INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

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NPTEL ONLINE CERTIFICATION COURSE

Biomedical Nanotechnology

Lec-04
Synthesis of Nanomaterials by Biological Methods

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Hello everyone today we are going to see the fourth lecture of this course. The title of this today's lecture is synthesis of nanomaterials by biological methods. So in the previous lecture we have seen how to synthesis nanoparticles by physical method and chemical methods. And in this lecture we are going to learn how to synthesis nanoparticle by biological method.

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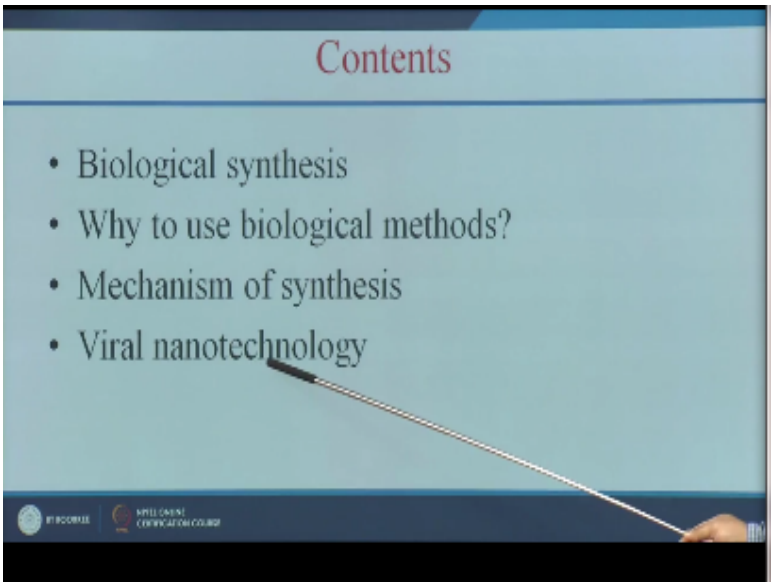

And also we are going to learn why we have to use biological methods, what are the advantages of using these biological methods and what is the mechanism behind the synthesis of this nanoparticle by biological method, and we are also going to see viral nanotechnology. In this we

are going to see how to use virus and make use of those virus nano structures for various applications.

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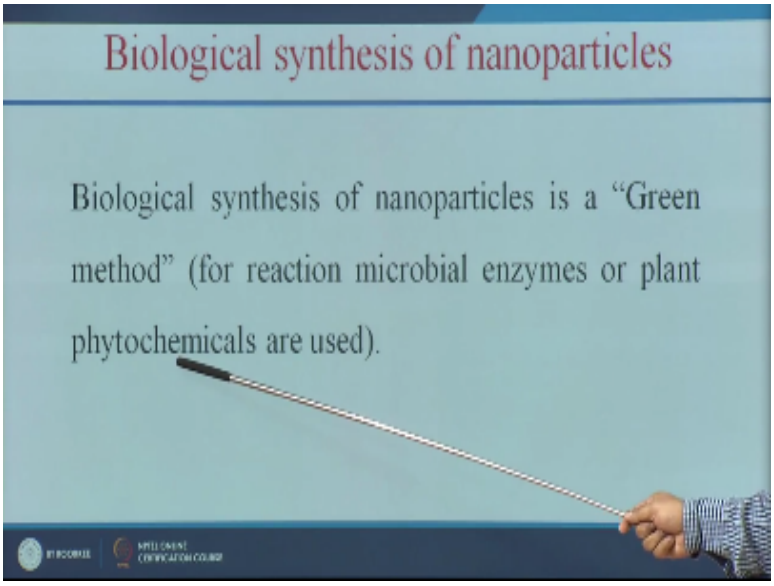

So first we will see what is biological synthesis it is a green method, it is similar to your chemical synthesis method only, but in this case we are not going to use the chemical reducing agent. Instead of using the chemical reducing agent we are going to use microbial enzymes or plant phytochemicals.

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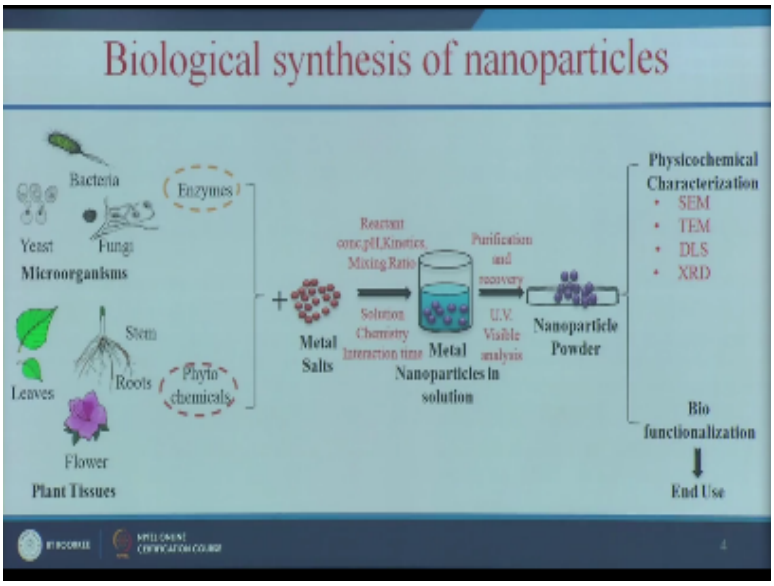

So this is the overview of biological synthesis and nanoparticles, so here we are going to use either microorganism or plant tissues and the enzymes present in the microorganisms will act like a reducing agent as well as it act like your stabilizing agent or capping agent. So similarly the phytochemicals act like a reducing agent and it also act like a capping agent for your nanoparticles.

So when you mix this bacteria or any microorganisms with the metal salt, so the enzymes will convert this metal salts into metal nanoparticles. So this nanoparticles can be further purified and characterized. So by using this scanning ultra microscope, transmission ultra microscope and various other methods we can characterize this nanoparticles. So the characterization of nanoparticles I will explain in the next lecture, so once you characterize these nanoparticles we can do the bio functionalization, that means you are going to add functional group to these nanoparticles and we can take it for further applications.

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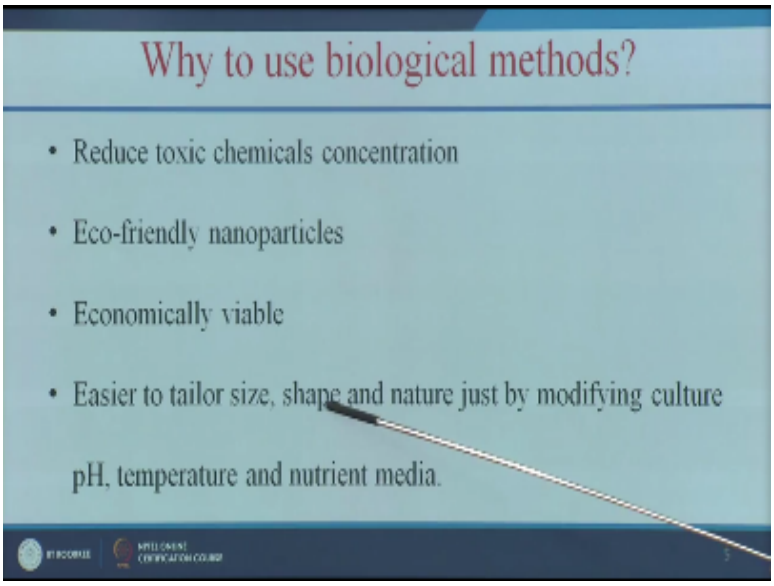

Why to use biological method, what are the advantages of using this biological method. So here we are not using any toxic chemicals, so it is a eco-friendly nanoparticles and it is economically viable, we are not using any costly chemicals, we are using this plants or microbes which is readily available and easy to tailor this size or shape and nature of the nanoparticle by simply modifying the culture conditions like pH, temperature or the nutrient media.

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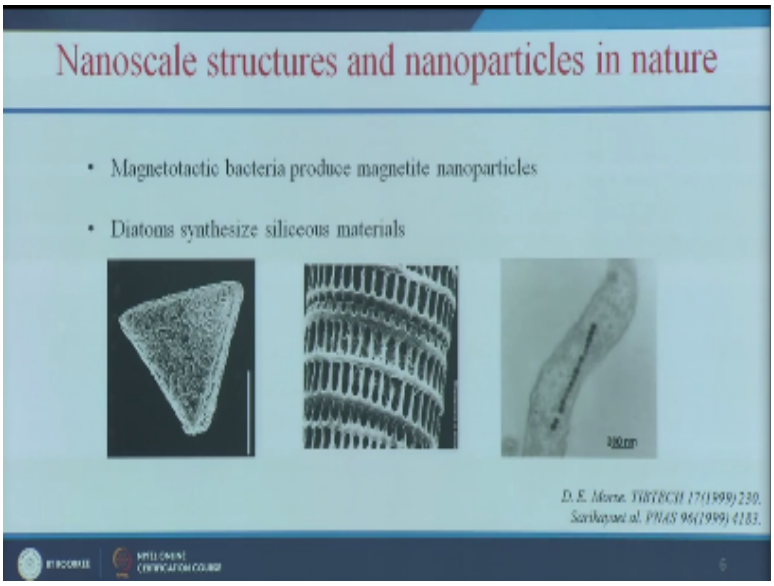

So these are some of the examples of nanoscale structures in the nature. So these are magnetotactic bacteria producing magnetic nanoparticles and also diatoms synthesis siliceous materials.

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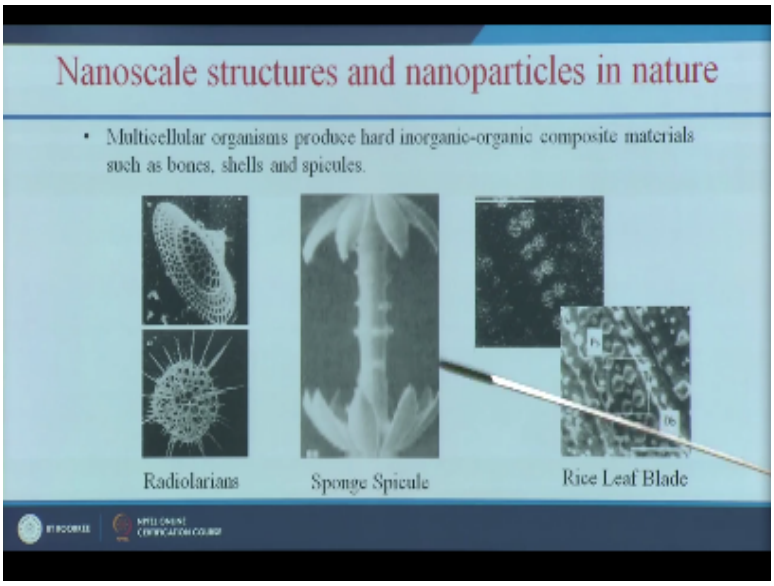

And also multicellular organism produce hard inorganic and organic composites examples are bones, shells and spicules. So these are some of the examples which is having nanostructures in the nature itself.

