Tissue Engineering
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Lecture - 08
Hydrogels - Part 2

Today, we will start with a small demo. This swelling itself will take some time, but we will just have the experiment start, while I start talking about smart Hydrogels.

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So, these are hydrogels. These are different kinds of hydrogels which we have prepared in our lab. The one which is here that is isabgol, one is konjac glucomannan, this is just PVA, and this is poly HEMA. These are the hydrogels which we have prepared, and you can just have a look. Isabgol is a polysaccharide; so, it is primarily arabinoxylan. What we do is, we buy isabgol commercially, and we just dissolve it in water to prepare this hydrogel. KGM is konjac glucomannan, which is again a polysaccharide. PVA and poly HEMA are synthetic polymers that are used for hydrogels.

We have prepared all of these; some of these will swell a lot, some might not swell as much. We have only a couple of beakers, but we do have a 6-well plate. So, what we will do is, we will cut a small piece of it and put it inside these well, 6-well plates, and we will pour the buffer.

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She has given me PBS. So, we will pour a little bit of this buffer, and we will see how the swelling has actually taken out. After it swells, we will take it out and blot it with a tissue paper, and then we can see whether the water actually comes out or not.

The loosely adhered water can only come out, if it is properly swollen and the water is closely interacting, it cannot come out. So, we will try and do this. What we will first do is, I will ask for volunteers who can cut it up into smaller pieces, and we will put it in this, and we will wait for how it swells, ok.

After we put up a small piece, you can feel the texture as well as the dry ones. So that after swelling, you will be able to see how it feels. So, that is PVA. It is quite strong, actually. So, one is PVA, and I already have poly HEMA.

Student: Sir, the other ones.

These are samples too. These are natural polysaccharides. As you can see, it is so much easier to cut; they are mechanically weak compared to what you had with PVA. Do not cut too much of that, or else it will swell a lot.

Student: Ok.

You can show it around to people; so that people can take it up and have a look. Here, I am not measuring any volume of the buffer, because that does not really matter. We just have to add enough, so that it can absorb.

Student: So, what buffer you are adding?

So, this is PBS. Let us see how well it swells and just leave it here. Today, we will talk about a special class of hydrogels, which are called as intelligent or smart hydrogels.

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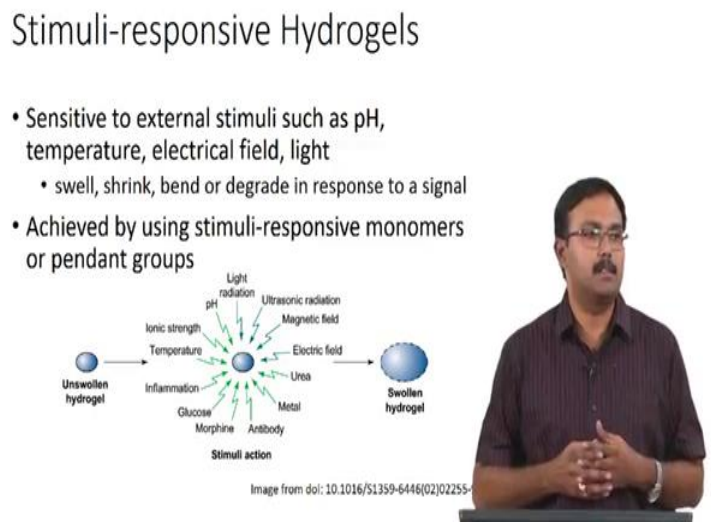

These hydrogels are stimuli-responsive hydrogels. See when hydrogel swell, they just swell when you put it in PBS right. So, that is what is happening to these. These when you put it there, they are going to absorb a lot of water and start swelling. Whereas, if you are talking about the stimuli-responsive hydrogels, they respond to external stimuli. The stimuli could be pH, temperature, electrical field, light, magnetic field, and so on.

