Nanobio Technology Enabled Point-of-Care Devices
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Lecture - 04
Nanomaterials for Healthcare Biosensing

Dear students, today I will start the Nanomaterials for Healthcare Bio-Sensings. Last class I taught Translation Health Research, how we can use the new technology for point of care device development right, if you want to bring some new technology, how you can use for like for translation. Now, let us you learn the new some fundamental things; like, now you can use the nanomaterials for development of the healthcare biosensings.

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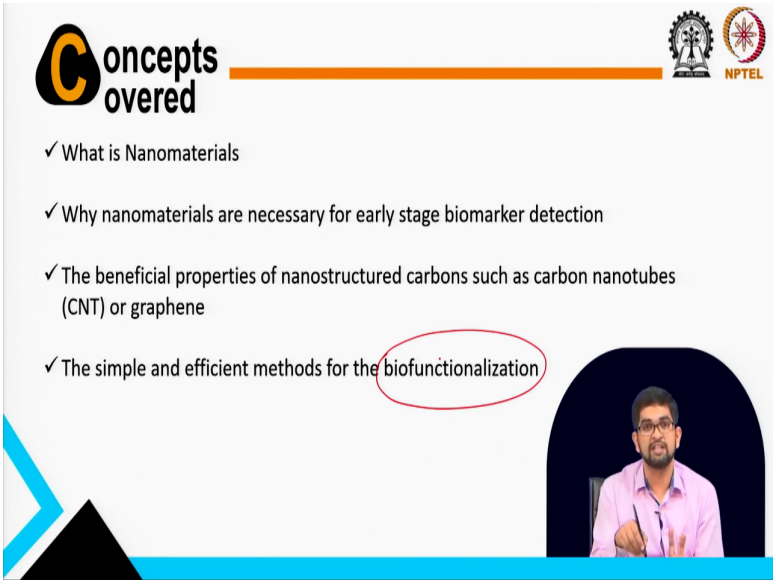

So, in this topic I will cover mainly what is the nanomaterials, then why nanomaterials is really necessary for early stage biomarker detections as. As this teaching topic is the nano bio

technology which we will use different nanomaterials itself for biosensor development and signal amplifications; so, that we can use this technology for point of care.

So, that also I will cover and I will tell you the beneficial properties of nanostructures some carbon based nanostructures like, carbon nanotubes, graphene. So, what is this that I will show you some structures also and how can you use this one for functionalizations of the surface than useful for biosensor development.

And the simple efficient method for biofunctionization; so, I will show you how to immobilize different kind of nanomaterial on the surface and then different bio nano biomaterials like antibody, aptamer or peptide. How we can immobilize on the sensor surface, because they are the receptor, those receptor can help to detect different biomarker.

So, first we have to immobilize on the sensor surface; so, we need some bio that is called bio functionalizations or bioconjugations. So, that we will immobilize based on some conjugation chemistry that I will teach you now.

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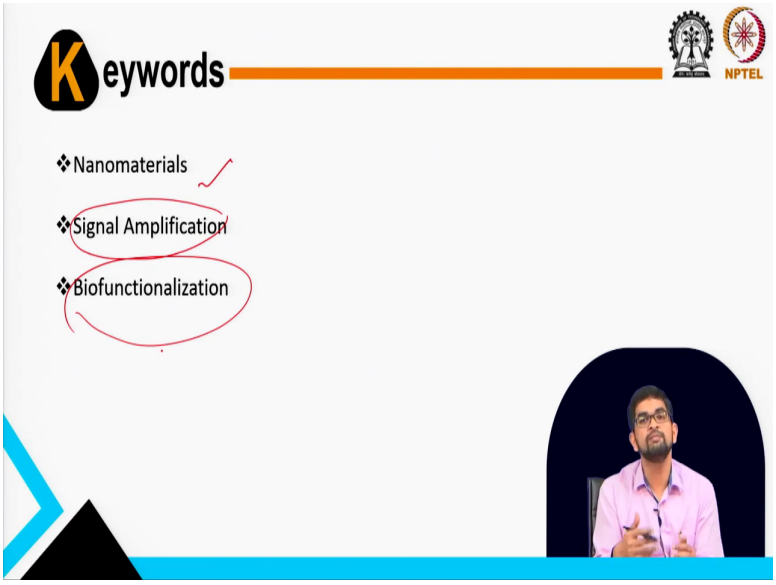

So, main keywords that is why this topic is a nanomaterial will amplify the signal based on the different nanomaterial, why we need signal amplifications that I will also teach you now and biofunctionalizations. So, biofunctionalizations means, how we can functionalize the different nanobiomaterial on the sensor surface using some different conjugation chemistry.

