Drug Delivery Principles and Engineering
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Lecture – 16
Hydrogels – III

Hello, everyone. Welcome to another lecture of Drug Delivery Engineering and Principles. We are currently talking about Hydrogels and we are going to continue that in this class like the last two classes.

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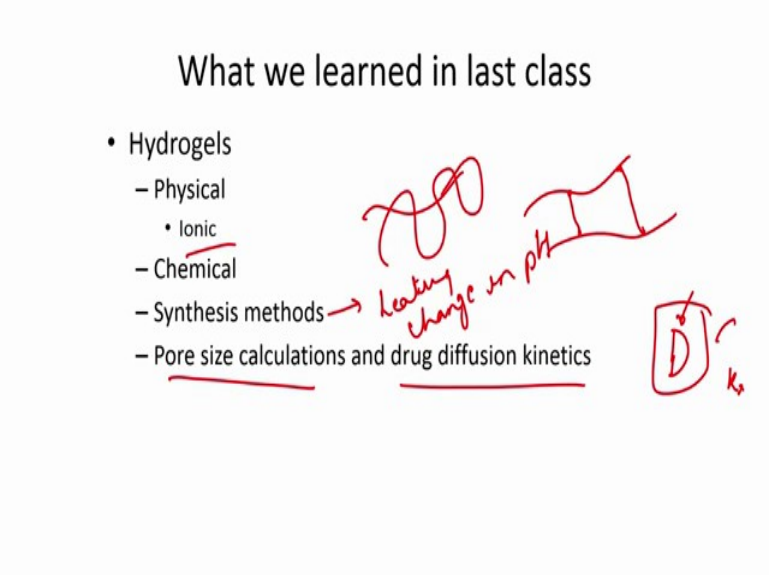

Let us take a quick recap on what we have learned in the last class. So, we have talked about hydrogels, we have talked about the physical hydrogels, which essentially we talked about ionic in this case, then we talked about chemical hydrogels, we talked about how these are formed. So, essentially what is the major difference between chemical and physical. Physical are essentially molecular chain entanglements whereas, chemical may have an actual bond that is present between these polymer chains.

And then we have talked about synthesis process. So, typically synthesis process for physical hydrogels involves either heating or change in pH or any other trigger that causes these chains to start interacting with each other and form these gels. Whereas, the chemical hydrogels, all you have to do is just add the cross linker or somehow activate

the groups; it could be either through light or it could be maybe adding glutaraldehyde or some other kind of cross linker that causes this trigger to happen.

Then we talked about some pore size calculations. So, very briefly we discussed this. We are going to discuss this more in future classes and then we talked about drug diffusion kinetics. So, how can we model, how the drug is going to diffuse. So, we found out that we can just use the Fick's law and all we have to really change is the diffusion coefficient D, which will then be affected by whether the gel is micro porous or macro porous.

So, this micro porous D does not really have much role to play here. Although there is some factors that get added up; whereas, if it is micro porous then along with these factors we add another partition coefficient Kr because these chain these drug molecules will now start to interact with that as well.

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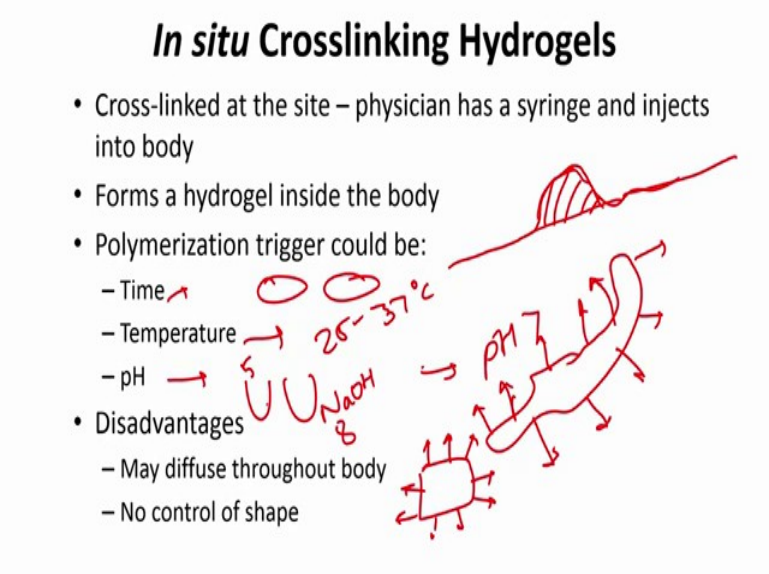

So, let us talk about in situ cross linking hydrogels. This could be any of those previous two hydrogels if we talked about, both physical or chemical, but what essentially in situ means is this that these gels can cross link at the site. So, these are very desirable when you talk about any kind of delivery because what you can do is you can have individual components. You can mix those two components together and once you mix those two components together the trigger is basically given for the polymerization to start and

these things polymerize within a certain amount of time. This could be over a period of seconds or a period of minutes.

So, why it is desirable? Let us say if I am a clinician, I am a doctor and I am trying to inject something through basically a syringe and want to have a hydrogel system to kind of have this drug release over time. So, all I can do is I can mix the two component together in the syringe and directly inject into the patient and what will happen is in that time or in that trigger this thing will polymerize and wherever it was inject it will stay there and sort of form a depot. So, these they call in situ because they form hydrogel inside the body.