In response to these stimuli, they will either shrink or swell or bend or degrade, and this can be used to tailor its applications in tissue engineering. This is prepared using a stimuli response of monomers or pendant groups, which are the side chains that would respond to certain stimuli. An unswollen hydrogel in the presence of certain stimuli can form the swollen hydrogels. This is the most common thing which we look at, like swelling; there is also response with respect to gelation and so on. In some cases, the gelation itself is dependent on how well it responds to stimuli.

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Environmental stimuli	Hydrogel	Mechanism	Applications
Electrical signal	Polyelectrolyte hydrogel	Change in charge distribution causes swelling and drug release	Actuator, artificial muscle, on/off drug release.
Thermal	Thermo-responsive hydrogel, e.g., poly (Nisopropylacrylamide)	Change in polymer–polymer and polymer–water interactions cause swelling and drug release	On/off drug release, squeezing device
pH	Acidic or basic hydrogel e.g. PAA, PDEAEM	Ionization of polymer chain upon pH change; pH change causes swelling and release of drug	pH-dependent oral drug delivery
Magnetic fields	Magnetic particles dispersed in the hydrogel matrix	Applied magnetic field causes pores in gel and swelling followed by drug release	Controlled drug delivery while the magnetic particles used form medical therapy.
Ionic strength	Ionic hydrogel	Change in concentration of ions inside the gel cause swelling and release of drug	Biosensor for glucose, used for medical therapy
Chemical	Hydrogel containing electron-accepting groups e.g. Chitosan -PEO	Formation of charge-transfer complex cause swelling and release of drug	Controlled drug delivery
Enzyme–substrate	Hydrogel containing immobilized enzymes	Product of enzymatic conversion causes swelling and release of drug	Modulated drug release in the presence of a specific antigen; sensor for immunoassay and antigen.
Light	Copolymer of PNIPAAm	Temperature change via the incorporated photosensitive molecules; dissociation into ion pairs by UV irradiation	Optical switches, ophthalmic drug delivery

Table from doi: 10.1002/9781118774

So, these are some of the common environmental stimuli and the type of hydrogel which can respond to these kinds of stimuli and the mechanism and its applications. So, when you have a hydrogel which can respond to electrical stimuli, it would usually be a polyelectrolyte hydrogel. When you have a polyelectrolyte hydrogel, there are ions that can respond to the external electrical stimuli.

When you have a change in charged distribution, it can cause swelling or drug release or biomolecule release in case of tissue engineering. This is used in the case of actuators, for engineering artificial muscles and also as on-off drug delivery vehicles.

With respect to thermal properties, they are called as thermoresponsive hydrogels. These thermoresponsive hydrogels respond to a particular temperature. Even gelation can be dependent on temperature. And the change in polymer-polymer and polymer-water interactions happens at specific temperatures, and this causes either swelling or shrinkage, which will lead to drug release or biomolecule release.

This again is used for on-off drug release and also for squeezing devices, in the sense that when the temperature changes instead of swelling, it just shrinks. It will just squeeze out whatever is present inside. So, that kind of mechanism has been used.

One of the common thermoresponsive hydrogels which we will talk about is poly N-isopropylacrylamide, it is also called as PNIPAAm. This is very well studied because of

the temperature at which it goes through these changes. So, we will go into details of that in the next few slides.

pH plays a crucial role. In your body, your pH is not the same. So, your pH can be different based on which part of the body you are talking about and which state of homeostasis your body is in. Under normal condition, your physiological pH is around 7.4 but does not mean that your pH is always 7.4. In case of injury or something, your pH will go down significantly. And, in certain regions in your body, like your stomach, your pH is significantly lower.

Using this difference to deliver molecules or cause gelation has been extensively studied. Acidic and basic hydrogels are the most common examples; polyacrylamide and PDEAEM are all molecules that have been shown to have pH-responsive properties.

Student: Sir, are these smart hydrogels made from special polymers, or can regular hydrogels be modified to have smart properties?

They have to have some monomer or a side chain that will respond to these stimuli. If you were just to take PVA, a regular PVA hydrogel cannot behave like that. But if you can create side chains which can provide stimuli responses, then you can do it. So, which would mean you are modifying PVA into some other polymer before you do it.