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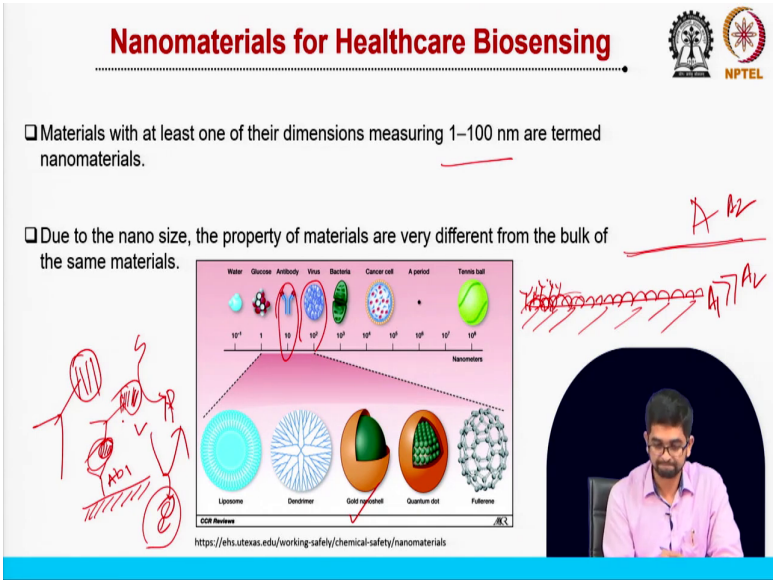

See you can see some images here nanomaterial for healthcare biosensings, what is the nanometer size, nanoparticle size? It is this nanometer range 1 to 100 nanometer range that term as a nanomaterials. You can see like the different sizes of the different nanomaterial, like the antibody their size that 10 nanometers, these in this range virus is around the 100 nanometers they are very very nanometer size.

So, like SARS Cov2 we are going to detect the, if you want to detect the whole virus; so, you should have some idea what about their size. Also, we are using different kind of nanomaterial like gold, gold nanoscale or something gold nanoparticle or carbon based some nanoparticle or platinum nanoparticle we will use for device fabrications.

So, why we need them? There is a some story, because due to that nanosize their property will be that is different than the bulk material. Like, if your sensor surface suppose this is

your sensor surface, if it is gold surface and if we immobilize the gold nanoparticle on the surface; so, their property will be different. Because, nanoparticles means because of their nanosize it is surface actual surface is much higher than the bulk material.

If it is the like just a flat; so, if you measure the actual surface area and if you measure now the nanomaterial coated surface area; so, actual surface area is much higher. So, if you see this is the A 1 and if it is just the flat if it is A 2; so, A 1 will be much much greater than the A 2. Not only this we are we are using the nanomaterial as I told you for functionalization of the different biomolecules that in the very high surface area you see. So, many antibodies we can immobilize on the sensor surface then the flat surface.

So, we will get the huge surface area that is one of the another advantage and definitely they are very highly conductive sometimes you may need the very conductive surface. So, nanomaterial can help and nanomaterial also can be useful for tagging like sometime your secondary antibody I can be tagged with some nanoparticle and that can act as a level and it can help for the signal amplifications.

Suppose, I will show you one design you know the sandwich allyser, I can show you if you do not know like if you have sensor surface. So, first your that is called a primary antibody, antibody 1 it is coated on the sensor surface. Now, you drop your sample; so, your target is here; now, you can add the secondary antibody right. So, this is secondary antibody that also you can tag with some nanoparticles.

So, nanoparticles they have lots of use, not only the sensor surface you can coat the nanoparticle also you can tag the nanoparticle with some antibody. And it can act as a level and it can react with some substrate I mean some chemical and it can form some product. And that product now it can be easily oxidized on the sensor surface and it will release the electron and that electron we can measure.

