Drug Delivery Principles and Engineering
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Lecture – 23
Nano and Micro particles – VI

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. So, we have been talking quite a bit about particles in the last few lectures and we are going to continue that discussion. So, just a quick recap of what we learnt in the last class.

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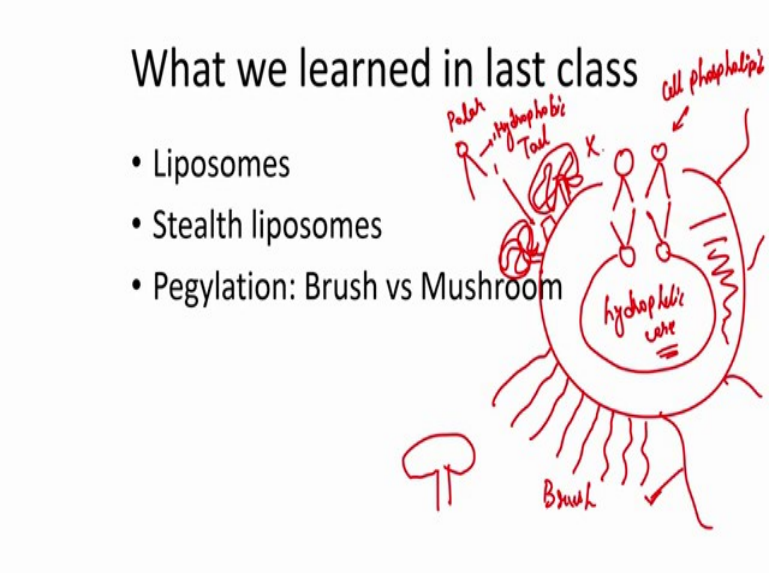

So, in the last class we talked about liposomes which we said are nothing but made up of phospholipids with a polar head group and a hydrophobic tail. And then there are several of them that gets aligned in a format like this essentially making a hydrophilic core as well as a hydrophobic domain right.

So, any of the drugs that are hydrophobic can get encapsulated in this region whereas, any drug which is hydrophilic can get this encapsulated in this region. And the lipids are fairly compatible- they are actually derived from the cell phospholipids. And these could be several different types -could be DPPC, DSPE there are several types of lipids that are commercially available which people can use to make these liposomes. And you can get

them to be charged, you can get them to be neutral they can be positively charged negatively charged, so all different variations are out there.

So, all of this gives a lot more tunability to liposomes than some other particles that we have discussed and that is why they are very widely used. Next we talked about stealth liposomes, so similar to the polymer-drug conjugate that we have talked about a few classes ago, a stealth liposomes can also be made by PEGylating the external surface.

So, you can have lipids that are conjugated to PEG or some other hydrophilic molecule or you can have some conjugation chemistry taking place between the PEG molecule and the lipid itself to get this PEGylation. And what that does? This again it will act as a molecular windshield wiper, so it is going to continue to move in all directions and make sure that if any foreign protein or cell is trying to attack this particular liposome it gets repelled away.

So, and that is the advantage and we saw that similar to the polymer drug conjugates when you conjugate PEG to these liposome particles and they have a very high residence time. So, and they can increase the residence time up to 10 times or higher and then we talked that PEGylation can also be in two different conformations.

If it is fairly well covered and the surrounding density is very high between different PEG molecules. Then these PEG brushes will tend to be close together and they cannot really collapse because the other brush is not going to let it collapse, so they will essentially be in this brush conformation.

So, it is like a brush with all the bristles are sort of standing and making sure that there is really no space for the molecule to go into the liposome or what can happen is you can have them slightly far apart. So, you can have a big PEG molecule, but then the density of the PEG is so low that it tends to collapse and sort of forms our structure like mushrooms. So, if you see mushrooms - mushrooms are essentially you will find in the literature like this in the nature. So, this is essentially that structure here that these mushroom like structures have form of these PEG brushes and their surface coverage is not as high.

So, what can happen is potentially a protein can come in and is able to attack the surface of the liposome. So, these conformations are not desirable if you are talking about

repelling of any sort of foreign object from coming in contact with this liposome, so they do not work as well as let us say a brush conformation. So, for most applications you would want a brush confirmation to be present other than a mushroom conformation.

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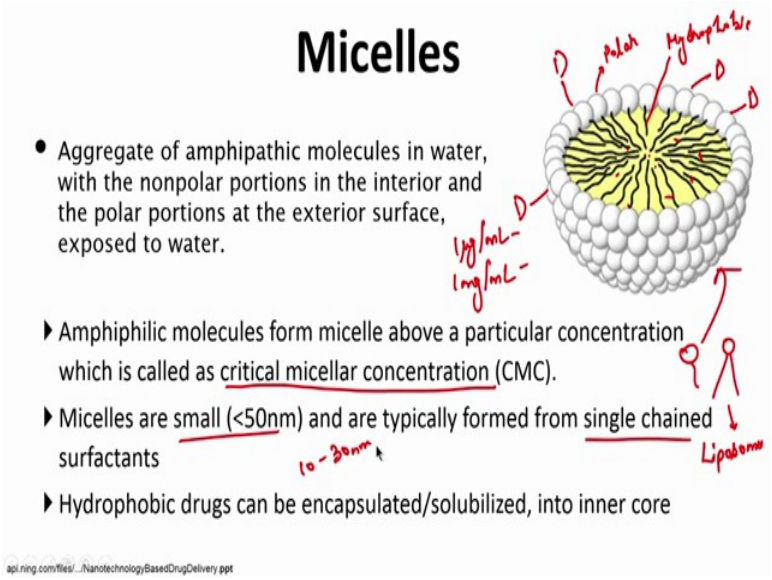

So, we are going to talk further about another class of particles which is called micelle. Like liposome they are also very similar they also have hydrophobic and hydrophilic domains. So, you have a polar head group and then you also have a tail which is hydrophobic and unlike liposomes these do not have bilayer that you saw earlier this is just a mono layer. And so there is no two domains there is no hydrophobic and hydrophilic domain inside the particle, there is only a hydrophobic domain.