Student: Will the surface modification help?

For a hydrogel, a surface modification would not be very helpful because hydrogel is the 3D structure. So, surface modifications are usually done for polymers that are being implanted. Whereas, in the case of polymers, which are going to be fabricated into something else, then you need a chemical modification of the entire thing. A surface modification alone cannot address the required needs.

What happens in a pH-responsive hydrogel is the ionization of the polymer chain occurs due to pH change, and this leads to swelling and further release of the molecule which you have loaded. So, this is primarily used in pH-dependent drug delivery systems.

Magnetic fields have also been explored as external stimuli. Magnetic particles that are dispersed in a hydrogel matrix can cause this kind of a magnetic response. The advantage of doing this would be by applying magnetic stresses; you might be able to alter the

pores in the gel or cause swelling, which will lead to the drug release. This can be used as an on-off drug delivery system. On-demand, you can provide a magnetic field to release the drug molecule, and otherwise, it will not be delivered. This kind of thing is extensively studied for cancer therapy, and so on.

Ionic strength has also been used. Here, depending on ionic strength, you will be able to control the release. And this is primarily used as biosensors for glucose, and people have tried to use this to sense glucose and then supply insulin and so on.

Chemical stimuli occur in the presence of other electron-accepting groups; there could be other chemical molecules that can trigger the swelling or change the physical properties leading to the release of the molecules loaded.

Enzyme responsive or bioresponsive hydrogels are the ones where there is an enzyme-substrate correlation. You would have hydrogels that are immobilized with the enzymes, and the production of this enzymatic conversion will lead to swelling or release of the molecule. It could also be the other way, where the enzyme acts on it for degradation to have it release. Your hydrogel could be a substrate for the enzyme, as well.

Light is also used for delivery. So, using optical or other light waves like UV radiation; to see whether that can alter the crosslinking of the hydrogels thereby you can change how the gelation happens and how the release happens, and so on.

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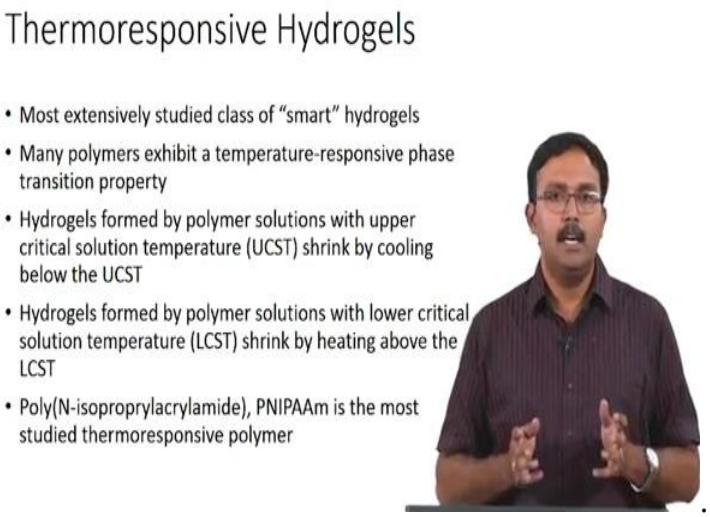

Thermoresponsive hydrogels are the ones that have been extensively studied. Many polymers exhibit thermoresponsive phase transition property. These polymers have something called UCST or LCST. UCST is the upper critical solution temperature, and LCST is the lower critical solution temperature. When a polymer exhibits a UCST, what happens is, it shrinks when you cool it below UCST, and it is present us chains when it is above UCST. And the other way around happens when the polymer has LCST.

Poly N-isopropyl acrylamide or PNIPAAm is one of the most commonly studied thermoresponsive polymers. Because it has a thermally reversible property with an LCST at 32°C in pure water.