And based on this number of the electron we can determine the concentration of the target, see. So, this is the another application of the nanomaterial that can be useful for the nanobio sensing design.

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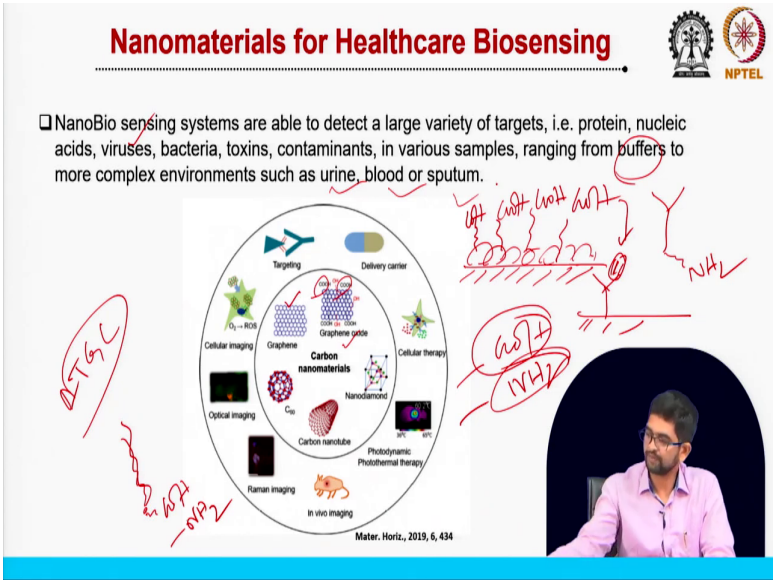

Let us come define nanomaterials here, I will show you like this is a carbon based different carbon based nanoparticle nanomaterial; like, the you can see the graphene, graphene oxide, like you can say different kind of the nano diamond. So, this kind of different carbon based nanomaterial you can use for biosensor development.

Suppose you have a like suppose this is a sensor surface; so, you can functionalize this surface with some graphene some graphene nanomaterial. So, you can answer why we need

the grapheme, because graphene or graphene oxide you can use. So, at one thing you can if you means from graphene if you go to graphene oxide or maybe reduce graphene oxide.

You can enhance the conductivity at the same time also you can function you can also functionalize the surface you can bring some functional group. Means, it is functionalized you can see they have lots of functional group like carboxylic group. So, you can I mean incorporating some functional group also on the surface. So, just you can drop cast on the sensor surface some this kind of carbon nanomaterial.

So, this kind of functional group will be very much helpful to immobilize different nanobiomaterials. Suppose antibody you want to immobilize on this sensor surface; so, this functional group will help to bind with the antibody, why? This antibody also contains some functional group it has pre amine group. So, this carboxylic group and this amine group we can now conjugate using some chemical or some basic chemistry that I will teach you in the next slide.

Now, that is why we need some nanomaterial also yeah clear; so, that is the utility of use different nanomaterial for the biosensor development. You can see here also we can detect lots of not only the protein, we can go for the nucleic acid, we can go for DNA, RNA detections also. Because, we can also immobilize on the sensor surface different aptomer.

So, aptomer just we can artificially we can synthesize it is like ATGC sequence you know already; so, using some different sequence some pacific bioreceptor we can develop some aptomer; so, we can develop. So, those sequence or DNA or different DNA we can functionalize with some carboxylic group or amine group; so, on the surface see we need carboxylic group or amine group.

So, if you are using carboxylic group on the bioreceptor, then your surface should have amine group. Or if your sensor surface have the I mean your bioreceptor content amine group, then you can be in the carboxylic and if it is carboxylic, then you can be in the amine group right

like vice versa. Then you can use some chemical and then you can functionalize clear this concept.

And this kind of all the technology we can use for you know also virus detections like COVID cases, we also use some rapid test kit like whole virus detections. So, here we can what we can do? So, on the sensor surface just we can immobilize a very specific antibody for the whole virus like SARS CoV two detections. That now, if you drop the sample then whole virus can be attached then we can go for different electrochemical detection. So, that all the electro chemical technique I will teach you in the next class, not today.