So, only the hydrophobic drugs can get encapsulated in this region whereas, the hydrophilic drug will have to be used by some other method. You have to use some other particle if you want to deliver a hydrophilic drug, so this micelles are mostly for hydrophobic drug.

So, what are Micelles? Micelles are nothing but these are aggregate of amphipathic molecules in water, with nonpolar portion, which is inside, which is of course, hydrophobic and the exterior is exposed to polar head groups which are hydrophilic. So, so these are essentially sort of detergent like molecules which have both hydrophilic and hydrophobic domains and although they are not as hydrophobic as the liposome lipids are.

So, if the lipids are extremely hydrophobic and have a very long hydrophobic domain and then they tend to form liposomes and if then they are not as hydrophobic they have some solubility in water and then they tend to form micelles. So, these amphiphilic molecules will form micelle above a particular concentration, which is called a Critical Micellar Concentration (CMC).

So, for any sort of mp filling molecule there is a CMC that is defined this could be let us say 1 mg per ml, this could be 1 microgram per ml or this could be something else. But for any amphiphilic molecule above these particular concentrations they will tend to form micelles below that they will be in the solubility limit.

So, they will still be soluble, but as you increase the concentration of these molecules in the water or the aqueous environment they will tend to then form these micellar structure. And as you can see from the structure itself these are essentially talking about single molecules and maybe 5- 6 of them at a length. So, micelles tend to be fairly small typically less than 50 nanometer and they form from single chained surfactants.

So, as I said most of these surfactants are single chained if they are double chained surfactants then the hydrophobicity is fairly high and they tend to form liposomes, but the single chain like we have seen in this figure then they tend to form micelles. So, that is somewhat a distinguishing feature between a micelle in a liposome and of course, we talked about at liposome can carry both hydrophilic and hydrophobic drugs. Because it has two different domains inside the particle whereas, in micelle you only have one single domain which can only carry hydrophobic drugs.

You can potentially still conjugate the drug on the surface and so that way it can allow you to carry hydrophilic drugs as well. But predominantly if you are looking for an encapsulation and all those kinds of things, then you have to look at a hydrophobic drugs with micelles. So, as I said the hydrophobic drugs can be encapsulated or solubilized in the inner core.

So, another major difference between micelle and liposome is the size itself. So, as we discussed the liposomes you can make them anywhere from 100 nanometer to all the way up to a few microns. Whereas, in micelles because they are found by self assembly and this very small molecules that we are looking at typically, the size of the micelles are

small and they are below 50 nanometer. So, around 10 to typically you will find them in the data to be able 10 to 30 nanometer.

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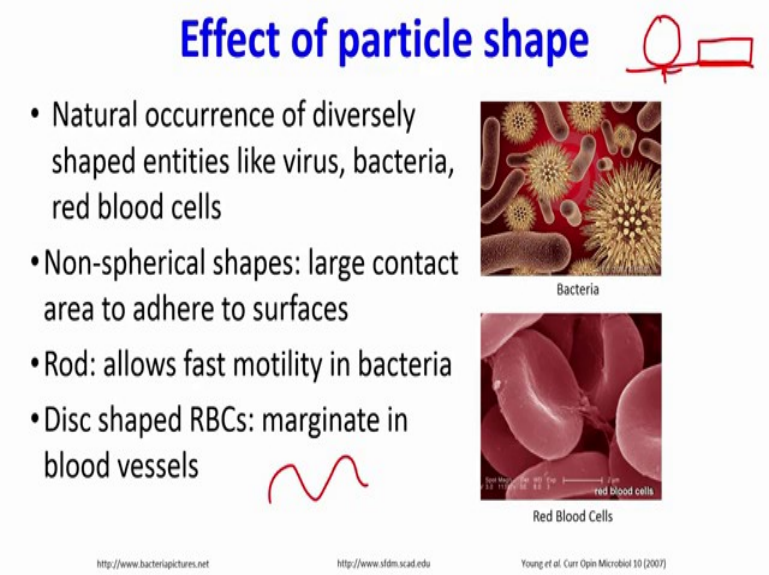

So, now, that we have studied these effect of different types of particles and their property how to sort of make them, let us talk about particles in general as to what is the different features of a particle that we can think of? So, one thing that immediately comes to our mind was size and we discussed about size as we went through all different things we talked about emulsion based methods in which we can vary the size depending on what amount of power and what amount of concentrations we are using.

Then the other thing that comes to mind is the particle shape. So, far most of our discussion we have talked about spherical particles, but if you look at the nature you will find that not everything is spherical. So, you have different spiky kind of viruses, you have bacteria which are rod shaped, you have bacteria which is spherical you have our RBCs which are essentially disc shaped particles.

And they have actually evolved over millions of years of evolution to maintain the shape and the shape also confers some advantages. So, then the idea is that can be a sort of mimic this property of the shape from nature and see if that can help us in various applications of drug delivery or tissue engineering in terms of getting more efficacy and patient comfort.

So, this is basically the motivation to vary the particle shape and of course, we see in the nature also, so why RBCs are disc shape? So, typically when a spherical particle flows through a vessel what you will find is in a streamline motion it will continue to go straight. So, if a particle is going straight it will continue to go straight, it will not really go towards the edge of the; edge of the vessel, but then what is the main job of RBC? The main job of RBC is to deliver oxygen.

