Drug Delivery: Principles and Engineering

Prof. Rachit Agarwal
Department of BioSystems Science and Engineering
Indian Institute of Science, Bengaluru

Lecture – 24
Nano and Micro particles – VII

Hello everyone, welcome to another lecture for Drug Delivery Principles and Engineering. So, we have been talking about quite a lot about particles in the past few classes; basically we are going to continuing this discussion and most likely will finish the discussion in today's class. Particles are again one of the buzzword in the field quite a lot of them being used for various applications.

And there is quite a lot of excitement about it and it is fairly obvious. Why? Because you do not have to do any surgery and over a free drug you get a lot more sustained release of the drug; so that the patients do not have to take tablets several times a day, you can just get one injection or one shot of particle either orally or by some other mechanism. And that may be enough to treat some minor disease or in case of chronic diseases also, you may only have to take particles maybe once or twice a month or something like that.

So, just depends on the application in the particles you are using, but there is a lot of excitement and there is a lot of tunability that it gives to the researchers; as well as the clinicians. And so we are going to continue that discussion, we had talked several things about particles, we have talked about how to manufacture them and we have talked about what are the different size ranges that are used in the literature.

And why they are used; we are talked about certain classes of particles that are very widely used polymeric being the first one we talked about. We talked about liposomes, we talked about micelles, then we talked about some of the physical properties of the particles that is desirable. So, size is of course, one; so, spherical particle there is certain size range that we want. So, if you want it to sustain the sort of flow in the blood vessel or remain in our body we wanted to be greater than 6 nanometer because 6 nanometer is essentially the kidney filtration.

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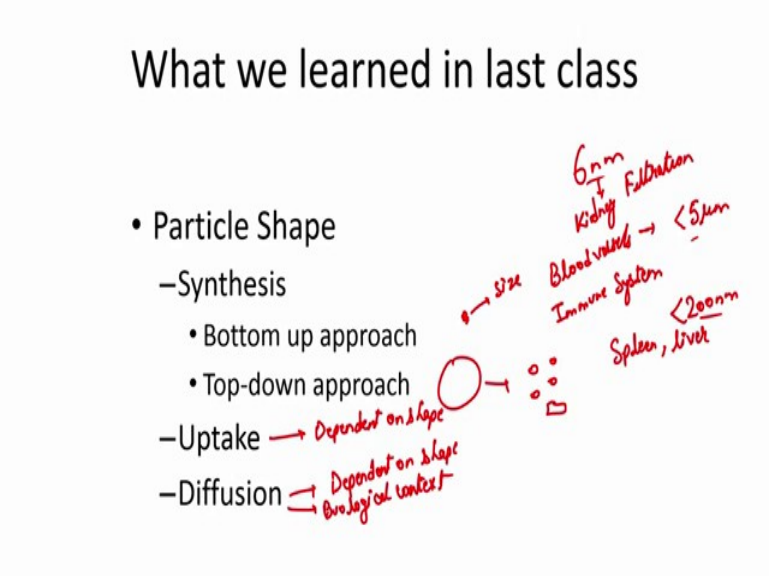

If we want it to be injectable in let us say blood vessels, then we want it to be closer to or at least less than 5 micron because the smallest vessels that we have are close to about 5-6 microns; so, we do not want them to get clogged.

And we know that our body immune system is fairly good in clearing certain things up and what we have found is if the particles are less than 200 nanometer will flow for quite a long time because typically spleen and liver or organs that will clear anything above the 200 nanometer.

So, these were some of the things that we talked about spherical particles, but now we have introduced a new concept and we are saying their particles which could be of different shape. And now when we are saying different shape we are basically saying that at least one of the dimension should follow these criteria, the other dimension could be different. And we are going to continue the discussion today as well to see what are the other properties we can change around while sort of still keeping in mind these limitations, but then circumventing them somewhat.

So, in the last class we talked about particle shape, we talked about what are the synthesis method; so they were two synthesis method that we talked about. One was bottom up approach where you make particles from single atom and sort of accumulate them to make a certain size. Or you can have a top down approach where you use some

sort of imprint lithography or you have bigger particle and then you break it down into smaller particles or different shape maybe.

So, these are some of the approaches we talked about. And then we talked about some of the uses of them we found that uptake for is dependent on the shape. We briefly discussed one research paper on this, but then several research paper out there which actually further corroborate this point. And then we further found out that even the diffusion can be dependent on the shape itself (in the biological context). So, this is what we talked about in the last class. So, now you are going to look further; we also talked about micelles. Now we are going to look further and talk about the charge of the particles.

