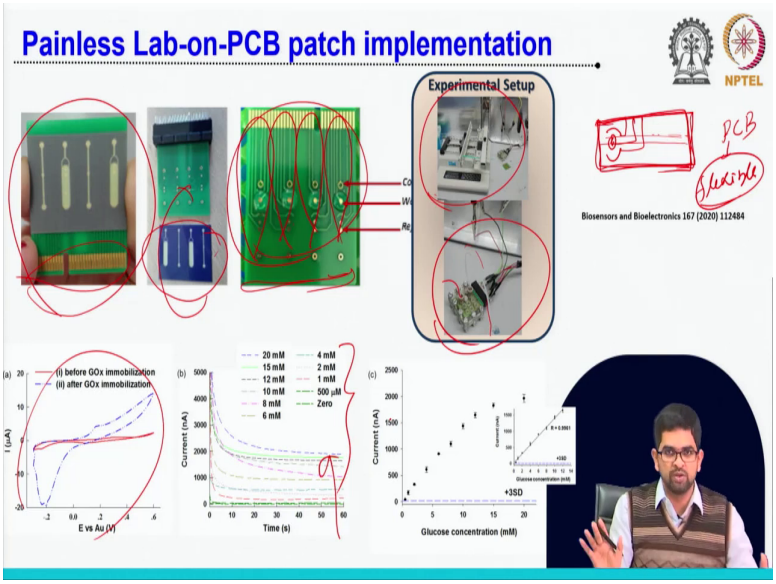

Let us show you how we can implement a patch right. So, I wanted to develop a very simple patch like bandaid right. And, here this patch we will use a PCB, but this PCB will be flexible PCB, flexible. Why I am saying flexible? That, these PCB substrate should be a flexible substrate, on that flexible substrate you have to print these all the these all the working reference counter electrode you can print, that can be done easily ok.

So, if you want to develop a wearable biosensor based on PCB, let us use a flexible substrate like polyamide sheet something, flexible polyamide sheet, you can try. See, something like this, then this is your microfluidics layer, you just put on this top. And, you can use some

glue, then you can fix your upper part is the microfluidic and down lower part you have your PCB ok.

So, this PCB and this microfluidics let put together with some adhesive and your systems will be looks like this. And, then you have the your adapter, this adapter you can try just integrate with the potentiostat and just for lab purpose. But, when you are thinking for patch development, that time I will show you one patch means something like this, may be like bandaid you know. Here, you can use like one working electrode, one counter electrode, one reference electrode like this.

And, may be here connection will be like this and here should be some microfluidic channel should go through this electrode, that I am going to show you in the next slide. But here this slide is a complete information you have, like see in the lab setting generally we are doing this is just a pump, by using this pump we can control the flow of some artificials like interstitial fluid, artificial sweat sample you can use. And, then you just flow through this inlet and outlet tube, this tube inlet and outlet.

Because, while you use the flexible patch, when you will keep it on skin then it will take the interstitial fluid or what is interstitial fluid you know that I am going to show you again. So, interstitial fluid it will collect or sweat sample it will collect, then it will go through the your sensor surface, then it will measure. This is this setup is the laboratory setup, now I wanted to show you the actually patch setup.

So, here is the data, that data actually you already know like glucose oxidase based on the glucose oxidase immobilizations. You can see the different different concentration, the glucose current is increasing then you can develop your own calibration curve, that concept you know already.

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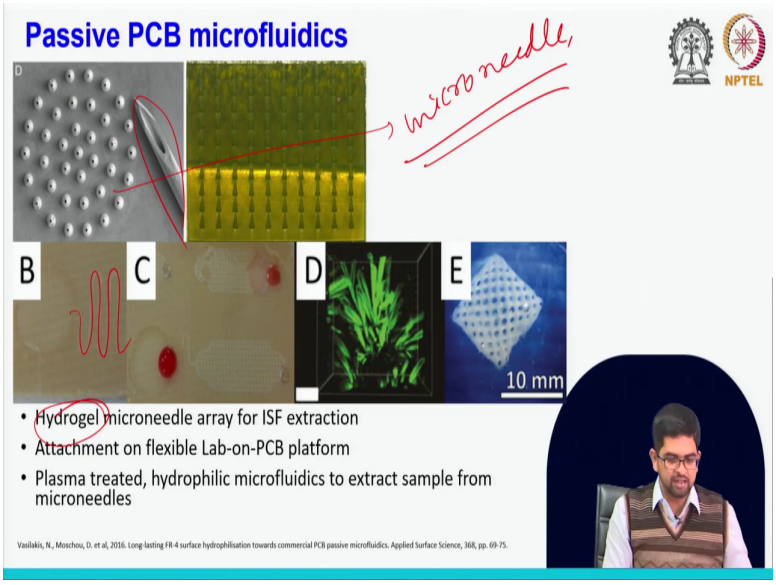

So, for this development of this patch based biosensor so, you have to collect the interstitial fluid, as I told you need some microfluidic like this. So, on this microfluidic there is some (Refer Time: 27:30) So, here your all the interstitial fluid means you can inject and it will go through. But, when it will be the real patch then it will collect, it will put on your skin it will collect the interstitial fluid, where you need some very small needle.

