INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

NPTEL

NPTEL ONLINE CERTIFICATION COURSE

Biomedical Nanotechnology

Lec-19
In Vitro Methods of Study Antibacterial and Anticancer Properties of Nanomaterials

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Hello everyone I welcome all to this 19th lecture of this course. This 19th lecture is on In Vitro methods to study the antibacterial and anticancer properties of nanomaterials.

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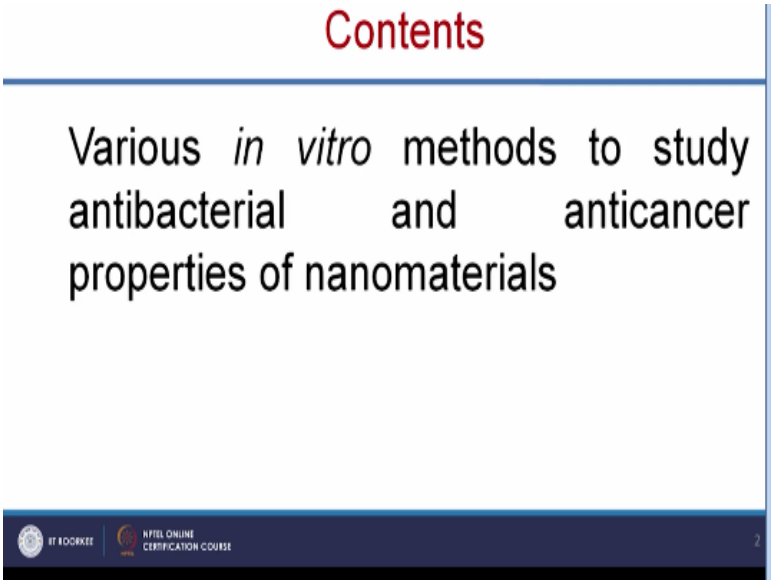

So in this lecture we are going to learn a various In Vitro method to study the antibacterial and anticancer properties of nanomaterials.

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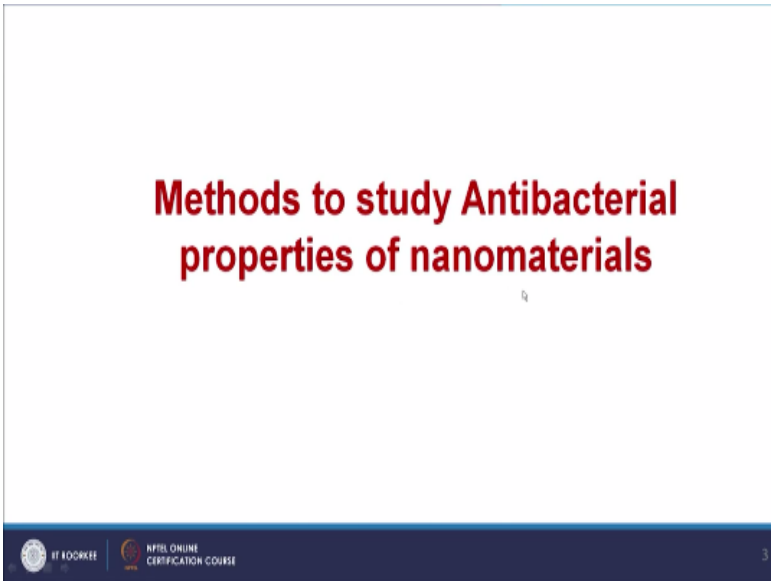

First we will study about what are the various methods available to study the antibacterial properties of nanomaterials.

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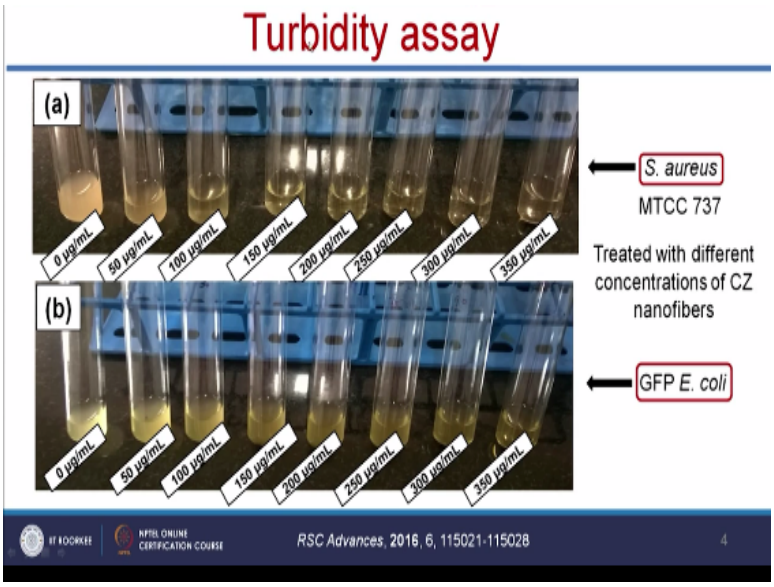

So the first experiement is turbidity assay okay. So in this we are going to visually monitor the turbidity of the bacterial growth okay. And whenever you make a nanoparticles the first thing is you have to select the suitable bacteria to study the antibacterial efficiency. So the ideal choice should be like one gram positive bacteria and one gram negative bacteria.

