

INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

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NPTEL ONLINE CERTIFICATION COURSE

Biomedical Nanotechnology

Lec -18

Cellular Uptake Mechanisms of Nanomaterials

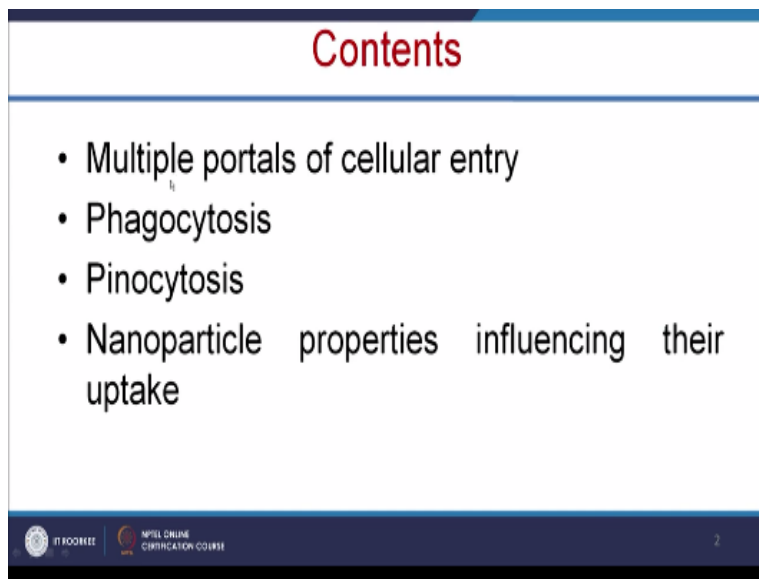
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Hello everyone I welcome all to the 18 th lecture of this course, so the 18 th lecture is cellular uptake of nanomaterials.

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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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Introduction

- The design of smart multifunctional nanosystems for intracellular imaging and targeted therapeutic applications requires a thorough understanding of the mechanisms of nanoparticles (NPs) entering and leaving the cells.
- NPs have been considered as effective delivery vehicles and extensively studied for delivering drugs, genes, diagnostics, and vaccines into cells of interest.
- Site-specific delivery of drugs and therapeutics can significantly reduce drug toxicity and increase therapeutic effects.



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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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Introduction

- For biological and clinical applications, the ability to control and manipulate the accumulation of NPs for an extended period of time inside a cell can lead to improvements in diagnostic sensitivity and therapeutic efficiency.
- Furthermore, elucidating the exocytosis and metabolism of NPs in cells could lead to a better understanding of NP toxicity (i.e., if the NPs are trapped in vesicles and leave the cells intact, they are unlikely to induce cellular toxicity).

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?

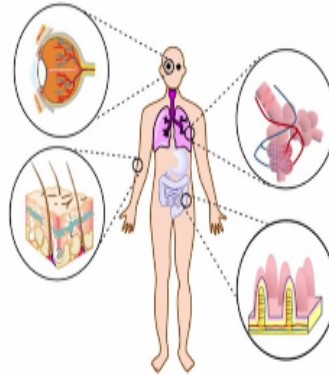
So it is better to understand the mechanism how to enter the cell and what is the subsequence process how is can reduce the therapeutic efficiency. Those things has to be understood thoroughly before we take the nano medicine to the clinical application.

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Entry of NPs in body by multiple routes

Major NP entry routes into the body:

- Dermal
- Ocular
- Respiratory
- Gastrointestinal systems



Source: Beddoes *et al.*, *Advances in Colloid and Interface Science* 218 (2015) 48–68



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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NPs in a biological environment: the ambiguous role of protein corona

- Once in a biological environment, an NP 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).
- The inner 'hard' corona rapidly forms in seconds and strongly adheres to the NP surface.
- The secondary 'soft' corona is found atop, which can take hours to equilibrate due to its high sensitivity to the external environment composition and conditions