So, if it wants to deliver oxygen it wants to be closer to the surface, so what has been found is if the particle is non spherical. And let us say the particle, instead of being a sphere, is a rod shaped particle - then it tends to tumble around as it flows. And because of this tumbling it is now exploring the blood vessel in quite high quantities and that way it can then deliver oxygen with much better efficiency then let us say a spherical RBC.

So, that is why you see the different sort of natural particles or of different shapes depending on what the role is in the nature and that is over a million of years of evolution that they have acquired that particular shape. So, again as I said for bacteria non spherical shapes also for a large contact area with the surface, so if I say that I have a surface on to which if a seed a spherical particle, the contact area is essentially just a little point right here whereas, if I am seeding a rod shaped particle you have a much much higher surface contact. And so, it will help this bacteria to adhere to a surface also better also the rod shape also gives faster mobility to the bacteria and we talked about disc shape particles being able to marginate in the blood vessels quite a lot.

So, there are obviously, some advantages of shape and then because of this we also want to explore what are some of these concepts that we can then sort of pick and use it and drug delivery vehicles. So, that we can do a better efficiency of drug delivery as well as much more controlled delivery.

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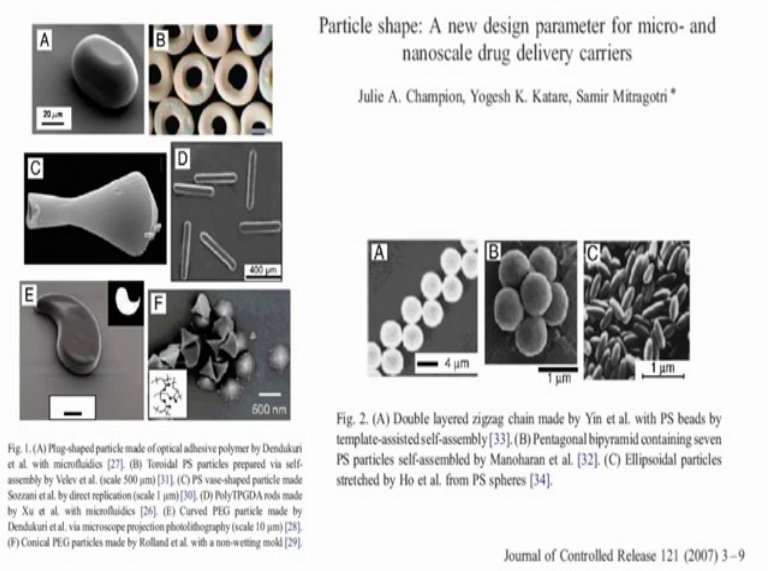

So, let us see, so how do we make different spherical shaped particles? So, there are several reports in the literature actually- shape has been more and more sort of considered as a paradigm that can be varied and can be used for different applications. So, just some examples of different shape of particles both nano- and micro- that have been formed.

So, you can you name it and there a shape for it there are spherical particle there are spherical particles aligned in a certain pattern, there are these rod shaped particles or ellipsoidal particles, you have these cone shaped particles. So, there are all kinds of particles that have been synthesized by researchers across the world and it has been strange and actually shown in certain applications that these shapes can actually be an important role in sort of increasing the efficacy of delivery.

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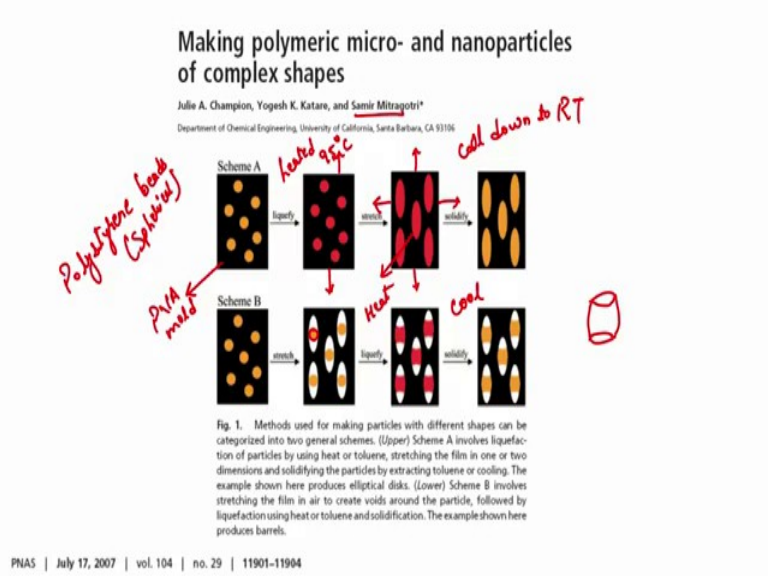

So, how do you make them spherical? So, that the thing is if you are going to use any sort of chemical methods what happens is due to the surface tension of the water and the minimization of the contact area between different oil and different fluid it always tends to be spherical. So, somehow we have got to mitigate that.

So there are two approaches which you can use to make spherical particles or non spherical particles; one is you can use a bottom up approach which basically means you do some chemical reaction and you grow a particle right from this individual atoms into a bigger structure up to what you want. Or you can do a top down approach is you have a big particle and then you break that down into smaller particles or somewhat synthesize the particle immediately in a size that you want, so let us look at it.

So, here is a group from Samir Mitragotri which are using polymeric micro nanoparticles of complex shape. And so the way they have done this is, in this particular paper they are using a polystyrene polymer and what they have done is they have made a mold of PVA. So, this is a PVA mold another polymer which essentially they have created a film and embedded these polystyrene beads which are spherical. So, you can synthesize them or you can buy them directly from commercial sources.

So, what they have done is they have entrapped these polystyrene beads in the PVA mold and then what they have done is they have heated it above the melting temperature of the polystyrene. So, polystyrene if you heat it up to let us say 95 degree Celsius it will

start to melt. So, now, what do you have is you have a PVA mold and in that PVA mold there are cavities which contain polystyrene in liquid form, then what you do is you apply a force on polystyrene mold on let us say a single dimension.