So in this experiment we have selected S-aureus okay as a gram positive model and equalized as a gram negative model. And we have treated with a different concentration of copper, zinc, nanofibers okay. So here you will be taking a equal amount of bacteria in the test tube and you will be adding different concentration of fluid nanomaterials to study the antibacterial efficiency of your nanomaterials.

And in the control that is where there is no nanomaterial so you can see here the growth of bacteria is visible by the turbidity okay. And with respect to concentration you can see here the turbidity is going down. So using this visual turbidity assay we can easily identify the minimal inhibitory concentration as well as minimal killing concentration or minimal bactericidal concentration.

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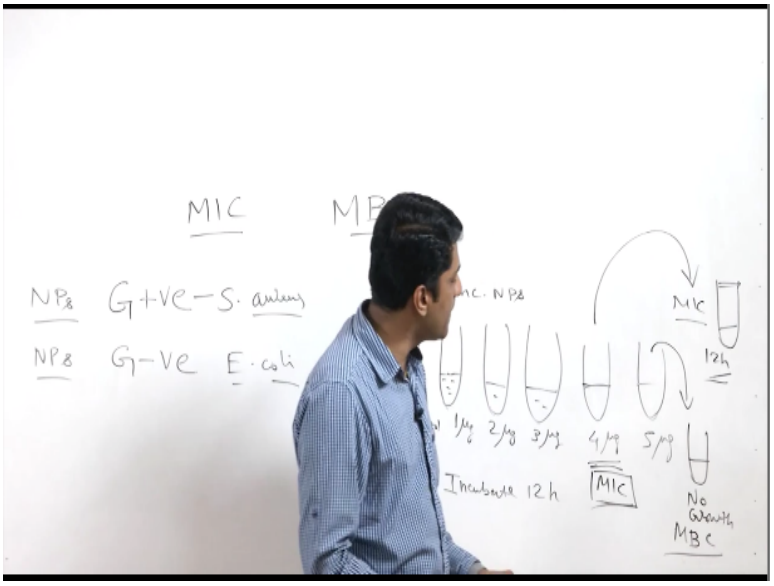

So let me knew how to determine the minimal inhibitory concentration and minimal killing concentration or minimal bactericidal concentration. So for this you will be selecting one gram positive bacteria okay, and gram negative bacteria. So when we add these nanoparticles to this gram positive bacteria and gram negative bacteria we can identify whether your nanomaterial is having antibacterial efficiency again to the gram positive or gram negative whether it is having a broad antibacterial efficiency or not okay.

So here we have taken gram positive bacteris S-aureus, and gram negative bacteria E-colin. So for identifying this MBC concentration, so what we have to do is, we have to add equal

concentration of bacteria to test with the Newton broth or the LD broth okay. So we can add 10^7 CFU that is Colony forming unit okay. So we are adding equal concentration of bacteria to various test tubes and you will be adding different concentration of your nanoparticles.

So this first one is your control, where there is no nanoparticles and here for example you are adding one microgram and here you are adding two microgram, three microgram okay, four and five microgram. So after that you will be incubating for 12 hours okay that O neg incubation. So after incubating you will see complete turbidity in the control where there is no nanoparticle, and you will be able to see that the turbidity will going down with respect to concentration okay.

So here at some concentration you would not see any bacterial growth okay. So we cannot see any bacterial growth at this concentration, so that is your MACR, MBC. So how do you confirm that it is MACR and MBC, so from this test you have to innoculate into a fresh test tube okay. So from the test tube where you do not see any growth from there you take the innoculum and add it to the fresh tube, and you incubate for 12 hours.

And after 12 hours if we are able to see the growth that means this concentration is called as minimal inhibitory concentration okay. And from this test tube again you can innoculate into the fresh test tube and here there is no growth, that means this concentration is called as minimal bactericidal concentration okay. So you are inoculating equal amount of bacteria and you are adding different concentration of nanomaterials okay.

And the test tube where you do not find any growth from there you are taking these inoculum and adding to the fresh tube with the medium okay. So if you are able to see the growth that concentration is called as inhibitory concentration. So from the another test tube where there is no growth you are inoculating to the fresh tube and if you are seeing there is no growth that means this concentration is called as minimal bactericidal concentration.