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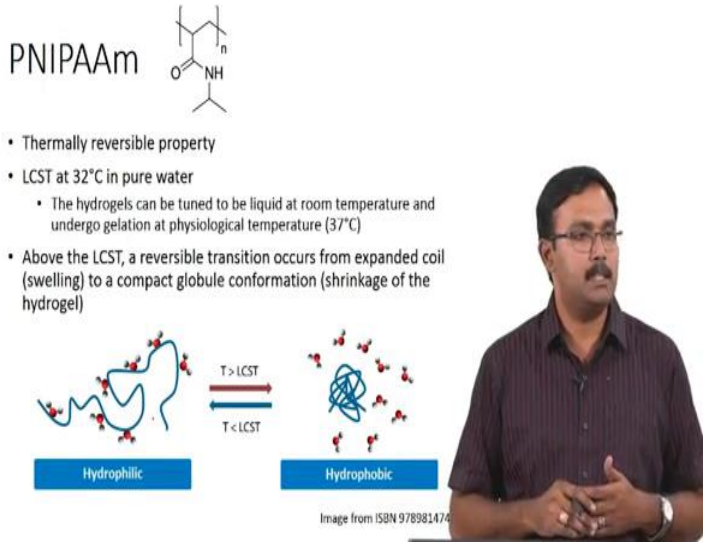

This 32°C is very close to your physiological temperature of 37, so that makes it an ideal candidate to work with. At room temperature, you are talking about 25°C, and at the physiological temperature, you are talking about 37, and this comes in between these two temperatures. So, this gives us a nice property which can help in using this thermoresponsive behavior in physiological conditions.

Above the LCST, what happens is, a reversible phase transition occurs, and the expanded coil is seen in the hydrophilic environment. So, what you see here, the expanded coils; when you increase the temperature above the LCST, it goes into a compact globule formation, which causes the shrinkage of the hydrogel. So, whatever molecule is loaded inside will get released. Thereby, this squeezing kind of effect is there for the molecule

to be released immediately. So, this is a thermoresponsive hydrogel that has been extensively studied in many different applications.

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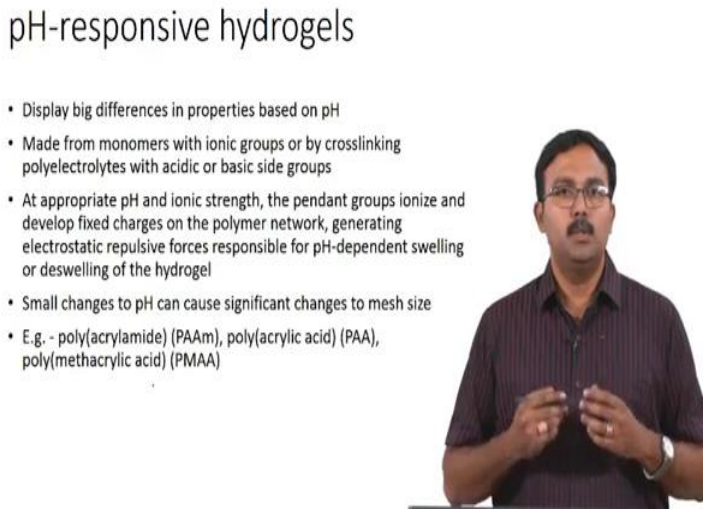

pH-responsive hydrogels display big differences in properties based on the pH itself. They are made from monomers that have ionic groups or using crosslinking with polyelectrolytes. Even with PVA, you can prepare it to be a polyelectrolyte. Depending on how you do the crosslinking, you can create a polyelectrolyte nature for the hydrogel, and that can help in pH-responsive behavior.

At appropriate pH and ionic strength, the side chains ionize to develop fixed charges. And this can cause either repulsive forces or attractive forces. Depending on whether it is attractive or repulsive forces, there will be swelling or de-swelling of the hydrogel. Even small changes to the pH can have significant effects on the mesh size. When the porosity changes, obviously your diffusion properties are going to change, and that will mean your release will also change.