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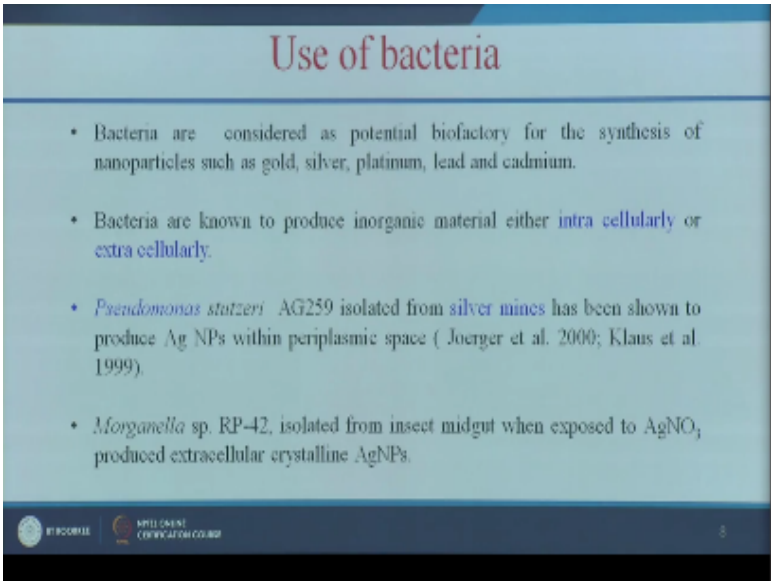

So let us see some example using bacteria how we can synthesis these nanoparticles. So the bacteria are considered as potential bio factory for the synthesis of nanoparticles such as gold, silver and other metal nanoparticles. So this bacteria can produce the nanoparticles either intra cellularly or it can produce extra cellularly. So what is intra cellular, intra cellular means inside this cell, what is extra cellular it can synthesis nanoparticle outside this cell okay.

So let us see some example the pseudomonas bacteria, the isolated silver mines, so it has the capacity to convert the silver nitrate into silver nanoparticles. And it is synthesizing the nanoparticles in the periplasmic species of bacteria. So from this you can get a idea, so you can also collect some soil sample near the copper industry or some other metal based industry. So those soil samples may have some bacteria which can convert your copper into copper nanoparticles.

Another example is morgonella species bacteria, so they isolate this bacteria from the insect midget and it is able to secret nanoparticles extra cellularly.

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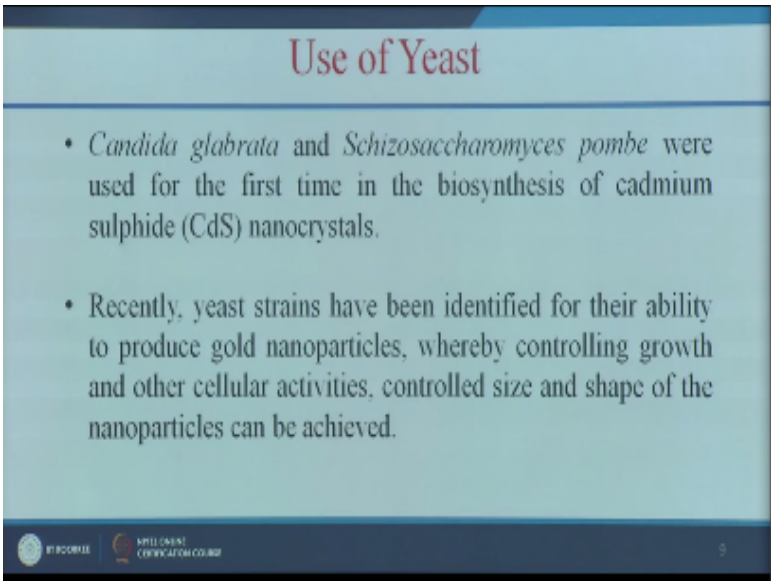

And we can also use yeast for making the nanoparticles for example the candida can be useful for synthesizing cadmium sulphide nanoparticles and also recently the yeast strains have been identified for making gold nanoparticles and other metal nanoparticles. Using the yeast we can have a controlled size and shape of nanoparticles can be easily achieved. And again you know the yeast is not very caused costly, it can be available in any shops, you can purchase the normal yeast and you can do your research you can simply add this yeast grow it when the presences of a metal salt so the enzyme present in the yeast can cover your metal salt into metal nano particle and you can do further characterization and application studies.

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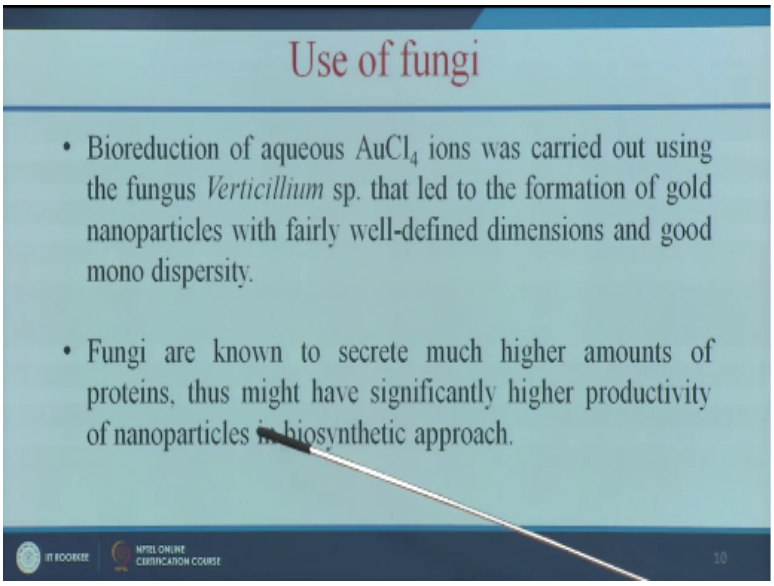

And we can also use a fungi the example is Verticillium spices sp that can be useful for making gold nano particles with very good mono dispersity okay and also why we have to sue fungi because the fungi are known to secrete much higher amount of proteins so thus might have high significant and higher productively on nanoparticles in this biosynthetic approach.

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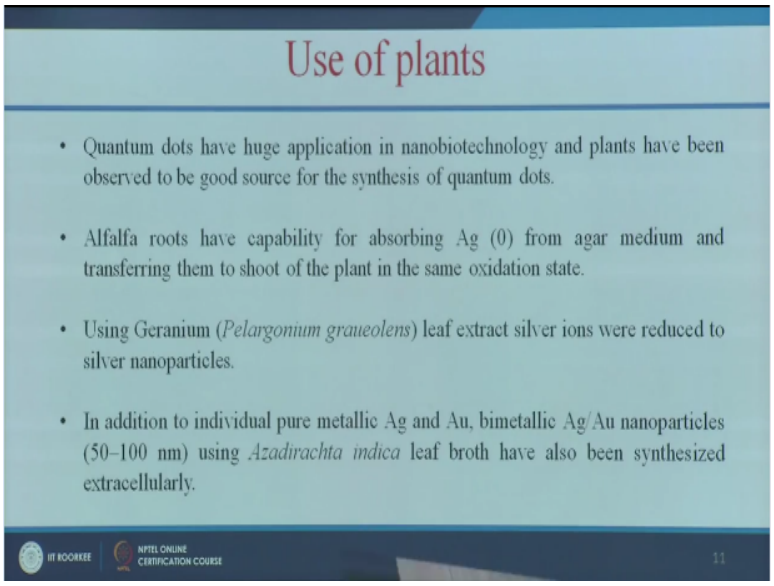

And we came also plants so the quantum dots it is a Florence's nano particles simple connected nano particles so the quantum dots have wide application in the nano bio technology and we can use a plant as a good source for synthesis this quantum dots. For example the alfalfa roots it has it capability absorb the Ag (0) the nano particle Ag (0) it can observe and from agar medium and it can transferring them to shoot of the plant in the oxidation state.

And also we can use geranium leaf extract and this can converts silver ions to the sliver nano particle in addition to that we can make silver, gold bimetallic nano particles using indicia leaf extract so which can be useful for synthesizing this bimetallic nano particles extracellular so similarly there are plenty of examples are available we can use any mycoses or any plant and we make the metal nano particles or any kind of nano particles.

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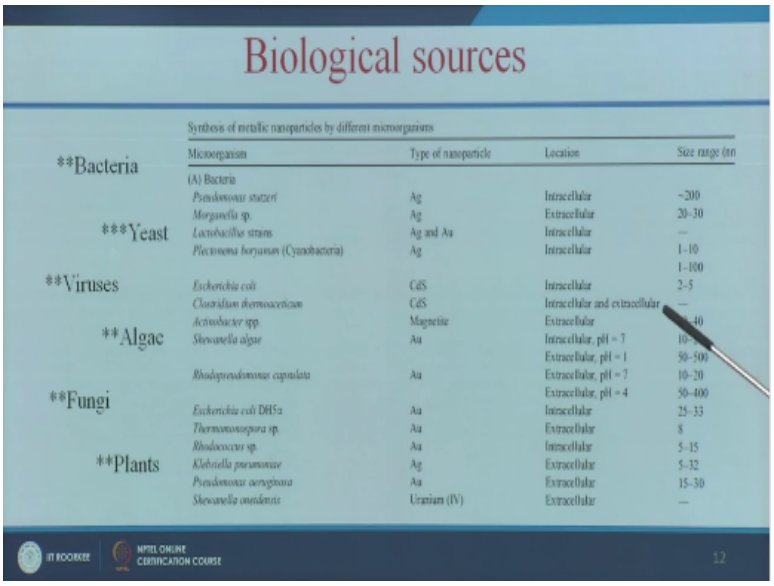

So this tabular column will give you idea which bacteria we can use and which metal nano particle can be synthesized weather it is intercellular and extra cellular and what is a size range of this nano particles.

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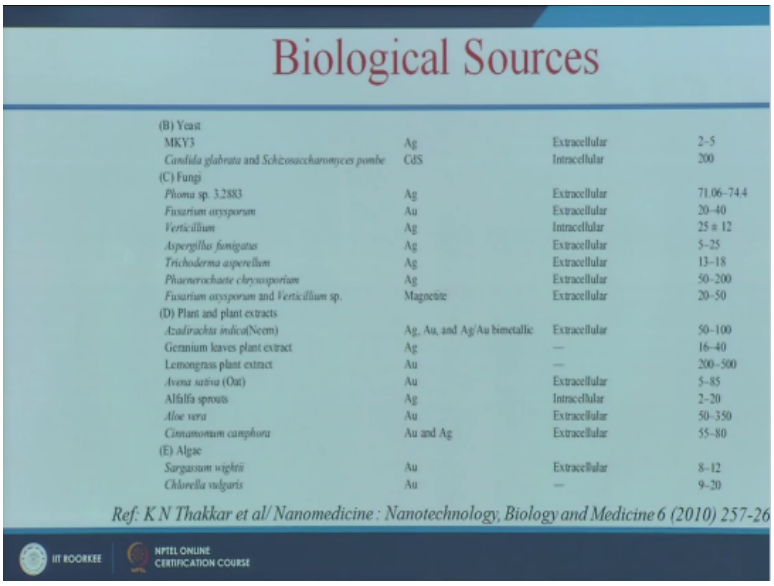

So this is a plant and plant extracts so you can use this kind plant extracts for example aloe Vera you can used it gold nano particle and it is synthesizes extra cellular and the size of the nano particle is 50 to 350 so similarly we have plenty of researches going on in the biomedical or bio synthesizes of nano particles.