And the polymerization trigger could be several types. So, one trigger is time. So, you all you have to do is just mix two components together and it may be like as I said few seconds 10 seconds, 20 seconds or it could be minutes, 2 minutes, 3 minutes and within that the polymerization will start. It may be temperature right, I mean I may not even have to do anything in this syringe. So, in syringe is completely stable I do not even have to hurry up, but I mean temperature let us say 25 degree Celsius, but when I injected into the body our body temperature is 37 degree Celsius. So, because now there is this change in temperature something got activated that causes the polymerization, the gelling to happen and that 25 to 37 degree Celsius trigger is enough that it done polymerizes let us say within a few seconds. Or it could be pH. Let us say and this could be again several types. Let us say in my two tubes that I am mixing together, the combined pH of the polymer here let us say is pH of 5 at which it does not polymerize.

The other tube basically just contains sodium hydroxide let us say with a pH of 8, but when I mix them together that leads to a pH of 7 and at the pH of 7 this thing starts to polymerize. So, this could happen it could be that I do not even worry about mixing with NaOH. I just inject it into the body and a body pH is about 7 anyways and so when this diffuses in, it can cause the polymerization to happen. So, any of these things can be explored in if you are talking about in situ gelation.

There are some disadvantages to it. Obviously, there is lots and lots of advantages, but there is some disadvantages. One is this will diffuse throughout the body right. I mean if I let us say inject it into a area which is highly fluidic let us say if it is in my stomach or in my peritoneal cavity or any place which has lots of diffusion and convection going

around then before it can polymerize it can just diffuse throughout the body. I do not really have a control of shape.

So, that is a big problem because as you know most of these diffusion kinetics will depend on what is the area through which it is coming out of the system. So, if the area is compact and the drug is only coming out from this much surface area, you will have a different release rate where whereas, in case of in situ cross linking what will happen is once injected into the body, it can take whatever weird shape it flows in before it polymerizes and now you have much larger surface area and much irregular surface area through which the drug can start to diffuse out. In this case, it is very hard for me to model how much drug is going to come out in a particular amount of time.

So, these are some of the challenges. Another advantage here is obviously, if I am looking to fill a defect; let us say; let us say an injury and some part of my muscle is gone and I want to fill it with a gel containing some cells, all I have to do is just put some liquid over it. The liquid will automatically take the shape, let us say if this was my muscle and there was some accident through which this is the shape that needs to be filled. Now, I do not even need to worry about designing the shape. What I can do is I can fill this with the polymer and once it gels it essentially just takes up the structure that I want.

So, there are some advantages to it, but obviously, it has to be looked at what sort of applications we are looking at before we can move forward with this. So, again this is very widely used in research and there is lots of hope for this to get translated into the clinic. We will talk about one major research paper on this to kind of understand this. The paper is going to look at different things as well. So, let us get into it.

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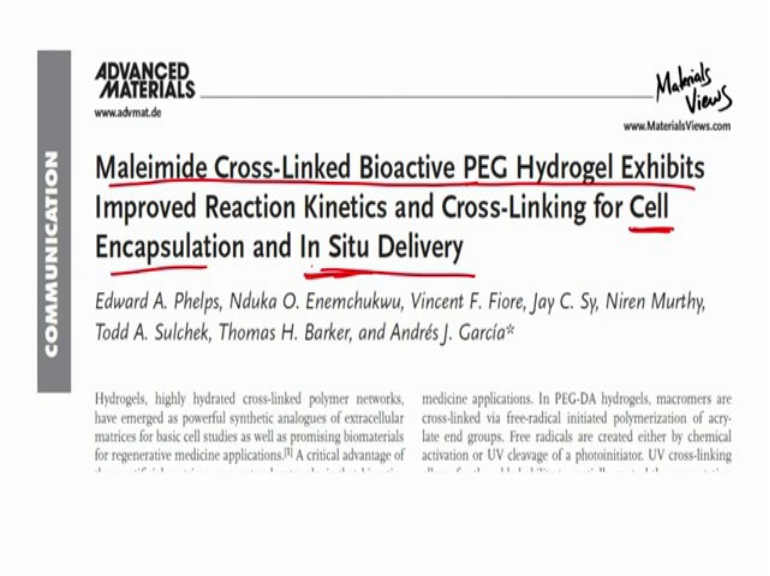

So, in this case this is a paper which is using an in situ cross linking, but then this is here what I am trying to do is basically give you some ideas to how these hydrogels are being used in the system.

So, in this particular paper what they are doing is they are using a maleimide based cross linking of a PEG hydrogel to improve the reaction kinetics in the cross linking to happen for both cell encapsulation as well as in situ delivery right. So, in this case the drug is actually cells and they want to make a system that they can just inject into the body and not have to worry about surgeries and all.