So, once you do that what is going to happen is because now this force is there you will have expansion of these pores in one direction. And hence created anisotropy in the system, because this is liquid it is going to fill up these pores and then you can cool it down to room temperature.

And what will happen you will now have a polymerized polystyrene which is of a ellipsoidal shape right. So, that is one of the methods that they have used to make these different shape particles, you can have a different scheme to you can first stretch it in that case you still have a solid polystyrene so it is not going to flow and then you heat it, so what will happen because you have heated it later and you only heat it for a certain amount of time you only get certain filling of these pores. And then you cool it back again and you now you get a different shape, now you are getting a shape which is more looking like more looking like a tabla for example.

So, just by imagining how you do this and how you are stretching this and of course, this is just one dimension I am shown stretching you can stretch it in the other dimension as well the x and y as well as the z direction as well. So, all of these can be done and you can stretch it in all three directions in different ratios and all of this will result in different configurations of the particles that will end up being made through this PVA mold.

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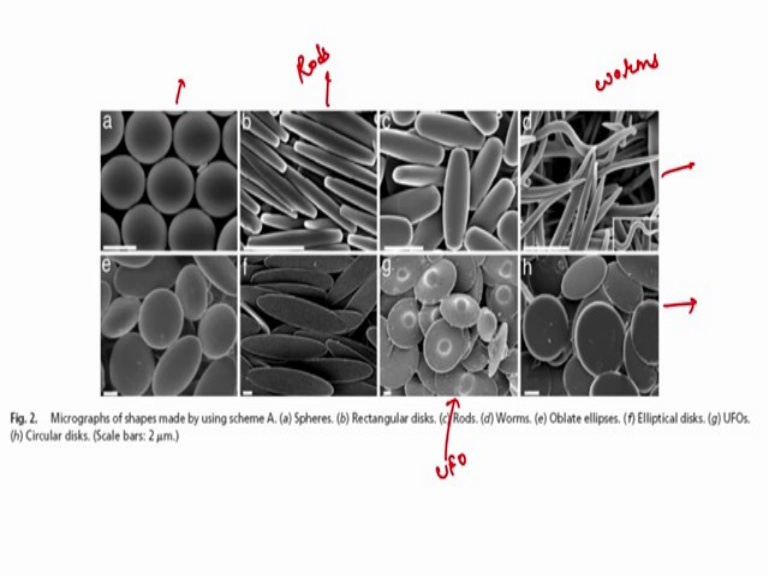

Fig. 2. Micrographs of shapes made by using scheme A. (a) Spheres. (b) Rectangular disks. (c) Rods. (d) Worms. (e) Oblate ellipses. (f) Elliptical disks. (g) UFOs. (h) Circular disks. (Scale bars: 2 μm.)

So, these group then went ahead and did this in different dimensions and now you can see they get all types of shapes here. They have a fairly spherical shape, they have sort of pancake kind of shape, they have shaped like worms they have shaped like long rods some sort of a like UFO like object. So, depending on how much they are stretching at various dimensions in various axis, they can get all kinds of different shapes. So, this is just one example of how you can sort of get these different shaped particles.

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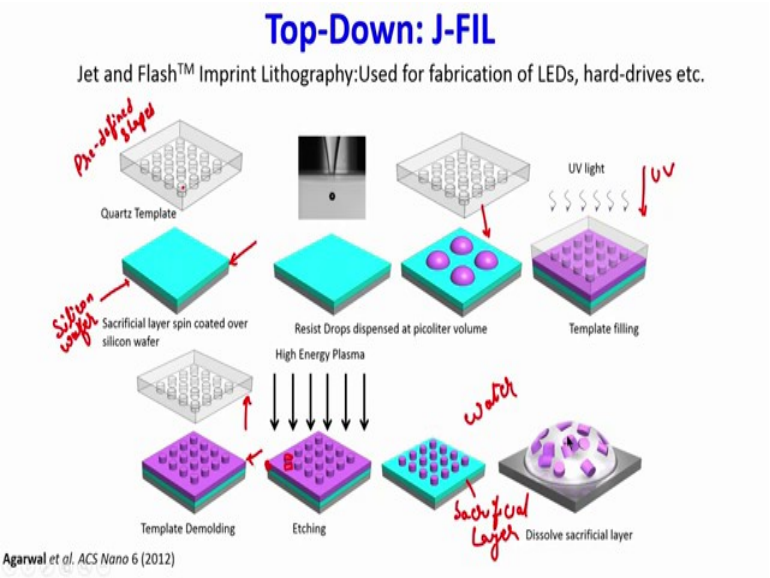

Another way you can go is the top down approach as was saying is to sort of build it. So, an example I will give you is from this paper that was published in ACS nano in 2012 and what it is they these guys have used and imprint lithography tools. So, it is a fabrication tool that is currently used for LEDs and hard drives manufacturing, and what they have done is they have a silicon wafer.

So, they have a silicon wafer and then they have a quartz template which already has these shapes that they want etched into it. So, you can get this through some ion beam etching and all we are not going to go into details for that, but you have some templates with predefined shapes and then you have some sort of a sacrificial layer which is spin coated.

So, you have a small let us say 10 micron, 20 micron thick layer which is called a sacrificial layer. And we will talk about why it is called a sacrificial layer, but it is something that can be dissolved in a certain solvent. So, then what you do is you dispense your pre polymer solution that you want to make these particles out of- you come down with a quartz template the reason the quartz template is used this quartz is fairly transparent to UV wavelengths.

And so, you come down what will happen is due to the capillary action all these cavities ever present in the quartz template will get filled. So, now, you essentially have a structure where all these cavities have been filled, then you come down and shine UV, the UV causes the polymerization of this liquid.