Some of the common hydrogels which are used are polyacrylamide, polyacrylic acid, polymethacrylic acid, and so on. So, these have shown to have pH-responsive behaviors.

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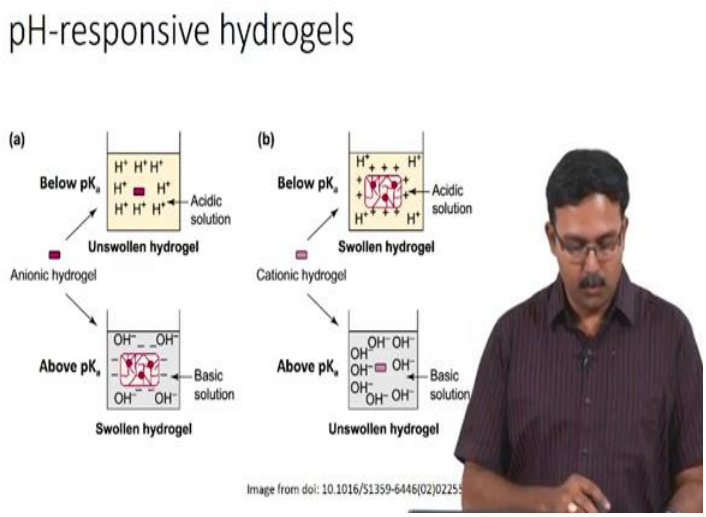

So, this is what happens. You can either have an anionic hydrogel or a cationic hydrogel. You can start with any ionic hydrogel. An anionic hydrogel below its pKa, basically stays in an unswollen form, and above it is pKa, it starts swelling, and it starts releasing whatever molecule would be present at a much faster rate.

Even in the unswollen state, there will be some leakage of the material; it will be only diffusion, and it will be a much slower diffusion because the mesh sizes are very small. In case of a cationic hydrogel, you will have the reverse, where below pKa, you will have the ions being observed. And therefore, the hydrogel being swollen and the drug being released.

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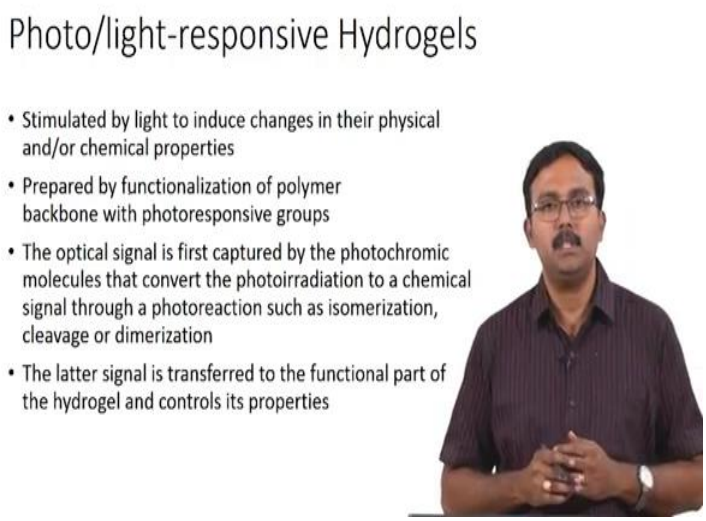

Photo or light-responsive hydrogels are stimulated by light to induce changes in their physical and chemical properties. This is prepared by the functionalization of polymer backbone with photoresponsive groups. Any polymer chain which has functional groups, you can add these photoresponsive groups, which will respond to different wavelengths of light.

The optical signal is first captured by the photochromic molecules, which are the side chains, and this converts this photo-irradiation to a chemical signal either through isomerization or cleavage or dimerization, which leads to different properties. One of the common parameters which are usually affected by light is the gelation itself. This photoresponsive hydrogel will form gels in the presence of a light source whereas, in the absence of it, it would not form these gels; it will just be in a liquid form. This signal, which is formed, is transferred to the functional part of the hydrogels, which then controls its properties.