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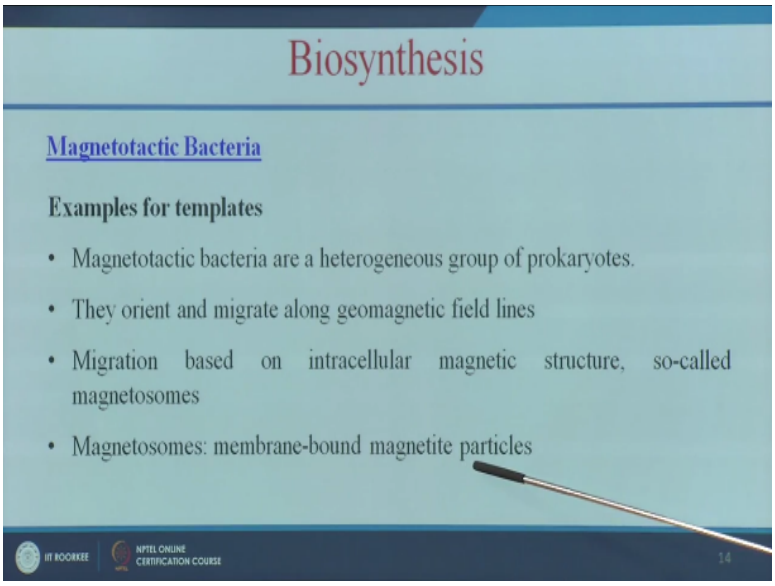

So let us see another example where we are going to see how we can use nano totatic bacteria for synthesizing magnetic nano particles so this magnetotatic bacteria or a heterogeneous group of prokaryotes and they orient and migrate along the geomagnetic field lines, it do not have it is large light moves according to the geo magnetic field line and if migration is based on the intracellular magnetic structure so the inter cellular magnetic structure so the intercellular magnetic structure is called as magnetosomes. So the magnetosome means it is a membrane bound magnetic particles.

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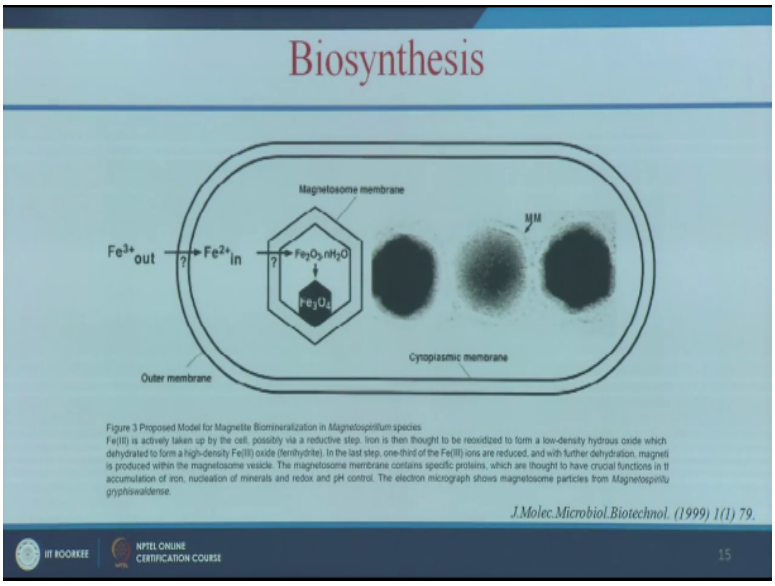

So this is a structure of this is bacteria you can see here so Fe3+ is you have to add to it this bacteria and this will convert into ion or say nano particles.

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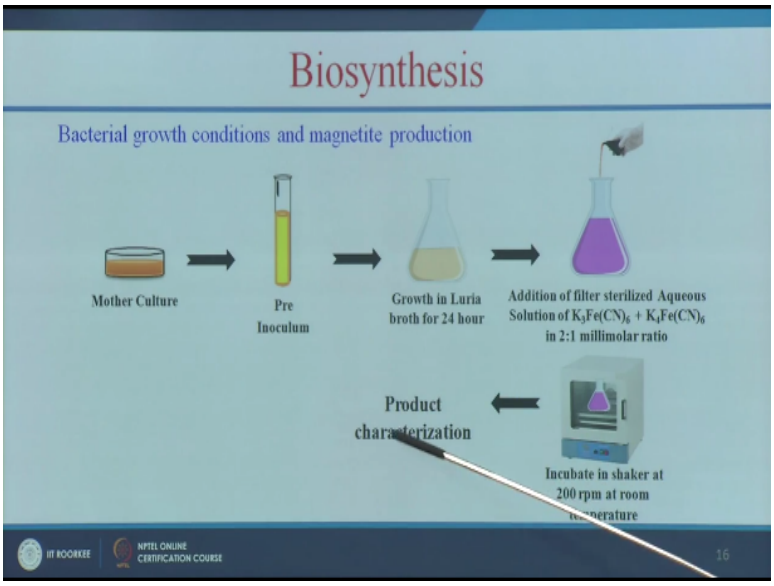

So how to note this bacteria so we grow this bacteria and transfer it to the test tube and grow it over night then you transfer to the growth LB growth in a conical flask and once the bacteria is grown you have to add the metal precursor we have to filters the laze the metal percussive and add to this in a 2:1 mill molar ratio so when you add it then you have to incubate in a incubator at 37 degree C for 200rpm and finally you will get the ionized nano particles which we can take it for further characterization.

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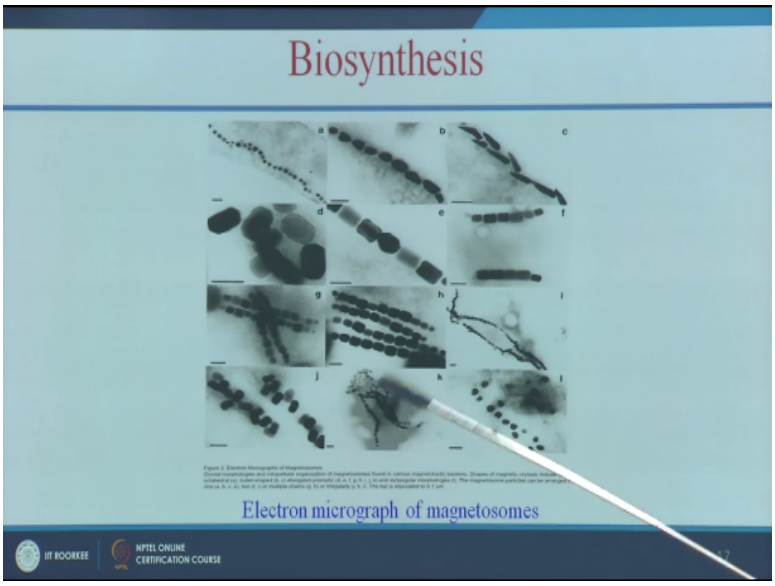

So here you can see the beautiful pictures of this magnetosomes which having this magnetic nano practices.

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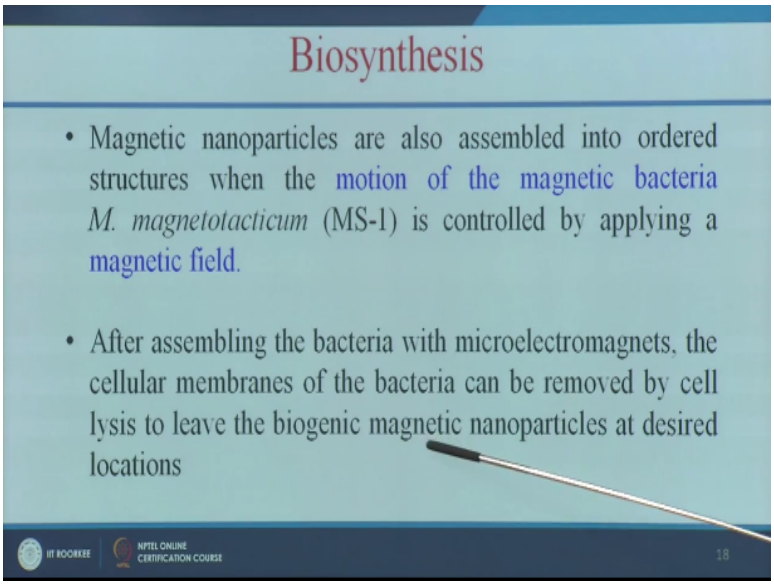

And another advantage of this magnetic bacteria is we can assemble this magnetic particles by applying external magnetic field by simply applying the magnetic field we can arrange this magnetic bacteria so after assembling the bacteria with the micro electromagnets what you can do is we can remove the cellular membranes how we can remove the cellular membranes we can add some lysis solution so that can lysis the cell membrane and it can leave the nano particle as in traced.

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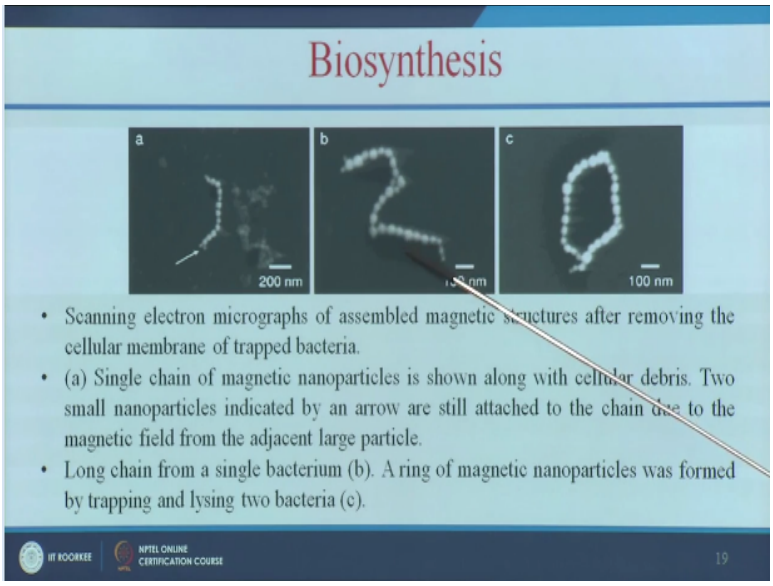

So this feature will clearly tell you so this is scanning electron microscope picture of magnetic structures assemble after removing the cellular membrane of trapped bacteria so they assemble this bacteria in such a way like a chain structure or like a ring structure once the bacteria is assembled they added the lysis solution so this lysis salutation will degrade this cell membrane and leave the magnetic nano particles in tacked so in figure a you can see here it is a single chain of magnetic nano particles along with the cellular debris these are the light cellular debris cell membrane derbies okay.