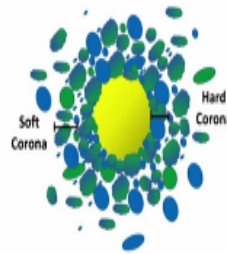
So once it enters the biological environment, so there is a formation of protein corona. Let us see what is protein corona? So in the biological environment the nanoparticle will adopt a biological identity with the formation of a protein corona on its surface due to adsorption of layers of proteins 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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NPs in a biological environment: the ambiguous role of protein corona

- The physical properties of NPs, including the size, shape and surface chemistry, all affect the final corona composition, and in turn the toxicity they mediate.
- It is the outer soft corona that is presented to, and directly interacts with, the cell rather than the original NP surface. This can pose a challenge while designing specific targeting NPs.



Source: Beddoes *et al.*, *Advances in Colloid and Interface Science* 218 (2015) 48–68

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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Multiple portals of cellular entry

- NPs are of similar size to typical cellular components and can efficiently intrude living cells by exploiting the cellular endocytosis machinery, resulting in permanent cell damage.
- Only specialized cells such as macrophages are capable of phagocytosis (a form of endocytosis in which the cell engulfs larger particles).
- Almost all cells, can internalize NPs by pinocytosis.

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.

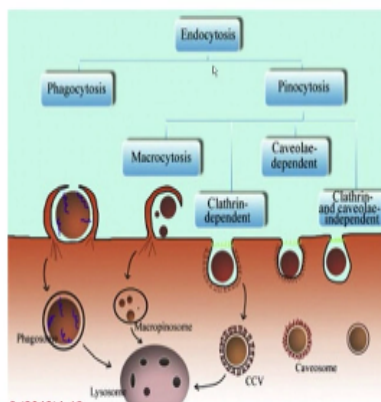
And almost all the cell can internalize nanoparticle by pinocytosis okay? So the nanoparticle goes to the body there is a cell called by macrophages so that will uptake the nanoparticle by a process called phagocytosis. The phagocytosis process means cell eating, so most of the other cell will taken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

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Nanoparticles internalization pathways in mammalian cells

- The picture briefly shows the classification of endocytic trafficking and different mechanisms of endocytosis.

- Abbreviation is: CCV, clathrin coated vesicle.



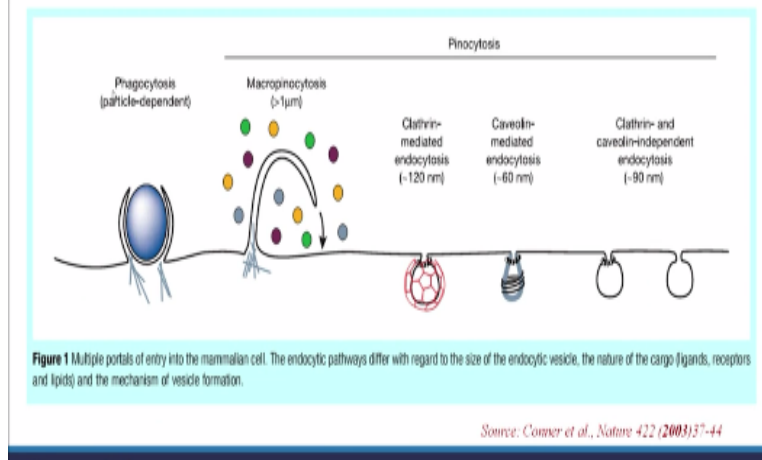
Source: Kou et al., Asian Journal of Pharmaceutical Sciences 8 (2013)1-10



So let us see the mechanism of endocytosis in details. So endocytosis is divided 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 clathrin and clathrin independent.