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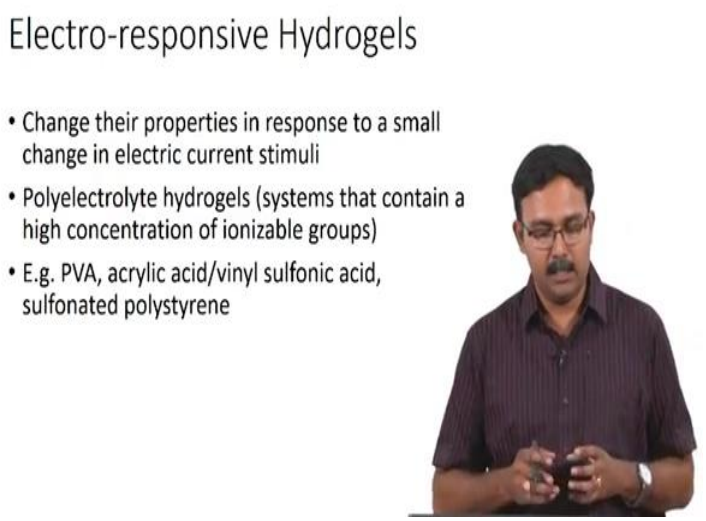

There are other hydrogels, as well, like electro-responsive hydrogels. These change their properties in response to changes in electric stimuli. Polyelectrolyte hydrogels are the ones that can be used for these because they have a high concentration of ionizable groups. So, PVA, acrylic acid, vinyl sulfonic acid, and sulfonated polystyrene are all some of the examples which have been prepared as polyelectrolyte hydrogels.

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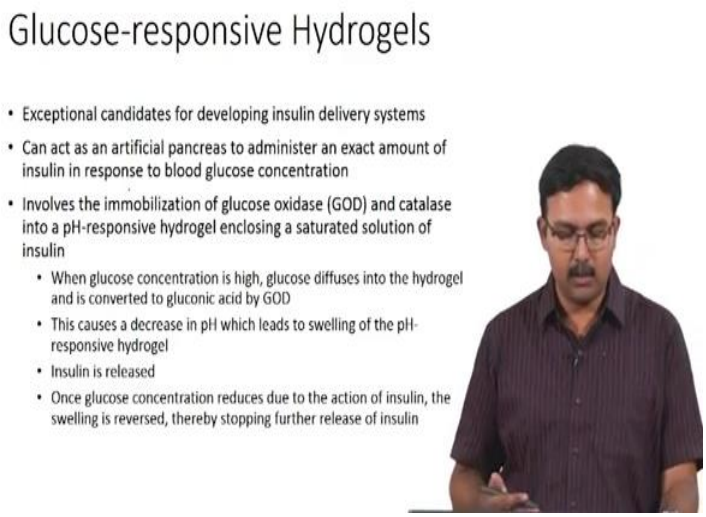

Another interesting class of hydrogels is the glucose-responsive hydrogels. These are interesting candidates because they can be used for insulin delivery systems. So, what

happens is, these act as the artificial pancreas, and they can swell in response to the presence of glucose. They will release insulin when there is glucose, and they will shrink again and stop the insulin release when there is no glucose.

So, what happens is you have to immobilize the glucose oxidase and a catalase into a pH-responsive hydrogel. And this is enclosed in a saturated solution of insulin. Now, insulin is loaded into the hydrogel that can sense glucose and respond to pH environments. When glucose concentration is high, the glucose will diffuse into the hydrogel, and it is converted to gluconic acid in the presence of glucose oxidase. Because this gluconic acid is formed, the pH is going to drop. This decrease in pH will cause swelling of the pH-responsive hydrogel. So, insulin is released. And once the glucose concentration reduces because of the insulin which is being released. So, there is no gluconic acid. So, your pH is going to increase, leading to the shrinkage of the hydrogel. Thereby, it does not release any more insulin. This is an excellent candidate that people are exploring for such demand-based insulin release. There are some publications on it, but I do not think it has gone to the clinical trial stage yet.