So and again you can see that there are two small nano particles indicated by this arrow so these are still attached to the chain due to the magnetic field of from the adjacent large particle and this is a long chain from a single bacterium and this is a ring of made in a particles found by trapping and lies in two bacteria.

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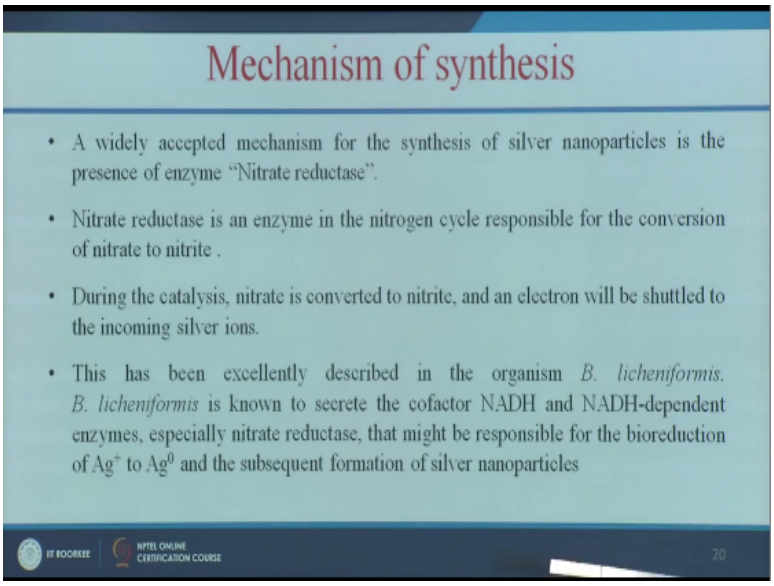

And let us see the overall mechanism of synthesis of nanoparticles so here the widely affected mechanism of synthesis of silver nanoparticle is the presence of enzyme called nitrate reductase as we know this nitrate reductase is an enzyme in the nitrogen cycle which is responsive for the conversion of nitrate to nitrate and during the catalysis the nitrate is convert to nitrate and in an electron will be shuttled to the incoming silver irons so this is where studied in the basis micro so this basis micro base known to secrete.

Cofactors like NADH and NADH dependent enzymes so this will be converting this Ag + to Ag0 and to the formation of silver nanoparticles.

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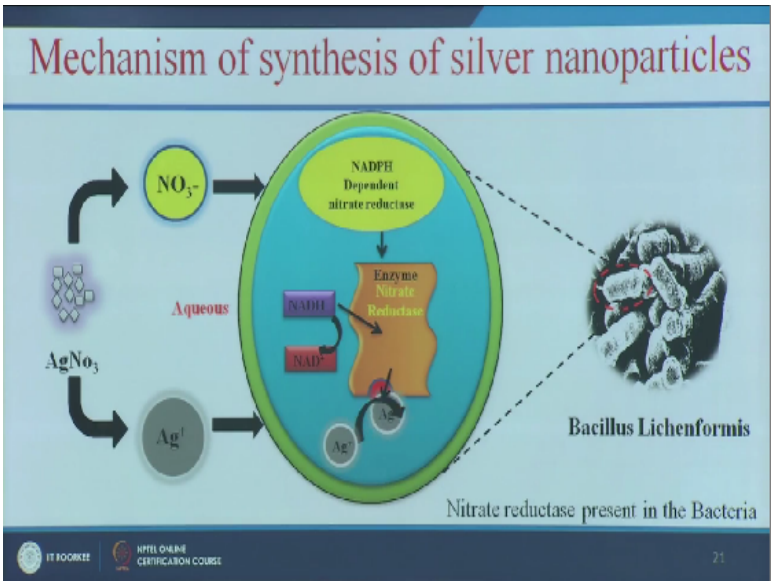

So this animation will give with idea the silver nitrate is connected to the Ag+ and this nitrate it enzyme and this NADH it going to convert this Ag+ to Ag0 silver nanoparticles.

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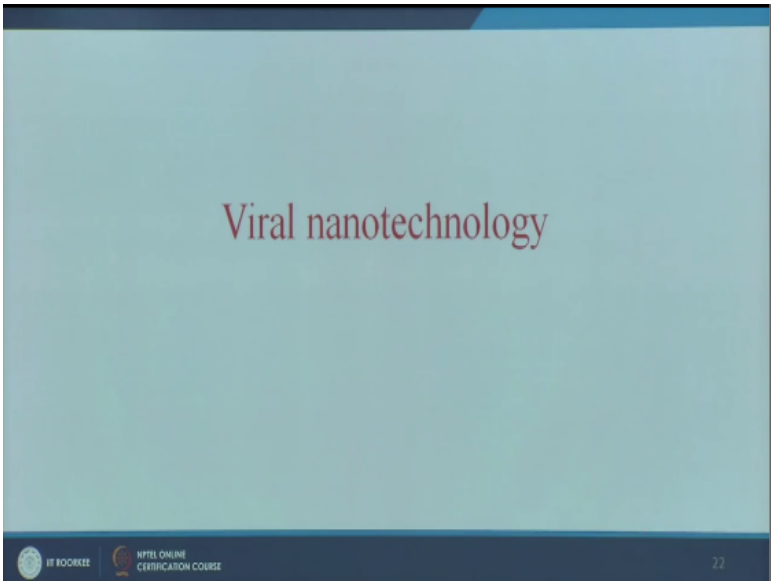

So let us known to the another topic of this lecture that is viral nano technology so perceive or same how to synthesis nanoparticles using bacteria fungi and also plant based extract and here we are going to see how we can use this virus for making various nano structures and could be have wide applications.

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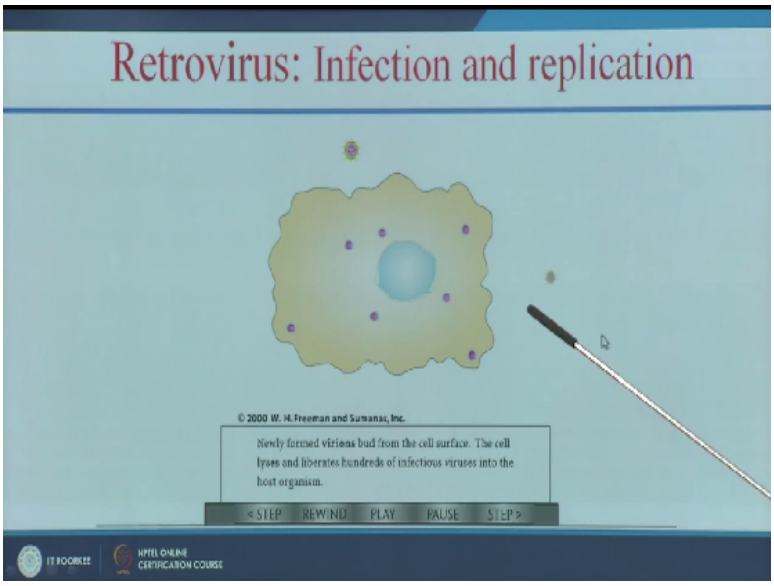

So let us see a small animation so this is a viral infection replication cycle of Retro virus so from this animation you can understand how the Recto virus come and bind the mammalian cells and how it is going to replicate see the first step is attachment of virus to the mammalian cells so once it attached this nucleic acid is it is relating the nucleic acid and the nucleic acid id made copies and this nucleic acid is going and integrating with the mammalian cells denote and it is making multiple copies of the viral genetic material.

And using that viral genetic material whether it is a DNA or RNA it is making the proteins which is record for making the complete virus structure so the synthesis is done so once a synthesis is done it is packing okay, and packing in the relating the viral materials so a single virus particles entering the cell and it is making millions of copies of virus and finally it is destroying the complete cell okay so this is a typical viral replication cycle so from this we can understand like a which are the components.

Important for the viral infection replication so let us see how we can use this virus structure from using it for various application.

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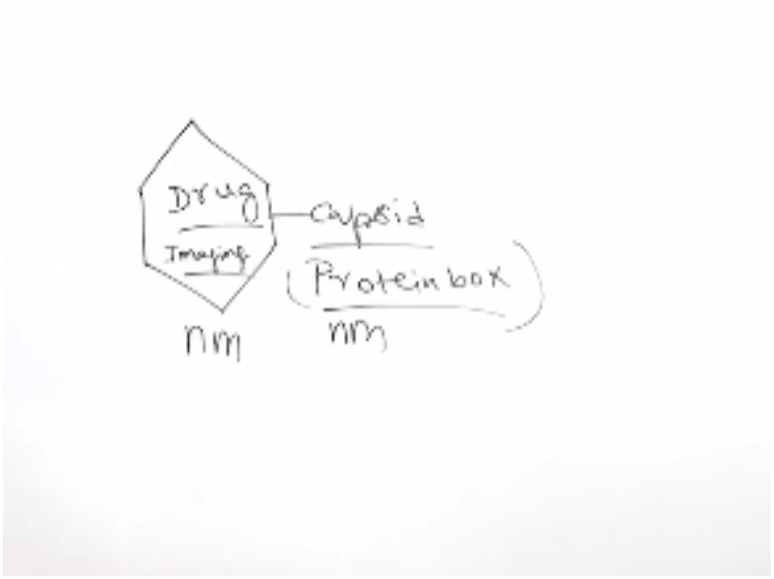

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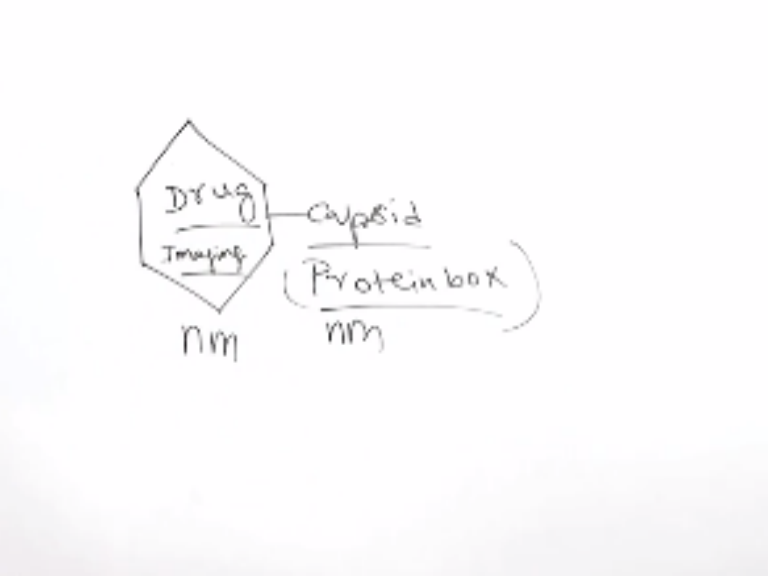

So this is the viral capsid so you can simply called like a protein box so it is made up of protein and as you know the size of all the virus is in the range of Nano meter and the main important component of any virus is, its genetic material either it is a DNA or RNA so once you remove this genetic material DNA or RNA it will be like a simple Nano sized protein boxed and we are going to use this Nano size protein box.