So, first I will show you the all the basic fundamental part, then I will come to the technique part how we can detect, what which technique we can use. See, we this bio sensor cases we can go for the different like media like different different from the buffer we can go for the urine, blood, sputum different different media we can use; so, there we can try for detections.

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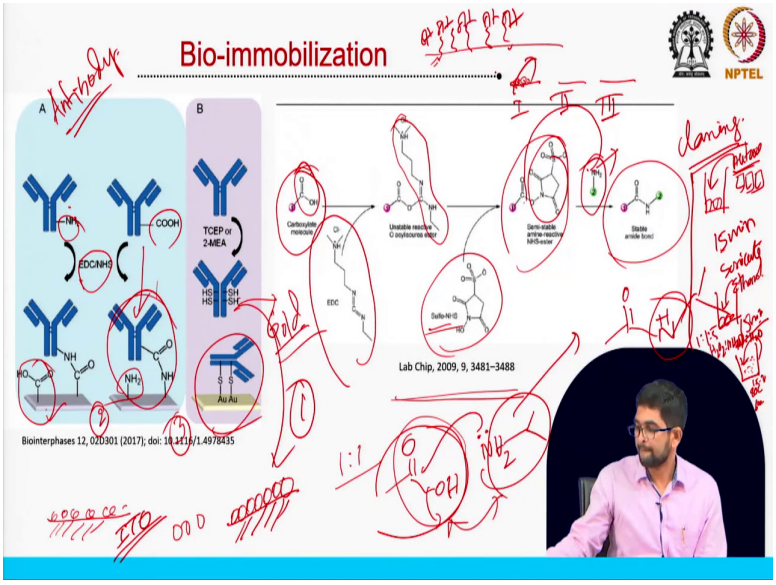

See now come to the immobilization part, the just now, I told you like if you have one biodeceptor like antibody. So, mainly I will always give you example based on antibody, because it is very simple example and everyone you know what is the antibody right. And they have the functional group free some amine group or carboxylic group they have.

So, based on this which function group you want to use on the sensor surface. So, this one your sensor surface, there you can functionalize some group based on some functionalized nano particle you can use ok. Also, chemically also you can functionalize, but that topic I will come later. Let us you have a nano particle like, graphene oxide it has some carboxylic group that we can use.

So, the chemical name now I am going to tell you that EDC and NHS, this is the chemical I can show you the basic structure of the EDC and NHS; so, this is the EDC structure and this

is the NHS structure. So, we are using sulfo NHS because it is very easily, we can make soluble in aqueous medium that is why we use the sulfo NHS sulfo functionalize group.

So, what we are doing suppose; so, your sensor surface you have carboxylic group, this example. Now, here we will add first EDC, but this EDC and NHS sometime we can use 1 is to 1 concentrations or sometime can vary also depend on your requirement. So, that actually sometime you need to optimize there is lots of publication that I also give some reference here where we borrowed this image.

You can see the lab chip where we this kind of technology already mentions. If you want to go for further study you can check the basic fundamental things, I am going to teach you that you can use the EDC and then this one will react with this carboxylic group clear. Then this functional group see it make this EDC group here, on your sensor surface.

But this surface is very unstable and reactive that is why we can use the sulfo NHS; so, that we can make some semi stable some surface that after reacting with the NHS. Now, you will add your primary antibody that already have NH 2 free amine group. So, this there is a nucleophilic attacked here and it will remove this functional group and amine group will bind, and you will get a stable amide bond.

So, actually the basic reactions is this one right C double bond O OH and you have the amine group with the antibody right. So, this come here and OH group release; so, basic amide group is forming right; so, basic amide group is forming that is your antibody. So, you can answer why we need the EDC NHS? Let us mix this carboxylic group and amine functionalize antibody and we will get the amide, you can say yes, they are thermodynamically they are feasible.

But kinetically they are really slow this reaction. So, we need some catalyst; so, they help this one. So, first they will react with this carboxylic group and they will form this intermediate stage and this will be very much reactive towards the amine group ok. And that is why we are getting very easily this amide bond; so, this is your first example.

Now come to the second example on your sensor surface you have the amine group; so, this is opposite. So, first we tried amine group from the antibody and carboxylic group on your sensor surface. Here we will now we are trying your carboxylic group on your antibody and amine group your sensor surface.