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Multiple portals of cellular entry

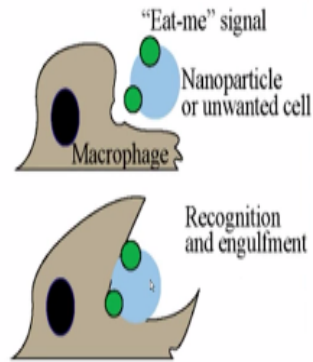


So these phagocytosis is a particle dependent okay? And this pinocytosis again updated into four categories like macro pinocytosis. So here the size of the vesicle will be more than one micrometer and this clathrin mediator endocytosis here the size of the vesicles 120 nanometer and this caviln mediator 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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Phagocytosis ('cell eating')

- Special endocytic pathway predominantly occurred in phagocytes, such as macrophages, neutrophils and monocytes.
- Relatively, large particles are more likely to take this way.
- NPs which adopt this way of entry into cells need to be recognized by the opsonin firstly, such as immunoglobulin (IgG and IgM), complement component (C3, C4, and C5) and blood serum proteins.



<https://maiseyeulab.xyz/portfolio/eat-me-imaging/>

So first one I phagocytosis, so here the specific endocytic pathway predominantly occurred in phagocytosis, such as macrophages and neutrophils 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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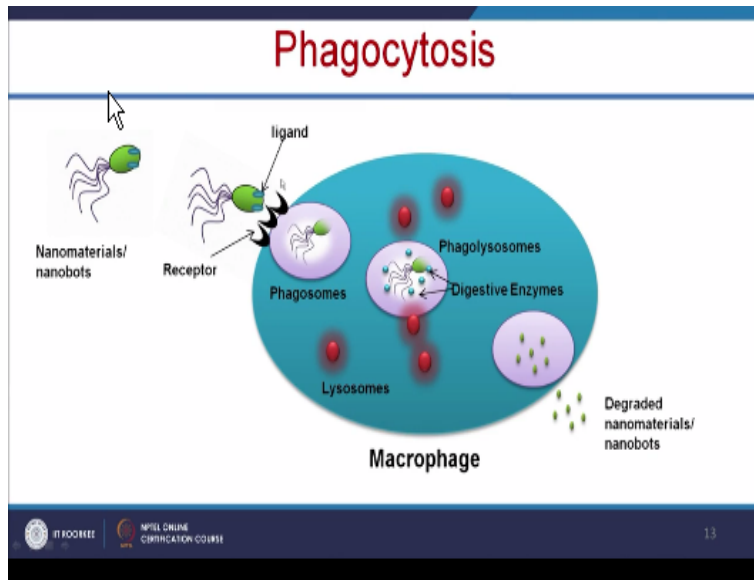
Phagocytosis

- Thereafter, the opsonized nanoparticles bind to the cell surface and interact with the receptor, inducing the cup-shaped membrane extension formation.
- The membrane extensions enclose the nanoparticles and then internalize them, forming the phagosomes which have a diameter of 0.5–10 μm . Finally, the phagosomes move to fuse with lysosomes
- But the cargo contained in the phagosomes will be destroyed by acidification and enzymolysis in the lysosomes. Therefore, to produce desired effects, nanomedicines must bypass this route to avoid degradation.

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 nanobots and it is having this option in 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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Pinocytosis ('cell drinking')

- Is a non-specific route where the membrane invaginates and engulfs the materials and then pinches off, producing vesicles of sizes between 0.5 and 5 μm .
- This mechanism, sometimes referred to as macropinocytosis for larger vesicle formation, is primarily used for the uptake of fluids and other essential generic materials required for the cell such as salts, glucose, and amino acids.

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.

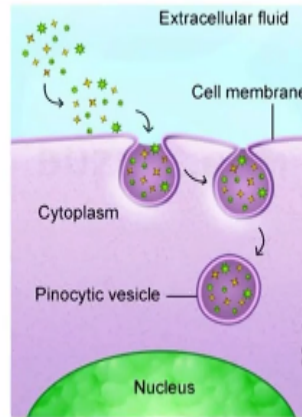
And it is primarily used for the uptake of fluids and other essential genetic material required for the such as salt, glucose, and amino acids. So this the normal process salts and amino acids okay? And if any foreign particles they can also taken up by this pinocytosis process

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Pinocytosis ('cell drinking')

It is classified to clathrin-dependent endocytosis, caveolae-dependent endocytosis, macropinocytosis and clathrin- and caveolae-independent endocytosis, based on the proteins involved in the pathways.