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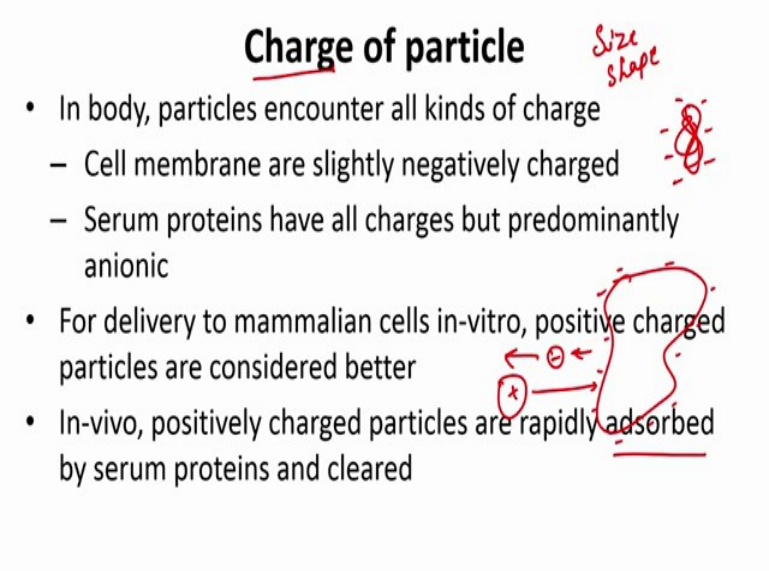

So, we have talked about size and we have talked about shape; the next property we are going to talk about is the charge. So what is essentially charge? Charge is nothing, but what is sort of the electronic structure or how much of the positive or the negative charge electrons are available on the surface of a particle.

So, as you know in body the particle will encounter all kinds of charge. So, the cell membrane that we have this is made of lipids that are slightly negatively charged. So, you can assume that all cells have slightly negative charge; the serum proteins that are flowing in our blood, they are also predominately anionic. So, most proteins that are flowing also typically carry negative charge; although this is not true with all proteins

there are proteins which are also positively charged, but predominantly we will find that most serum proteins are negatively charged.

So, now that we know that this membrane is negatively charged if I want to deliver something to the cell, I would probably want to have something that has a positive charge right. So, there is an electrostatic interaction; it gets attracted towards the cell. What will happen if I have a negative charge? Even though the particle may want to come close to the cell, there will be an electrostatic repulsion that will cause the particle to move away because of this negative charge and negative charge repulsion.

So, for positively charged particles the uptake of the mammalian cells is much higher than the negatively charged particle. But then what happens in vivo is let us say if I inject it into a human or let us say an animal. Because I said that the serum contains lots and lots of proteins which are negatively charged; these proteins tend to interact with the particle quite a lot and they will tend to adsorb onto these serum proteins.

So, we are going to talk about adsorption in much more detail in next class, but this is a phenomena that will start to occur. And some of these serum proteins are used by immune system to sort of recognize; if there is for an object and that will cause the clearance of your particles much more rapidly than say a negatively charged or neutral charged particle.

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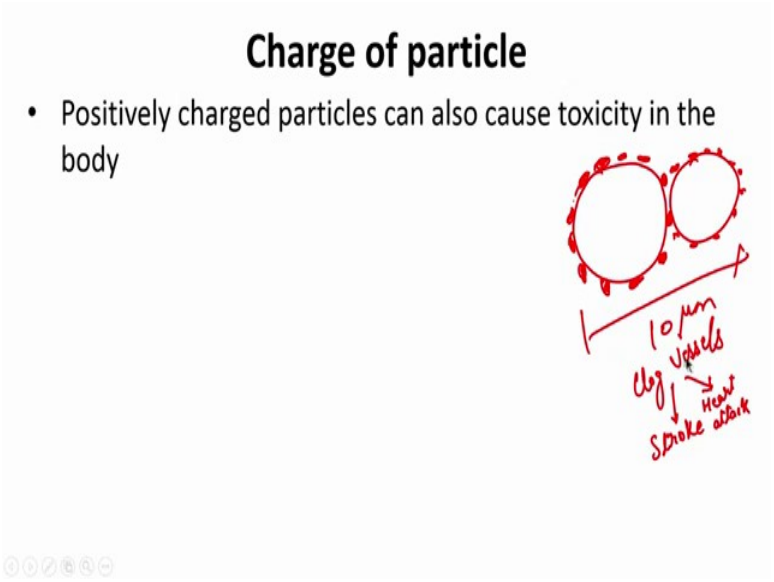