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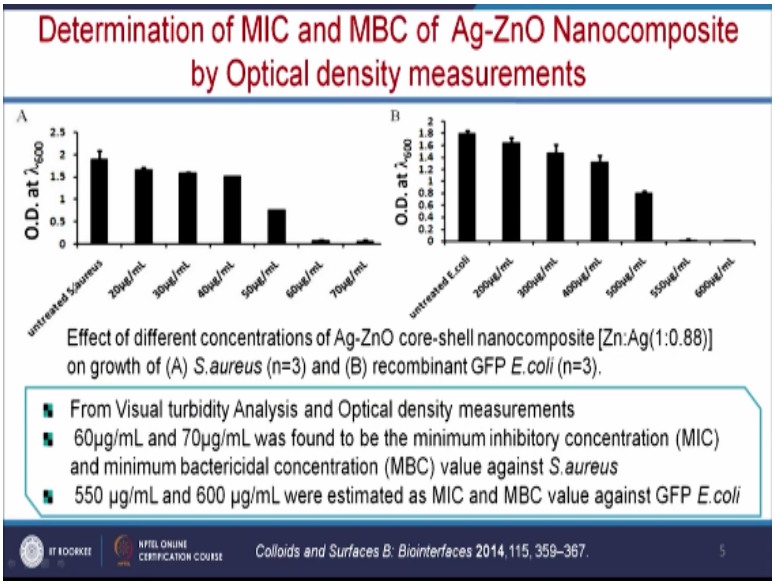

And this minimal inhibitory concentration, minimal bactericidal concentration can be also studied by optical density measrement that is called as OD okay. So whenever you estimate the DNA or protein will be measuring that observance at 260 hours and observance at 280 nanometer, because your DNA or protein is observing. But whenever you use that bacteria we are using the term OD, that is optical density, because here it is not observing it is scattering the light okay, that is the difference between observance and optical density.

So here this is a S-aureus bacteria, untreated S-aureus bacteria and it is treated with a different concentration of nanomaterials. So in this case that is Ag-ZnO nanocomposite okay. So you can see here with respect to concentration the bacterial growth is going down. And this is for gram positive bactyeria, and this is for the gram negative bacteria E-coli. So you can see here this minimal inhibitory concentration as well as the minimal bactericidal concentration is different for both the bacteria.

So as I told you like it depends on the bacteria whether it is gram positive or gram negative the antibacterial concentration will vary. Again similarly it depends on the bacterial strains also, the antibacterial concentration or effciency will vary okay. So in this example you can see here, so 60 microgram is your MAC concentration for gram positive bacteria, and 70 microgram is the MBC, that is minimal bactericidal and concentration.

And in case of E-coli it is 550 microgram as MIC and 600 microgram as a MBC value okay. And here we are measuring this n=3 that means this experiment is repeated for three times okay, three

independent experiments then you will get the, this kind of bar chart okay. So you will plot the average of the value and also you will plot this kind of standard deviation error bar. So here we have done with different concentration of nanomaterials.

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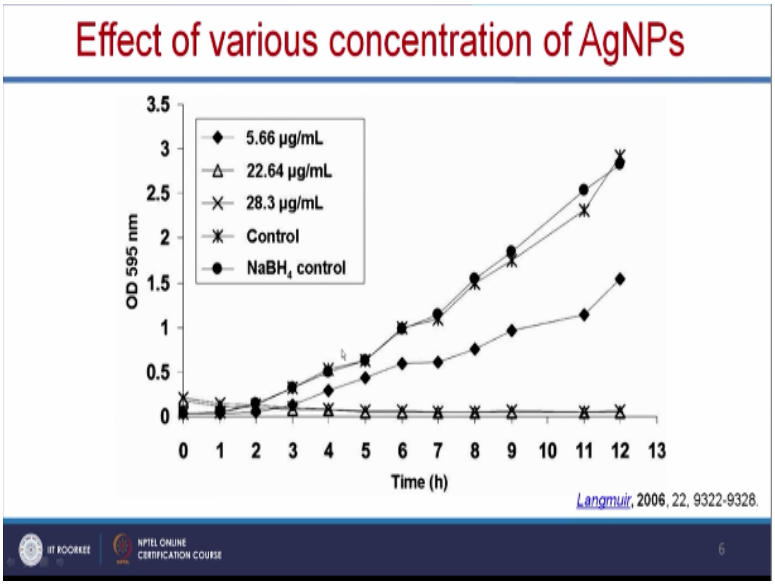

So in the next slide we can see here, so this with respect to time point, different time points okay. So effect of various concentration nanoparticle with respect to at different time points. So here you can see here, so in the control where there is no nanomaterial, so the growth is gradually increasing and in this nanoparticle preparation we have used the sodium borohydride as reducing agent.