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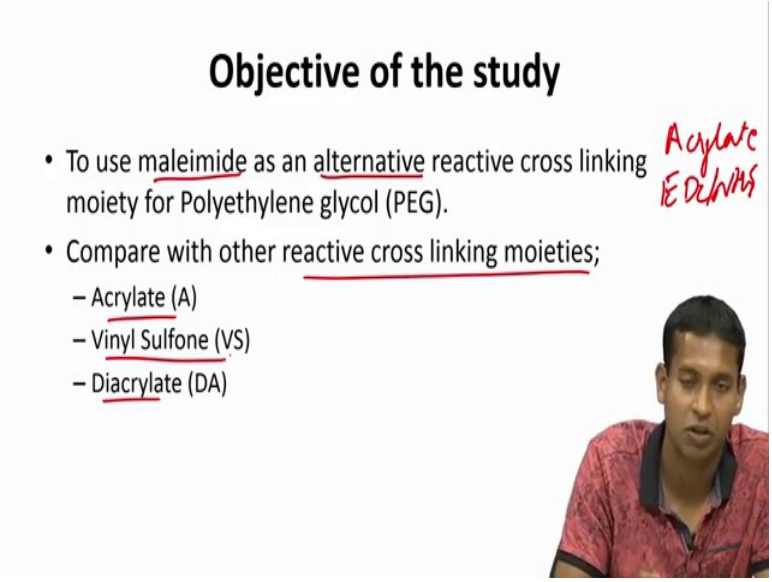

So, in this case the objective of the study is to use a maleimide as an alternative cross linking moiety to polyethylene glycol and when I say alternative there is try different types of cross linking methods and see which is very widely used.

So, before this paper came out people typically were using acrylate or let us say some EDC NHS or some other use some other form of coupling, but now this paper is proposing using a maleimide base cross linking. So, what they have done is they have compared the cross linking moieties which is again as I acrylates, diacrylates and vinyl sulfone which is another functional group.

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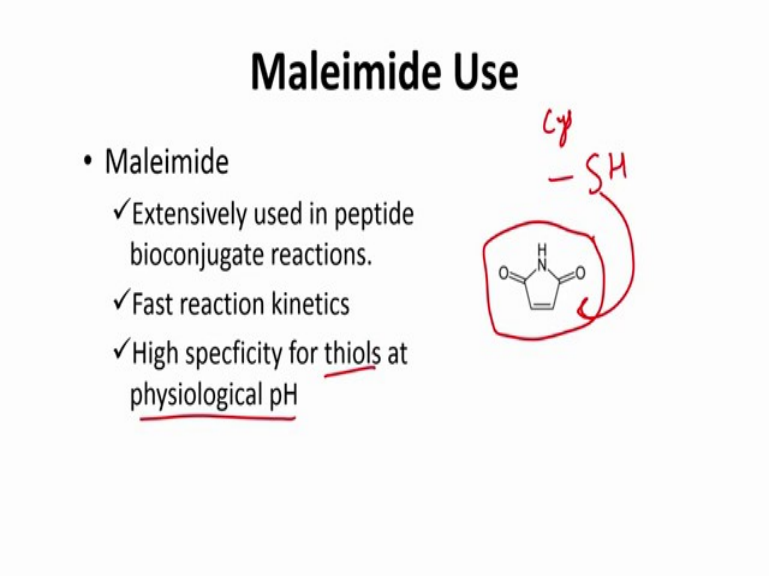

So, maleimide as we have discussed in the past is a structure like this in presence of thiols that this reacts with the sulfhydryl group. So, thiols all will have a sulfhydryl. So, something like cysteine or any other thiol group might be present due to some other molecule and this will directly react with this. So, this is again very extensively used in peptide bioconjugate reactions, but then here they are now proposing it to use it with hydrogels.

The reaction kinetics is very fast as you will see in the rest of the data in this paper. This has very high specificity for thiols. So, at physiological pH this is again for most of the biological applications we are looking at physiological pH. So, this maleimide group has a very high affinity and specificity for thiols. So, it would not have too many reactions going around.

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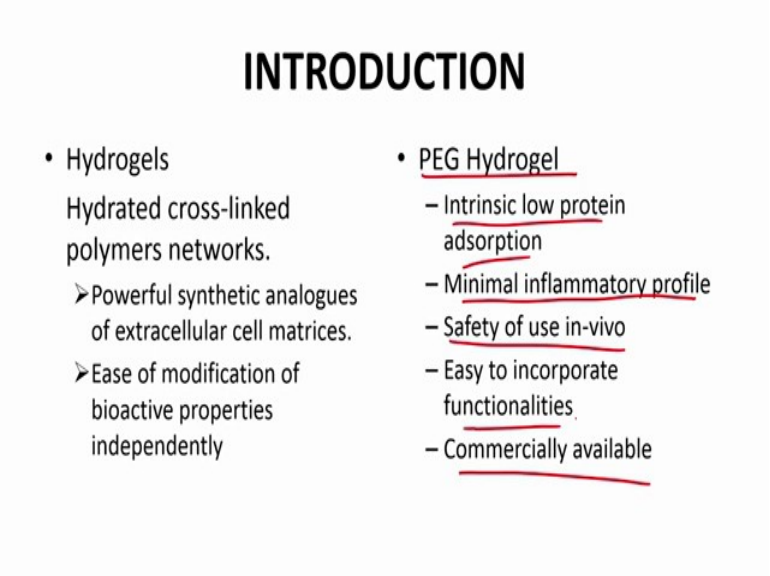

So, let us see what they have done in this paper. So, they used hydrogels. These are again as I said these are hydrated cross linked polymer networks. They are kind of a synthetic analog to the extracellular matrices in which the cells typically reside. So, this is a very similar structure as I told in the last two classes and there is ease of modification and you can modify them. They will result in some bioactive properties which are independent of what you are encapsulating in them.

And in this case they have use PEG hydrogel. Again as I said PEG is very widely used. So, and somewhere reasons we already know that it is very low protein adsorption. It is actually used in different areas to prevent the protein adsorption from happening. It minimally causes any inflammation. Although we have talked about some PEG antibodies weak form, but in general if there is no PEG antibodies it causes very low inflammation.

