INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

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NPTEL ONLINE CERTIFICATION COURSE

Biomedical Nanotechnology

Lec -18
Cellular Uptake Mechanisms of Nanomaterials

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Hello everyone I welcome all to the 18 th lecture of this course, so the 18 th lecture is cellular uptake of nanomaterials.

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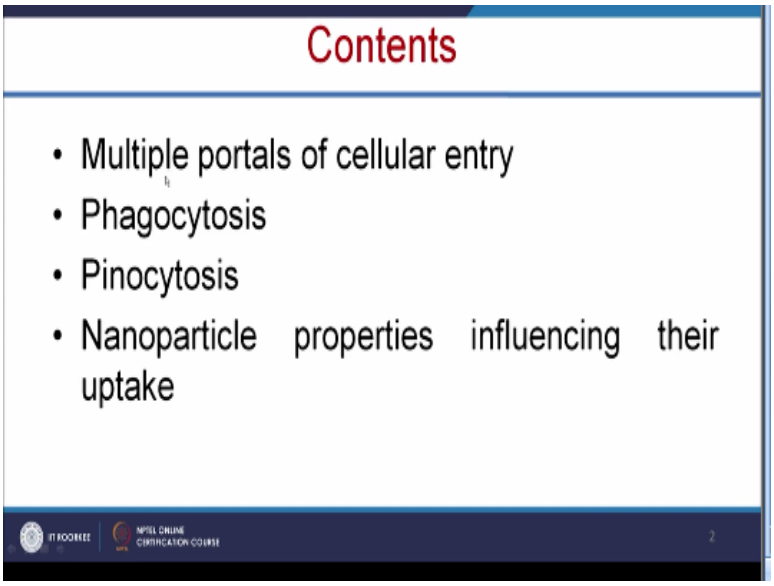

So in this lecture we are going to learn what are the various multiple portals available of cellular entry and also how the nanoparticle can enter the cell to phagocytosis pinocytosis process and here we also going to learn nanoparticle properties influencing their uptake.

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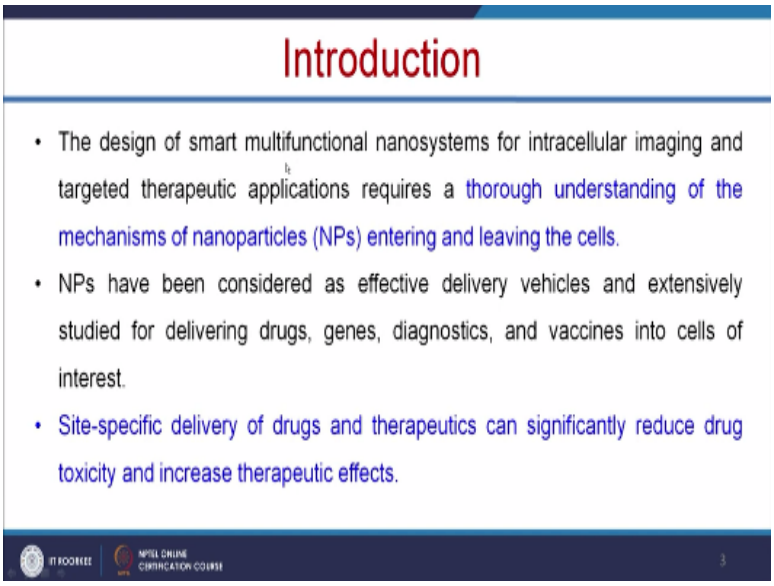

So the design of smart multifunctional nanosystems for intracellular imaging and a targeted therapeutic applications require a thorough understanding of the mechanism of nanoparticles okay? So because the nanoparticles is a considered as one of the important delivery vehicle for delivering the drug and genes because the site specific delivery of drugs and therapeutic can significantly reduce the drug toxicity and increase the therapeutic effect.

So here when we designing an nanomaterials is better to understand the complete mechanism how it is taken up this cells. Because if we understanding the complete mechanism then we can easily target to the particular cell and also we can release the drug to increase the therapeutic efficiency.

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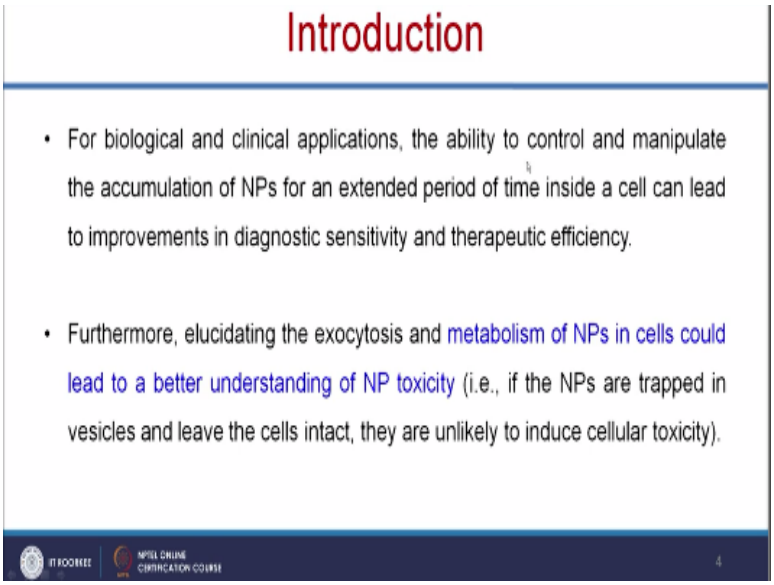

And for biological and clinical application so the ability to control and manipulate the accumulation of nanoparticle for an extended period of time inside the cell can lead to improvements in diagnostic sensitivity and therapeutic efficiency, For example if we are making a nanomaterials for diagnostic application to enter the cancer cell and it has stay for more time. So that we can get more signal and we can detect the cancer in the early stage.

And also these elucidating the exocytose and metabolism nanoparticle in the cell so lead to a better understanding of nanoparticle toxicity. That is nanoparticles of trapped in the vesicles and lead the cell intact, their unlikely to induce cellular toxicity. For example if a nanoparticle entering to a cell okay? And it is not entering to the nucleus and cytoplasm and it is leading the cells intact then it would not induce cellular cytotoxicity to kill the particular cell okay?

So it is better to understand the mechanism how to enter the cell and what is the subsequence process how is can reduce the therapeutic efficiency. Those things has to be understood thoroughly before we take the nano medicine to the clinical application.

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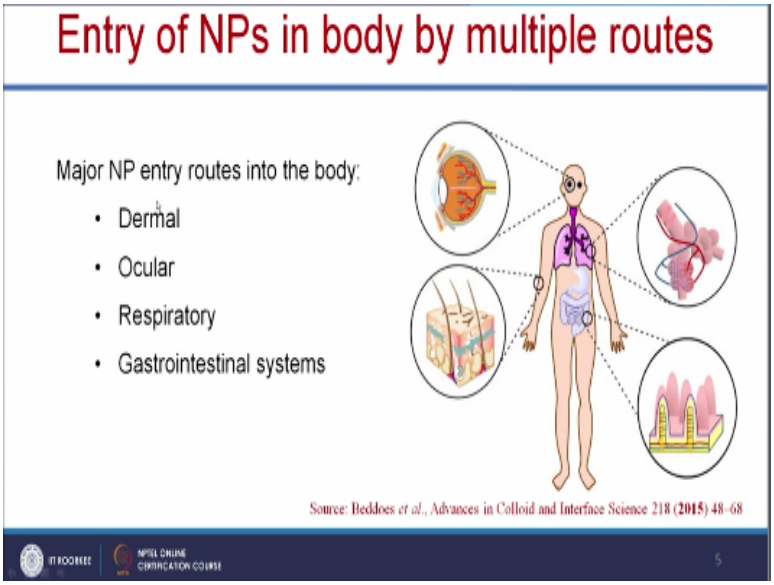

So let us see how this nanoparticles enter the body, so here the nanoparticle enter to the dermal ocular respiratory as well as the gastrointestinal systems.

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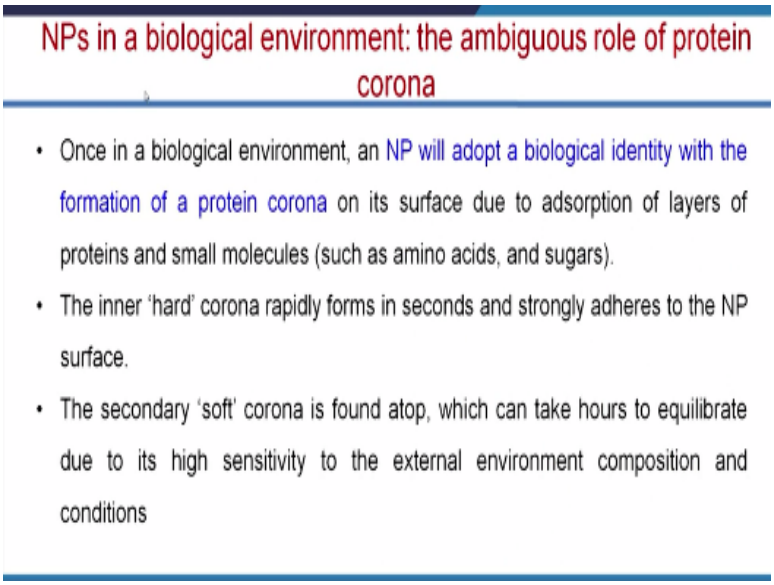

So once it enters the biological environment, so there is a formation of protein corona. Let us see what is protein corona? So in the biological environment the nanoparticle will adopt a biological identity with the formation of a protein corona on its surface due to adsorption of layers of proteins and small molecules such as amino acids and sugars. And the inner hard corona rapidly forms in seconds and strongly attached to the nanoparticle surface and the secondary soft corona is found atop.

So which can take hours to equilibrate due to its high sensitivity to the external environment composition and conditions? So once a nanoparticle enter into the cell then what happens is like there will be a formation of protein corona. So proteins and amino will form a layer on top of these nanoparticles. The first layer is called as hard corona which strongly attach to the nanoparticles. And followed by the there will be a soft corona okay?