So, positively charged particles tend to adsorb so much and that is why also cause toxicity. So, if I have a positively charged particle there will be lots and lots of protein that will get adsorbed on the surface. And the structure of the protein will change, the function of the protein will change, the way these guys can coagulate with another particle and so on and so forth. So, their actual size once it goes in the blood may change and it may cause toxicity; maybe it will become larger than 5 micron maybe this will become 10 micron and that will clog blood vessels.

What will happen if it clog vessels? If the vessel is going to the brain and if you clog it; the brain will not get enough oxygen and it will result in a stroke or it will cause a heart attack. What if the vessel was going directly to the heart? The heart will not get enough oxygen it will start pumping and that will result in heart attack. So, these are some of the considerations that we have to keep in mind while you are talking about charge.

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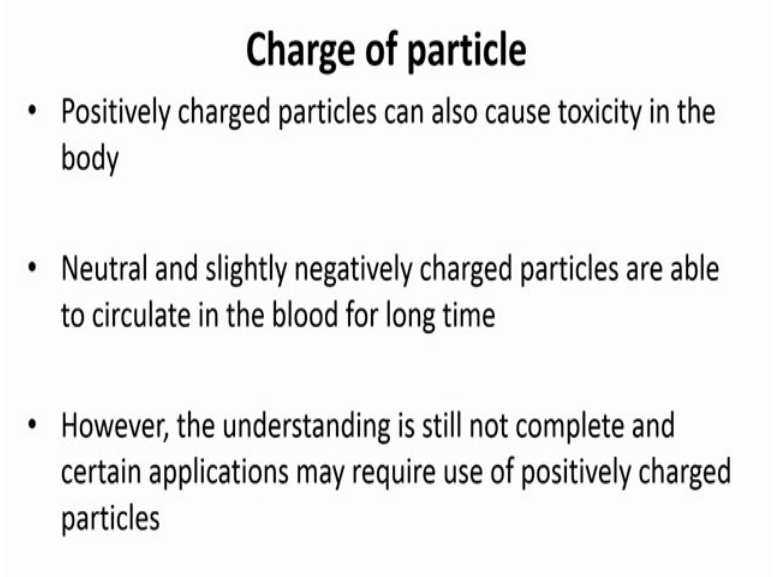

So, neutral and slightly negative charged particles are typically preferred when you are talking about in vivo delivery. Because the positively charged particles can cause toxicity plus it does not really stay for quite a bit of time in the blood. And so even though for let us say if you want to just give some drug to the cell which is outside the body environment; you probably want to prefer a possible charge particle, but for a new applications you may want to look at slightly negative or neutral charged particles.

But then again the understanding is still evolving every day; there is new and new research coming out sort of challenging all these concepts and proposing new concepts. So, it is still fairly dynamic field, but the general consensus is for a longer circulation you want neutral to slightly negatively charged particle to flow in the body.

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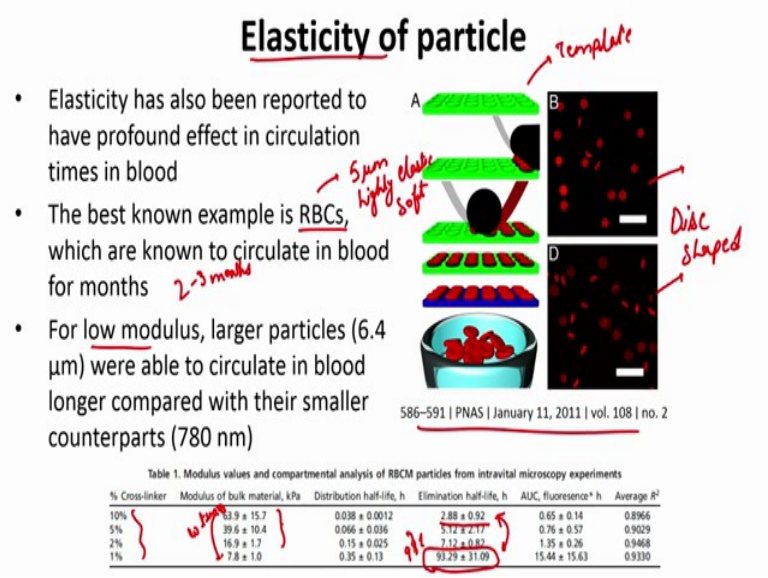


So, the fourth property we are going to talk about is the elasticity of the particle and this is again a fairly nascent field; not much has been done in this area, but more and more people are now starting to look at the mechanical properties of the particles that they are using.