So the reducing agent is not toxic, yoi can see here where we added the reducing agent there is no inhibition of growth, it is also growing similar to the control. And you can see this one, this one is the low concentration of nanoparticles that is 5.66 you can see here in presence of low concentration of nanoparticle, so the growth is gradually decreased okay. And here when we use the MIC or MBC concentration you can see that there is no growth, the growth is completely inhibited or arrested.

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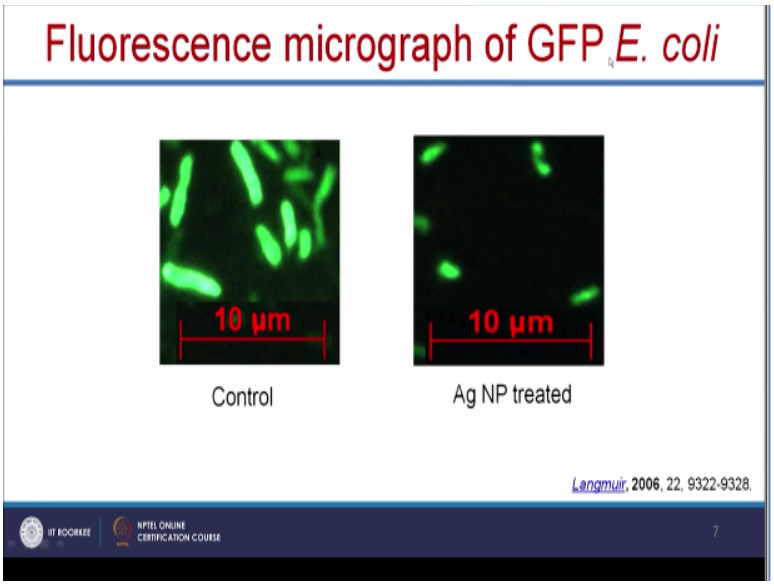

So in our experiement we have used Green Fluorescent protein expressing E-coil okay. So the advantage of using this green fluorescent protein expressing E-coil is when you use the wild type bacteria, so to monitor the effect of this nanomaterials you have to strain the bacteria using gram stain. So when you use the gram stain, there is a lot of washing steps, so there is a chance for artifacts or positive or positive negative cells.

So to avoid that we can use this green fluorescent protein expressing E-coli. So here ou can easily monitor the control vessels, similar nanoparticle treated E-coli, you can see here, here the morphology is normal and in the treated one the morphology is, the cell is damaged okay.

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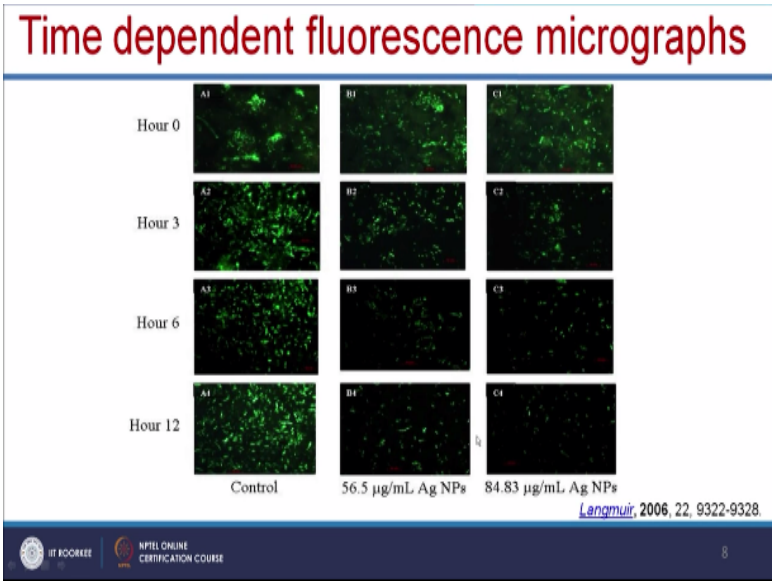

And here we can also do that time dependent studies, so at zero hour you can see here control and cellular nanoparticle different concentration you can see here equal amount of bacteria. So the test need to time, so in control where there is no nanomaterials, the growth is gradually increased that where there is a nanoparticles the growth is inhibited okay, and another image of using this GFp E-coli is, we can also estimate the fluorescent intensity okay.

So there is another way to confirm the antibacterial efficiency, you can see here with respect to different concentration of nanoparticle the fluorescent intensity is also going down. So as I told you that every experiment you have to repeat for 3-5 times and you will get the average then you have to make the standard deviation error bar okay. So and these stars on the top of this is statistical analysis okay.