Safety of use in-vivo; again it has been used very widely for last few decades and there lots of commercially available products that have used different functionalities to incorporate into them. So, all of these are a plus to this.

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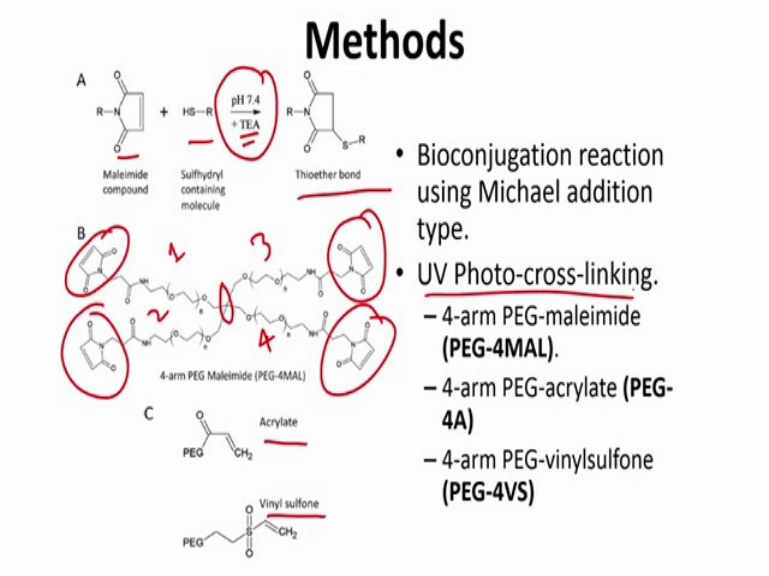

So, here are some of the methods that they have used. As I said these maleimides will actually react with thiols to form this thioether bond and what they have done is they have purchased the PEG commercially available which has the four acrylate groups, it is a 4-arm PEG. So, here is the cross link between the four arms of the PEG. So, this is arm 1, arm 2, arm 3, arm 4 and each of these arms contains a maleimide group at the end. So, and then instead of maleimide they also have done similar modifications and I have got an acrylate and vinyl sulfone which are two of the groups that were used quite a bit at the time when this paper was published.

And then again this reaction happens at a pH of 7.4. TEA is kind of a catalyst to this and so for acrylate the polymerization happens on the basis of a UV cross linking and the vinyl sulfone also reacts with thiols to form bonds.

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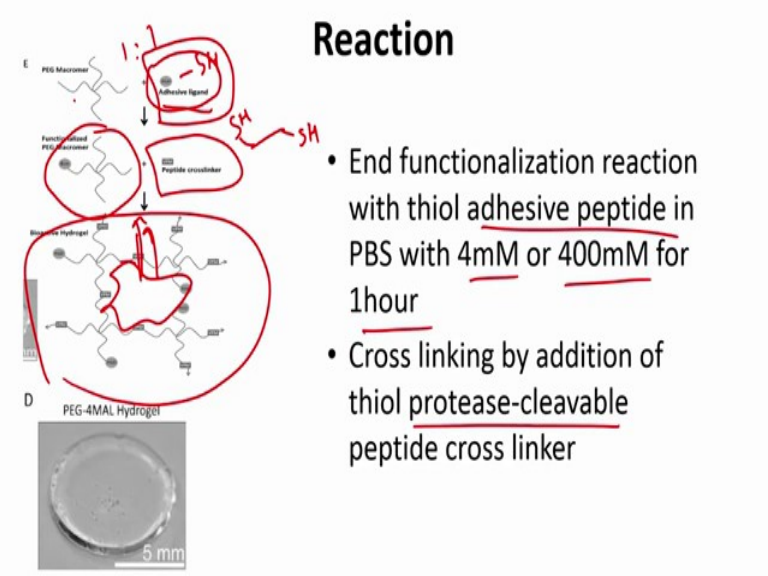

And so, that is what they have done they have done reactions. The reaction that they have done is they have a PEG macromer, they put an adhesive ligand. Why is this required? What do you mean by adhesive ligand? This is a ligand that reacts or that actually interacts with cells and causes the cell to adhere to it. As we said that PEG does not allow any adherence. So, they have put some adhesive ligands on here that will result in the PEG to be cell compatible. The cells can now bind to it.

And so, once they do that, they get a structure like this, where let us say at whatever ratio they are making let us say they are putting this in a ratio of 1 is to 1, then each of these PEG macromer will result in on an average will have one of this adhesive ligand and then they have another peptide cross linker. So, this is kind of a cross linker which contains thiols. So, this one also contains thiol, but it is only a single thiol. So, it can react once, but it would not form a gel, but in this case now they are talking about a peptide which contains thiol at both ends.

So, now it can essentially cross link in a whole network like this. And they have used different conditions they have used the adhesive peptide at 4 milli molar or 400 millimolar, they have used for different times up to an hour and then the cross linking is done by a peptide which is also cleavable which is a protease cleavable, so, that means, the cells can secrete proteases. So, let us say the cell wants to move around and it is entrapped in this giant mesh, so, if a cell is here and it wants to move out from here it can

just secrete some protease, degrade this and this will open up and then the cell can move out.

And, so, all of this resulted in this hydrogel structure as you can see here it is they have formed it in a disk shape. So, it just took the shape of the disk that they formed in and it resulted in hydrogel.