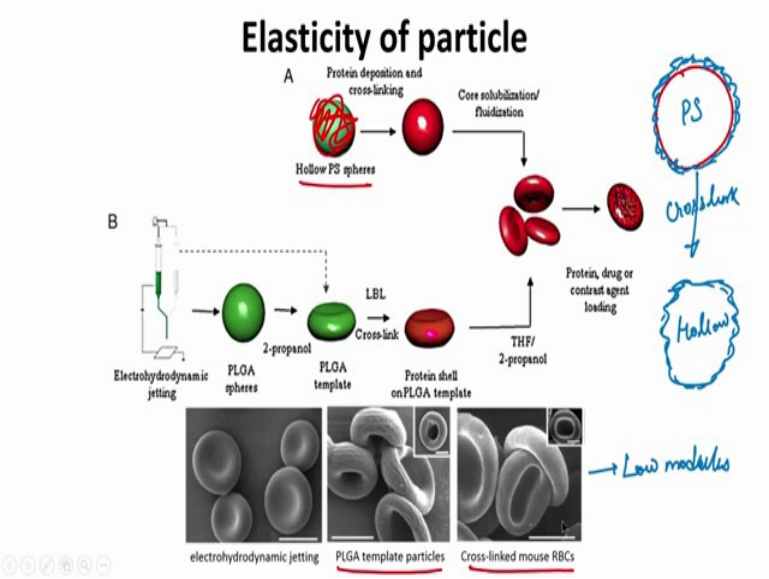
So, again elasticity has been reported to have profound effect on how much the particle circulates in blood; actually the best known example of this is a natural particle which is RBC right. So, RBC we know is about 5 micron and they are highly elastic and very soft. And they have been known to circulate in the blood for about 2 to 3 months.

So, this is by far as much circulation time as you will ever get with any synthetic particle. And what they have is they have a very low modulus and so what people have now done is started making particles which like RBCs have a very low elastic modulus and I have a large size and then studied how their effect is when they compared it with the RBC.

So, compared to a synthetic particle versus a natural RBC cell that is circulating. And so here is an example, here these guys have used again top down approaches; so in this paper they have reported these top-down approaches, so in this case this is the template.

And what they have done is they have a pre polymer mixture and they roll this pre polymer mixture to fill these templates and then cause the polymerization to happen. And then they can dissolve this template itself to sort of get your individual particles and as you can see the particles are fairly disc shape. And here they have reported sort of the bulk properties of this. So, depending on the amount of cross linker that they have added; so they can vary the cross linker from 10 to 1 percent their bulk material modulus will also change.

So, here they have been able to change it by almost an order of magnitudes; so 10 times. And with that they also see that the half life has changed; so if you look at the half life, you are talking about a very elastic particle having a half life of only about 3 hours whereas, something that the very low modulus has a half life of close to about 95 hours or 93 hours. So, you can see what a jump just the modulus had on the circulation time. (Refer Slide Time: 12:24)



And so this has been reported and one of the reasons for this will come in a few slides, but then there are other methods as well.

So, here is another method to make these low modulus particle; in this case again they have used a hollow polystyrene spheres; what they have done is they have created

proteins to adsorb onto these. So, because the proteins will adsorb on any exposed surface; what they have is they have this proteins which are essentially coated.

So, let us see if I make this particle and then this particle gets coated with several proteins and then what do you do? You cross link this protein. So, now whatever these proteins were present on the surface well get cross linked and will form bonds between the neighboring proteins and hence becomes very stable; then you come with a solvent that is going to dissolve this polystyrene.

So, then what you end up with is nothing, but a very soft hollow protein structure which is cross linked on the surface and is hollow. So, essentially extremely low elastic modulus and that you can then use to sort of get these RBC shape depending on the size. So, because it is hollow it just collapses and you get these RBC doughnut shape particles which are very low modulus.