So if you have more stars that means your data is more statistically significant okay, is simple to understand. And here you can see here, so this control one the bacteria is more and with respect to concentration of nanoparticles the bacteria number of going down.

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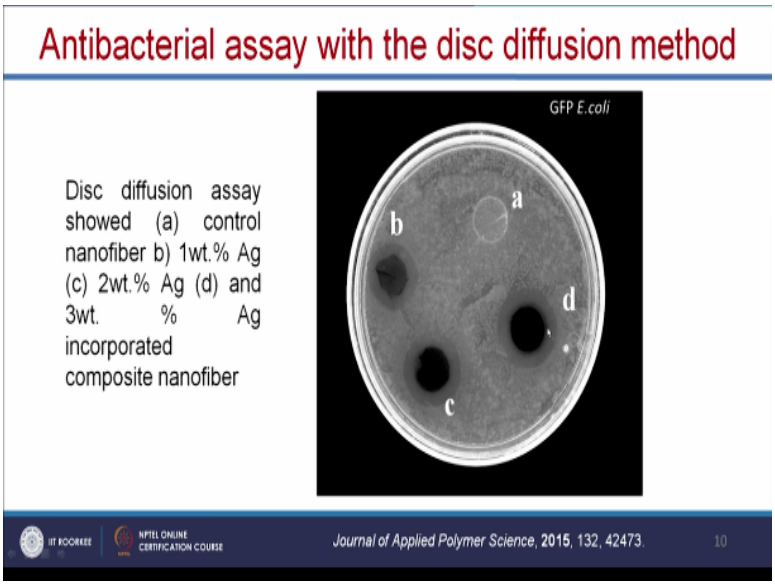

So the next method is antibacterial assay with the disc diffusion method okay. So here in the bacterial plate, so you will be adding the lawn of bacteria, by spread light method okay. So onve you made a lawn of bacteria, then you will be adding this uniform size of nanofiber disc or if you are hacving a nanoparticle solution you can make a sterile water made paper okay, uniform size and you can dip it into the nanoparticle solution and you can place it on the top of the bacterial plate, where there is a lawn of bacteria.

And allow it to stay for overnight incubation okay, so after overnight incubation you can see the clear zone of incubation, that means these are nanofiber or the nanoparticle loaded disc it is inhibited in the growth of the bacteria, so this is called as zone of incubation. So by measuring the length of the zone of incubation we can calculate the antibacterial efficiency of your nanomaterial.

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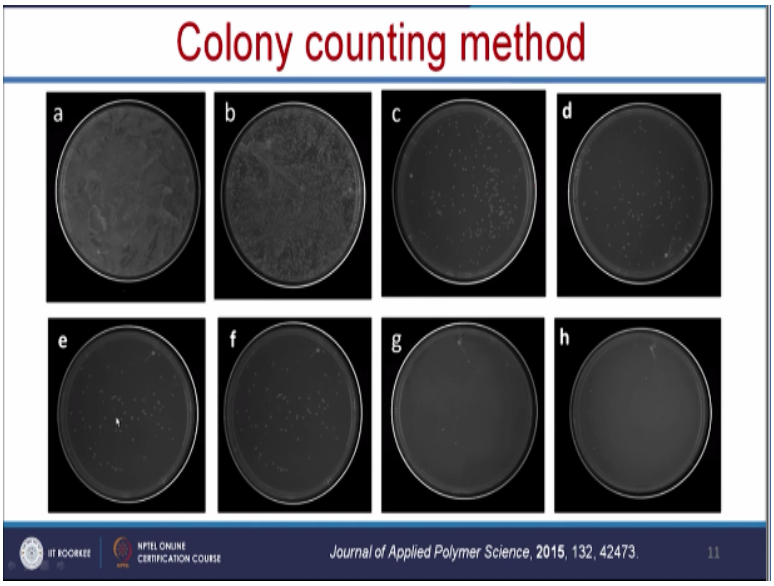

And another method is colony counting method, so it is the same thing like where you did the visual turbidity method from there you can inoculate into the bacterial plate okay. So you can by spread threading technique you can spread that bacterial solution the top of this plate, and here will be incubated the plate for overnight then you can count the number of colonies, you can see here with respect to concentration the bacterial colony is going down.