Now, you can answer how you can then modify your surface amine group, because I told your nanomaterial has the carboxylic group not amine group, yes, we can modify again the amine group also on your nanomaterial. Some dendimer also available that you can immobilize on the sensor surface that has the amine group ok, you just dropcast those material on the sensor surface.

Then you will get the amine group on the sensor surface and again you just use the EDC, NHS and you will get the functionalized antibody coated sensor surface and that can this is ready. Now, ready for the detections, now you can drop the sample and can detect. Another example number 3, I will tell you how you can functionalize like your antibody can be modified with thiol group, you can use TCEP or 2 MEA and you can modify the thiol group and there is a strong interactions with thiol and gold.

So, if you have any gold nanoparticles; suppose, if you have a sensor surface that is modified with gold nanoparticle or maybe just gold plate. Or sometime you can modify on a gold nanoparticle because it is very cheaper. Because, gold nanoparticle you can easily you can make with gold salt that technique also I will teach you today like how you can make the gold nanoparticles.

Then just you can dropcast and you can get a gold nanoparticle modified sensor surface. Now, thiol and gold they have very strong interactions you just drop this thiolated antibody and you will get antibody coated sensor surface. Now, one things I am going to tell you and I always saying that let us dropcast some nanometer on the sensor surface, but before that one technique you have to follow.

Whenever you are getting any sensor like we are dropcast what is the sensor which sensor surface will you use? Suppose, we are going to use a glass surface that coated with indium tin oxide nanoparticle that is commercially available that is called ITO. ITO this is very much commercially available Indium Tin Oxide coated glass surface it is very stable; there we can modify your antibody.

So, this ITO then you can modify with some nanomaterial also like some graphene oxide or maybe some reduced graphene oxide or maybe gold nanoparticle. But, before that one thing you have to remember your sensor surface maybe it contains; so, much too much impurity.

Because, when we are receiving because it is commercially available, we are using from the industry, they may send or maybe they sometimes they touched on the sensor surface our skin may contain different our oil skin or different kind of organic impurities that may come on the sensor surface it can cover the active area.

It may cause maybe some reproducibility problem that suppose you make like 1, 2, 3 sensor surfaces. If some sensor surface already coated with some impurity, then these all 3 sensor surface may not behave same, but similar they may not behave because some active surface already cover with some impurities; so, we have to remove these impurities.

How we can remove these impurities? You have to go for some cleaning we have to go for some cleaning procedure. What is the cleaning procedure you have to follow? That is I am going to say tell you. So, first you have to clean your all the electrode surface, let us take all the electrode like small small electrode chip you can keep inside the beaker, then you can put like acetone and then you can sonicate it 15 minute and sonicate.

Why I choose acetone? Because it is non polar and your organic contaminant also not much polar. So, non polar non polar they have some interaction, they can easily remove the organic impurities. So, I choose first acetone, then slowly I will increase the polarity of the solvent. So, after 15 minute sonication then we will remove the acetone then again I will keep all the

electrodes in a beaker where I will use like some ethanol alright, it is politely little higher then again 15 minute I could sonicate.

Now, I can make a mixture of a solution which mixture? I can take here hydrogen peroxide, ammonium hydroxide is to water hydrogen peroxide, ammonium hydroxide and water mixture. Which ratio? I can use this one 1 is to 1 is to 5 ratio, this mixture is a very highly polar mixture and it can remove maximum the impurities from the sensor surface.

So, these solutions I can prepare and I can put all the your sensor chip inside these solutions. And again, I can sonicate or maybe even you can boil not boil you can use like 80 degree Celsius temperature you can heat these solutions around like 60 minute or you can even you can sonicate it 15 to 30 minute, then almost all the contaminant will be removed.

And another thing once your sensor surface removed all the contaminant, your surface become too much hydrophilic. So, if your surface contain like one can be contaminant there actually hydrophobic, hydrophobic means your if you drop some water, you can see like circle like if you measure the contact tangle this is a very high contact tangle it is the hydrophobic it means.