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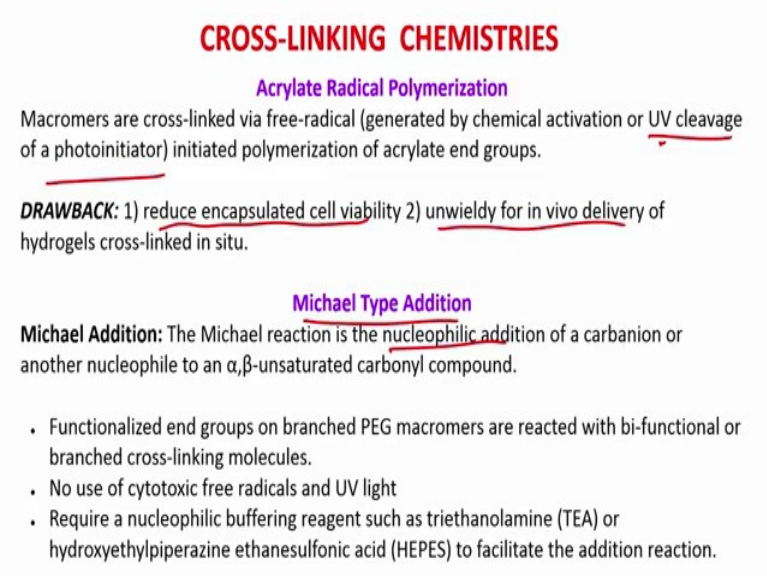

Let us talk about some of the cross linking chemistry that they have used. So, as I said the acrylate is radical polymerization. So, if you have some UV based methods, some photo initiator is used, that will result in polymerization to happen. Remember the drawback here is of course, the UV itself is toxic to cells and the photo initiator is also toxic to cell. So, you can actually have reduced cell viability there. So, that is a problem that was identified with acrylate so, quite a bit of time.

And it is not really very conducive to do an in situ delivery and we will talk about that it does not really happen very fast. The reaction is very slow and in the reaction is slow and if you inject it, let us say it is if it takes 30 minutes and then 30 minutes all your hydrogel components will get just diffused out into your body and it really would not form a depot at the site you are injecting it at.

And then the other reaction is the Michael type addition and so, this is basically a Michael reaction where a nucleophilic addition happens on another nucleophile. So, this is what they have used to react the maleimide to the thiols.

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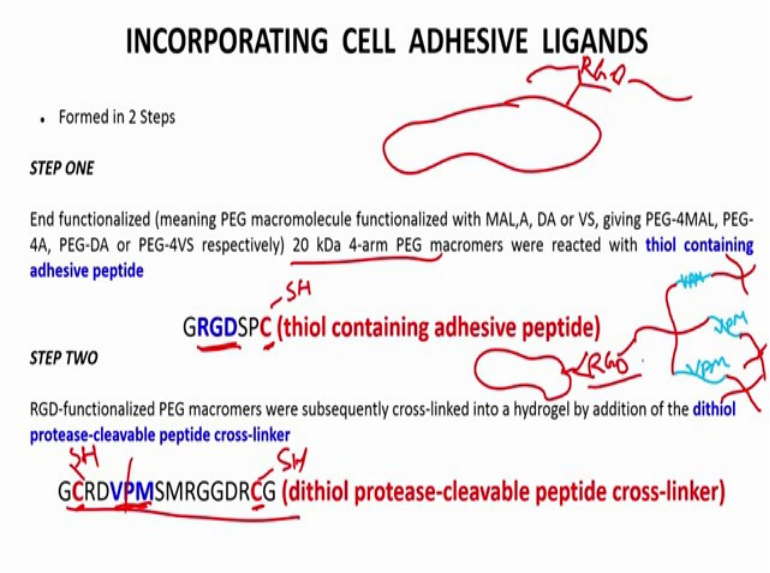

So, again, as we said they have incorporated cell adhesive ligand so, one of the ligands have been incorporated is this RGD molecule. This is a molecule that is very widely found in fibronectin or vitronectin and other ECM molecules through which these cells have ligands and when they find RGD in a polymer chain they can just bind to it and kind of tether themselves from there. So, this is what they have used.

They have also modified this to also contain a thiol so that there is one sulfydryl group present through which it can react and then the RGD group is still available for the cells to bind to. So, that is what they have done. So, they used 20 kDa 4-arm PEG with thiol containing adhesive peptide. And then in a step two, what they have done is the degradable peptide is this. In this case this is the whole peptide and this VPM site is where the cells will cleave it.

So, when the protease are in the solution they will cleave this VPM peptide, once you see it and then again they modified it with two cysteines each containing a thiol and so, again what that does is that allows you to sort of have a control on the system where now you can have polymerization happening from this as well as its degradable. So, I hope this is clear.

So, now, you are talking about let us say this is our 4-arm chain one is containing RGD that is capable of binding to a cell and the other arms are then cross linked through this VPM. So, if I use another color. So, now, you have a chain containing VPM from the rest of them which will then be bonded to another 4-arm PEG. So, that is how the whole structure is formed. So, this is zoomed in to one of these and that is how it is. So, now, the cells can actually degrade this VPM moieties to move around as well as bind to this RGD structure.