For synthesizing various Nano materials and also we can use it for various therapeutic as well as other applications. So instead of nucleic acid we can put our anti acid drug or you can put a imaging agent for diagnosing various diseases so thus we are converting the normal virus for various applications.

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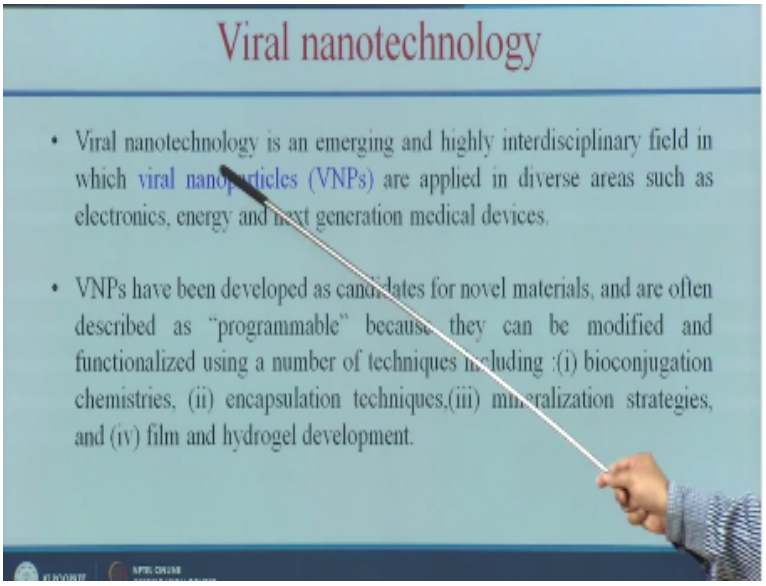

So let us see what is viral nanotechnology, so the viral Nano technology is an emerging field it is a highly interdisciplinary field where we make the viral Nano particles and we can you it for applications in various areas for example electronics, energy as well as net generation medical devices, okay. And here we are going to use that bio Nano particles and we can tune the viral Nano particles like we can program this bio Nano particles.

We can easily modify it using that techniques like bio conjugation chemistry, encapsulation techniques and minerals strategies and also film and hydrogen development we will see one by one in the subsequent slides.

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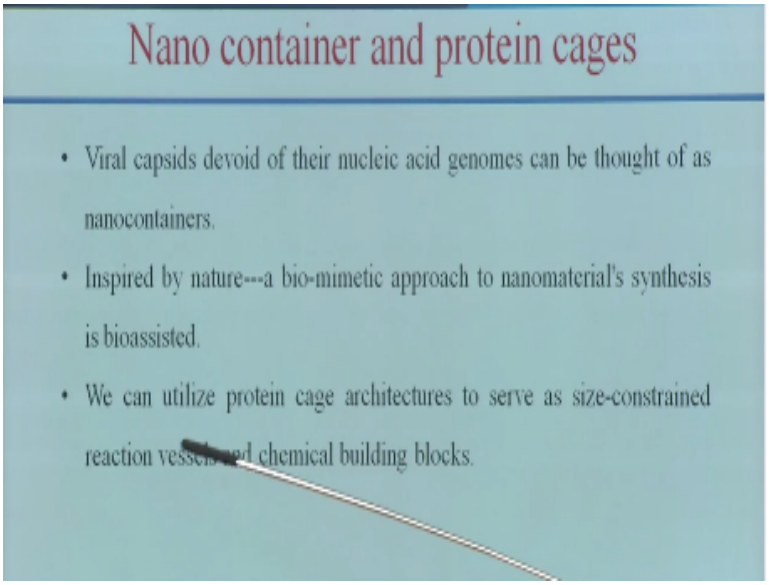

So here the viral capsids divide of their nucleic acid genomes can be thought as a Nano container, as I told you earlier the viral capsids without the genetic material it can be use as a kind of Nano container, so again scientist got the idea from the nature it is a bio-mimetic approach for making the Nano particles.

So we can use the protein cage architecture and it can act like a size constrained reaction vessels, so this protein cage will act like a reaction vessel and it can synthesize Nano particle in the smaller Nano size vessel.

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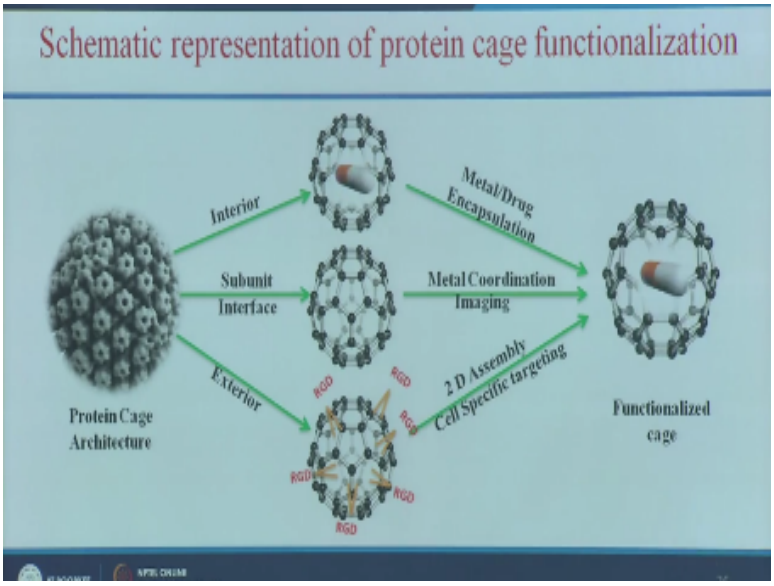

So this is the schematic representation of protein cage and so this is the protein cage architecture it is a viral Capsids you can assume this structure to collide with the football okay, so you can here see here it has a interior sub build interface as well as exterior so in the interior we can add any kind of therapeutic molecule we can add any anti acid drug or any other therapeutic molecule.

And in this sub build interface we can add gadolinium metal ions so this will be usual for imaging application we can use it for cancelling imaging and again in the exterior we can add RGE peptides these are small peptides made up of RGN, glassine and aspartic acid so this is the 3 amino acid peptide so which will enhance the cell attachment, so a single Nano particle having multiple function.

So this is called as multi functional Nano particles so it can be having a therapeutic value and it is having this imaging application and also it is spells self specific targeting it can easily bind to the cell using this RGD peptides.

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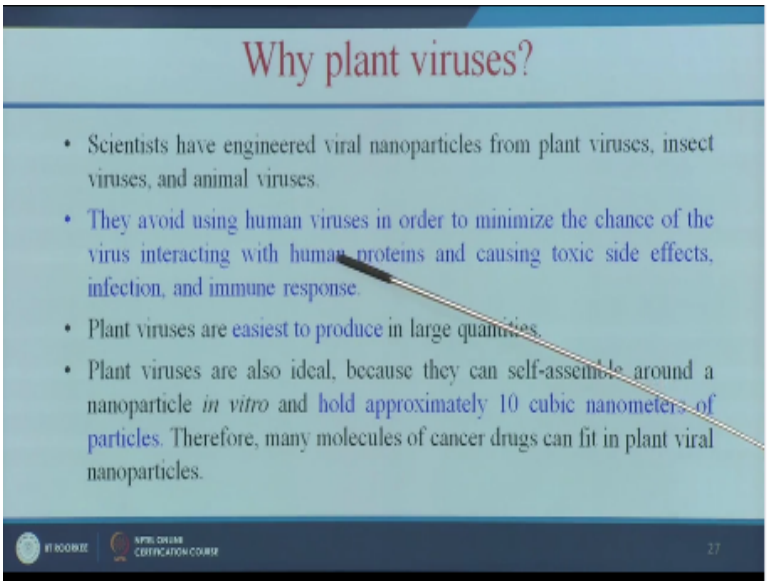

And in this case viral nanotechnology we are going to use only the plant virus we are not going to use the animal virus so why we have to use plant virus, so scientist have engineered viral Nano particles from plant virus, insect virus and animal virus but they avoid the human virus because when use the human virus it will induce human response or any kind of toxic side effects so in order to minimize those chances of virus interacting with the human proteins so they are using the plat virus and another important thing is if you want to make the animal virus you have to use the animal cells, again the cause of the virus will be high as well as in some cases you have to scarifies the animal to isolate the virus.

But in this case it is very easy to produce in large quantities, you are going to add the virus to the plant leaf and this plant leaf is going to made n number of millions of virus, okay. And another advantage of using the plant virus is it can hold 10 cubic nano meters of particles, so the size of the plant virus is bigger and so that we can load more amount of anti cancer durg or any kind of therapeutic molecule inside the plant based viral particles.

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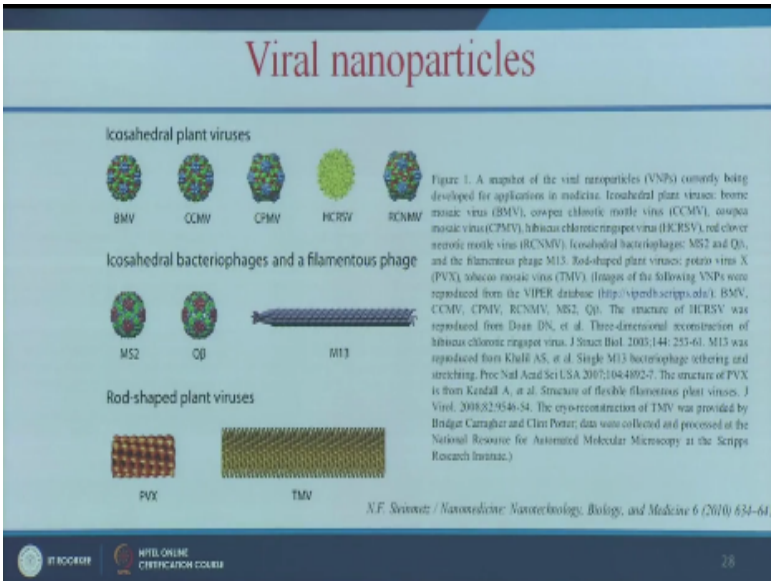

And another advantage of this plant virus is you can see these are all are plat virus okay, so all the names like TMV means tobacco mosaic virus so similarly all the names of virus is mentioned here and if you see that the plant virus have different size and different shape so it depends on the application you can select the plant virus and take it for further applications.