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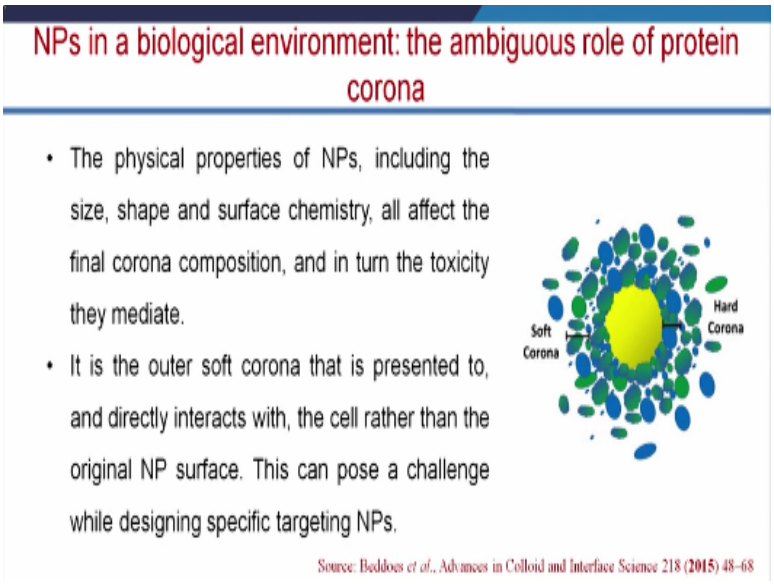

So here you can see the physical properties of nanoparticles including the size shape and surface chemistry, which is going to effect the final corona composition okay? And based on that corona composition toxicity effect will be varied okay? So you can see here this is you hard corona which is tightly attached to the nanoparticle surface and followed by that you are having the soft corona.

So the soft corona is outer soft corona that is presented to and directly interacts to the cell rather than the original nanoparticle surface. So this can pose a challenge while designing specific targeting nanoparticle. So here so this soft corona is going to interact with your cell not the nanoparticle charge or anything. So based on the soft corona it is going to give the therapeutic effect. So when you define any nanoparticles for a targeted delivery so you have to consider the effect of this soft corona also.

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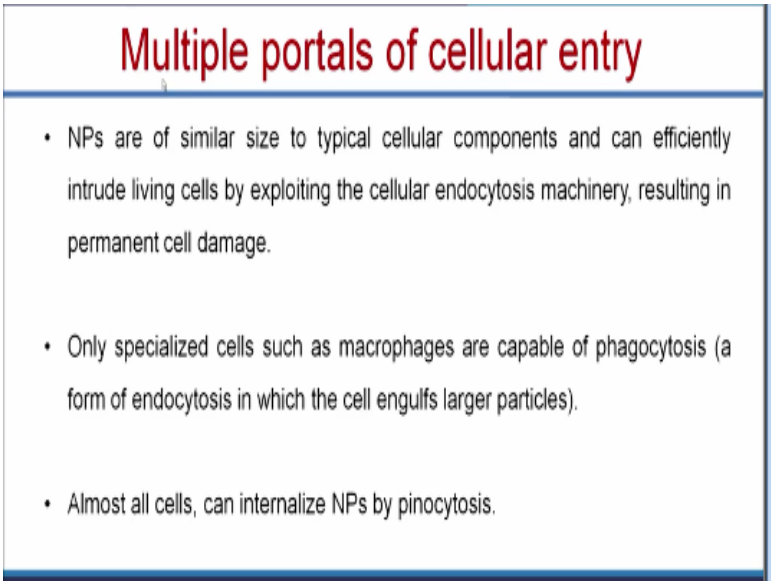

So let us see the multiple portals of cellular entry so nano particles of similar size to typical cellular components and can efficiently intrude the living cells by exploiting the cellular endocytosis machinery and it can result in the permanent cell damage. So only specialized cell such as macrophages are capable of phagocytosis a form of endocytosis in which the cell engulfs large particles.

And almost all the cell can internalize nanoparticle by pinocytosis okay? So the nanoparticle goes to the body there is a cell called by macrophages so that will uptake the nanoparticle by a process called phagocytosis. The phagocytosis process means cell eating, so most of the other cell will taken up the nanoparticle process called pinocytosis. Pinocytosis means cell drinking.

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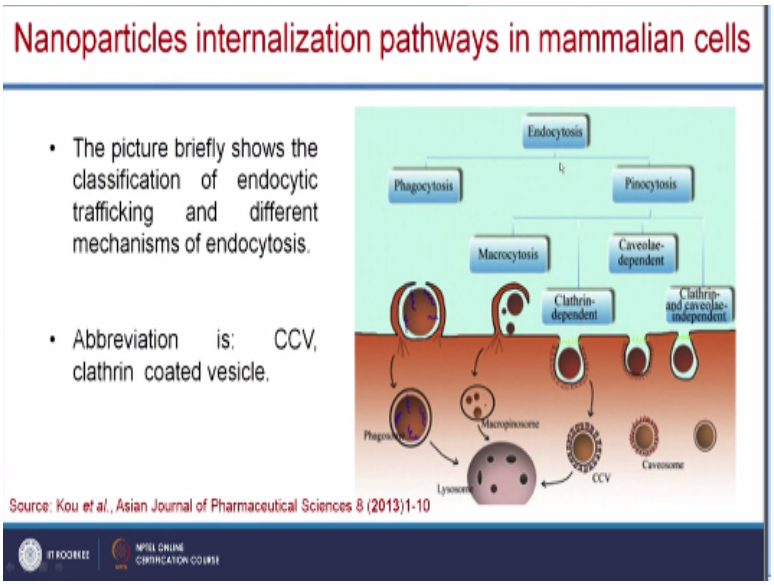

So let us see the mechanism of endocytosis in details. So endocytosis is dived into phagocytosis and pinocytosis. As I told earlier the phagocytosis process means cell eating process and Pinocytosis means cell drinking process. So under this pinocytosis again there are subclass like macrocytosis and clathrin dependent and clavilly dependent and clathrin and clavilly independent.

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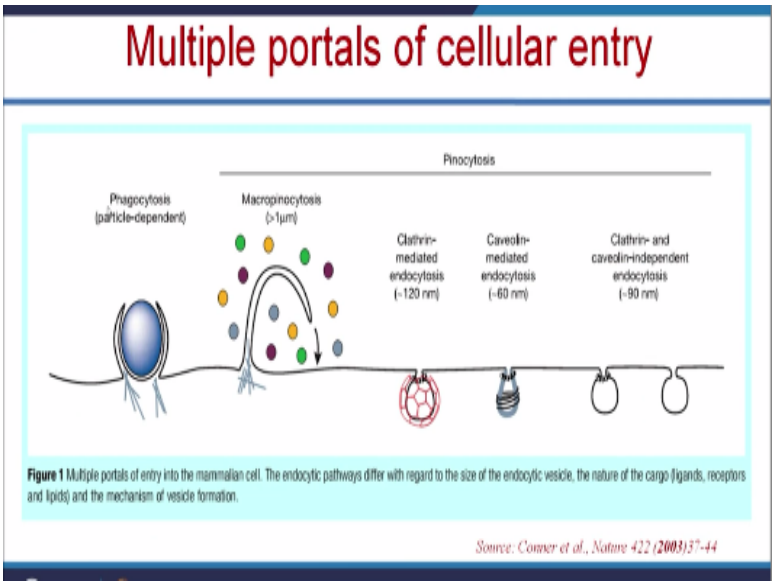

So these phagocytosis is a particle dependent okay? And this pinocytosis again updated into four categories like macro pinocytosis. So here the size of the vesicle will be more than one micrometer and this clathrin mediator endocytosis here the size of the vesicles 120 nanometer and this caviln mediator endocytosis the size is 16 nanometer and the case of clathrin and cavilen independent endocytosis the size of the vesicles 90 nanometer. So let us see the mechanism in detail.

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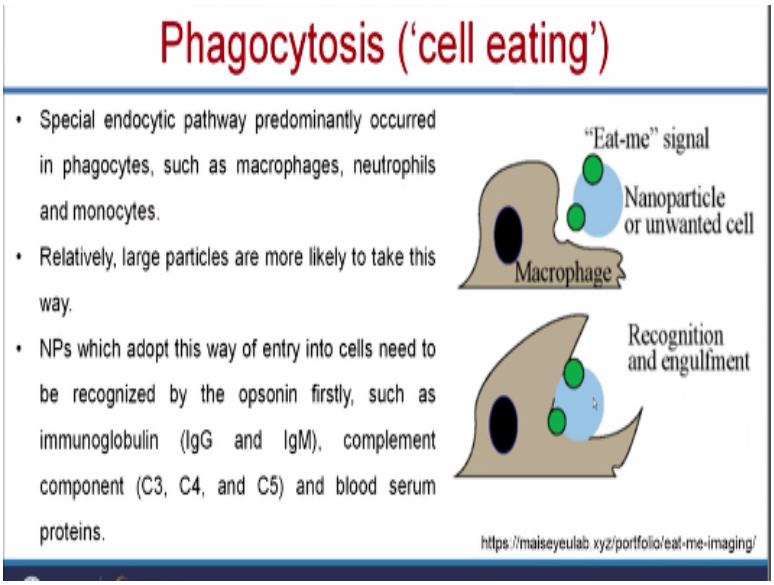

So first one I phagocytosis, so here the specific endocytic pathway predominantly occurred in phagocytosis, such as macrophages and neutophils and monocytes okay. So these are the cells will be involved in this phagocytosis process. And relatively larger particles are more likely to take this way. So the bigger size particles will be taken up by the cells by phagocytes process. And nanoparticles which adopt this way of entry into cells need to be recognized by the optionin firstly.

So the option are like immunoglobulin or compliment component and blood serum protein okay? The nanoparticle enter into your body what happens is these immune system is try to come and attack it. So these immune system as to break activator for the top of the nanoparticle there are some options will be attached, options is like antibodies are component protein or blood serum proteins that will be attach to the nanoparticle.

So once it attached this macrophages will go to the nanoparticle and it will ingles. So here you can see this is your nanoparticle on the top of the nanoparticle some signal options are attached okay? Once it attached so this macrophils will go and eat that particular nanoparticles.