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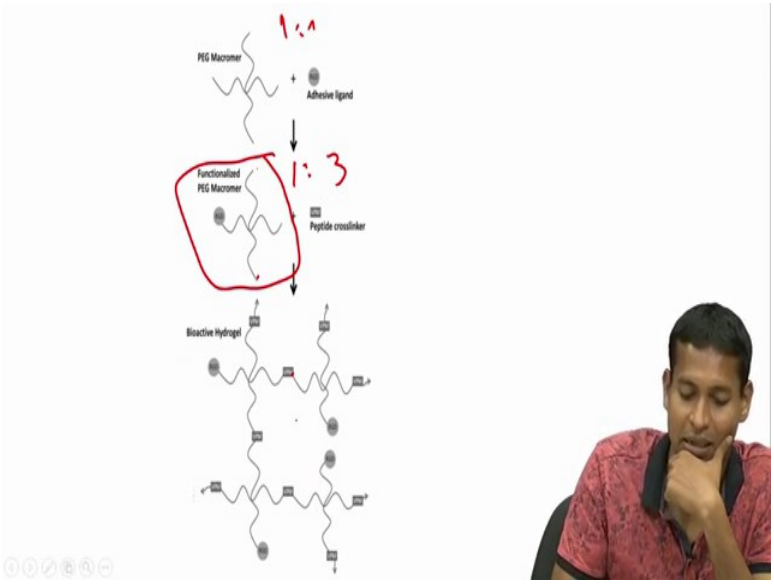

So, here you go, it is just the same thing that I just draw. So, you first react it at a 1 is to 1 ratio. So, you essentially get right functionalized with RGD and then you mix this with the ratio let us say 1 is to 3. So, now, you have three sites on which all the VPM gets bonded as well it starts cross linking throughout the network.

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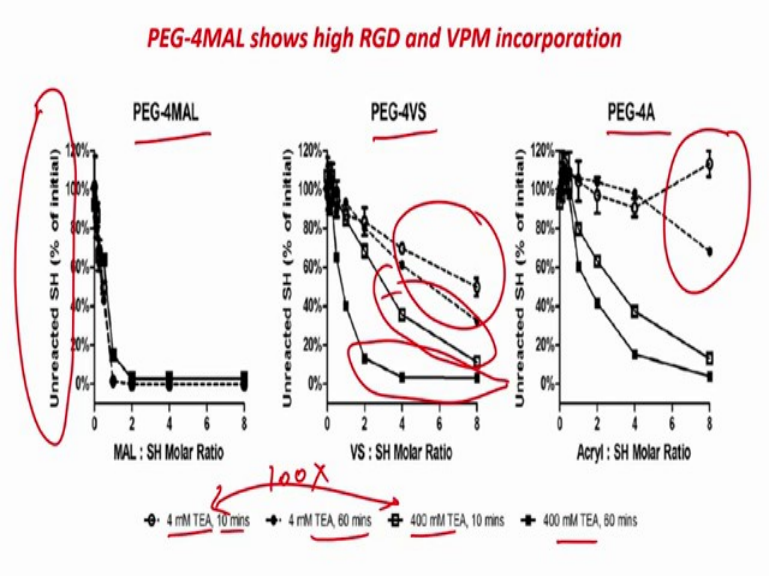

So, now let us look at what the data they achieve from this. So, first of all, the PEG-4MAL shows higher RGD and VPM incorporation. So, this reaction is more efficient that is what they are trying to propose. So, let us look at that. So, in cases of PEG-4MAL what they see is even if they have low amount of the TEA which is a catalyst here, even in 10 minutes you almost get all the reactions. So, on the y-axis you have the unreacted thiol. So, they measure how much of the unreacted thiol is present. So, if they were stoichiometrically mixed, so assuming that all the thiol should have been finished, that is what they see that almost they get 100 percent thiol conversion and the same thing happens at 60 minutes. Whereas, if you look at look at PEG vinyl sulfhone or PEG acrylate at least for the concentrations of TEA for 10 and 60 minutes you only see very little incorporation. So, I mean you are talking about only 40 percent or in this case case very little.

If now you increase the TEA concentration which is again a catalyst here, you get to 100 times. So, I mean this is essentially we are talking about 100 times increasing the catalyst concentration even then you are seeing not a very good incorporation only after quite a bit of time you start seeing almost 100 percent reaction and the same thing applies with a PEG-4-acrylate. So, clearly shows that this reaction chemistry is with much more efficient to continue with this particular system.

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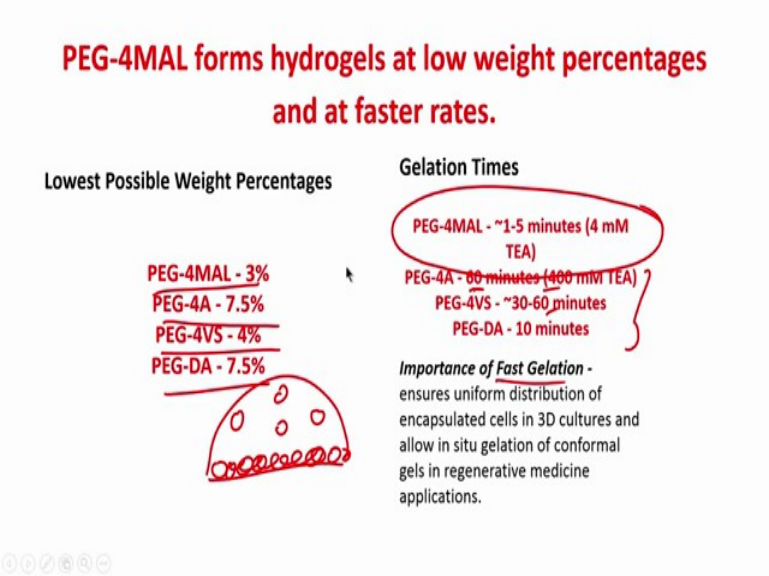

Then, what is the different polymer concentrations we can use to form this hydrogel. So, what is the lowest possible weight? So, and if you continue to reduce the polymer chains you may not get a hydrogel at a certain point of time. So, they found that with the PEG-4MAL even a 3 percent gel can be formed whereas, with acrylate you can only get a gel at a minimum of 7.5 percentage of this polymer and with PEG-vinyl-sulfate you get at 4 percent and then the PEG-DA is also forming at least at 7.5 percent.