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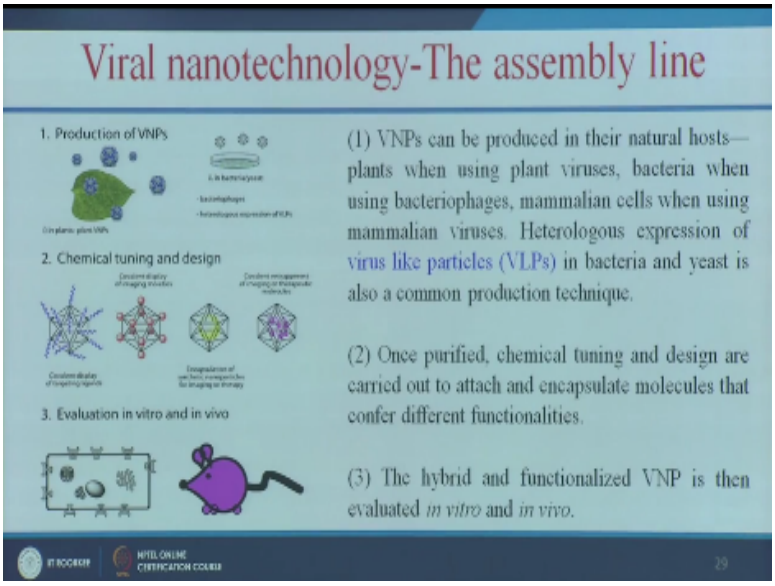

And this is the complete viral nanotechnology assembly line, so if you want to make the viral nano particle the first step is you take the plant virus added to the plant leaf so it is going to make millions of copies of plant virus and purify the plant virus once you purify the plant virus. The second step is you have to chemically modify it okay, how to you modify by you have to remove the nucleic acid from the plant virus and you can put your therapeutic molecules and also you can add some kind of ligand so that it can go and spherically bind only to the cancer cells not to the healthy cells.

So once you done by this you have to evaluate this efficiency of this nano particle so how do you evaluate you have to use the in vitro and in vivo. Now what is in vitro, in vitro means in lab condition so we can use the cell lines mamalian cell lines and you can study the therapeutic efficiency once you have studied and optimize the concentration then you can take it to the animal models that is in vivo, in vivo means inside in living system.

So we can use the mouse model or any other animal models and we can study the therapeutic efficiency of your viral nano particles.

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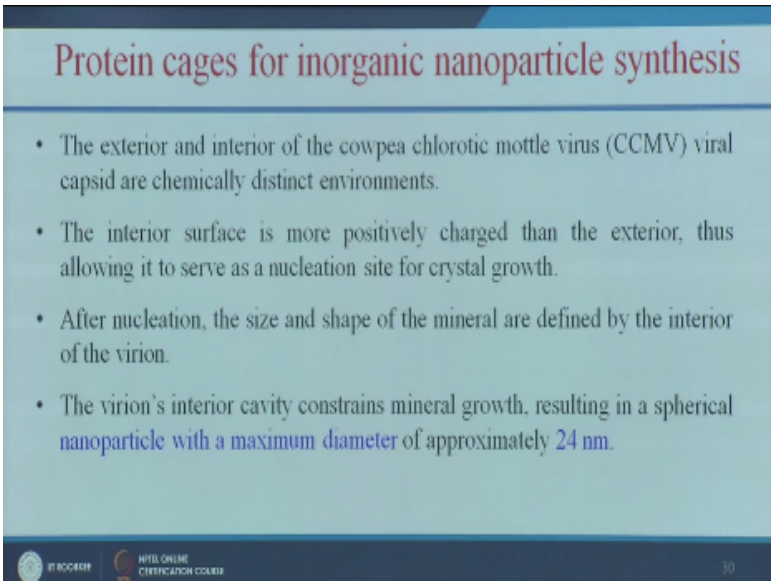

And we can also use this protein cage for inorganic nano particle synthesis, so the beauty of this virus is the exterior and interior of this cowpea mottle virus CCMV this viral capsid have it distinct environment, the interior surface is more partially charge then the exterior. So this will allow and serve a same nucleation side for crystal growth, so as I told you in one of second lecture so nucleation is very important process for nano particle synthesis.

So after the nucleation the size and shape of the mineral are depend by the interior of the virion and it can make a nano particle of maximum size of 24 nano meter and also it can make a uniform size nano particles.

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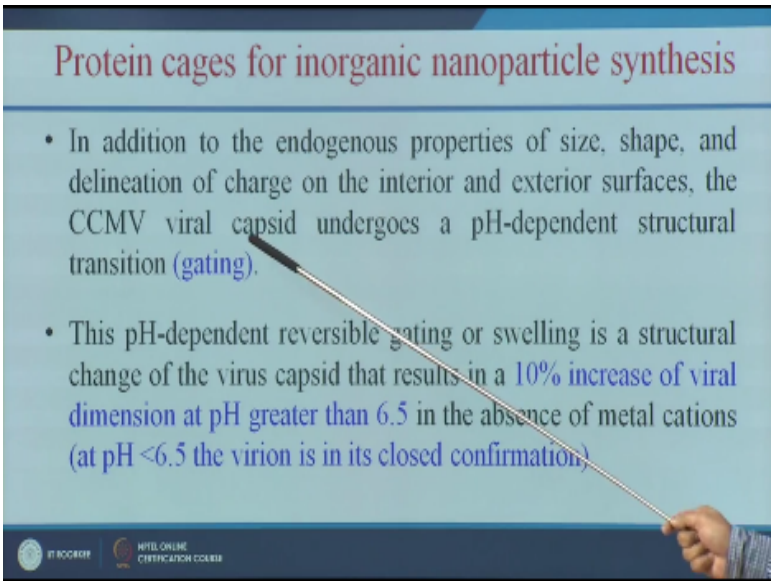

So another important property of this CCMV viral capsid is it has a property of gating, so what is gating it is a pH-dependent structural transition that is called as gating. So if the pH is greater than 6.5 there will be a increase 10% increase of viral dimension and it can easily allow the any materials to go inside.

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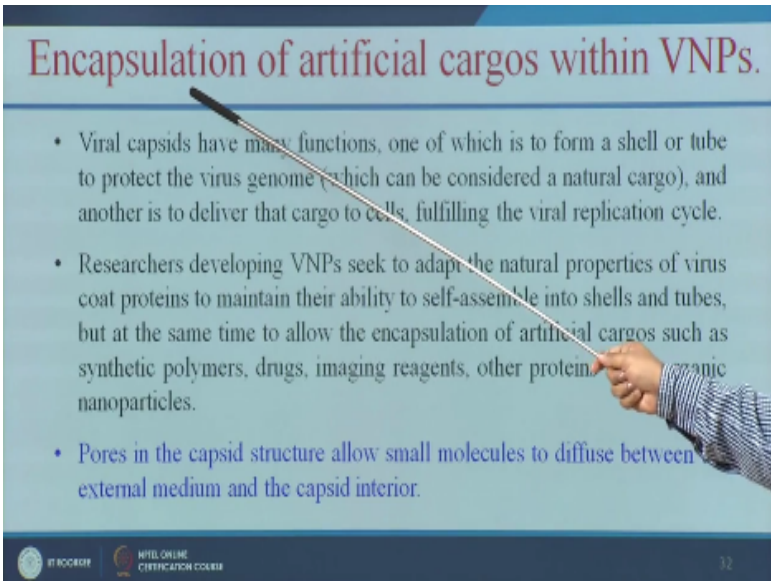

And the next one is we can encapsulate artificial cargo within the viral nano particles, so as I told you earlier the viral capsid have many functions okay, so one of which is to protect the virus genome and all you know this virus genome or any genetic material like DNA, RNA it has the negatively charge okay, so we can make such kind of nano material which have the negatively charge and so that this virus can easily encapsulate those negatively charge material into the viral protein capsids, okay.

And these pores in the capsid structure allow small molecule to defuse between the external medium and capsid interior.

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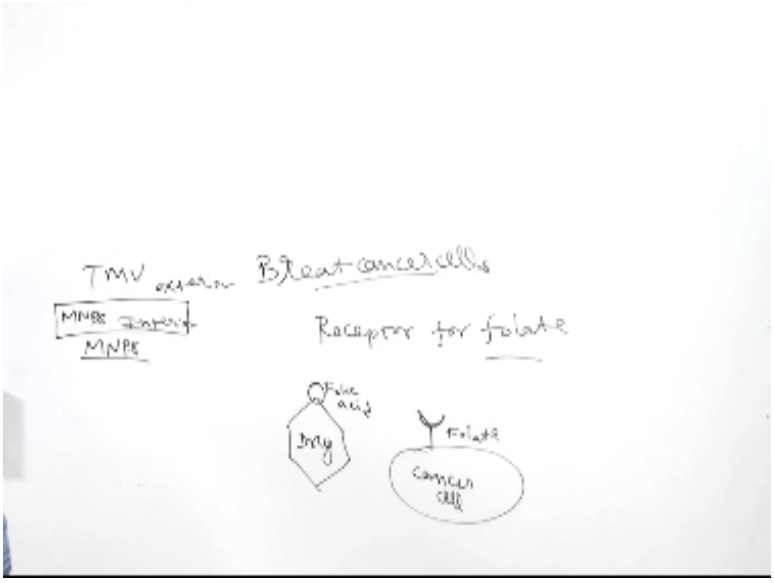

And next one is artificial polymer as I told you that the viral nano particles having genetic material and again the genetic material is DNA, RNA any genetic material like DNA, RNA it will be negatively charge so we can make similar kind of polymer which will have the negatively charge so this is our polymer which is negatively charge at the same time it is carrying the anti cancer drug. So it acts like a artificial nucleic acid and it will be packed inside the viral structure.