<http://www.buzzle.com/images/buzzle/pinocytosis.jpg>



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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Clathrin-dependent endocytosis (CME)

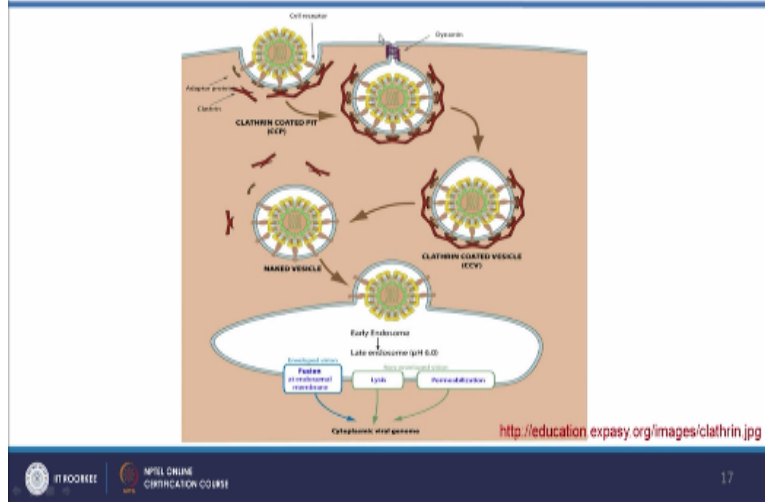
- Present in all mammalian cells, occupying an important part in cellular entry.
- After nanomaterials interact with receptors on the cytomembrane, a kind of cytosolic protein named clathrin-1 polymerizes on the cytosolic side of the plasma where the cargo is internalized .
- After wrapping the nanoparticles inside, the vesicle is pinched off through the GTPase activity of dynamin, forming a clathrin coated vesicles (CCV) . With energy supplied by actin, CCVs move towards inside the cells, and the route is regulated by the cytoskeleton . The clathrin coat is shed off in the cytosol.

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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Clathrin-dependent endocytosis (CME)



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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Clathrin-dependent endocytosis (CME)

- Where is the destination of the vesicles?: It may be associated with the receptor that NPs ligands attach to. For example, low-density lipoprotein particles are internalized through LDL receptor and transferred to lysosomes for degradation; while, iron-loaded transferrin is engulfed via transferrin receptor and recycled to the cell surface .
- This route can be blocked by its inhibitors or some other factors, such as chlorpromazine, a hypertonic medium or potassium depletion.

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 transferrin that will be engulfed via transferrin 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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Caveolae-dependent endocytosis

- Common cellular entry pathway
- Pathogens including viruses and bacteria select this way to avoid lysosomal degradation (as it can bypass lysosomes).
- Caveolin, a protein exist in most cells, plays a dominate role.
- Three isoforms of caveolin in mammalian cells exists: Caveolin-3 is muscle specific, while caveolin-1 and -2 are abundant in most non-muscle cells (such as endothelial cells, fibroblasts and adipocytes) and absent in neurons and leukocytes.

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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Caveolae-dependent endocytosis

- By binding to the receptors on the plasma membrane, **nanoparticles** or pathogens, like Simian virus 40 and cholera toxin, **can interact with the receptors to induce the formation of the flask-shaped vesicles, which are cut off from the membrane by dynamin.**
- Caveolae vesicles traffic to fuse with caveosomes or multivesicular bodies (MTV) which have a neutral pH.
- The caveosomes containing nanomedicine move along with microtubules to the ER.