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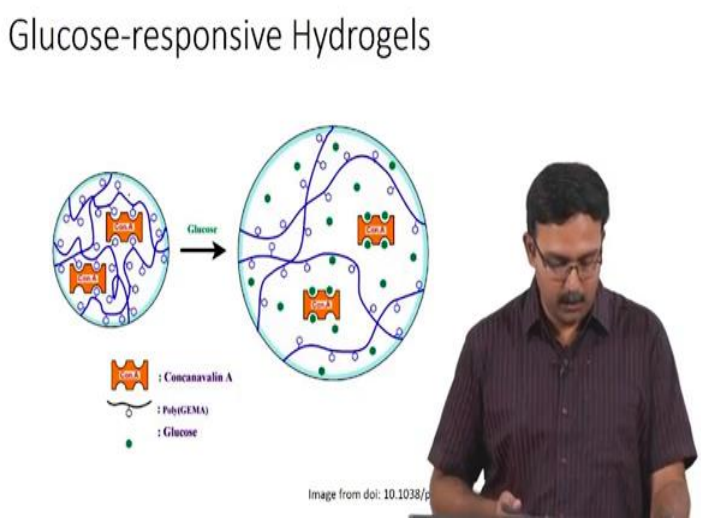

This is how it works. So, this is from one of the recent papers. What they have done is, loaded, something called Concanavalin A and in a poly GEMA hydrogel. When glucose is present, it results in swelling, and that releases whatever molecule loaded to it.

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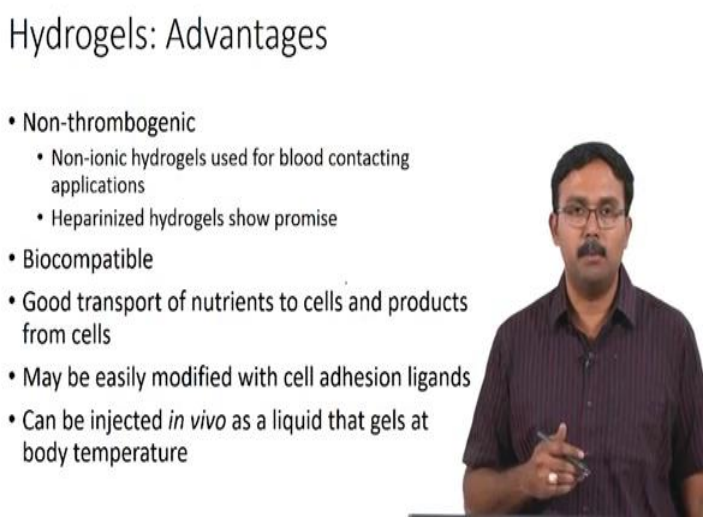

So, why hydrogel? Whatever we looked at with respect to hydrogels still now we looked at all the basics of hydrogels, we saw why hydrogels are used and what are the different applications which they have used in. We have not specifically looked at tissue engineering; we just looked at multiple things where hydrogels are used and multiple types of hydrogels. So, what do you think are the advantages of hydrogels?

So that is one thing. You can easily engineer it. So, there is enough variability to it that you can engineer it for desired physical properties.

Student: ECM, wound healing.

Similar to ECM.

Student: Wound bandages something like that.

Wound bandage would be an application where you can use hydrogels; why do you think it can be used for wound bandages?

Student: Because we can put a drug in it; antibiotic or antibacterial.

Yeah.

Student: Drug in it to prevent infection.

That is one thing, and moist is another thing. Providing a moist environment is important for wound healing, and also it can absorb the wound exudates. The wound exudates many a times can have matrix metal proteases, which will degrade some of the matrices which are being formed. So, it is better to have these absorbed. Depending on the level of exudates, having hydrogels that can swell moderately or significantly can be chosen. So, it is similar to ECM, and that is one thing.

Student: Variation and structural properties might it have various structure-property.

Ok, you can prepare different kinds of; yeah sure. So.

Student: Sir, dynamics like swollen states.