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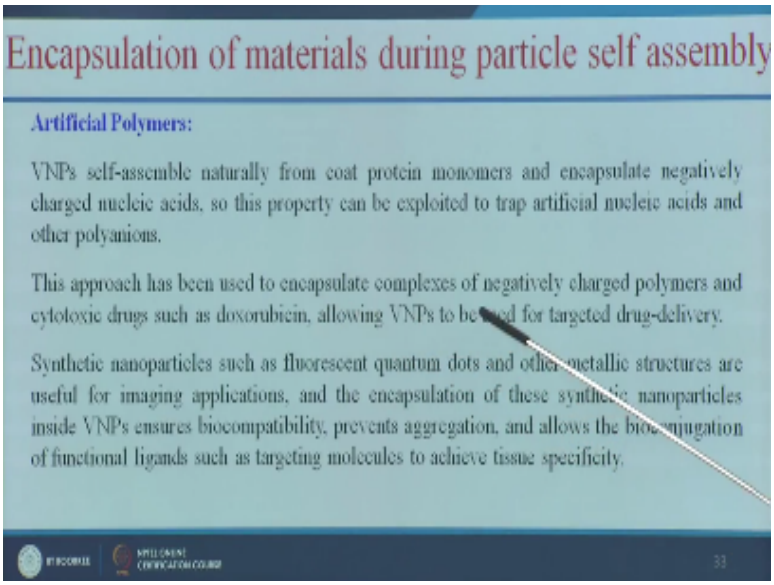

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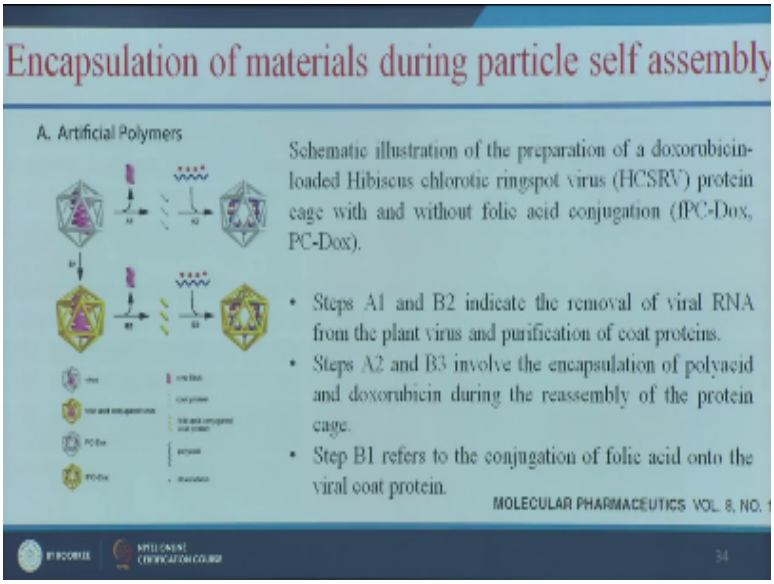

So here we can see here how we can encapsulation the materials during particle self assembly, so as I told you earlier so here we can take the plant virus for example we can take the plant virus from hibiscus ring spot virus okay and the first step is here to remove the nucleic acid from this virus once we remove the nucleic acid from the virus and the second step is we have to encapsulate the poly acid and doxorubicin the doxorubicin are anti cancer drug and poly acid is a negatively charge polymer.

So which will resemble like your nucleic acid of the particular virus, so what happens is this during the self assembly instead of this it will take this artificial polymer which is having the anti cancer direct and it will pack this polymer in to this viral capsule, and again we are going to add folic acid to this while code protein in this step B1 okay. So when you add this folic acid it will be targeted only for the breast cancer.

So mostly the breast cancer cells express folic acid, so breast cancer cells express receptor for folic acid so when you make your nano particles with the anti cancer drug and when you are adding a molecules folic acid, so it can go that cancer cell which is having receptor for folic. So it can go and specifically bind only to the breast cancer cells not the healthy cells. So we can make a targeted nano particle for cancer therapy using this virus nano particle.

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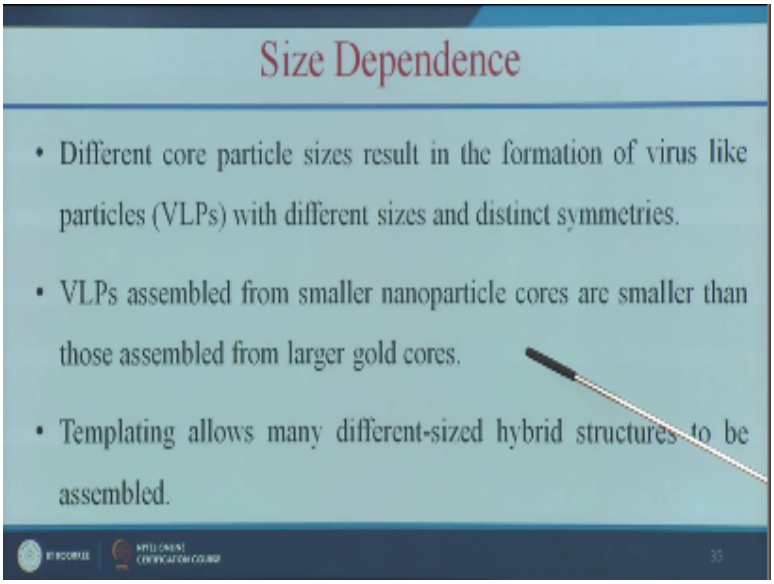

And next one is size dependence so we can uses this virus are say size constraint vessels for making different size nano particles okay.

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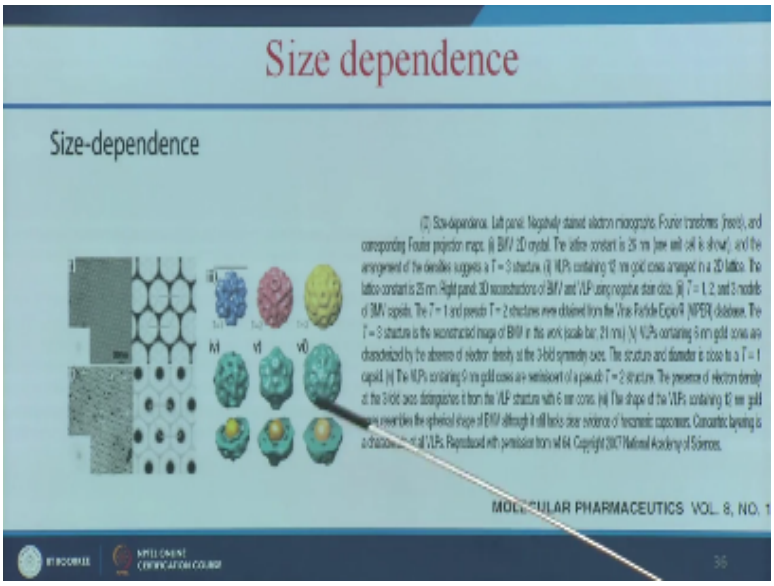

For example here you can see here these are different types of virus and each comes with different kind of size, so when you synthesis nano particles will get a uniform size at the same time based on the virus caps it protein size maximum capacity it will make the different size nano particle for example you are having two or three types of plant virus so one is five nano meter size and other one is 10 nano meter size.

So when you synthesis nano particles using this virus it can make five nano meter particle and it can make 10 nano meter particle so it can make a uniform size nano particle okay, here you can see here all the virus particles are very uniform size so you can make a uniform size particle at the same time it act like a size constraint vessel to make the small size nano particle.

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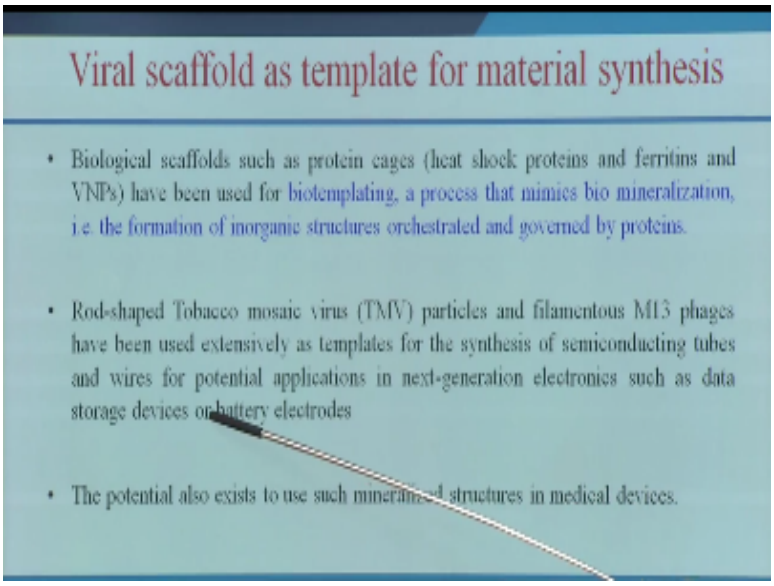

And also this viral scaffold act like a template for material synthesis. So what is bio templating it act a template it is a process that mimics the bio mineralization that means formation of in organic structures by the proteins. So here we can take the example of rod shape Tabacco mosaic virus it will impact only the Tabacco plant and also filamentous M13 phages that means it will impact only the particular type of bacteria okay.

So we can use these as a template and we can make a semi conducting nano tubes or nano wires which could be useful for next generation electronics such as data storage device or battery electrodes, let us see how we can use it.

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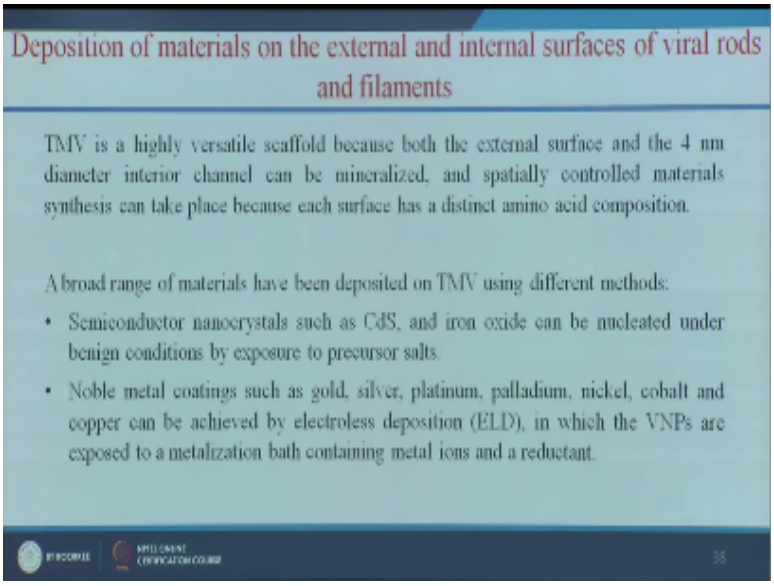

So this TMV virus is the highly versatile virus because it has the external surface and internal surface and both the external surface and the internal surface it has the capacity to mineralize okay. So the interior is only 4 nano meter and again we can deposit broad range of material on this TMV for example you can deposit semi conductor nano crystals in to inside the TMV and outside we can code with the different kind of metal nano particles. So as I told you earlier.

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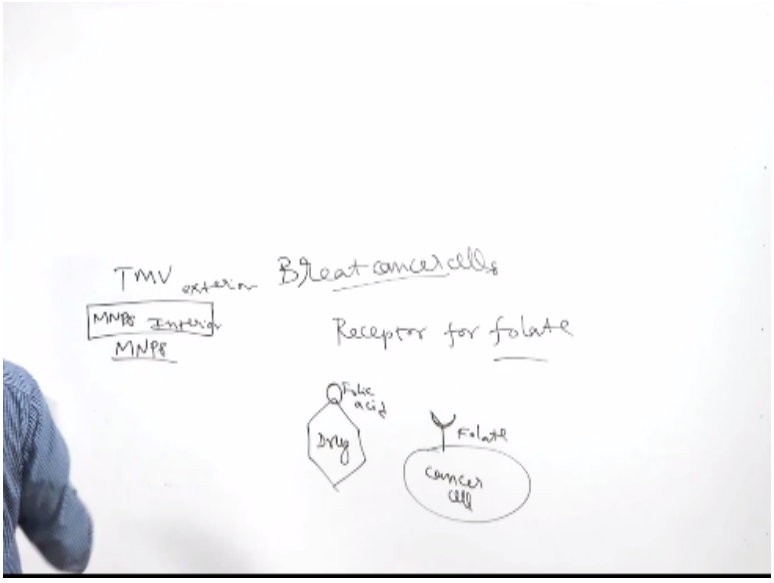

So this TMV is the rod shape virus so it has the different kind of exterior and different kind of inertial, so based on that we can deposit various type of metals inside and another kind of material on the exterior moment so it can act like a kind of nano tubes and nano wire.