So, if you now clean the surface your surface will form lots of hydroxyl group lots of hydroxyl group; so, because of this your surface become too much hydrophilic. Now easily this hydrophilic surface can be useful for modifications of the nanomaterial that you have, like reduce graphene oxide or other nanomaterial you can easily drop cast here and dry it you can use for sensor So, that is a before using your sensor surface let us clean it all the ways, then only you can get more reproducible data ok.

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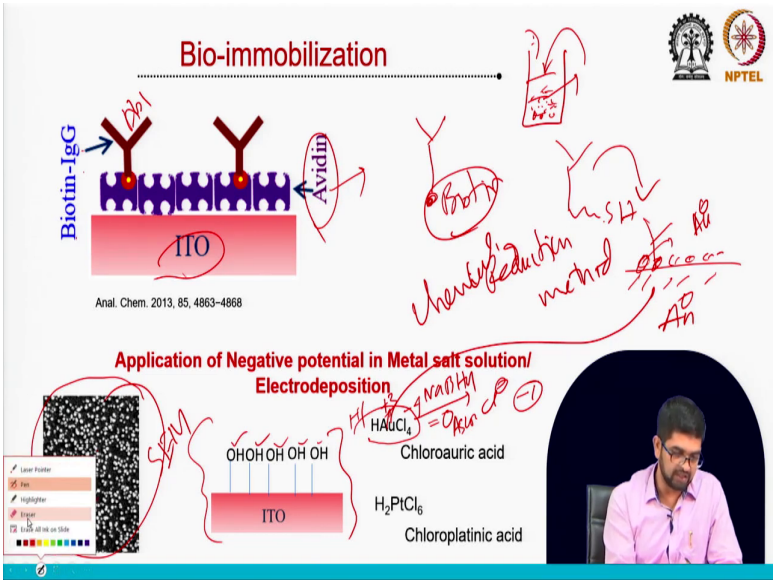

Now, another bi functionization technique I will show you like after cleaning that I told you the ITO electro. After cleaning you can immobilize on the sensor surface avidin this is another bi-molecules. Why I use avidin Avidin or stepped? Avidin in also we can use? Because, if you use a antibody that is already conjugated with biotin and avidin and biotin they have very strong interactions non-specific strong interactions.

So, biotinated IgG means your antibody, primary antibody. If you have the antibody coated surface just drop this antibody, it will strongly it will wound on the surface. So, you will get primary antibody coated sensor surface by using this, this avidin biotin interactions or you can use stepped avidin they are similar avidin or stepped avidin, this is another technique for bi-immobilization ok.

Now, I will come here that I told you previous slide that we can modify the gold nanoparticle also sensor surface for immobilization of thiolated antibodies. Suppose you have the antibody that is thiol group right and your sensor surface contain lots of gold nanoparticle. So, if you drop this thiolated antibody because of this thiol and gold interactions; so, you will get antibody coated sensor surface.

So, now you can understand how we can modify your sensor with gold nanoparticles. See there is two possibility of modifications of gold nanoparticle on the sensor surface, first technique I will tell you. So, I can see this is a SEM image, Scanning Electron Microscope image of the gold nanoparticle on the ITO surface. You can see the small small nanoparticles there are all the gold nanoparticles that can be formed by the help of two technique.

One is called the reduction method reduction; this is reduction method that is chemical reductions method. How we can use chemical reduction method? See you can see the salt like HAuCl 4, this is the gold salt chloroauric acid. You know the how to calculate the oxidation number of this molecule. You can see the chlorine cl minus it is minus 1 charge you know and hydrogen is the plus 1 their oxidation state.

So, here is the minus 4 total here plus 1; so, because molecule is neutral; so, A gold should be plus 3 right, then the total charge will be 0 right that you know already. So, gold nanoparticle means they are all are Au maximum they are Au 0 stage right. So, you have to form gold 3 to gold 0, how so you have to reduce it? So, you can use some reducing agent, suppose here you can use like sodium, borohydride or you can use ascorbic acid this kind of reducing agent you can use.

So, you can reduce this salt in a beaker you can take this chloroauric acid and you can add the reducing agent also at the same time you can have some stabilizer like citric acid something they will help not to aggregate each other. So, you will get like different different like lots of gold nanoparticles solutions you will get.