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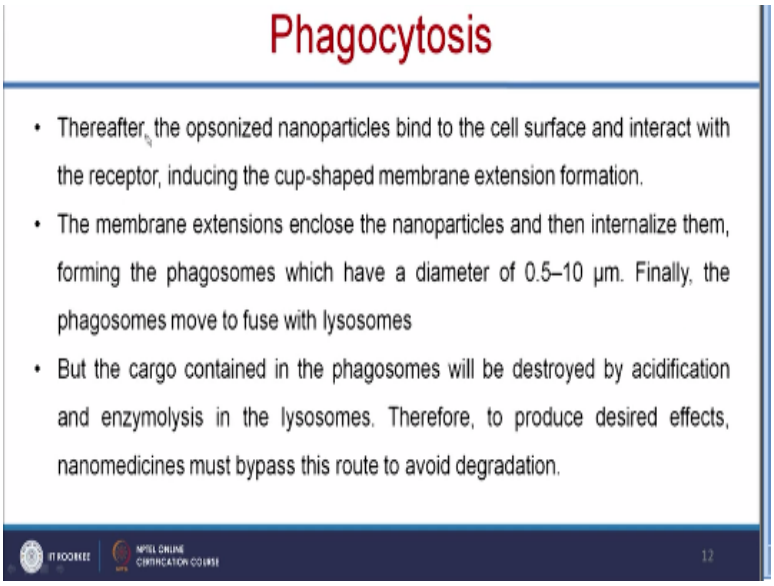

So let us see the phagocytes in details so these options is nanoparticles by bind to the cells surface interact with the receptor and it will inducing the cup shaped membrane extension formation okay? So these membrane extensions enclose the nanoparticles and then internalize them forming the phagosome. So which have a diameter of 0.5 to 10 micrometer, and finally phagosome moves to fuse to with the lysosomes okay?

So then it cargo contain in the phagosomes will be destroy and acidification and enzymolysis in the lysosomes. Therefore, to produce the desired effects, nanomedicine should bypass this route provide the degradation. So if you are making for some therapeutic application so that has to escape from this phagocytosis process. Then only it can lead the target cell and it can deliver the drug.

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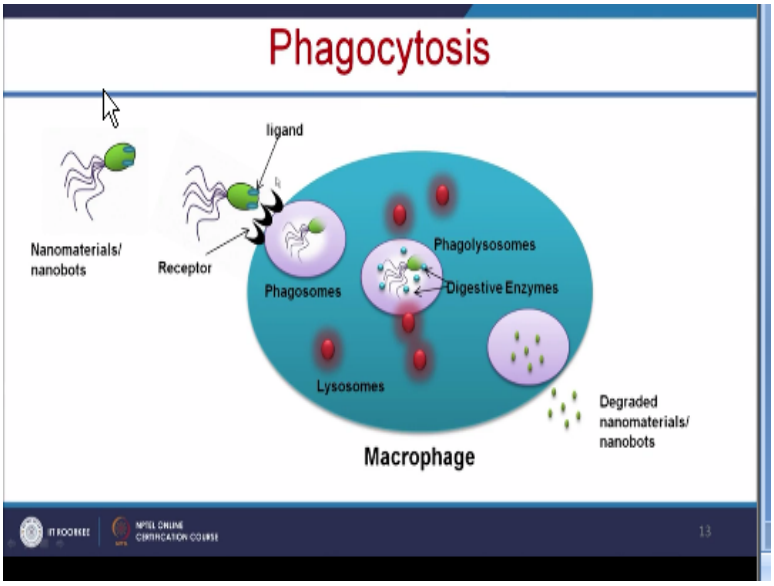

So you can see the phagocytosis in detail, so this is your nanoparticle or nanoboards and it is having this optionin okay? That is your ligand and when binds to the macrophils which is having the receptor so and it form a vesicle, so that is called as phagosome. And this phagosome will fuse with the lysosomes okay? And inside the lysosomes you are having lot of digestive enzymes so and this will degrade the this nanomaterials.

Then it release the degraded nanomaterials, so if a nanoparticle is caring a therapeutic molecule like a anti cancer drug anything so if it is taken up by these macrophage cells by the phagocytes process then it will degrade the nanoparticles the it cannot induce the desire therapeutic effect.

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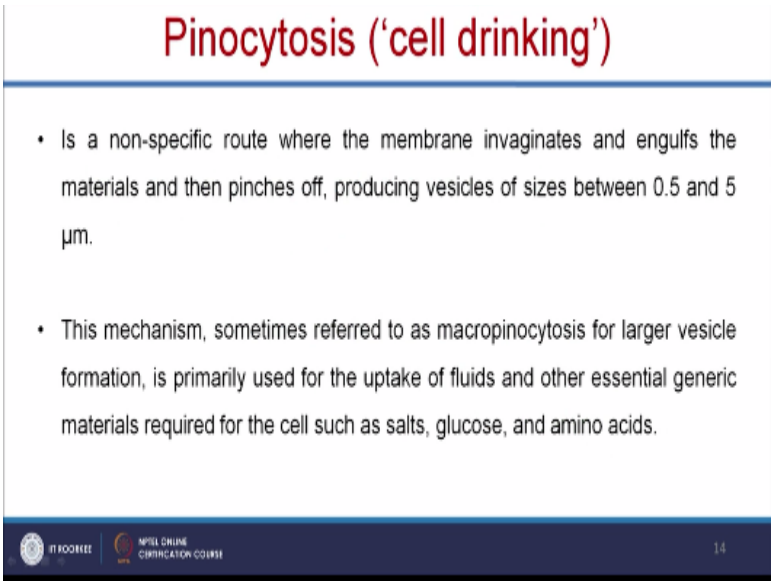

So let us see the next mechanism that is called as pinocytosis okay? So it is called as cell drinking. So it is a non-specific route where the membrane invaginates and engulf the materials and then pinches off, producing vesicles of sizes between 0.5 and 5 micrometer. So this mechanism sometimes referred to as macropinocytosis for larger vesicle formation.

And it is primarily used for the uptake of fluids and other essential genetic material required for the such as salt, glucose, and amino acids. So this the normal process salts and amino acids okay? And if any foreign particles they can also taken up by this pinocytosis process

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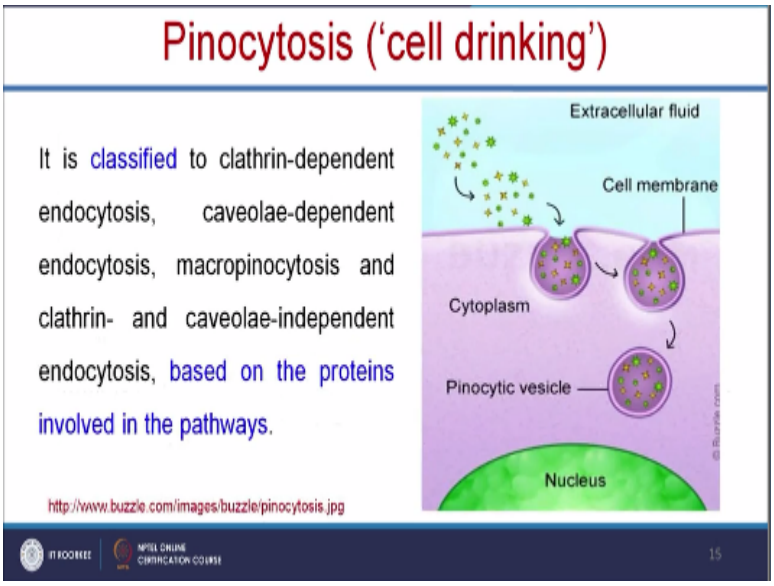

And again this pinocytosis process classified into clathrin dependent caveolae dependent macropinocytosis or caveolae and clathrin independent based on the protein involved in this pathway.

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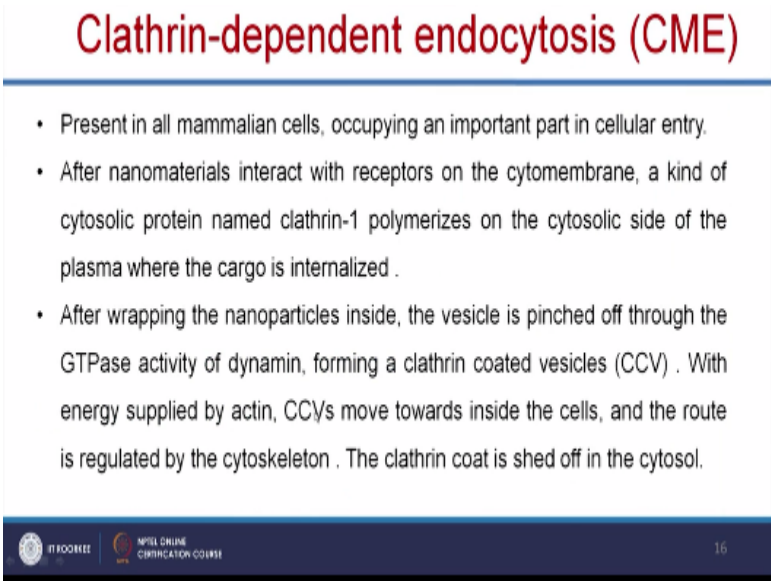

So let us see clathrin dependent endocytosis that is CME, so it is depend in all membrane cells it is palying a major roll in the cellular entry. So after nanoparticles interact with the receptors on cytomembrane and a kind of cytosolic protein made clathrin will polymers on the cytosolic side of the plasma where the cargo is internalized so after wrapping the nanoparticles inside so the vesicles is pinched off thorough the GTPase activity of dynamin, and forming at clathrin coated vesicles.

So with energy supplied by actin this CCVs move toward inside the cells and the route is regulated by the cytoskeleton. So this clathrin coat is shed off in the cytosol to release the therapeutic molecule.