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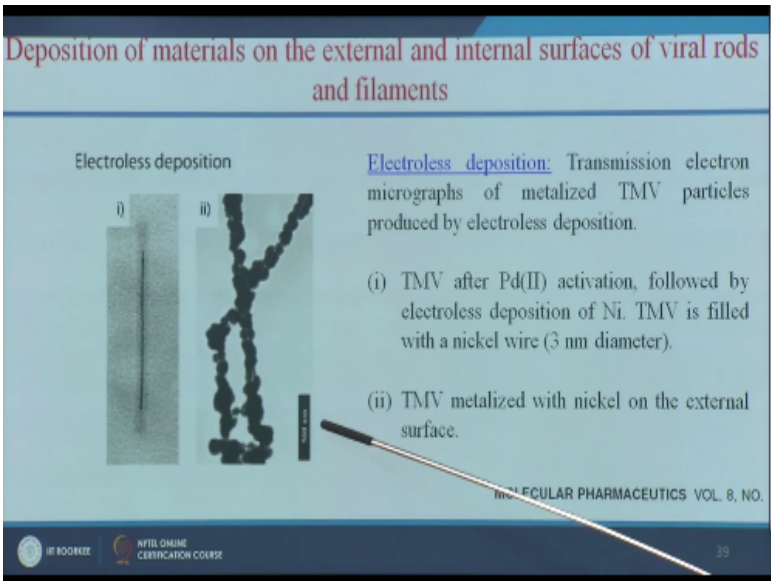

So here you can it is the electro less picture of tobacco masses wire so we can use it for electro less depositions okay. So you will be using this virus particle and we can add this pd on the top and inside we can add the nickel it is filled with the nickel and in this figure they add the nickel on the external surface. So if you combine two methods at the nano scale it will give you very good different kind of new properties.

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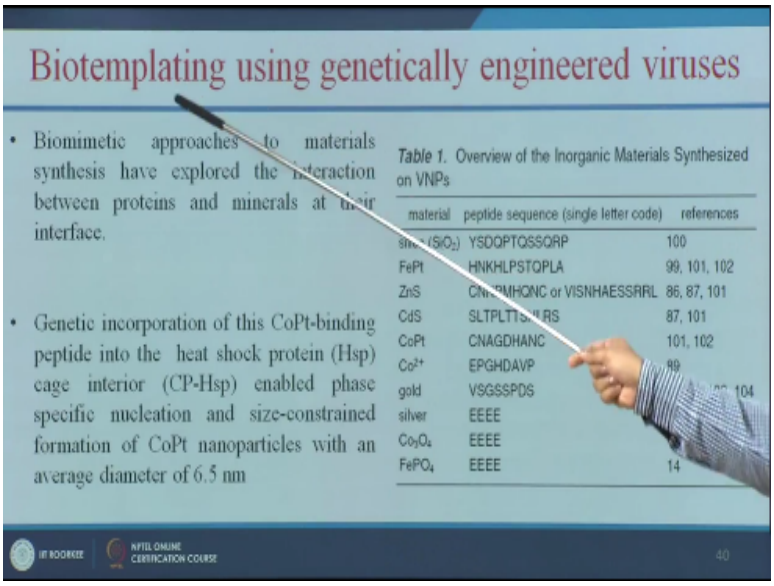

Some normal properties will express and we can also enhance the Biotemplating property by genetically engineered viruses. So genetically thing is that you are modifying the genetic virus and we can modify it. For example if the virus is expressing this kin d of peptide so that is most specific for the silver.

For example if the virus is expressing this kind of peptide sequence it is more specify for the silver nano particle. If the virus is expressing these kinds of peptides it is more physic for gold so we can modify the virus by genetic engineering and we can make it for further application.

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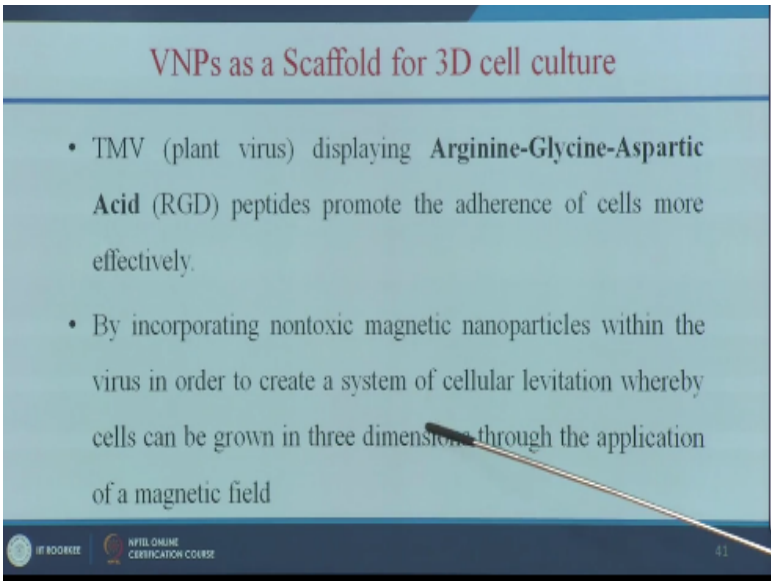

And also we can use this virus has a scaffold for 3D cell culture okay, so I will explain you what is 3 d cell culture. So here we are going to use the TMV plant virus and it will be displaying the Arginnie Glycine Aspartic that is RGD peptide which I explained in my earlier slides okay. So that will promote the attachment of cells more effectively.

Okay and we are going to incorporate the nontoxic magnetic nanoparticles within the virus to create a system of cellular levitation, so where we can grow the cell in third dimension and it will exactly mimic your body condition.

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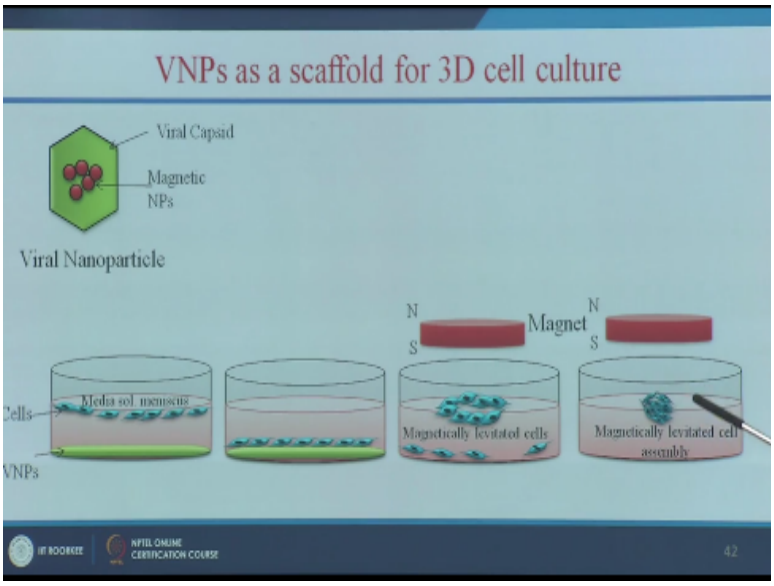

Usually in the lab we grow the cancer cells in the 2 dimensional peptidicious so usually these cells attached to the bottom of the petty place and it grows nicely but again if you see this inside body the organs are 3 dimensional so when we have the 3 dimensional agnomen or culture we can study the efficiency of drugs and we can Cole it with the studies.

So we can use the viral nanoparticles for making the 3D cell culture how we can make it so we can make use of viral particle. So we can take the virus and again this is not the tobacco that will be rod shaped it is just to give you the idea. So in this virus we are going to add our nontoxic nanoparticles.

So this virus is having the RGD peptide so this RGD peptide fell attachments so this bio nano particle coated this the bottom of your cell culture plate then you are adding the cells. So when you add the cells the cells will bind to these and when you applied to this filled it will elevate. So why it is elevating because it is having nanoparticle inside so when I apply this magnetic field all the cells will be lifted, and when we grow these cells in the same condition these cells will become a 3dimensional cell culture. So when you add if you want to test any efficiency of drugs you can add to this 3dimensional cell culture for that it will closely mimic your nvo animal studies.

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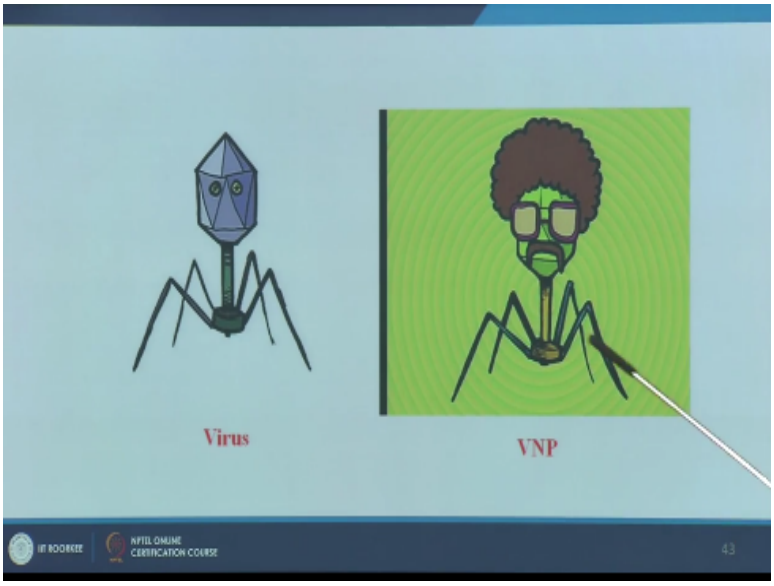

So in this lecture we will learn how we have converted this villain virus which causes the infection to a hero virus. This virus could be useful for not only for bio medical application like theoretical but it can be also used for other applications in electronic and other field.

So the summary of this lecture so we have learn like how the biological nanoparticles as open it is door to the world of nanoparticles with a easy preparation of nanoparticles and next toxic nanoparticles and also we have learn what is the mechanism of the synthesis but still more effects need to be done and understanding the mechanism of nanoparticles formation.

So once I understand the mechanism of nanoparticles formation we can still find and tune the size and shape of nanoparticles by simply changing the parameters and other thing is we can also use the genetic in techniques to improve the particles and also to control the corrosion and again bio particles could be exploded for various diagnostic applications and it will have application in various diseases and I told you earlier it is like nanoparticles. I end my lecture here thank you all for listening this lecture see you in another interesting lecture.

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