Now, after cleaning the surface, like this surface as I told how to clean the surface; so, after cleaning the surface like ITO I just taught you like a different method cleaning; so, it contains lots of hydroxyl groups see. Now, you can drop cost this gold nanoparticle here just drop cost and dry it and you will get the gold nanoparticle modified the sensor surface. This is one technique another technique that I am going to tell you that is; so, let us remove this I am going to teach you.

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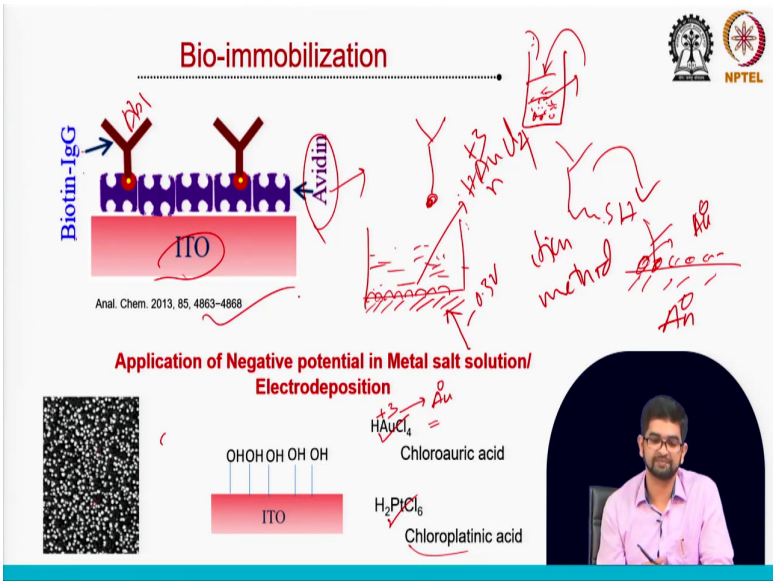

Now, another technique that is the electrodepositions technique where we will use the negative potentials let us show you. So, suppose you have a sensor surface now, as I told you that here gold is the plus 3 oxidation stage from here you have to make gold 0 that you can do; you can do by applying some negative potential. So, anyway you have to supply some

electron right; so, by applying some negative potential you can easily form the gold nanoparticle, see.

So, only electrode surface you can use gold salt, this is the electrode surface you can use the gold salt; so, it is HAuCl 4 which is plus 3 oxidation stage. Here you can apply some negative potentials, how about the minus 0.3 volt you can apply. When you are applying negative potential; so, from here nanoparticle will form and they will cover the sensor surface. This method is called electrode deposition by applying negative potential ok.

So, this is for the gold nanoparticle, at the same thing you can try like if you need the platinum nanoparticle. What will what will you use naturally? Like here, gold salt you can use some platinum salt gm see H2PTCL6 this is a chloroplatinic acid. Same way you can reduce it or you can apply some negative potential on the sensor surface you will get the nanoparticle, very basic very simple things I am teaching you.

Then and you can think about for other nanoparticle generations also, and modification of the sensor surface do you really need gold or do you need platinum or something else like iron based that. Now, you can design that is different kind of like core cell based nanoparticles also like now proposed.

You can go for some reference paper that I already put different references during the teaching all the references you please go through. Now, you can check that there is lots of other nanoparticles also available, you can use for you can just synthesize them and use them for sensor surface modification.

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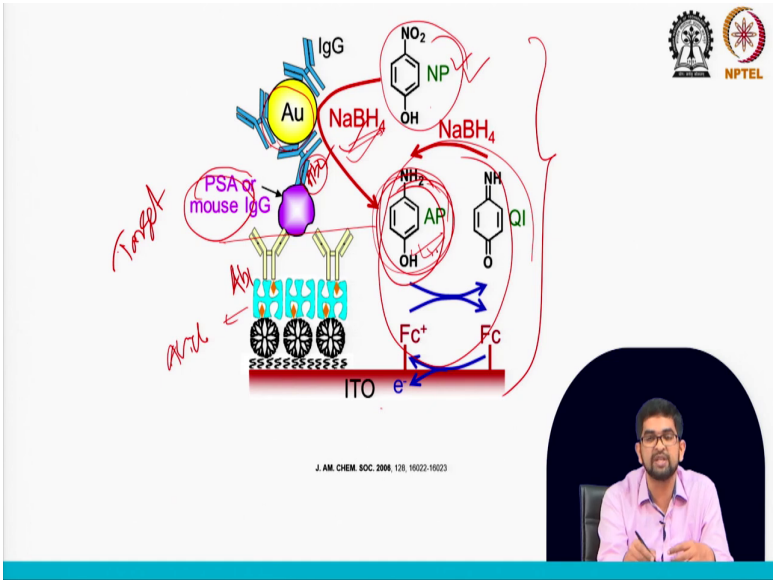