So, once it is polymerized now they will maintain the shape because there is a structure to it now, then you can just take the template away. Now, you have these small standing structures which are all connected by the way with the extra fluid that was there. So, it has created a film with some structures on it and then you can come with a very directional high energy plasma or something to sort of etch this away.

So, what will happen is this top portion will get eroded away, this portion will get eroded away and the portion that was below the particle will become a part of the particle and you get freestanding structure over your sacrificial layer. So, this remember is a sacrificial layer, and then you can just come in with whatever solvent the sacrificial layer is soluble in let us say water. And that way the sacrificial layer dissolve when you will have particles in the suspension.

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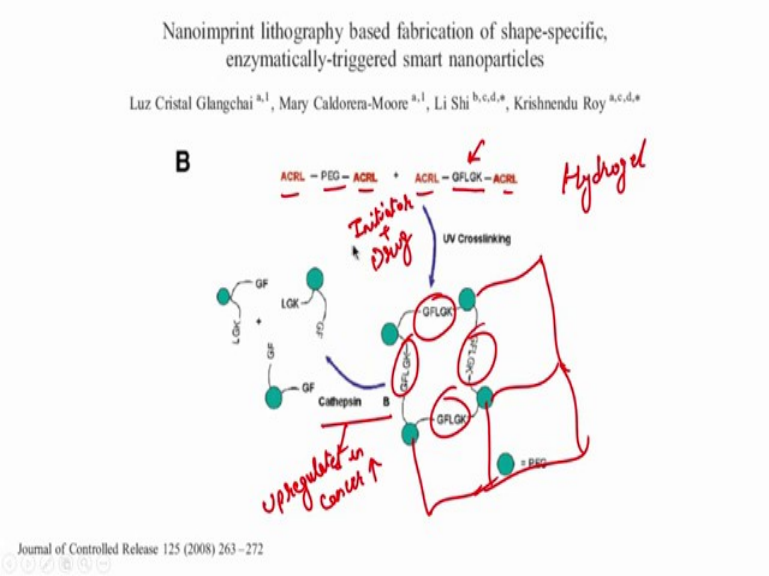

So, these authors then went ahead and use this method in this case for the pre polymer solution they have used a hydrogel based system. So, this is essentially nothing, but hydrogel and what they have is they have a PEG with a Di-acrylate. And then they also have a peptide which also the di-acrylate and I will come back to the peptide again, but what will happen if you shine UV with some initiator?

Let us say they have added a initiator then what will happen is the initiator will start up free radical reaction there will be a radical form and acrylates as we know can react with another acrylates using a free radical reaction. So, this will result in a polymer network being formed, so this is just one unit, but essentially there will be several units of this polymer and so on and so forth.

Which all have if you mix them in a 50 50 ratio or a 25 75 ratio depending on what ratio you have you will end up getting this peptide also cross linking the polymer itself. And now this peptide itself is degradable by a certain proteases, let us say Cathepsin B, so Cathepsin B might be upregulated in a disease.

So, we know some of the Cathepsins are upregulated in cancer, so because it is upregulated in cancer once these particles reaches a cancer site they will degrade faster compared to any other site. So, if your drug which was also added at the time of pre polymer solution is encapsulated within this network. Then what will happen is once it reaches the cancer site more and more drug will come out at the site and that way you

will have a lot more efficacy of your particles because it is responsive to the cancer tissue.

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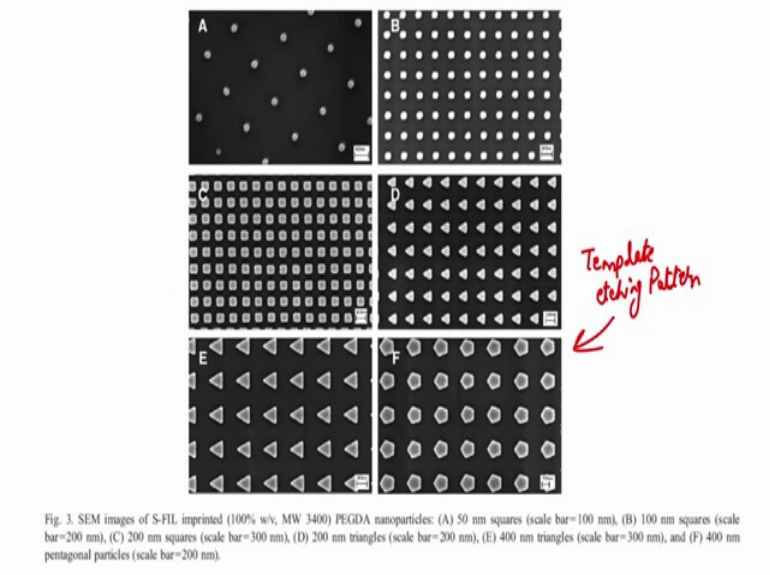

Fig. 3. SEM images of S-FIL imprinted (100% w/v, MW 3400) PEGDA nanoparticles: (A) 50 nm squares (scale bar=100 nm), (B) 100 nm squares (scale bar=200 nm), (C) 200 nm squares (scale bar=300 nm), (D) 200 nm triangles (scale bar=200 nm), (E) 400 nm triangles (scale bar=300 nm), and (F) 400 nm pentagonal particles (scale bar=200 nm).

So, these groups then went ahead and made different shape for particles, so essentially all these shapes are defined by the template shape itself. So, depending on what shape you etched in the template etching pattern, that will essentially define what shape you end up getting. And that way you can get all kinds of shapes that you want in a very nice array that is being shown here.