So, here is just an example where they are showing their actual particle and here are cross linked mouse RBCs they look very similar. So, what the authors are reporting here is they have been able to sort of mimic the RBCs using this particular method. So, this is another alternative to what we discussed in the previous slide.

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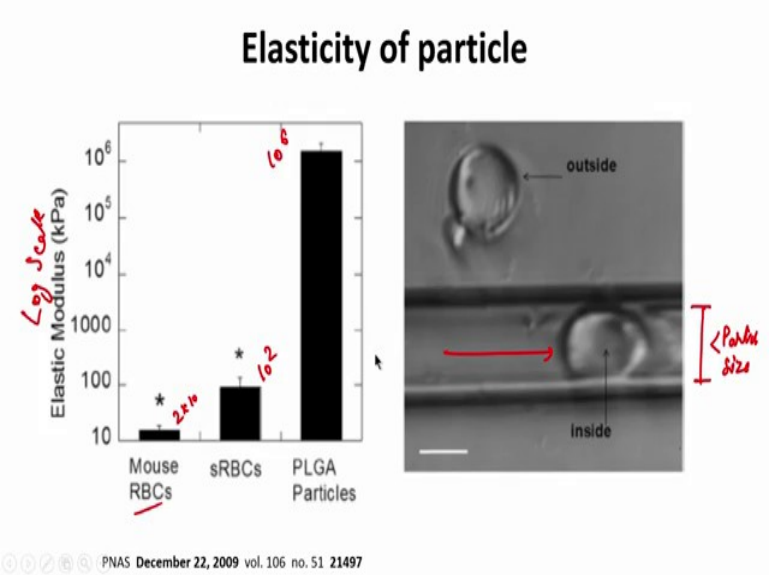

And then what this show is the elastic modulus of the original PLGA particle is fairly high; so this is on the log scale. So, you can see you are talking about in the order of 10 to the power 6.

But once they have done their method and cross linked protein and dissolve this PLGA particle they get down to about 10 to the power 2. This is just similar to about what is reported here as to 2 into 10; number 20. So, still they have not been able to get down to the mouse RBCs, but they still are able to reduce the elastic modulus quite a lot.

And here what they are showing is its fairly elastic, it can deform, it can regain the shape. So, now they have flown in through microfluidic channels which are actually smaller than the particle size. And what you can see is this particle and can actually squeeze through these microfluidic channels; just like the blood vessels will cause the RBC to squeeze through them.

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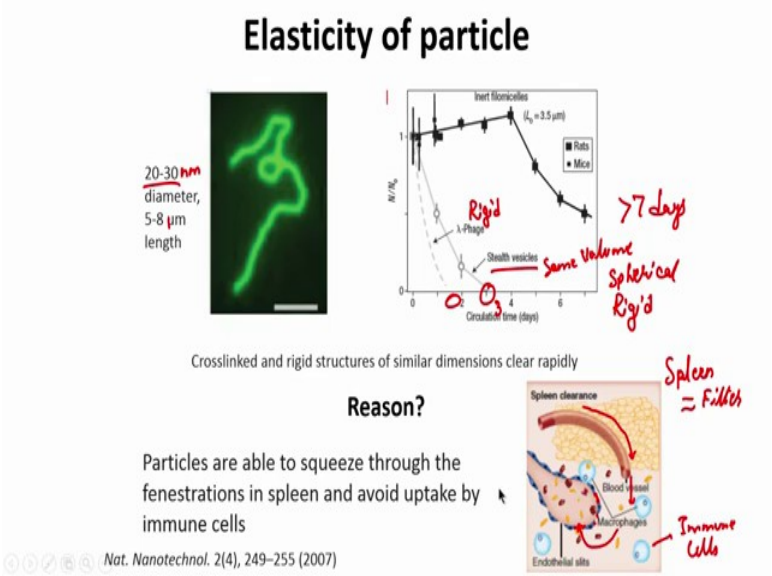

So, we can mimic these properties; another example of making elastic particles is filo micelles. And these are nothing, but these are polymeric particles which are 20 to 30 nanometer in diameter and they are about 5 to 8 micron in length.

So, here is just a fluorescent image of this. So, it is nothing, but this is just like a thread or a one like particle and what they have shown is; if you can cross link the different regions and make it rigid or you can leave it like this. And if you see their circulation

time, so if you use a lambda phage which is very similar in structure, but is a fairly rigid molecule.