Now, I will come a very good example, until now I told the sensor surface modification with some nanoparticle right. Now, the similar way the nanoparticle I already told you that you can modify this one as a tag or as a label which is a secondary antibody also you can label with some nanoparticle. So, this is the primary antibody 1 this is the antibody 2 right that I told you.

So, why we need this modification with the nanoparticle? It can help you to produce, but to generate an ultra sensitive biosensor. See this is just like a like sensor surface like ITO surface, there we just modify the surface with some this is just avidin coated. And this is a biotinylated antibody that modification now I am not telling you because I already taught you.

So, until now your primary antibody coated sensor surface ready right, any technique you can use for primary antibody modifications. Now, you just immobilize you will drop first you will drop your sample that sample may contain your target. So, here PSA your mm IgG this is nothing just a target that is a generic name I put here any target. If it is cancer anti any kind of antigen then you have to use the pacific antibody right. So, here the secondary antibody I use pacific that conjugated with gold nanoparticles.

Now, these gold nanoparticles can help with reacting with some chemical that is the nitrophenol chemical, if you use some reducing agent NA BH4 that can produce the amino phenol. Actually, these chemical can help to produce the signal, but this chemical cannot be formed if gold nanoparticle not there. So, basically gold nanoparticle actually helping as a catalyst to produce this amino phenol by reacting with the amino phenol in the presence of sodium borohydride.

If there is no gold nanoparticle this chemical will not form and this reaction will not happen and you will not get the signal amplification on your biosensors surface. Anyway, the next class I will teach you this kind of all the signal amplification, but here just today I just want to complete today's lecture by using this nanoparticle modification in the secondary antibody that can help as a tag.

And it can produce some like a reactive species only if it is present, if it is not there then your sensor will not work. So, that like this way your nanoparticle can help also to generate the signal very pacific way; so, this is another example ok.

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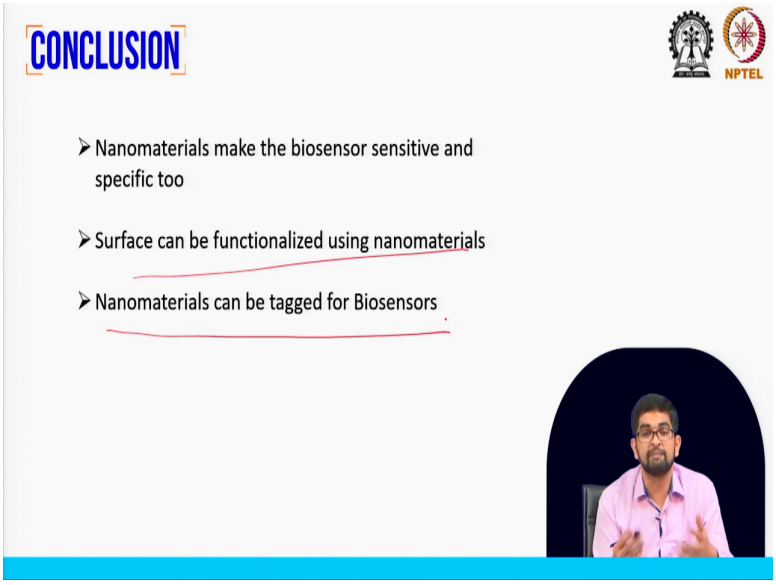

So, what is the conclusion for today's lecture? So, nanomaterials make your biosensors more sensitive also pacific too, because if nanobarticle present only then only reaction happen otherwise no. So, like this way one nanoparticle can help also surface can be functionalized your surface that I told you how we can modify the surface using nano bio nano material and nanomaterial can be tagged in the biosensors right. So, next class again I will teach you like signal amplification using nanoparticle.

Thank you very much.