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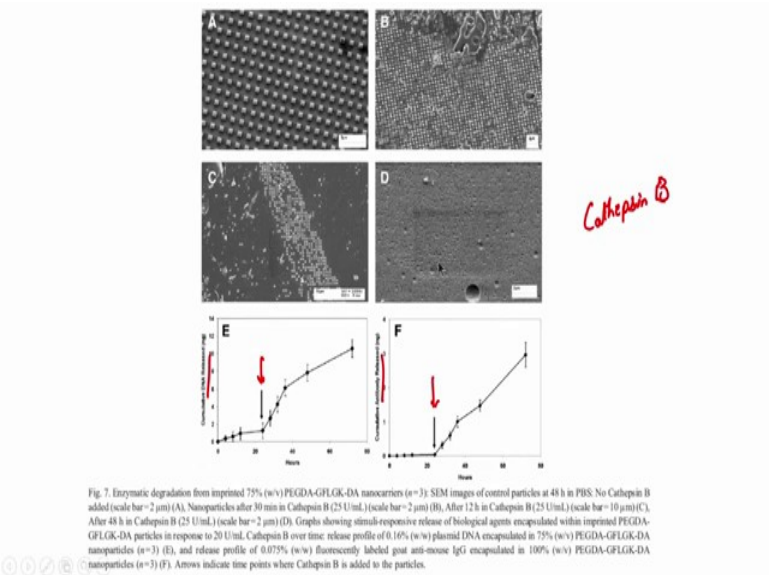

Fig. 7. Enzymatic degradation from imprinted 75% (w/v) PEGDA-GFLGK-DA nanocarriers (n=3): SEM images of control particles at 48 h in PBS: No Cathepsin B added (scale bar=2 μm) (A), Nanoparticles after 30 min in Cathepsin B (25 U/mL) (scale bar=2 μm) (B), After 12 h in Cathepsin B (25 U/mL) (scale bar=10 μm) (C), After 48 h in Cathepsin B (25 U/mL) (scale bar=2 μm) (D). Graphs showing stimuli-responsive release of biological agents encapsulated within imprinted PEGDA-GFLGK-DA particles in response to 20 U/mL Cathepsin B over time: release profile of 0.16% (w/w) plasmid DNA encapsulated in 75% (w/v) PEGDA-GFLGK-DA nanoparticles (n=3) (E), and release profile of 0.075% (w/w) fluorescently labeled goat anti-mouse IgG encapsulated in 100% (w/v) PEGDA-GFLGK-DA nanoparticles (n=3) (F). Arrows indicate time points where Cathepsin B is added to the particles.

And then the group then for the showed that if you treat that with let us say Cathepsin B as I said these networks are susceptible to Cathepsin B degradation, because Cathepsin B is going to cleave the peptide. So, what do you see is over time as Cathepsin B is incubated ultimately all the particles are gone, they have degraded.

And in this case they are shown that there was some DNA or some antibody that was encapsulated within this polymer network . Before adding additional Cathepsin B there is very minimal release or no release at all and once they have added Cathepsin B quite a bit of amount of the polymer or the drug has released.

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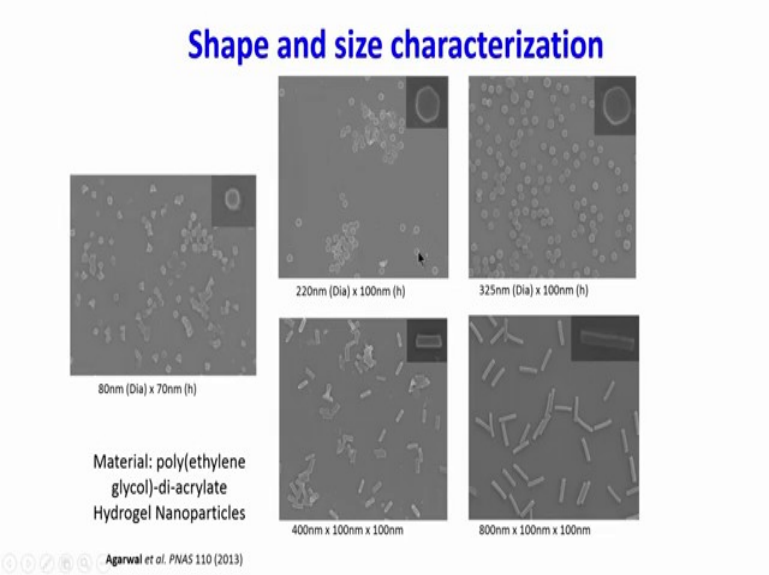

And then you can get these individual particles, so this is an SEM image showing the particles by themselves from all kinds of sizes.

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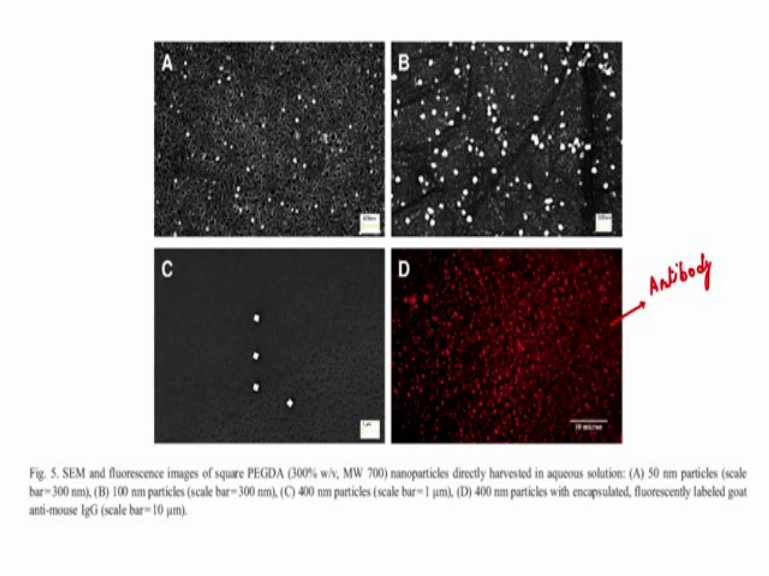

Fig. 5. SEM and fluorescence images of square PEGDA (300% w/v, MW 700) nanoparticles directly harvested in aqueous solution: (A) 50 nm particles (scale bar = 300 nm), (B) 100 nm particles (scale bar = 300 nm), (C) 400 nm particles (scale bar = 1 μm), (D) 400 nm particles with encapsulated, fluorescently labeled goat anti-mouse IgG (scale bar = 10 μm).