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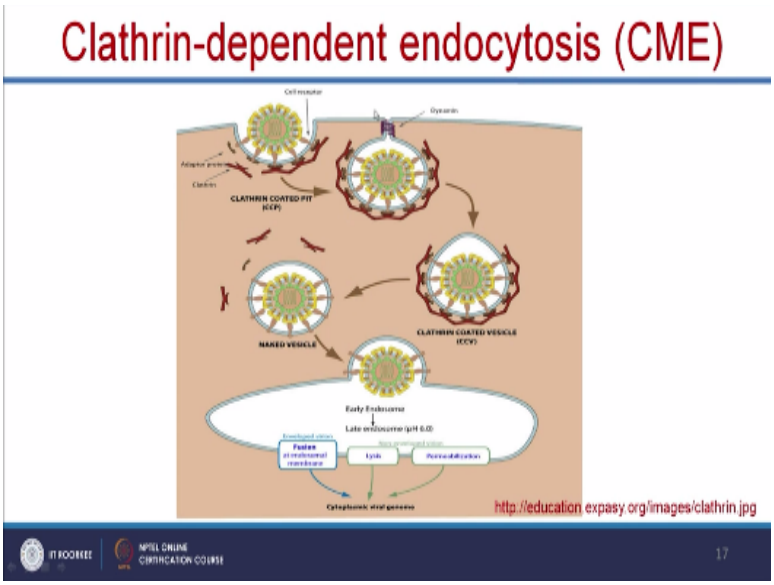

So we can see here this is your nanoparticle so it is coming and binding to the cell and this is a Catherin put in it is attaching to this membrane and it is forming a clathrin coated to it okay? And it forming like this kind of vesicles and once a your nanoparticle is entrapped to this vesicle and vesicle can be close to the help of dynamic and this will be taken inside the cell and there your clathrin will be released.

And only the naked vesicles will go into the endosome and there it will be degraded and it will release the your therapeutic molecule to the cytoplasm of the nucleus.

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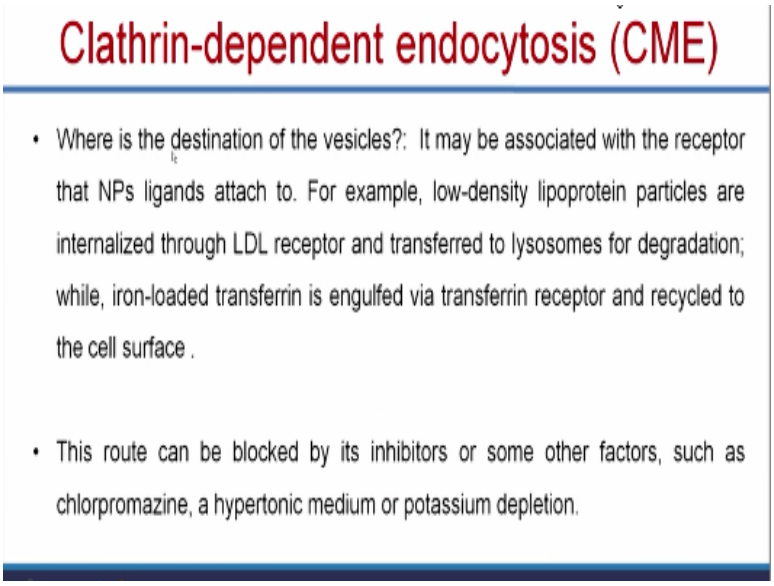

So where is the destination of the vesicles? As I told you earlier it may be associated with the receptor that nanoparticle ligands attach to. For example if you have the low density lipoprotein particles and it should be internalized through the LDL receptor. And it will transfer to the lysosomes for degradation. But if we are having iron loaded transferring that will be engulfed via transferring receptor and recycle to the cell surface.

And this route can be block by inhibitors like chlorpromazine or a hypertonic medium or potassium depletion. So by using this inhibitors we can inhibit the pathway CME pathway. And we can understand the nanoparticle is following this CME pathway or not.

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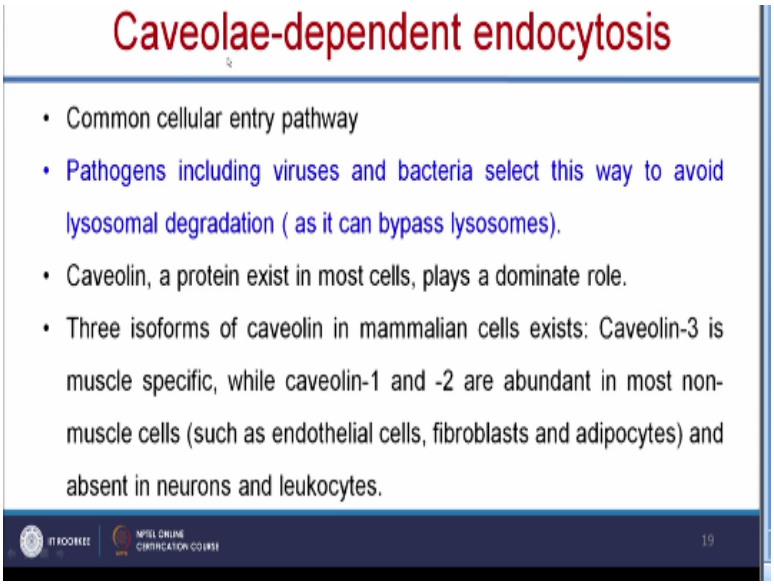

Next one is caveolae dependent endocytosis, so this the common cellular entry pathway. So most of the pathogens including viruses and bacteria select this way to avoid the lysosomes and degradation so it can bypass the lysosomes, so when you made the nanoparticles if it is following this caveolae dependent endocytosis pass way that is better it is escaping from this lysosomes degradation so can release the therapeutic molecule into the cytoplasm or the nucleus. So here the caveolae is a protein exist in the most cells, and it play a major role in the caveolae dependent endocytosis. So there are three forms of caveolae, caveolae 1, 2, 3 and it dependes on the cell and lipids from the application from this protein comes and play major role.

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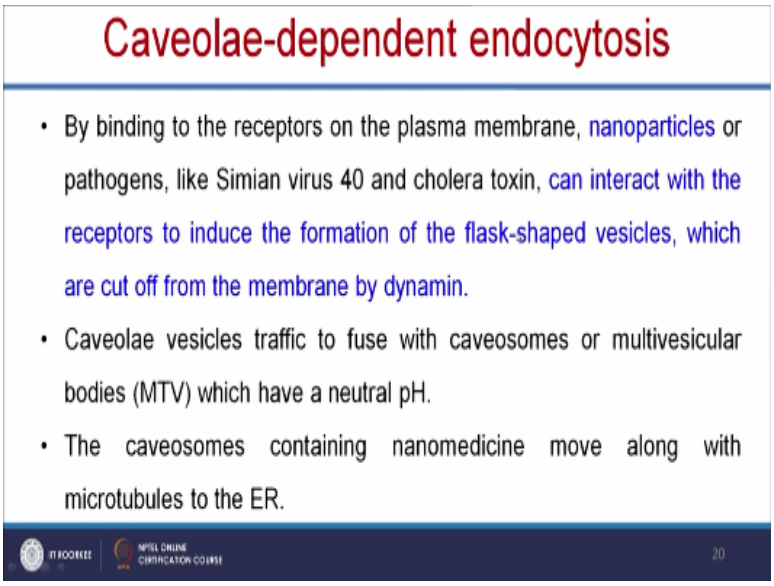

So let us see the caveolae dependent endocytosis in details, so once the nanoparticles of the virus which can come and interact with the receptors that will induce in formation of flack shape vesicles okay? So which are cut off from membrane by the dynamin and this caveolae vesicles traffic to fuse with caveosomes or multivesicular bodies which have neutral pH. So this caveolae containing nanomedicine moves along with microtubules to the endoplasmic reticulum.

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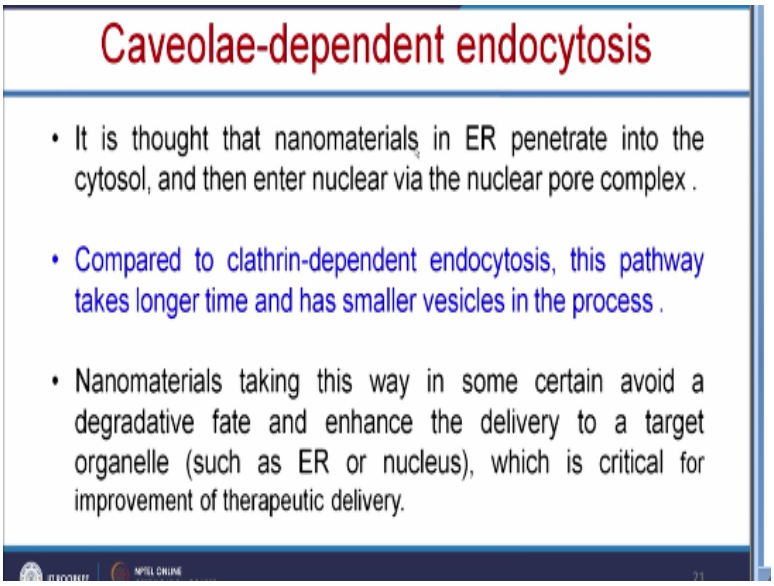

And here this nanomaterials in the endoplasm reticulum penetrated in the cytosol okay? And then enter he nucleus through the nuclear pore complex. So compare to the clathrin dependent endocytosis this pathway takes longer time and a smaller vesicles in the process. And the nanomaterials taking this way is it will avoid the fate and it will enhance the delivery to a target organelle such as a endoplasm reticulum of the nucleus.

So which is very important for improving the therapeutic delivery? For example if we care making nanoparticle for a delivering anticancer drug or any other therapeutic molecule so if you take this caveolae dependent endocytosois pathway so it can escape from the lysosomes. And it can escape from lysosomes and it can lead the cytoplasm of the nucleus therapeutic efficiency.