And the gelation times are also very different. So, the PEG-4MAL forms within 1 to 5 minutes whereas, the other takes up to 30 minutes and longer durations. So as I said, they are not very conducive for in-situ gelation because if it is taking 60 minutes to form a gel, then by the time it is injected in 60 minutes all the PEG polymer will be all dispersed throughout the body. So, and not only that if you are encapsulating cells and if you let us say doing it in a cell culture dish what will happen you took gravity the cells are now settling.

And, so, if let us say if this is your gel 3D structure. Let us say this is your gel 3D structure. All your cells will be kind of populated at the base and the rest of the gel will have a very sparse population because of the gravity these cells are settling. So, that is why you will not have a very good distribution of the cells either if the gelation time is not fast.

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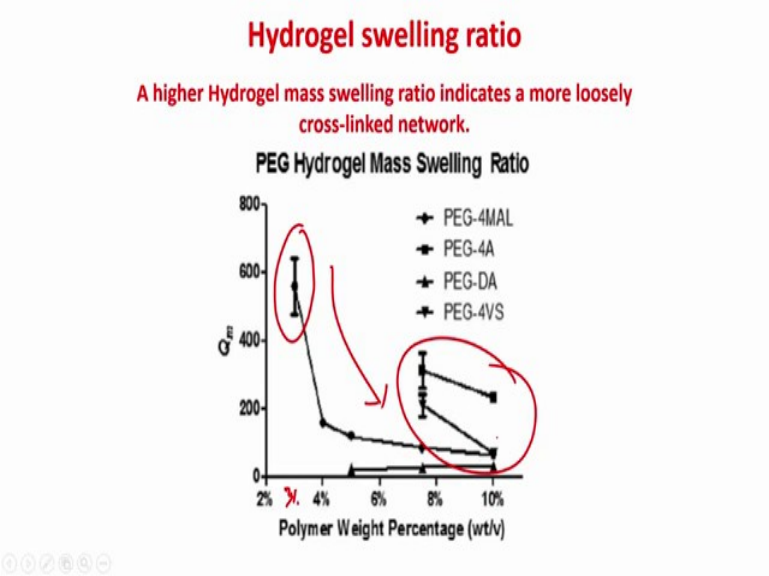

Then they went ahead and looked at the hydrogel swelling ratio. So, what they found is that the PEG-4MAL had a very high swelling at lower percentage. So, this was the 3 percentage which is the minimum they can get and as you increase the polymer concentration the swelling decreases. Whereas, in other cases, you do not really have much swelling happening because you are requiring quite a high percentages.

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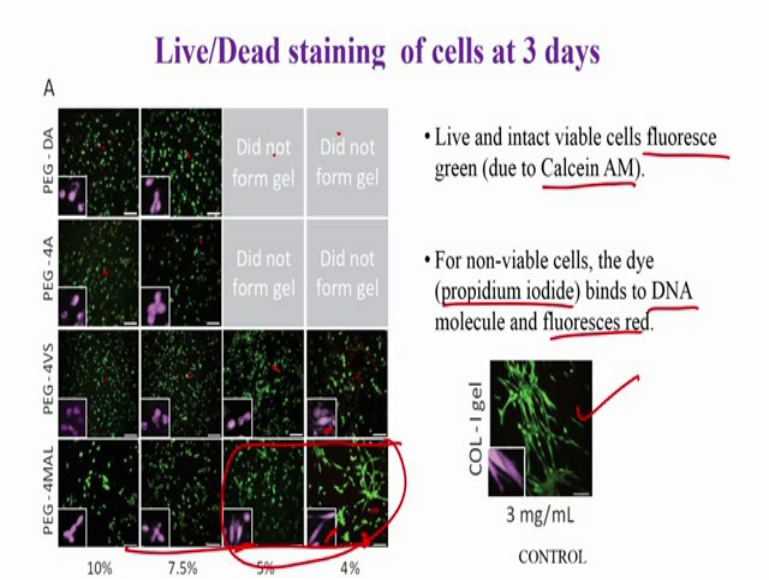

And so, then they went ahead and encapsulated some cells for 3 days and here is the data for that. So, what are we looking at now is on this row direction, you are seeing each

condition. And so, if you can see, so, the bottommost is a PEG-4MAL and on this column you are seeing essentially what percentage gel was used. So, this goes from 10 to 4 percent and what do you see here is that the PEG-DA does not really form gel at the 5 and the 4 percentage as was shown before like the same case with the PEG-4A.

And the PEG vinyl sulfone does form gels, but if you look at all these cells, they all look fairly rounded. The only time you are seeing a little bit of an elongation is on this one, but all the rest of them they look fairly rounded; that means, when the cells are out, they mean they cannot really tether to something. So, when the cells are tethering they start stretching, they start elongating and spreading onto the surface or onto a gel, but in this case you do not see that.