You can get various drugs in these particles, so this is showing different drugs that are being encapsulated. So, in this case you have encapsulated some antibody, but the group has also shown encapsulation of doxorubicin or any drug that might be relevant for certain application. And so, this is the fluorescence image.

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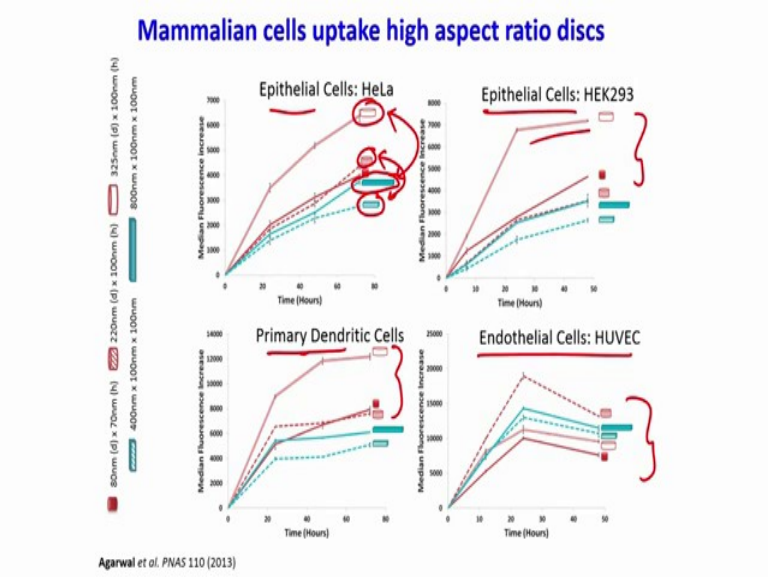

And then you can do an uptake with the cells to see if I want to deliver things to mammalian cells, how do mammalian cells then take these things up, so the group has

used several different cell lines. So, in this case they have used 2 epithelial cell lines, 1 dendritic cells line and other endothelial cell line.

And what you see is that different shapes even though they might be of similar volumes in this case this smaller disc and this larger this smaller rod are equal volume similarly this larger rod and this larger disc are of equal volume. So, even though they are of equal volume you see that their amount of uptake in the cell is very different. And because everything else is same- the material is same, the charge of the particle is same ,the volume of the particle is same ,the size of the particle is same -the only thing that is different is the shape. So, the authors were able to conclusively say that because of the certain size or certain shape a certain geometry and you get a difference in the uptake. So, certain shapes are better for delivery to the cancer cells or epithelial cells in this case than say others. And what they also found was very interesting is for let us say epithelial cells and dendritic cells you have the same trend; however, endothelial cells do not really follow this. All the discs have been shown in red in all the three cell lines you see that the red is above the rods; however, this is not true with endothelial cells where it does not seem to be much different. So, not only the shape will help you to get more in the cell, what it also does is it also makes it selective.

So, let us say if you want to target epithelial cells, you may want to use the disc shaped particles whereas, if you want to target the endothelial cells you may want to use some other sort of particles and not may be disc shape particles. So, there was a fascinating result.

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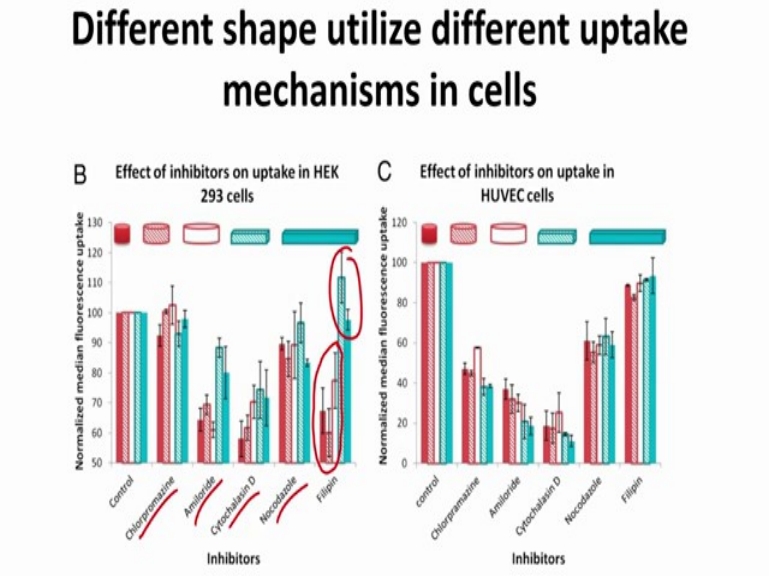

And then further to that they further looked into what mechanisms are causing this and what they found using by different inhibitors I am not going to go into details of what these inhibitors do. But essentially the cells have different pathways through which the particles are uptaken and all of these inhibit some of these pathways and what they find is that different shape particles get up taken by different mechanisms.

So, if you look closely here you find that disc shape particle uptake is affected when filipin is used whereas, it is not affected for the rod shaped particle. So, it also shows that there is a another mechanism through which disc shape particles are going in the cell. And so I guess the crux of the matter from all this is shape can play a very important role and it should be considered when we are talking about drug delivery and all.