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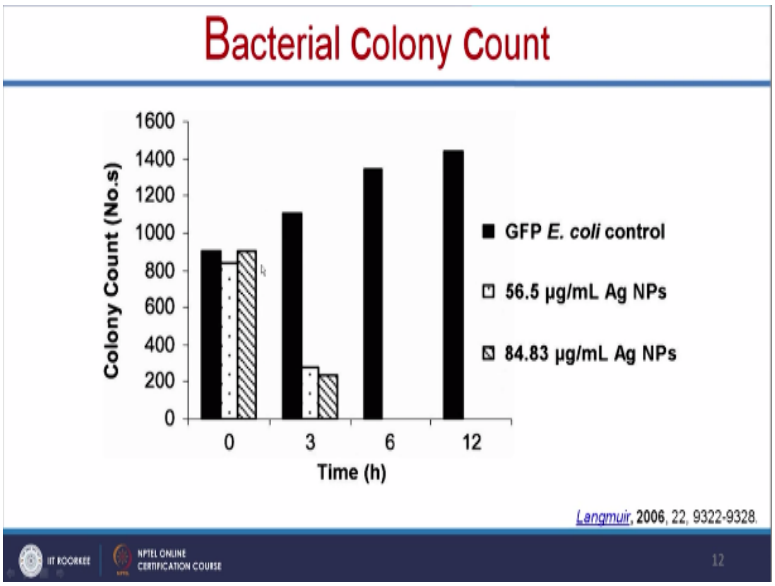

So this is the control where there is a huge number o colonies okay, lawn of bacteria and with increasing the concentration of nanoparticles, the number of bacterial colony is going down. So we can easily count that bacterial colony and you can plot it.

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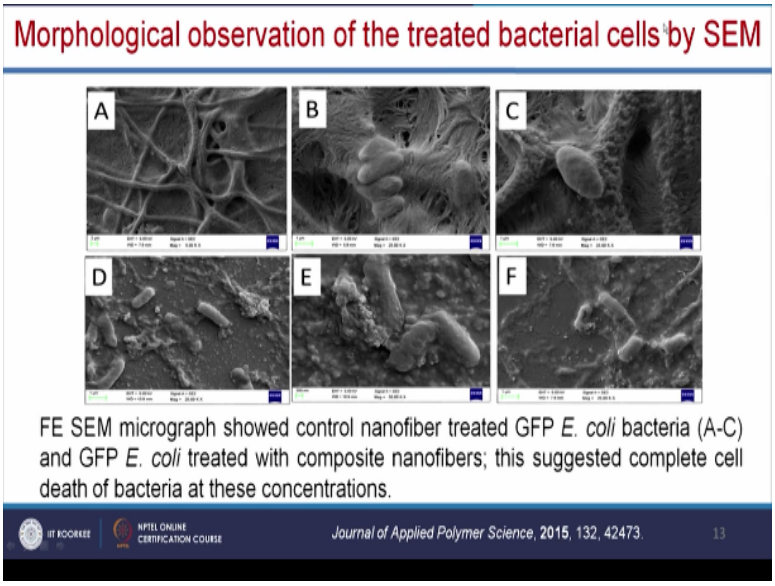

And we can also observe the morphology of the treated bacteria by using scanning electron micorscope, you can see here the control untreated the bacteria is having normal morphology, rod shape, E-coli okay. And in case of treated one you can see here the complete morphology is damaged, the E-coli is damaged and it shows confirm the death of the bacteria okay.

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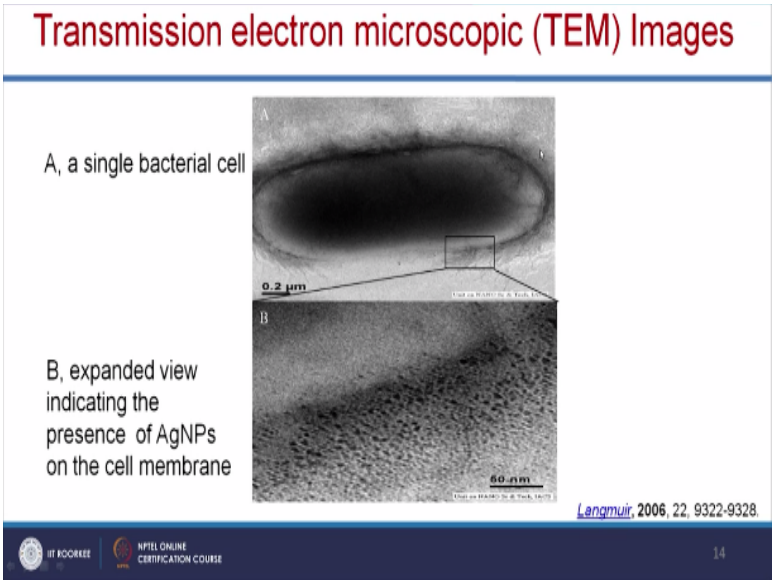

So next one is, we can also use the transmission electron microscope to understand the bacterial cell that mechanism. So you can see here this is a single bacterial cell and this is the expanded view of the bacterial cell and you can see here your cellular nanoparticle this black color spots cellular nanoparticles, the cellular nanoparticles bind into the cell wall and it is damaging the cell wall of the bacteria and killing the bacteria.