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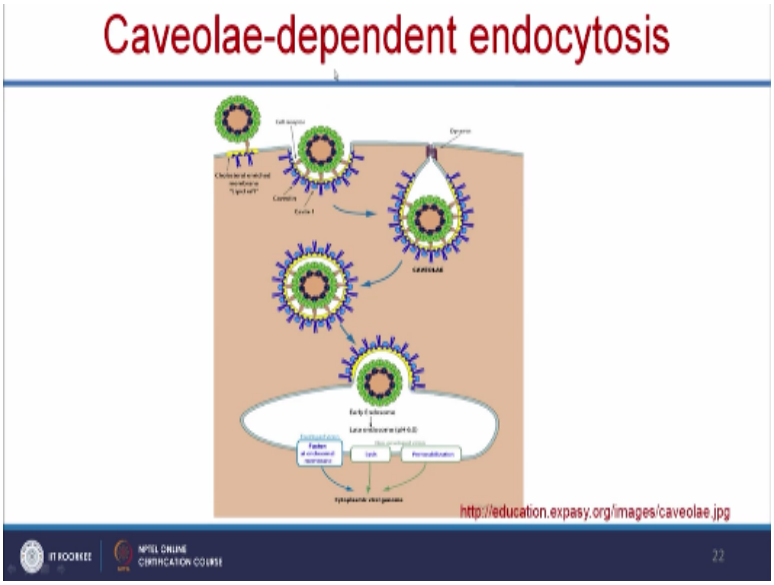

So in this caveolae so the nanoparticle come and bind to the cell okay? And mainly this colestral enriched membrane will be there at the receptor side and here you can see this is your V shape this is your caveolae protein and another protein is caveolae that is in blue color okay. And its forms like flack shaped caveolae and this dymin will come and help in enclose in vesicles and this vesicle will go and biond to this endosome and the material inside is nanoparticle will be released into the cytoplasm as well as the nucleus and the material inside is nanoparticles will be released into this cytoplasm as well as the nucleus.

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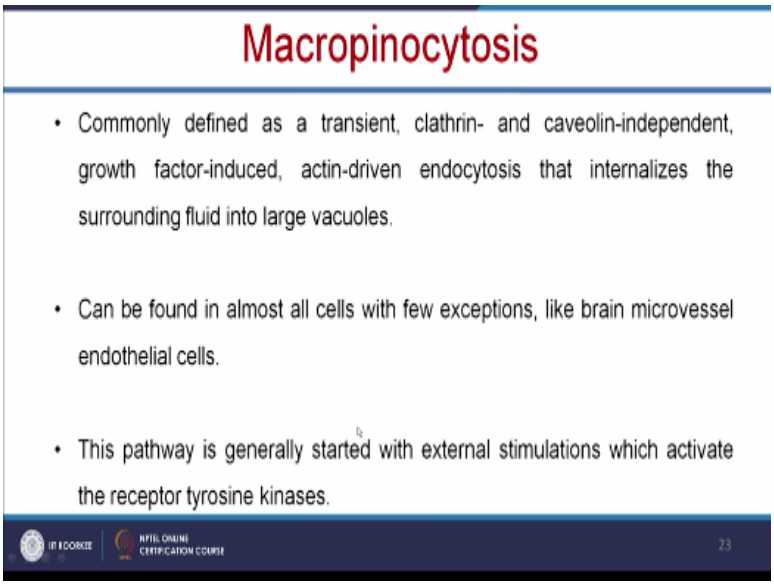

So next process is macropinocytosis so it is commonly dependence that transient and this is a clathrin and caveolin-independent growth factor-induced or the actin-driven endocytosis okay. So that can internalizes the surrounding fluid into large vacuoles. And it can be found in almost all the cells okay and with few exceptions like brain microvessel, endothelial cells, and this pathway is generally started with an external simulation which activate the receptor thyrocin kinases.

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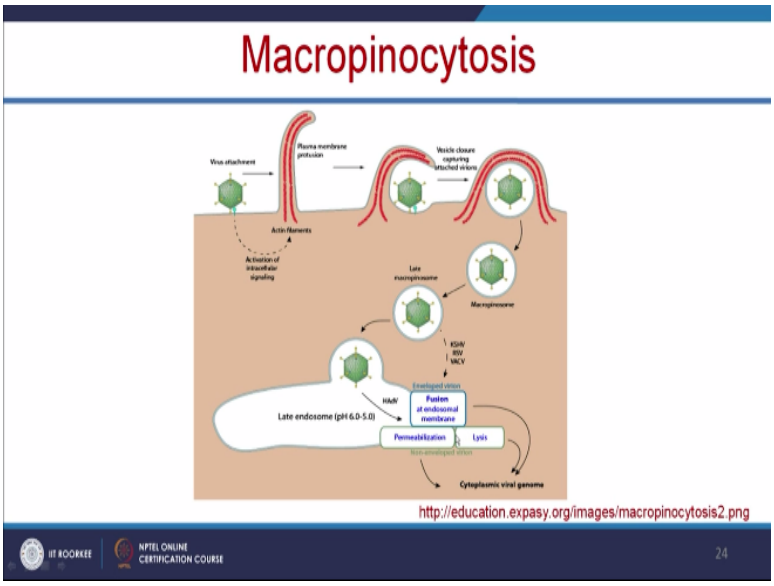

So let us see the macropinocytosis in details, so when the virus or your nanoparticles come and attach the cell, so it will activate the intracellular signaling, which leads to the protection of actin philaments, this actin philaments will make like this protution and this protution will cover your nanoparticles and it forms a macropinosomes. And this macropinosomes will reach your endosome and there it will be releasing your nanoparticles with a therapeutic molecule and that can be taken up by the cytoplasm or the nucleus.

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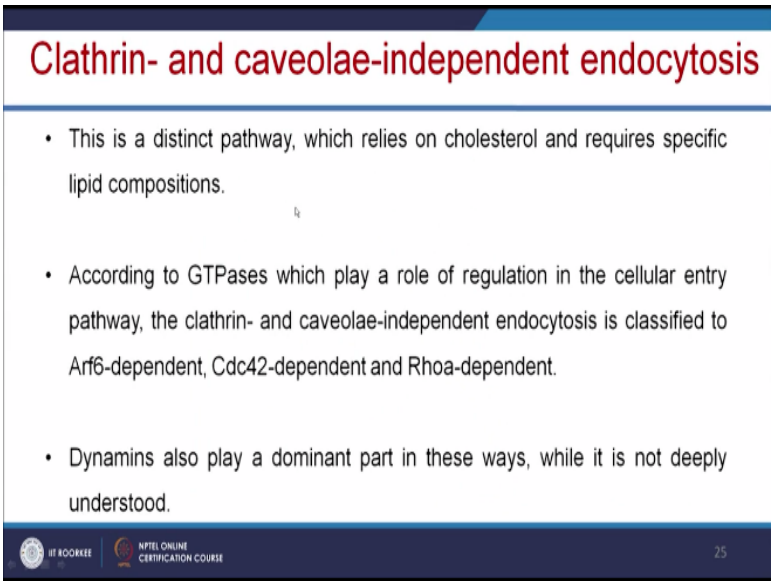

So in this clathrin and caveolae independent endocytosis so which is mainly released on the cholesterol and requires a specific liquid composition. So according to GTPases which play a major role in regulating these cellular entry pathway, so this clathrin and caveolae independent endocytosis is classified into Arf6 depeendent, Cdc42 dependent or Rhoa dependent. And here dynamin also play important role, but the mechanisms is not completely understood.

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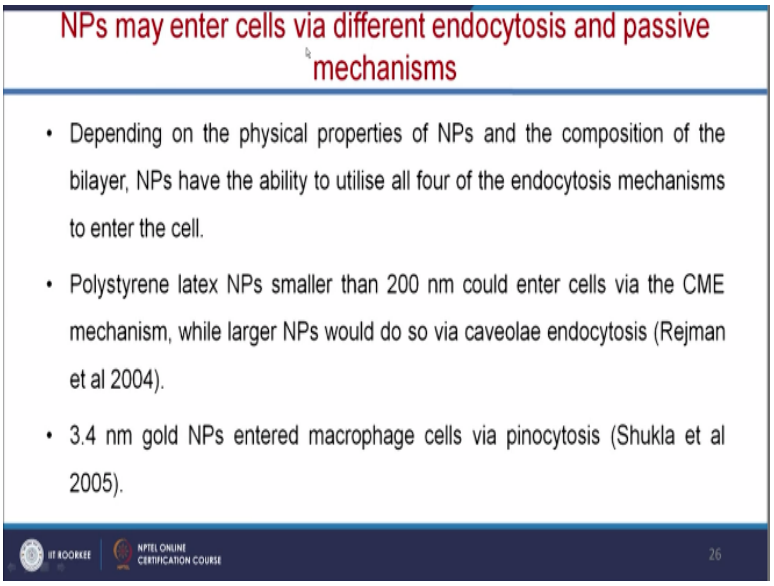

So let us see some of the examples, so the nanoparticles may enter these cells through different endocytosis and passivey mechanisms so depending on the physical properties of nanoparticles and the composition of bilayer okay. So the nanoparticles have the ability to utilize all four of the endocytosis mechanisms to enter the cells. So some of the example we see here, so polystyrene latex nanoparticles smaller than 200 nanoparticles could enter the cells through CME mechanism and the larger nanoparticles will enter the cells through the caveolae endocytosis.

And the gold nanoparticle with the diameter of 3.4, nanometer can enter the macrophage cells through the pinocytosis.

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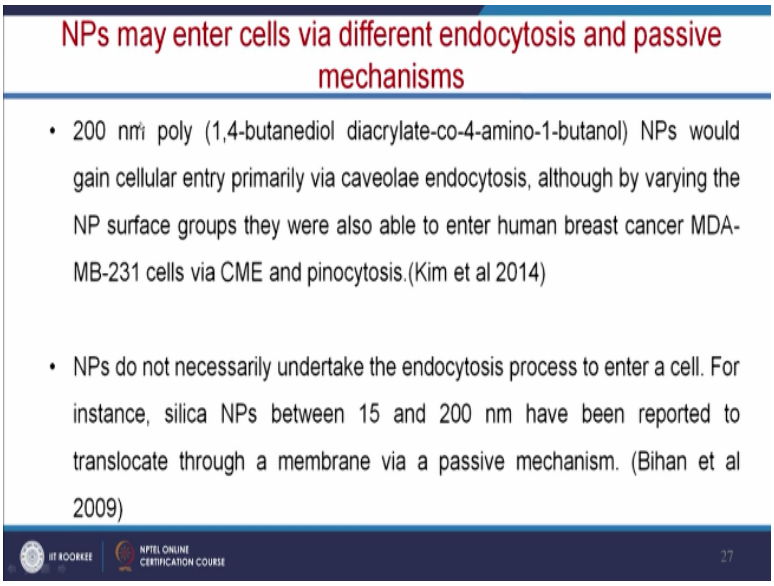

And 200 nanometer polymeric nanoparticles would enter these cells through caveolae endocytosis. And by changing the nanoparticle surface groups and it can also enter the cancer cells by CME as well as pinocytosis process. So based on the surface charges again the mechanism of cellular optic will be varied. So here the nanoparticles do not necessary undertake the endocytosis process to enter a cell.