So, you may think that this is the is it then I mean you can say invasive or non-invasive. But, when you use the small very means micro label, very small needle that needle can help you to collect the interstitial fluid under the skin and you will not feel much pain. It is you can say invasive, but minimal invasive not that much invasive. We are saying these are non-invasive also, you can say or minimal invasive; while you are using this micro needle, this is the micro needle.

So, it looks like this way. Actual needle is really really painful, I means while you are collecting the blood samples. But, when you use the micro needle, its not that much big. It will not affect; it will not damage your nerve system. So, it will not feel much pain. So, it will be looks like this, then you can integrate this because when it will come to your skin, then it will collect the interstitial fluid. Then, that industrial fluid will go through the microfluidic channel, then it will come to the sensor surface.

We can develop a new technology that is hydrogel based microfluidics. This hydrogel microfluidics is really really important for patch development for this kind of painless or minimal invasive patch biosensors, that easily you can attached on your flexible PCB. You can collect the interstitial fluid and you can get the different concentrations of glucose in the interstitial fluid. So, this is the images of the micro needles.

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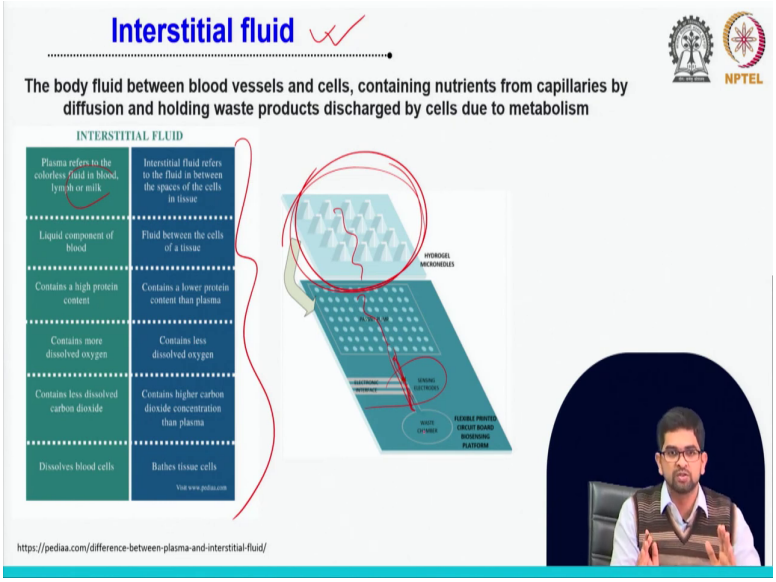

And, that micro needle suppose this is the micro needles right, that micro needle see it can absorb the my interstitial fluid and then go through the see here is the channel, it will come to the your sensing part, your sensor part and then your sensor is ready for detections. And, the this information that is I wanted to share you, you already know interstitial fluid, this is just a body fluid and just under the skin.

And, there is a difference between the interstitial fluid and plasma, that I already mentions here. So, plasma means just you may get colorless from the blood, but interstitial fluid just below the skin. So, just see this difference between the interstitial fluid and the plasma, that is just for your more you may get more information's. But, using the micro needle, this interstitial fluid can go through the like this channel and you can detect the glucose. This is just one patch development.

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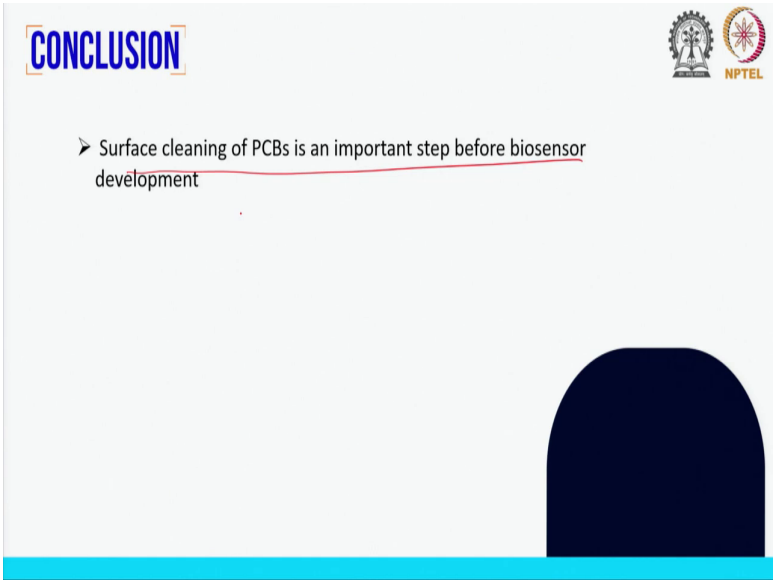

I will show you again this all the whole systems with the electronic part again next class. But just I wanted to tell you in this class that surface screening of PCB is really really important for biosensor development. I just started with the PCB for biosensor for glucose detections, then I will show you more example for biosensor development using the PCB.

But today's class you just learned that PCB can be useful for detections, many (Refer Time: 31:15), but you have to careful there surface cleaning method and surface roughness, their main cyclic voltammetry you have to measure before the electrochemical measurement ok.

Thank you, that is all for today's class.