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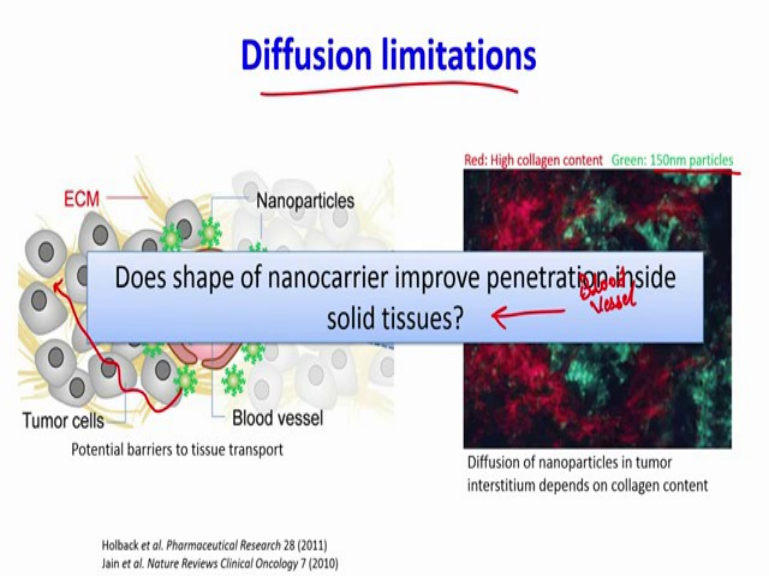

Here is another example of how shape can be a role, so this is essentially a cancer tissue. So, we know in cancer there are limited blood vessels, so there is a diffusion limitations from the blood vessels. So, let us say from delivering a drug it may not be able to go far away from the blood vessel.

So, let us say if a drug is coming out from these vessels and then it has to now diffuse all the way to the farthest cell away from the blood vessel. So, this is just a pictorial representation of a real example where you have delivered 150 nanometer particles which are labeled as green and the cancer is being labeled by collagen as red.

So, what you see is here is a blood vessel and what you see is all the particles are essentially localizing near the blood vessel itself and as you move further away the amount of particles is decreasing, so this is a big limitation. So, another group tried to explore the use of shape of particles to see the diffusion is better. So, can the shape improve the penetration inside solid tissues.

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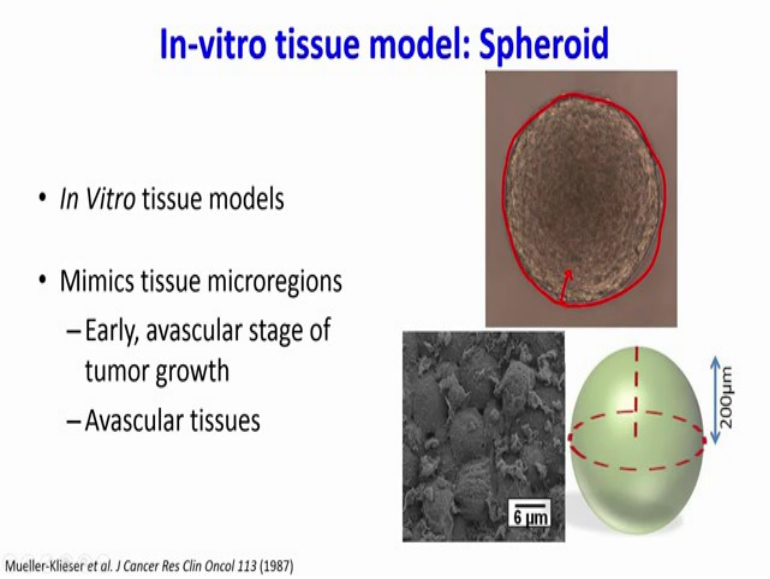

So, what they did is they use an in vitro tissue model called spheroid which is essentially nothing but aggregation of cells which are avascular. So, you can then consider that there is a blood vessel running right across this spheroid and anything (because this is essentially medium) which is further going in is away from the blood vessel.

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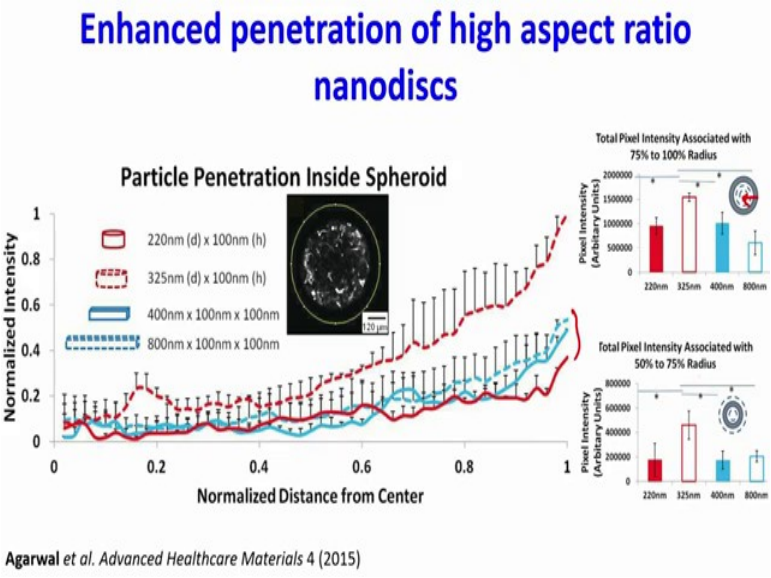

And what the found is that again the disc shaped particles were able to penetrate quite a bit deep into these tissues even though the same sized rod shaped particles do not do as well. And this is just a quantification showing that, yes ,in different regions further in

from the blood vessel you get further and further increased amount of penetration of certain particles. So, well stop here and we will continue with further description of the particles in the next class.

Thank you.