For example, silica nanoparticles between 15 to 200 nanometer, have been reported to translocate through a membrane through the passive mechanism.

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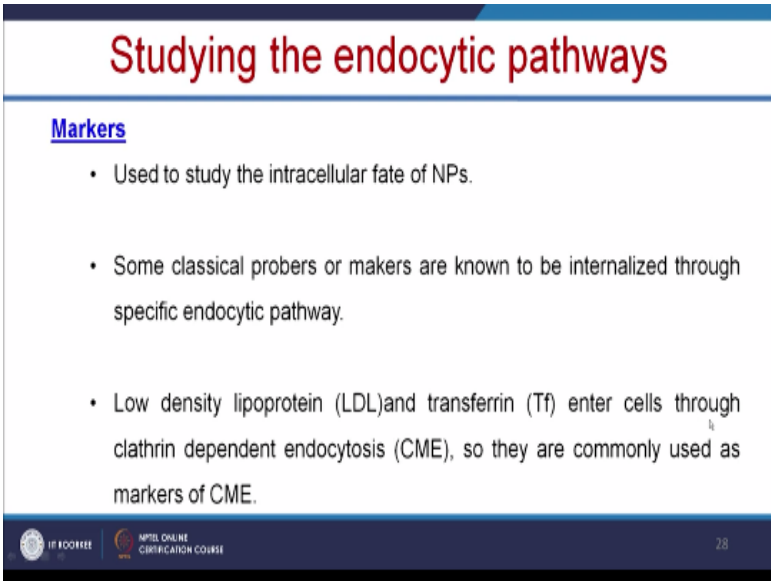

So if you want to study whether your nanoparticle is following endocytic pathways, so we can use the markers to study the intracellular fate of this nanoparticles. Some of the classical probers or markers known to be internalized through the specific endocytic pathway. For example, this LDL, this low density lipoprotein and transferring enter the cells through the clathrin dependent endocytosis. So they are commonly used as a markers for CME.

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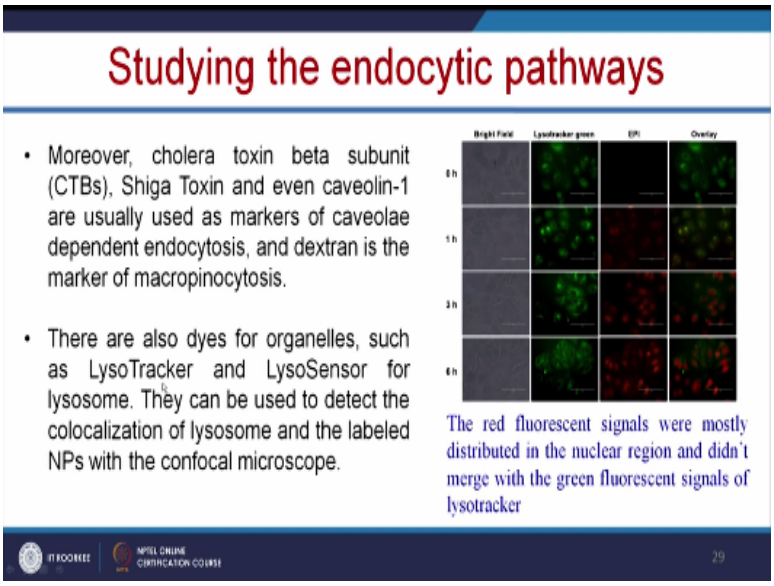

And also we can use this like cholera toxin or shiga toxin okay, and even caveolin – 1 so which are usually used as a markers for caveolae dependent endocytosis and again we can also use the dextran is the marker of macropinocytosis so when you attach this dextran to this particular protein and we can understand weather it is entering the cells though this particular path way or not and there are several dye available for specific organelles in the cell for example LysoTracker and LysoSensor for lysosome okay.

So this LysoTracker dye will satin only the Lysosome and they can be used to detect the colocalization of lysosome and the labeled nano particles with the confocal microscope so let us see in this picture we have used the cancer cell lines and it is trained with the lysotracaker green okay so this LysoTracket green will stain only the lysosome of the cells and this a em is a an anticancer drug and which as the intrinsic red color fluorine and when we over lay this images we can see here this anticancer drug is entering the nucleolus and your lysosomes that is a lyso tracker green that is on the cytoplasm okay.

And here the all the red fluorescent signals were mostly distributed in the nuclear region ands ddi not merge with the green fluoresce signals of the lysotracker so it shows that this nano particle is escaping from the lysosome and it is entering into the nucleus to deliver the particular theoretic molecule.

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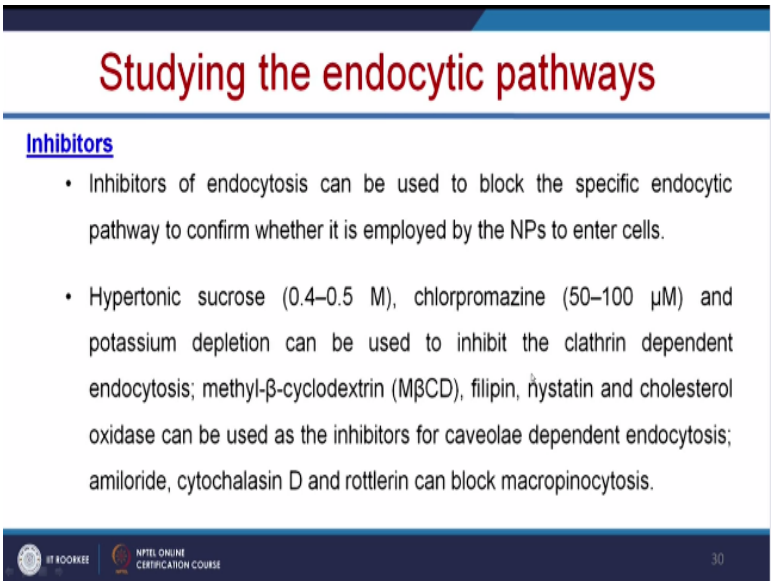

So there are several inhibitors also available which can used to block the specific endocytic pathway to confirm whether it is employed by the nano particles to enter cells so these are some of the examples like hypertonic sucrose or chlorpromahnzine and potassium depletion so which can be used to inhibit the clathrin dependent endocytosis and this chemical can be used to inhibitors the caveolae dependent endocytosis and this molecules can be used to block the macropinocytosis.

So by using these kind of chemicals we can block the particular endocytic pathway and we can add our nano particles and we can check whether our nano particles is following this particular pathway are not.

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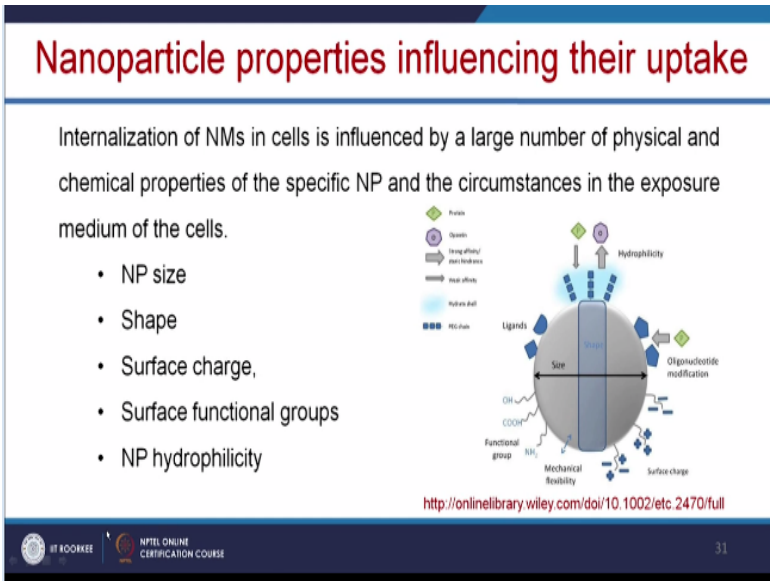

And here the nano particles properties play a major role in this cellular optic for examples the nano particles size, shape, surface charge and surface functional groups and also nano particles hydrophilicity so these are the various properties which is going to play a major role in this cellular optic.

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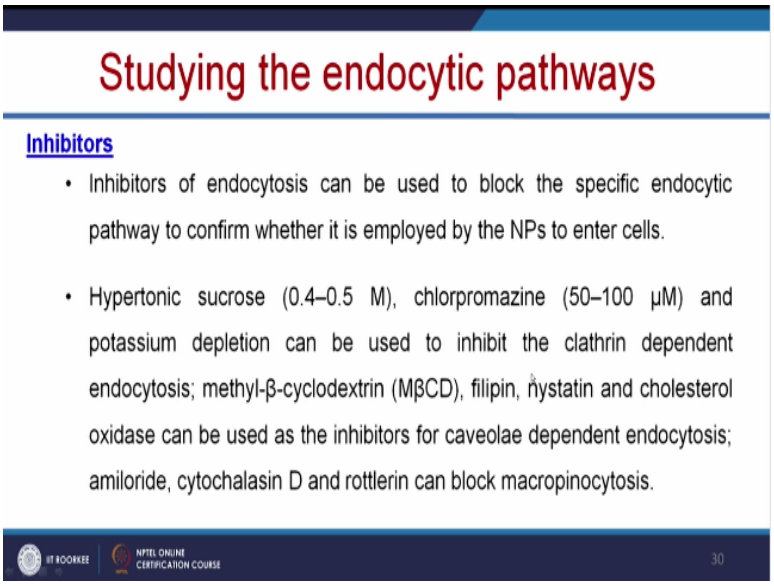

So let su see how the size plays a major role in cellular optic so the size of the vesicles that contain nano particles varies with the specific pathway and the particles size should be small enough to enter the vesicles and the size range from 10nm to 500nm and limited up to 5 micrometer, so it depends on the size the particles will take the different kind of cellular entry and the larger particles are mostly likely to be engulfed by macropincytosis and the size of the vesicle involved in the clathrin mediated endocytosis is ~100nm and the size involved in caveolae mediated endocytosis is 60-80nm.