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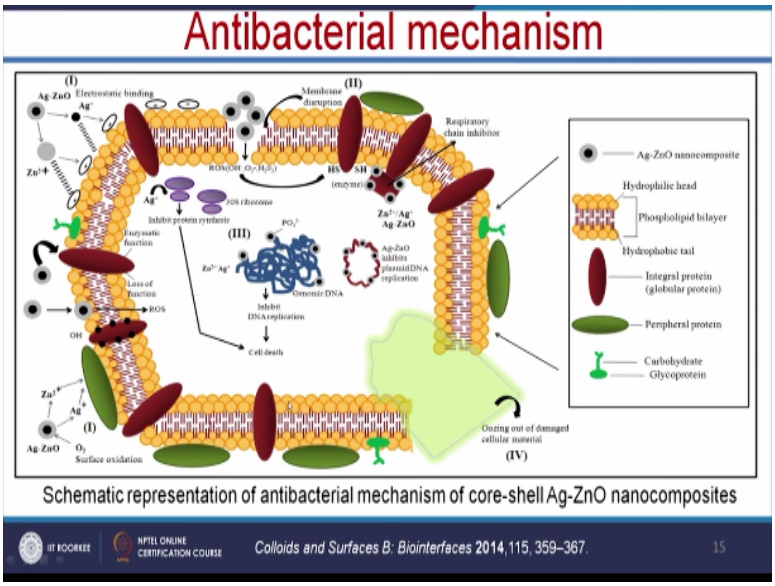

So based on the literature so this is the possible antibacterial mechanism, so most of the metal nanoparticles will be having positive charge and negative charge and our cell bacterial cell is

having negative charge. So this positive charge nanoparticle will come and bind through ultrastatic interaction okay. And it will induce reactive oxygen species, so and again some of the nanoparticles will go inside the bacteria and bind to the DNA and that will inhibit the DNA replication as well as they inhibit the protein synthesis.

And finally it will damage the cellwall okay and all the materials will be using out from the damaged cell okay. So these are the various mechanisms nanoparticles will follow to kill the bacteria.

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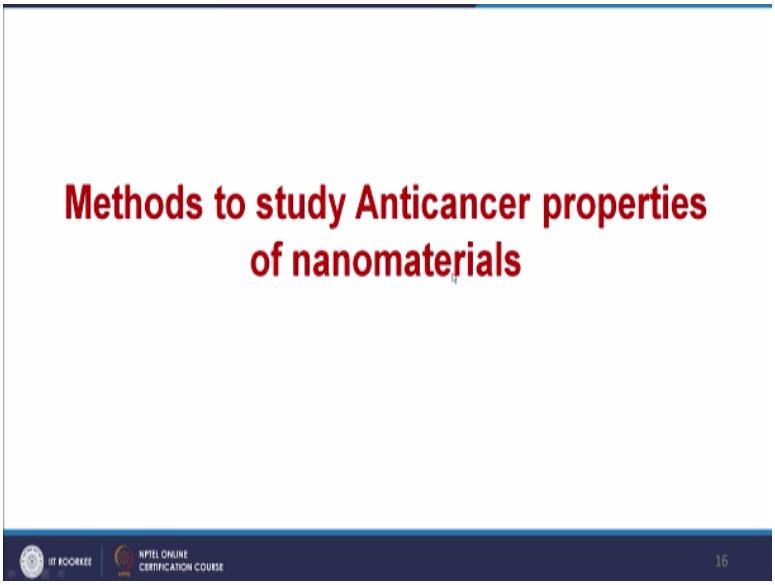

So let us see what are the various methods available to study the anticancer properties of nanomaterials.

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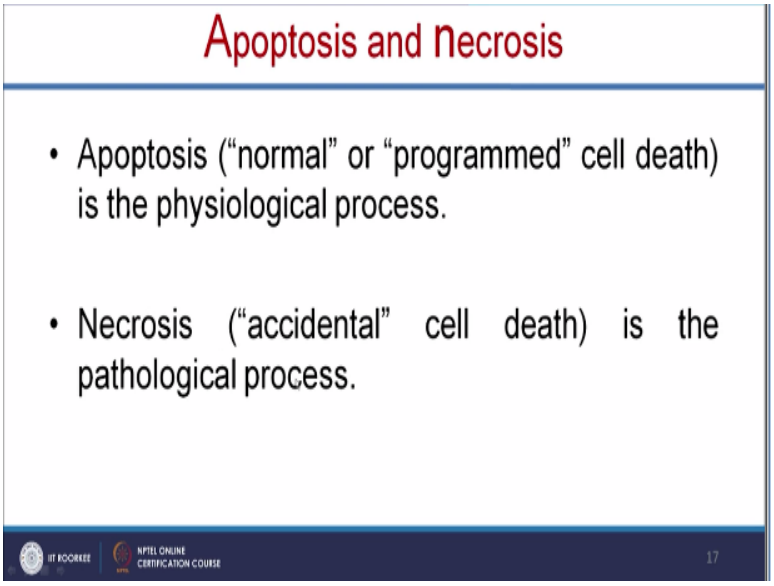

So before we change anticancer properties of nanomaterials we have to understand two types of cellular apoptosis and necrosis. So apoptisis are normal or programmed cell death is the physiological process, and necrosis is the accidental cell death is the pathological process. So apoptisis is similar to normal death of the person and the necrosis is similar to the accidental death of the person.