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 flask 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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Caveolae-dependent endocytosis

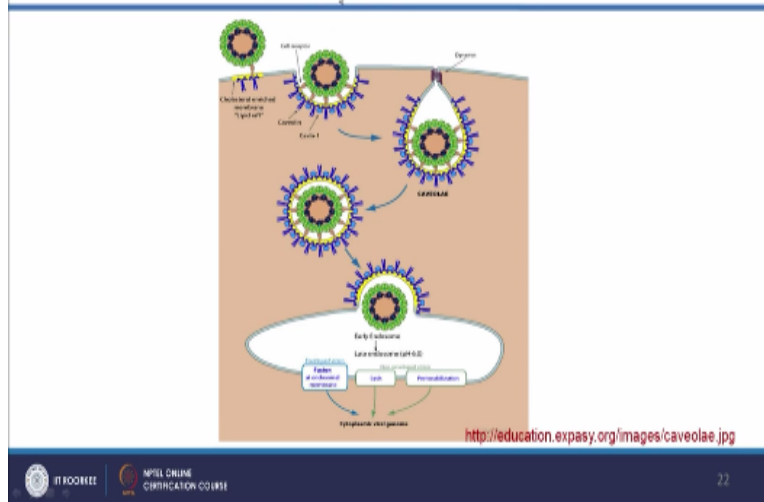
- It is thought that nanomaterials in ER penetrate into the cytosol, and then enter nucleus via the nuclear pore complex .
- Compared to clathrin-dependent endocytosis, this pathway takes longer time and has smaller vesicles in the process .
- Nanomaterials taking this way in some certain avoid a degradative fate and enhance the delivery to a target organelle (such as ER or nucleus), which is critical for improvement of therapeutic delivery.

And here this nanomaterials in the endoplasm reticulum penetrated in the cytosol okay? And then enter the 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 endocytosis 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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Caveolae-dependent endocytosis



So in this caveolae so the nanoparticle come and bind to the cell okay? And mainly this cholesterol 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 flask shaped caveolae and this dynamin will come and help in enclose in vesicles and this vesicle will go and bind 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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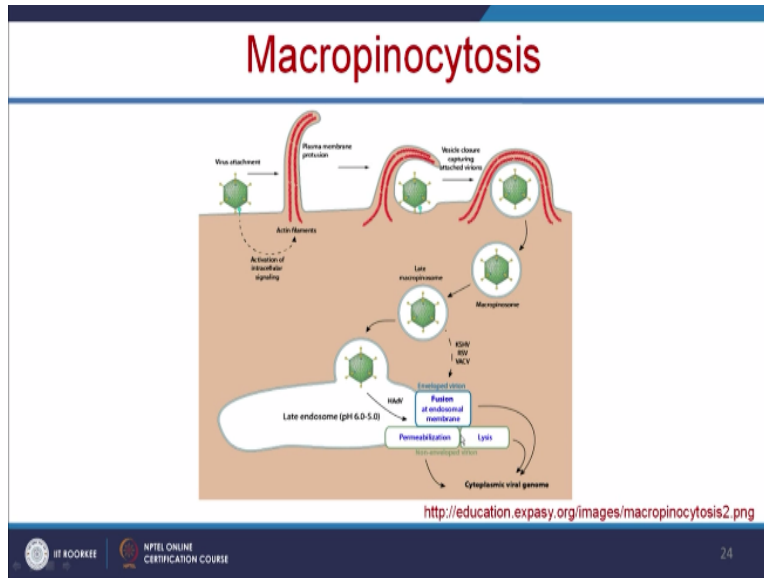
Macropinocytosis

- Commonly defined as a transient, clathrin- and caveolin-independent, growth factor-induced, actin-driven endocytosis that internalizes the surrounding fluid into large vacuoles.
- Can be found in almost all cells with few exceptions, like brain microvessel endothelial cells.
- This pathway is generally started with external stimulations which activate the receptor tyrosine kinases.



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 filaments, this actin filaments will make like this protrusion and this protrusion 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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Clathrin- and caveolae-independent endocytosis

- This is a distinct pathway, which relies on cholesterol and requires specific lipid compositions.
- According to GTPases which play a role of regulation in the cellular entry pathway, the clathrin- and caveolae-independent endocytosis is classified to Arf6-dependent, Cdc42-dependent and Rhoa-dependent.
- Dynamins also play a dominant part in these ways, while it is not deeply understood.



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 dependent, Cdc42 dependent or Rhoa dependent. And here dynamin also play important role, but the mechanisms is not completely understood.