So, this is a rigid that gets cleared out by 2 days; so by one and a half day this get completely cleared. If you use stealth vesicles which are of the same volume, but they are spherical and are rigid; you see even then you can maximum get it to 3 days. But when they use this filo micelles; they have been able to circulate for longer than 7 days, this is greater than 7 days.

Again just an example of showing how elasticity can cause this effect; so if they now if they use the same filomicelles and cross link it internally and this drastically drops down to something like a lambda phage where it gets cleared within a day or 2. So, what is the reason for all this? I mean, so we have talked about by elasticity is being or we talked about how elasticity is able to change the circulation time and able to flow quite a lot in our body, but what is the major reason that this is happening?

And the major reason is the spleen. So, spleen is essentially nothing, but a filter for out body and what happens in a spleen is typically the blood which is flowing through the spleen will come out into the tissue from the blood vessels and then will go back into the circulation. So, if this was let us say a spleen vessel in the blood will empty itself into the interstitium of the spleen where there are lots of immune cells residing around.

And then the blood vessel then there are other blood vessels which are fairly leaky and from these leaks all the blood will go in and squeeze through. So, if you have a large rigid particle it will not be able to squeeze through these gaps. And will just get entrapped in this region, where all these immune cells will clear it away. Whereas, if you have a soft and a large particle even though it might be larger than these gaps, it will still be able to squeeze through them and hence will have a longer circulation. So, this is just one of the mechanism through which we find that elasticity the particle plays a very important role in that circulation type.

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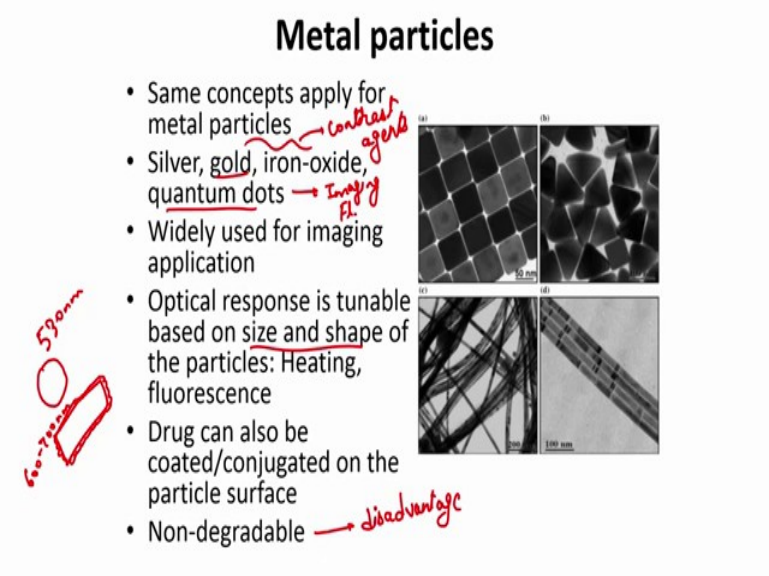

So, far we have only talked about polymer and lipid particles. Another class of particles we are going to talk about is metal particles; typically as you might have seen through this course we have not really talked much about hard implants or hard particles like metal.

The reason for that is of course, even though they have been fairly successful and we are going to talk some more about them in the future classes. The problem is again you have to do a resurgery and typically; unlike polymers they do not really allow you to degrade the matrix and release the drug over constant time typically the drug is just coated on the surface or they are for structural support.

So, they are not as widely used in the literature at least in the research scale. But then on the metal particles there is still quite a lot of applications and because these are small particles, they have been used for contrast agents, they have been just the surface coating of a drug will also cause enough volume of the drug or enough concentration the drug to be developed and so we will talk about metal particles.

So, in this case you see some of the images and again metal particles; since they form by crystallization, it is very easy to get different shapes of particles in very large quantities using bottom up approaches and so essentially the same concepts apply for metal particles. So, you can have some of the ones that have widely used a silver and gold;

gold again is one of the most widely used - you also have iron oxide, both of these are being used as contrast agents.

You have quantum dots which is used quite a lot with imaging, mostly fluorescence imaging. The quantum dot also has some limitations because some of the metals that are used could be toxic, but then the field is evolved enough that they have been able to sort of make sure that; these are non-toxic at least for the time being that they are required for.