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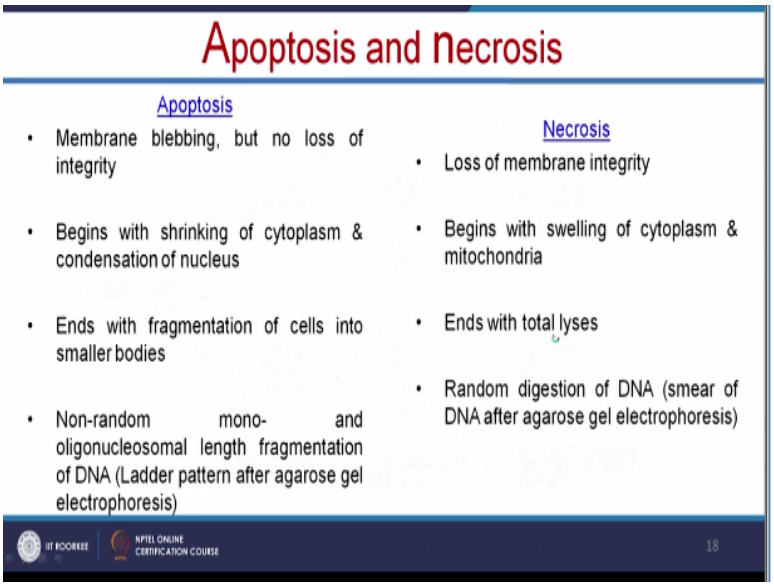

So let us see the different between apoptisis and necrosis. In apoptisis membrane blebbing will occur and there would not be any loss of integrity. And it begins with shrinking of cytoplasm and

condensation of nucleus. And it ends with fragmentation of cells into smaller bodies. And non-random monno and oligonucleosomal length fragmentation of DNA happen in this apoptosis and in necrosis there may be loss of integrity and it begins with swelling of cytoplasm and mitochondria.

And it ends with total lyses okay and here there will be random digestion of DNA you will find smear of DNA in the gel electrophoresis. So let us see these in detail okay.

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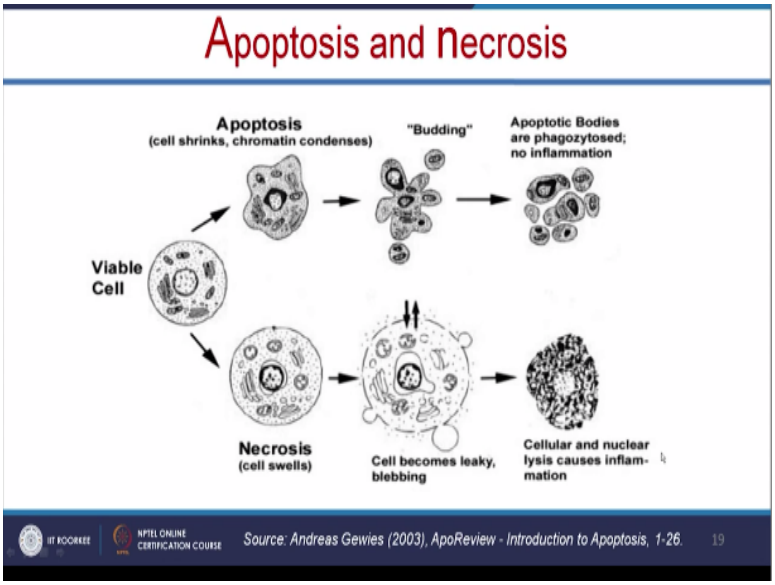

So this is our viable cell, so doing apoptosis what will happen the cell will shrink and the chromatinal contents okay, it forms a budding and this apoptotic body will be formed okay, that will be phagozytosed, so there would not be inflammation. Wherein case of necrosis cell will be swelling and the cell becames leaky and blurry, so all the mitochondria and these things they will be list and it will cause inflammation.

The cellular nuclear lysis will leads to inflammation, the simple example is like if you want to break a wall you can use the simple hammer and break the wall. And you can reuse the bricks to build the ne wall, it is similar to your apoptosis.

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