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NPs may enter cells via different endocytosis and passive mechanisms

- Depending on the physical properties of NPs and the composition of the bilayer, NPs have the ability to utilise all four of the endocytosis mechanisms to enter the cell.
- Polystyrene latex NPs smaller than 200 nm could enter cells via the CME mechanism, while larger NPs would do so via caveolae endocytosis (Rejman et al 2004).
- 3.4 nm gold NPs entered macrophage cells via pinocytosis (Shukla et al 2005).



So let us see some of the examples, so the nanoparticles may enter these cells through different endocytosis and passive 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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NPs may enter cells via different endocytosis and passive mechanisms

- 200 nm poly (1,4-butanediol diacrylate-co-4-amino-1-butanol) NPs would gain cellular entry primarily via caveolae endocytosis, although by varying the NP surface groups they were also able to enter human breast cancer MDA-MB-231 cells via CME and pinocytosis. (Kim et al 2014)
- NPs do not necessarily undertake the endocytosis process to enter a cell. For instance, silica NPs between 15 and 200 nm have been reported to translocate through a membrane via a passive mechanism. (Bihan et al 2009)



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 uptake will be varied. So here the nanoparticles do not necessarily 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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Studying the endocytic pathways

Markers

- Used to study the intracellular fate of NPs.
- Some classical probes or markers are known to be internalized through specific endocytic pathway.
- Low density lipoprotein (LDL) and transferrin (Tf) enter cells through clathrin dependent endocytosis (CME), so they are commonly used as markers of CME.

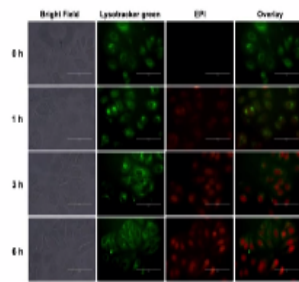


So if you want to study whether your nanoparticle is following endocytic pathways, so we can use the markers to study the intracellular fate of this nanoparticles. Some of the classical probes or markers known to be internalized through the specific endocytic pathway. For example, this LDL, this low 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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Studying the endocytic pathways

- Moreover, cholera toxin beta subunit (CTBs), Shiga Toxin and even caveolin-1 are usually used as markers of caveolae dependent endocytosis, and dextran is the marker of macropinocytosis.
- There are also dyes for organelles, such as LysoTracker and LysoSensor for lysosome. They can be used to detect the colocalization of lysosome and the labeled NPs with the confocal microscope.



The red fluorescent signals were mostly distributed in the nuclear region and didn't merge with the green fluorescent signals of lysotracker

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 stain 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 lysotracker green okay so this LysoTracker green will stain only the lysosome of the cells and this anticancer drug and which has the intrinsic red color fluorescence and when we overlay this images we can see here this anticancer drug is entering the nucleolus and your lysosomes that is a lysotracker green that is on the cytoplasm okay.

And here the all the red fluorescent signals were mostly distributed in the nuclear region and did not merge with the green fluorescence 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 therapeutic molecule.

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Studying the endocytic pathways

Inhibitors

- Inhibitors of endocytosis can be used to block the specific endocytic pathway to confirm whether it is employed by the NPs to enter cells.
- Hypertonic sucrose (0.4–0.5 M), chlorpromazine (50–100 μ M) and potassium depletion can be used to inhibit the clathrin dependent endocytosis; methyl- β -cyclodextrin (M β CD), filipin, nystatin and cholesterol oxidase can be used as the inhibitors for caveolae dependent endocytosis; amiloride, cytochalasin D and rottlerin can block macropinocytosis.

So there are several inhibitors also available which can be 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 chlorpromazine and potassium depletion so which can be used to inhibit the clathrin dependent endocytosis and this chemical can be used to inhibit the caveolae dependent endocytosis and these molecules can be used to block the macropinocytosis.

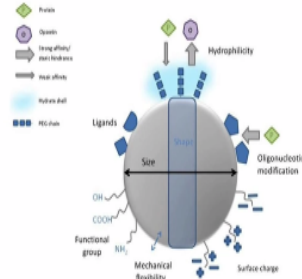
So by using these kind of chemicals we can block the particular endocytic pathway and we can add our nano particles and we can check whether our nano particles are following this particular pathway or not.