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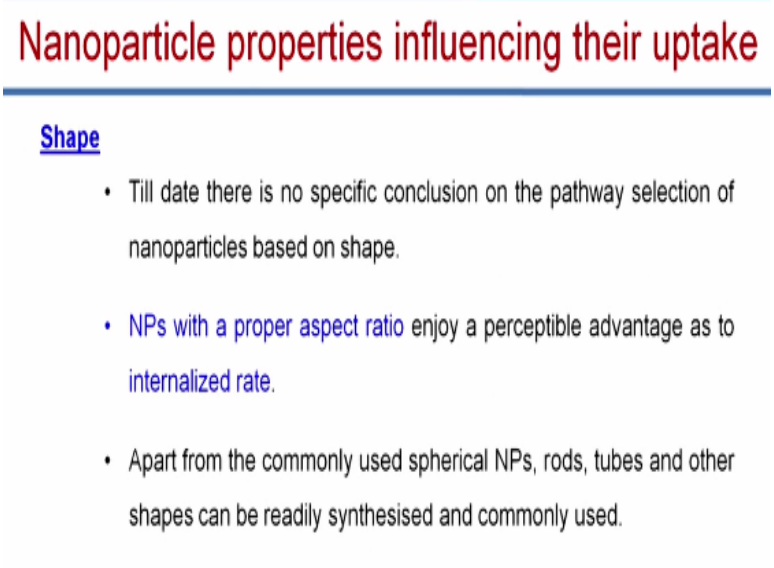

And let us see the shape so and till there is not specific conclusion on the path way selection of nano particles based on the shape some of the reports showing that rod shape particles have more cellular entry when compared to the spherical shape but still lot of researches going on there is no proper conclusion.

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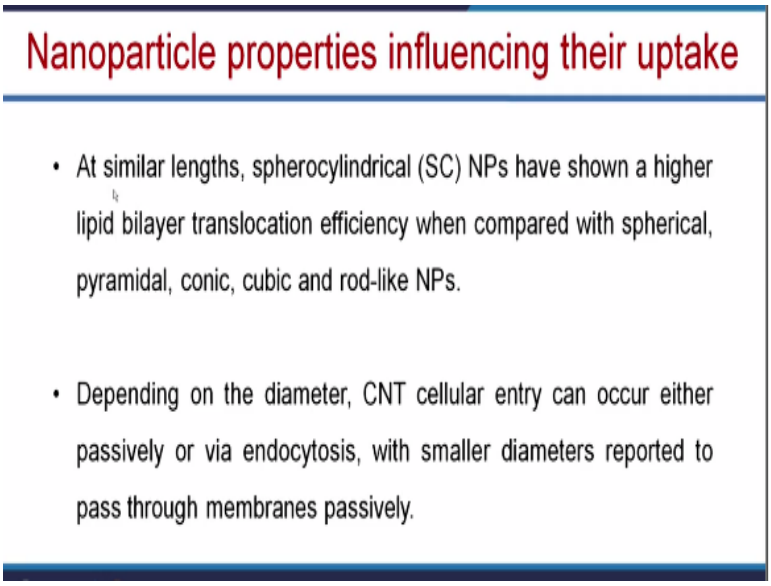

So at similar lengths spherocylindrical nano particles have shown a higher lipid bi-layer translocation efficiency when compared with the spherical or pyramidal or conical nano particles and depending on the diameter of carbon nano tubes the cellular entry of the carbon nano tubes can occur either passively or endocytosis, okay, So with a smaller diameters reported to pass through the membranes passively.

And again when you use the carbon nano tubes okay or nano rods depends on the size and depends on the diameter of the nano particles or nano rods so it can select the particular path way to enter into the cell.

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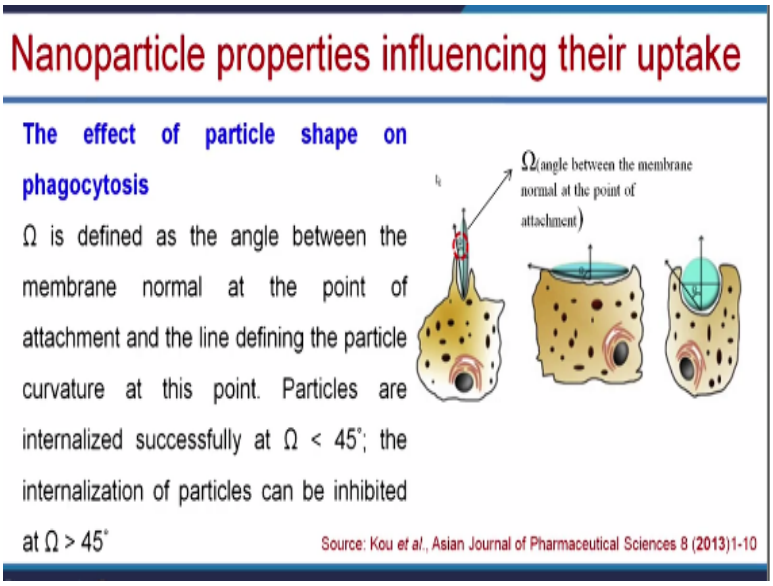

So let you see the effect of particle shape on the phagocytosis, so this omega is an angle between the membrane normal at the point of attachment suppose if the omega angle is less than45 so the particles can be internalized successfully and if the omega angle is more than 45 the internalization of the particle can be inhibited.

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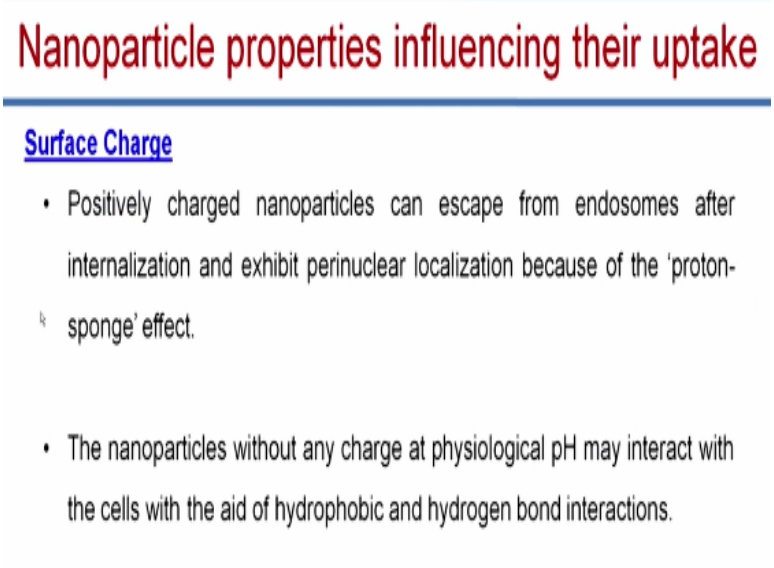

And let us see the role of surface charge, so here the positively charged nano practices can escape from endosome after internalization and it will exhibit a pheri-nuclear localization because of the protons sponge effect and here the nano particle without any charge at physiological PH may interact with a cells with the aid of hydrophobic and hydrogen bond interactions.

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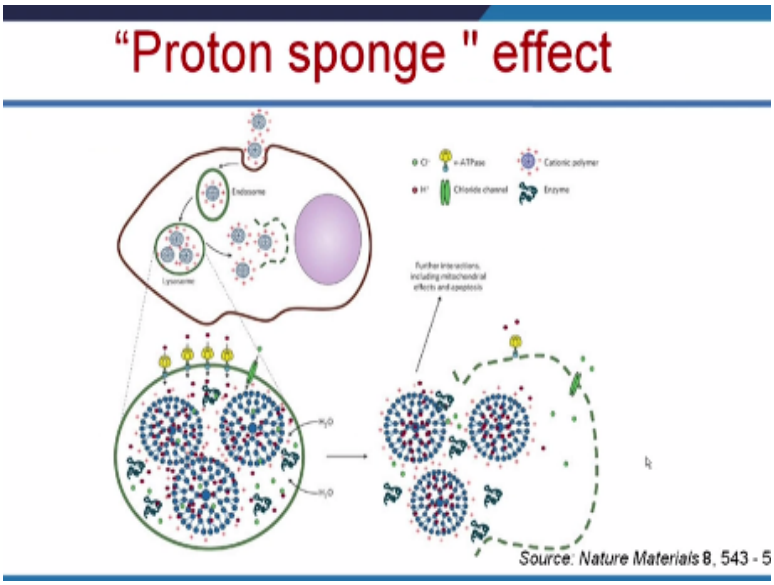

So let us see what is the proton sponge effect, so the positively charged nano particles will attach to the cells and it forms the endosome and this endosome will fuse with your lysosome okay and it forms a endolysosome and this endolysosome due to the more amount of positive charge in the endolysosome it will break and it will release the nano particles into the cytoplasm and this nano particle can release the drug to the nucleus and it can induce the therapeutic effect. So this is called as proton sponge effect.