So, one of the advantages with the all these particles is their optical response is fairly tunable. So, you can get different types of optical response based on the size in the shape. So, for gold particles for example, if you have a rod shape particle versus a spherical particle; you will find that the rod shaped particle has absorbance in close to about 600 to 700 nanometer whereas the spherical shape is about 530.

So, you can essentially tune that and depending on the length of the rod, you can further start to tune this. And then as I said, since the surface area now is quite large compared to the volume; for at least for the particle scenarios when you get to nano regimes, you can still load enough drug for drug delivery as well; so, those are some of the advantages with the metal particles.

One of the disadvantage of course, is non degradable; so this is a disadvantage in most cases. If I continuously get an injection of these metal particles, they cannot be cleared out from our body because they are big they can degrade. So, what happens? They will just accumulate in my body in over time they might reach toxic levels.

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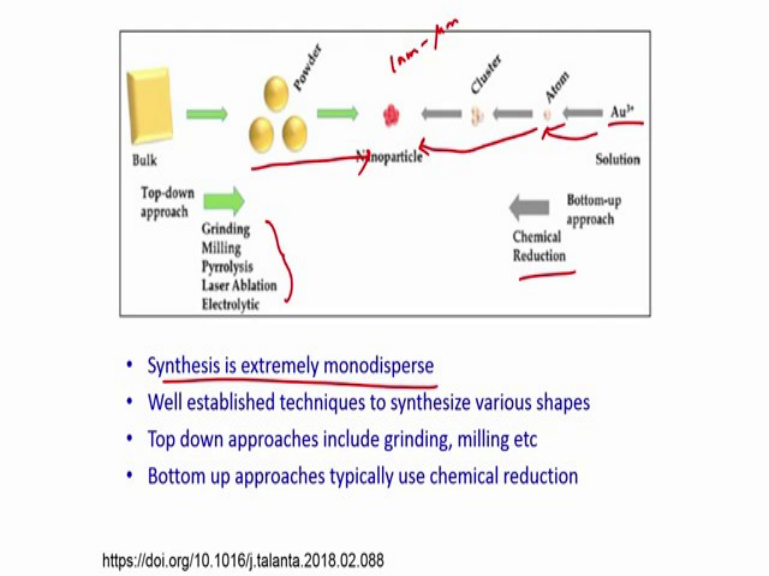

And in terms of the synthesis first of all the synthesis results in extremely mono dispersed particles. I mean we are talking about maybe variation of let us say 1 nanometer in each dimension at max.

So, in that ways they are extremely monodisperse and good for research application. And again like the polymeric particles we talked about when you are talking about synthesizing them in different shapes and different sizes; they are well established methods. You can start from salts, you can reduce them down either by a chemical reduction or some other method to an individual atom which will then start clustering. And you can grow these clusters up to the size range that you want and people have shown this for all kinds of sizes, all the way down from 1 nanometer to micron levels.

So, that is not a problem; you can take a bulk metal, you can do some physical methods or top down approaches, you can use laser ablation, you can grind it, you can mill it, you can reduce it further down to whatever levels you want. And so both these methods are well accepted and well used in the field. So, top down approaches, as I said, will include grinding and milling; in bottom up approaches typically requires chemical reduction.

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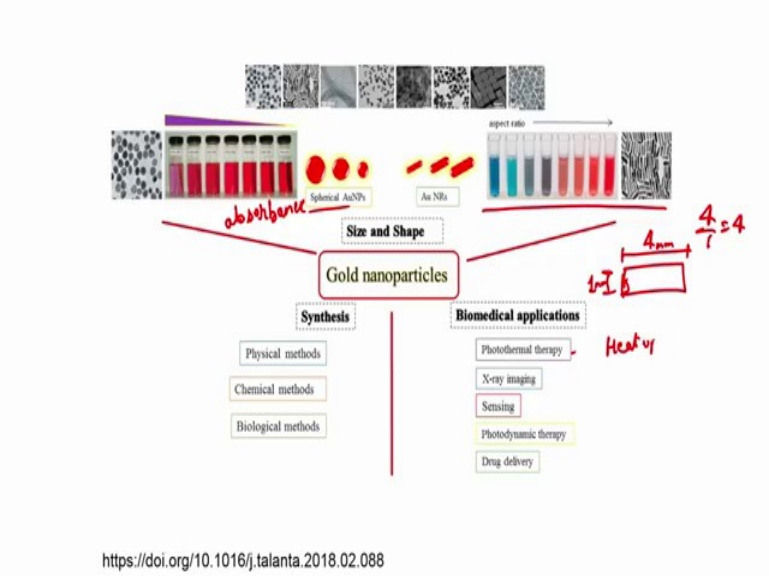