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Nanoparticle properties influencing their uptake

Internalization of NMs in cells is influenced by a large number of physical and chemical properties of the specific NP and the circumstances in the exposure medium of the cells.

- NP size
- Shape
- Surface charge,
- Surface functional groups
- NP hydrophilicity



<http://onlinelibrary.wiley.com/doi/10.1002/etc.2470/full>



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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Studying the endocytic pathways

Inhibitors

- Inhibitors of endocytosis can be used to block the specific endocytic pathway to confirm whether it is employed by the NPs to enter cells.
- Hypertonic sucrose (0.4–0.5 M), chlorpromazine (50–100 μ M) and potassium depletion can be used to inhibit the clathrin dependent endocytosis; methyl- β -cyclodextrin (M β CD), filipin, nystatin and cholesterol oxidase can be used as the inhibitors for caveolae dependent endocytosis; amiloride, cytochalasin D and rottlerin can block macropinocytosis.

So let us see how the size plays a major role in cellular uptake 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 macropinocytosis and the size of the vesicle involved in the clathrin mediated endocytosis is \sim 100nm and the size involved in caveolae mediated endocytosis is 60-80nm.

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Nanoparticle properties influencing their uptake

Shape

- Till date there is no specific conclusion on the pathway selection of nanoparticles based on shape.
- NPs with a proper aspect ratio enjoy a perceptible advantage as to internalized rate.
- Apart from the commonly used spherical NPs, rods, tubes and other shapes can be readily synthesised and commonly used.

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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Nanoparticle properties influencing their uptake

- At similar lengths, spherocylindrical (SC) NPs have shown a higher lipid bilayer translocation efficiency when compared with spherical, pyramidal, conic, cubic and rod-like NPs.
- Depending on the diameter, CNT cellular entry can occur either passively or via endocytosis, with smaller diameters reported to pass through membranes passively.

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.

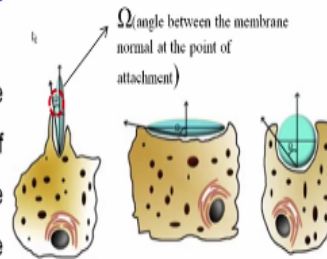
And again when you use the carbon nano tubes okay or nano rods depends on the size and depends on the diameter of the nano particles or nano rods so it can select the particular path way to enter into the cell.

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Nanoparticle properties influencing their uptake

The effect of particle shape on phagocytosis

Ω is defined as the angle between the membrane normal at the point of attachment and the line defining the particle curvature at this point. Particles are internalized successfully at $\Omega < 45^\circ$; the internalization of particles can be inhibited at $\Omega > 45^\circ$



Source: Kou et al., Asian Journal of Pharmaceutical Sciences 8 (2013)1-10

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 than 45 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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Nanoparticle properties influencing their uptake

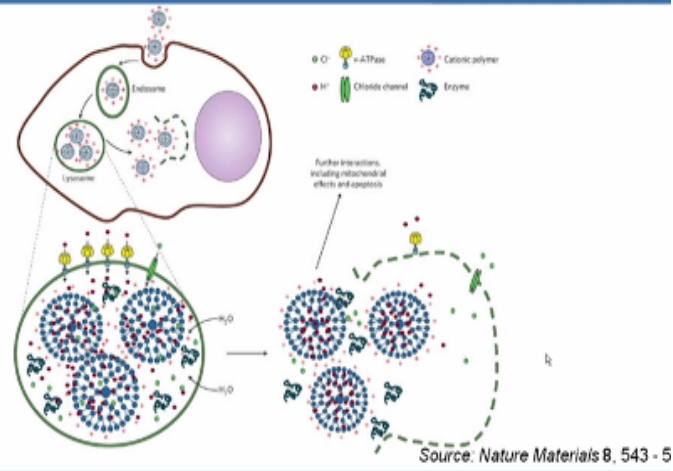
Surface Charge

- Positively charged nanoparticles can escape from endosomes after internalization and exhibit perinuclear localization because of the 'proton-sponge' effect.
- The nanoparticles without any charge at physiological pH may interact with the cells with the aid of hydrophobic and hydrogen bond interactions.