Whereas, if you look at PEG-4MAL at higher concentrations, yes, the cells are sort of rounded, but as you are decreasing the concentration of the polymer at 4 and 5 percent you see them very well elongated. This is this is how a typical ECM matrix will look like. This is a collagen gel which is the natural ECM matrix that we find in our body and so, this is how the cells typically look. And then the only two conditions that are able to mimic that are these two. So, the rest of them are not even able to do any kind of mimicking like collagen.

So, the stains here used are essentially Calcein AM, which is a dye that is permeable through the cell membrane and it goes in. And once it is there it is reduced inside the system to a byproduct which then has a fluorescence. So, only the live cells fluoresce. And then you have a non viable stain which is a propidium iodide, which is a dye that is unable to diffuse into the cells are alive, but the membrane is compromised. It diffuses in and binds to the DNA and then gives the fluorescent red. So, you can see which one are live and dead. So, all of these are most of them are showing green so, which means that they are live cells.

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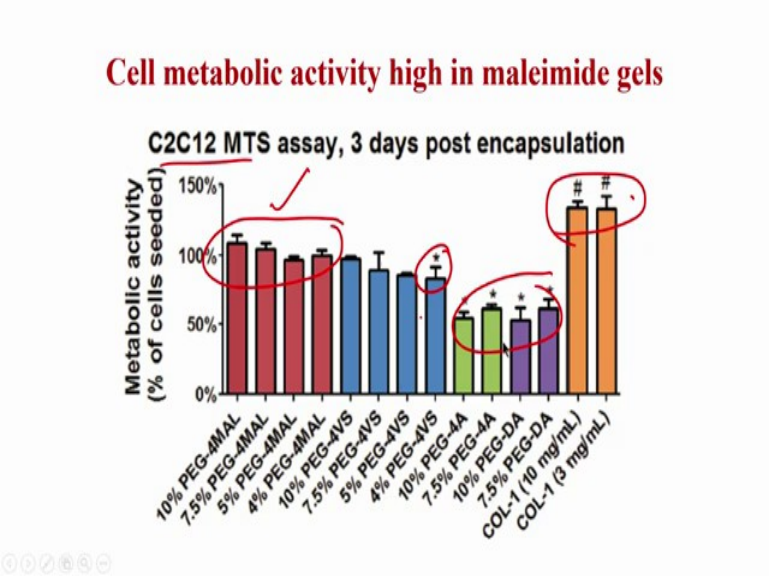

Then, they further went ahead and did some metabolic activity. So, they found that metabolic activity is high in maleimides. So, here you have maleimides in the red and all of them you see that compared to a 2D control, so, in these are all compared to a 2D control in which there is no gel, you find that they are fairly well metabolically active whereas, the metabolic activity decreases in other conditions and this is again just a collagen control. Collagen of course, is a higher metabolic activity for these cells.

These are C2C12 which are essentially some muscle myoblast cells and you see that even though the activity is not as good as collagen, but compared to the other synthetic polymer chemistries, they have a much better metabolic activity.

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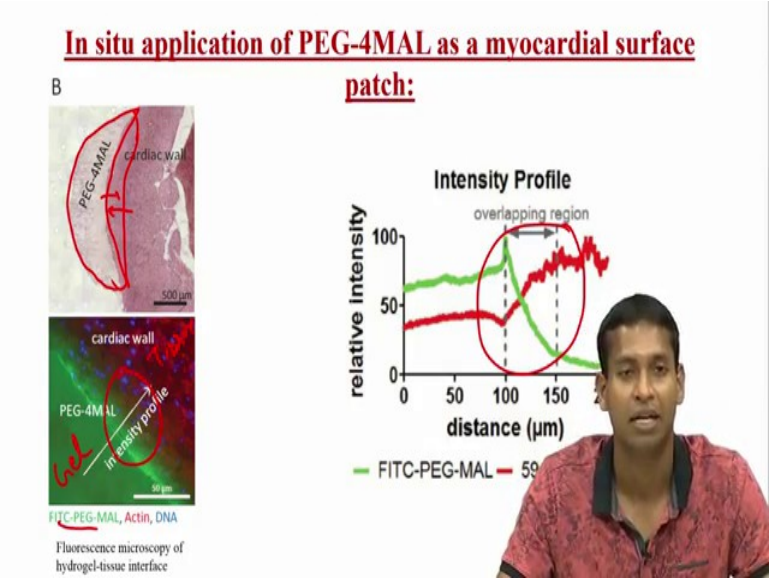

And then let us see what happens when you apply this in-vivo. So, what these authors have done is they then put it on a mouse heart; so, on a live animal. So, this is what happens. So, when you just dispense the little bit of liquid onto these mouse heart the beating heart. What you see is then the liquid was then spread for a little bit, but then it started polymerizing. So, you get a polymerized gel taking the shape of the external surface of the cardiac wall.

And not only that what the authors have done they have encapsulated some FITC in here so, which fluorescence green. So, this is all gel, this is all tissue, the cardiac tissue in this case and what do you see is there is some sort of an interface. So, the gel is was actually able to diffuse in and polymerize and then the cells were also able to move in here. So, what do you kind of see is an overlapping region. So, you do have this overlapping region in which these cells are interacting with the tissue and this is a very compatible surface.

So, that is basically all on the in situ hydrogels for this class. We will continue further with a hydrogel discussion and some more computation of the network pore size and all in the future class.

Thank you.