And then their uses, especially gold is very widely used for contrast agents and for fluorescence. So, the spherical nano particles depending on the size range they will have different colors and different absorbance. And then similarly nano-rods will also have different aspect ratios, which is the ratio of length to width. So, if this is 1 nanometer and let us say this is 4 nanometer and then aspect ratio is nothing, but 4 by 1 which is 4. So, depending on the aspect ratio you will get different fluorescence and absorbance for the nanorods also. So, they have been used for various applications, they have been used for photo thermal therapy; so these things will absorb light and will actually heat up.

So, this will heat up and the local temperature around these particles will increase to very high levels - up to let us say 60 degree Celsius, 70 degree Celsius and these can be then used to kill whatever cells are in the surrounding. So, you can imagine a scenario where these metal particles are accumulating; let us say in a tumor tissue. And then you externally give them some light; let us say it is a skin cancer and it is just topically applied particles, then you can just give them some light which will cause the disruption of the cancer cells; the death of the cancer cells because of the local heating of these particles and so that is one way.

And they have been used for X-ray imaging because of course, they are non-transparent to X-rays. So, wherever they are accumulating they will give a lot more contrast at that region and they have been used in sensors quite a lot; photodynamic therapy as well as

drug delivery. So, you can conjugate drug on the surfaces and release that out over time and to get enough concentration of the drug.

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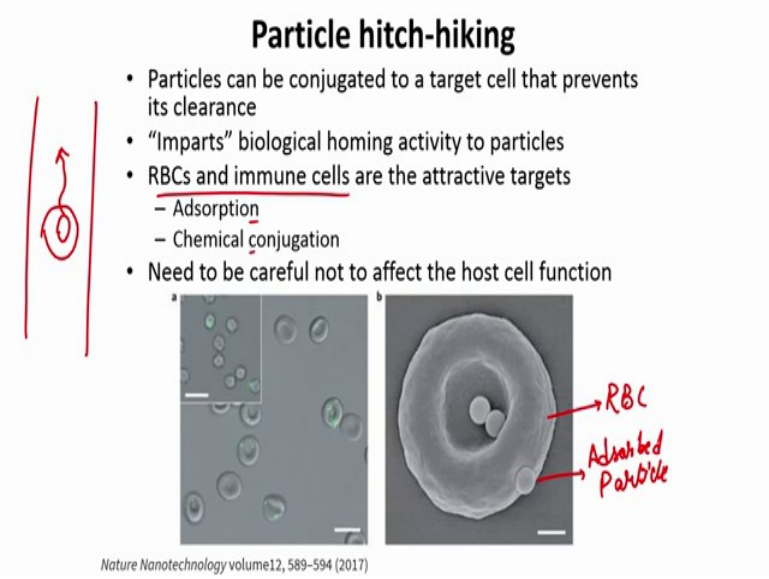

Another concept if we are going to talk about this particle hitchhiking. So, this is nothing to do with the particle property, but this is something that people are using to sort of make sure the particles are circulating for longer.

So, what is done here? That you can actually if the particles are just flowing alone in the blood vessel. So, let us say if this is a blood vessel and this is your particle just sort of flowing alone; what can happen is any immune cell can come and sort of engulf this. But what happens if I conjugate the particle to let us say something which the body considers to be "self" - Let us say the body's own cell, let us say the RBCs. So, what will happen is now the immune cells are not going to attack the RBCs because they think that this is one of their own and your particles can then circulate and eventually it is going to degrade to release whatever drug they are carrying.

So, RBCs and immune cells themselves a very attractive target, various methods are used such as adsorption or chemical conjugation to these. Although we need to be careful not to affect the function of the host cell itself. So, here is an example; here you see that they had these green particles which they have then adsorbed onto the RBC. And then these have been shown because of this adsorption; they can circulate in the body for lot longer than let us say the individual particles them self.

So, what you can do is you can isolate some blood, incubate your particles let them adsorb onto the RBCs. And then you can just infuse it back into the patient and these particles will then continue to circulate as long as that RBC circulating or they degrade. So, we will stop here; in the next class we will talk about protein adsorption.

Thank you.