And let us see the role of surface charge, so here the positively charged nanoparticles can escape from endosome after internalization and it will exhibit a peri-nuclear localization because of the proton sponge effect and here the nanoparticle without any charge at physiological pH may interact with a cell with the aid of hydrophobic and hydrogen bond interactions.

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“Proton sponge” effect



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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Nanoparticle properties influencing their uptake

- Neutral particles coated with hydrophilic polymers can prevent interaction with the cytomembrane leading to less absorption.
- Anionic nanoparticles may be endocytosed through the interaction with the positive site of the proteins in membrane, and they can be highly captured by cells because of their repulsive interactions with the negatively charged cell surface.

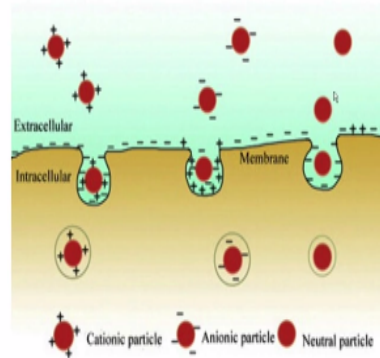
And here the neutral particles coated with hydrophilic polymers can prevent the interaction with the cytomembrane leading to less absorption and anionic particles may be endocytosis through the interacting with the positive side of the proteins in the membrane, okay and they can be highly captured by 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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Nanoparticle properties influencing their uptake

Nanoparticles which possess different charge or no internalization into cells.

1. Cationic particles strongly interact with the membrane and enter cells rapidly.
2. Anionic particles bind the positive site at the membrane and enter cells.
3. Neutral nanoparticles also can get into cells.



Source: Kou et al., Asian Journal of Pharmaceutical Sciences 8 (2013) 1-10

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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Nanoparticle properties influencing their uptake

- Cationic NPs, mainly enter cells through CME, while some others show that they utilize macropinocytosis or caveolae- and clathrin-independent endocytosis or even multiple pathways including caveolae mediated endocytosis.
- The anionic nanoparticles are more likely to use caveolae-dependent endocytosis.
- In addition, the neutral nanoparticles show no clear preference for specific routes.

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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Nanoparticle properties influencing their uptake

Surface hydrophobicity and hydrophilicity

- Hydrophobic NPs have higher affinity for the cell membrane than hydrophilic ones, leading to an improvement of cell uptake in the kinetics and the amount.
- Hydrophilic polymers used to modified nanoparticles, such as polyethylene glycol (PEG), poly (N-vinyl-2-pyrrolidone) (PVP), poly(amino acids) and dextran, form a 'cloud' to suppress the interaction between the nanoparticles and lipid bilayer of cells.

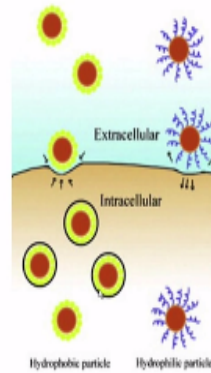


So let us see the role of surface hydrophobicity and hydrophilicity so this is hydrophobic nanoparticles have higher affinity for the cell membrane than hydrophilic ones so leading to an improvement or cell uptake in the kinetics and the amount And this hydrophilic polymers use to modified nanoparticles 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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Nanoparticle properties influencing their uptake

- Chemical composition at the NP surface determines the surface hydrophobicity which influence the route of cell uptake.
- Nanoparticles with different hydrophobicity present different affinity with the cell membrane.



Source: Kou et al., *Asian Journal of Pharmaceutical Sciences* 8 (2013) 1-10



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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Summary

- Role of protein corona
- Multiple portals of cellular entry
- Phagocytosis
- Pinocytosis
- Nanoparticle properties influencing their uptake



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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