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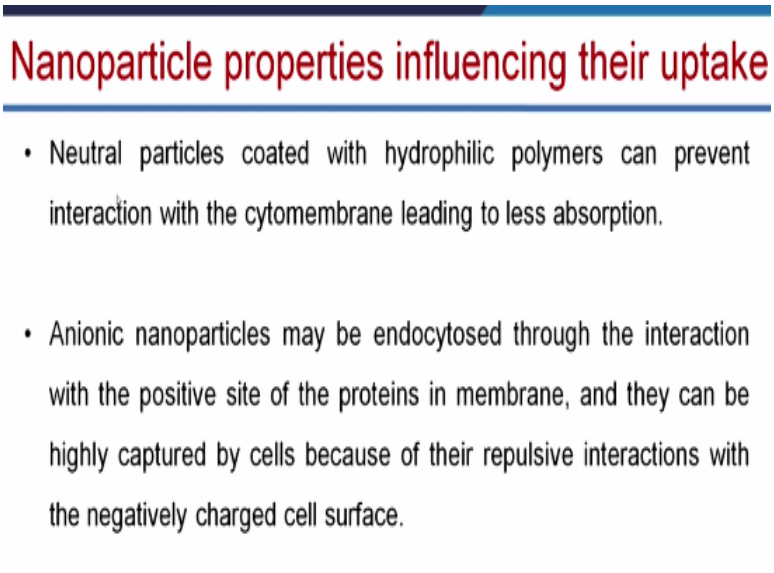

And here the neutral particles coated with hydrophilic polymers can prevent the interaction with the cytomembrane leading to less absorption and anionic particles may be endocytosis through the interacting with the positive side of the proteins in the membrane, okay and they can be highly captured by the cells because of their repulsive interaction with the negatively charged cell surface. So the positively charged nano particles can easily attach to the cells as you know that cells have the negatively charge and this anionic nano particles so that be taken up by the positively charged protein receptor on the cell surface.

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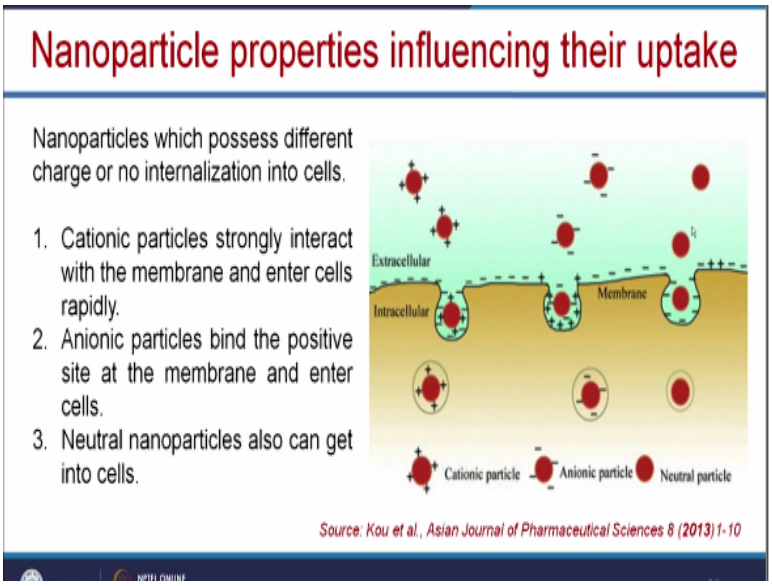

So this cationic nano particles strongly interact with the membrane and enter the cells rapidly and this anionic particles bind the positive site at the membrane and enter the cells and again neutral nano particles can also enter into the cells to the various process.

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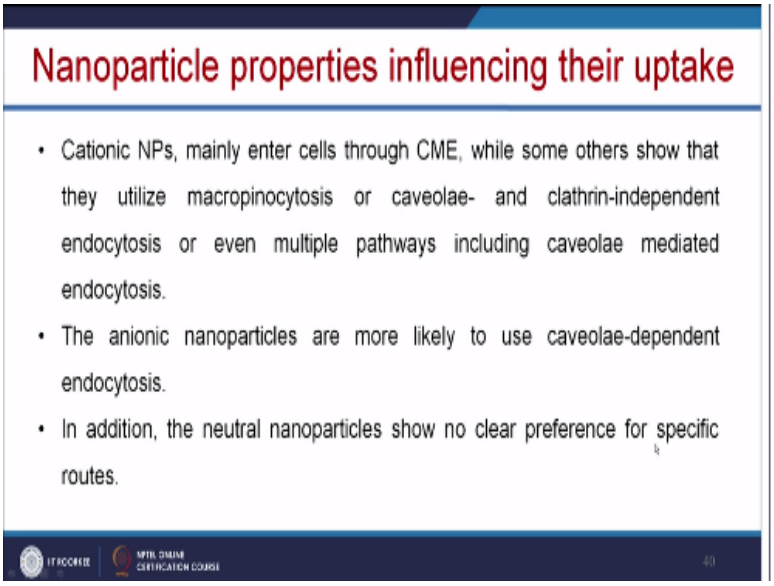

So here this cationic particles mainly enter the cells through CME and some results show that they can utilize macropinocytosis or caveolae and clathrin independent endocytosis or it can even follow multiple path ways including caveolae mediated and endocytosis, and mainly the anionic nano particles so these are likely to take up the caveolae dependent endocytosis and again the neutral nano particles there is no clear preference for specific routes.

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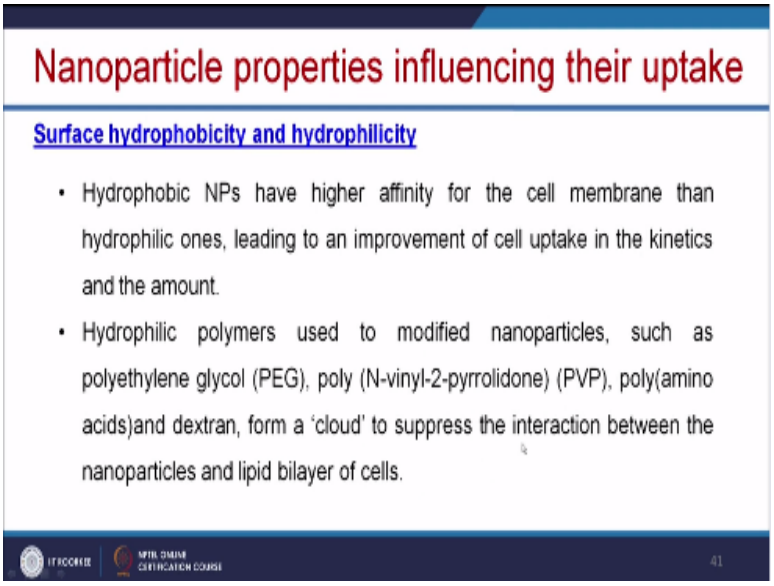

So let us see the role of surface hydrophobicity and hydrophilicity so this is hydrophobic nano particles have higher affinity for the cell membrane then hydrophilic ones so leading to an improvement or cell uptake in the kinetics and the amount And this hydrophilic polymers use to modified nanoparticles such as polyethylene glycol PEG or the PVP and dextran so which form a cloud to suppress the interaction between the nano particles and the lipid bilayer of cells so thus it will increase the prolong circulation of the particular nanoparticles in the system.

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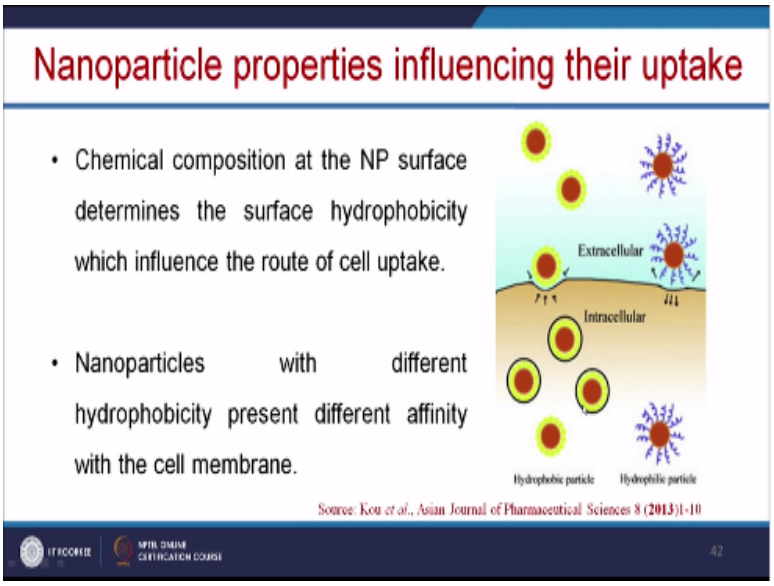

And again the chemical composition at the nanoparticles surface, also determines the surface hydrophobicity and which influence the route of cell or uptake and nanoparticles with different hydrophobicity present different affinity with the cell membrane so we can see here if we have the hydrophobic particle or the hydrophobic particles so we will be taken up by the cells differs on the affinity with the cell membrane, So as a summary of this lecture in this lecture we have learnt what is a role of.

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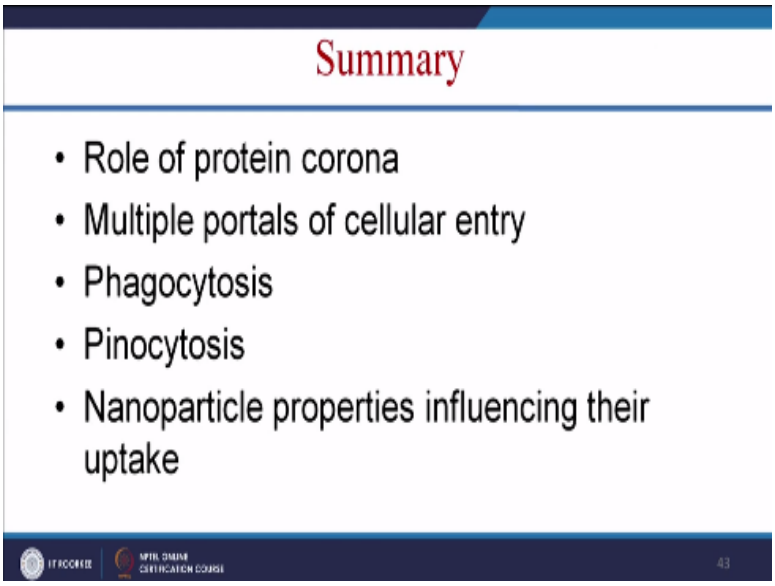

Protein corona and what are the various multiple portals available for cellular entry of the nano particles and we also learn the details of phagocytosis and pinocytosis process how the nanoparticles can I use this process to enter the cell and we also learnt how this nanoparticles properties influence their cellular uptake so in my lecture here and thank you all for listening this lecture as you see all in another interesting lecture.

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