Ok. So, it can be responsive to the environment. That is one advantage of using something like this. So, what I have here is, some of these things are there. The swelling is an important factor that actually helps in the transport of nutrients and the removal of toxins. Easily modified with cell adhesion ligands; so, that is the engineering part of it. You have a lot of options to change it, and non-ionic hydrogels are usually non-thrombogenic. So, this is not something we specifically talked about. However, ionic hydrogels are thrombogenic. So, it can actually trigger blood coagulation.

If you were to use calcium alginate, it would trigger blood coagulation. But, if you are using something which does not have ionic properties, it is non-thrombogenic, which means it will not cause blood clotting, which is a good thing for blood-contact applications. It is usually biocompatible and similar to the ECM and so on.

You also have injectable hydrogels. So, we did not talk about it in detail. Even the thermoresponsive hydrogels, if I am going to look at it from an application standpoint, I can have it as a liquid at room temperature at 25°C. And if I inject it into your body, in your body, it will form a hydrogel. So, it will just be a simple injection for me. Hence, site delivery is actually easier.

If I were to give an injection with a liquid, usually what will happen? It will get dispersed all over your body, which is not a good thing. Here it will form a gel, and it will stay there. If you are talking about drug delivery to a specific site or if you are forming a scaffold that has specific site where cells can migrate. So, this is not going to

get leached out. It is going to form the hydrogel there. So, those are some interesting applications for injectable hydrogels.

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What you think would be the disadvantages?

Student: Mechanical properties.

Yeah, the mechanical property is one serious problem. You saw some of these hydrogels, which are really soft right. So, they are like a sponge. Hence, mechanical properties can be an issue. What else?

Student: For the injectable one, you cannot really control this scaffold geometry.

Ok, yeah; so, it will depend on the site of injection. It will just form a mold kind of thing, but yeah, you would not have proper control over it.

Student: Sir, I cannot really imagine this injectable hydrogel, I mean, where do you inject it?

To the site where you want to inject it; See, not all injections are just over your skin right. You might have injections that will reach much deeper into your body. So, if I want to create an injectable hydrogel and use it for making a liver, I would inject it into the portal vein of your body. So, I probably use a bigger gauge needle and inject it to the site where I want. Injection just means minimal penetration, instead of cutting a person

open, I can just use an incision, small incision kind of thing. So, that is what it is. It is not always mean the small needle, which we are talking about ok.

Student: Sir, we just if the smart hydrogels which are there, they respond to a particular condition, but is there is any cap over how much, like an upper limit kind of thing over this limit they did not respond over. They would not respond to a particular concentration of the local application range.

Yeah, obviously, there will be some effect, up to a certain level, they will swell, beyond a point they cannot swell right. So, it will stop. If you keep increasing glucose concentration, it is not going to keep swelling continuously. It will have to stop at some point. Like for, glucose as an example. Even if you have other things, there will always be some range within which they will work, and that will depend on the material itself.

Student: Can we degrade it in the body?

Yeah degrading; yeah, it can get degraded, but degrading is not a very bad thing as long as you can control the degradation, it is not a bad thing.

Student: If hydrophilic, will cell adhesion be the problem?

If it is very hydrophilic, cell adhesion can be a problem, yes. But people, what people do is they try to attach ligands to it and use proteins which have these cell adhesion ligands along with the material and so on, those are ways to overcome it.

A couple of things are, they are difficult to handle, and sterilization can actually affect its properties. These hydrogels are quite fragile. And they need to be sterilized properly, and you cannot put it at 121°C for 45 minutes and hope it will survive.

But, there are ways to sterilize it, and when you are sterilizing it, one of the things would be UV sterilization. But UV sterilization you might not be able to get to the pores because it is a 3D structure, and UV sterilization might not be the most effective. There are other things like ETO sterilization, where gas is pumped through this. There are other ways to do it, but it gets tedious, and you do not know how that affects the chemical and mechanical properties of the hydrogel. So, that can be a challenge with respect to hydrogels.

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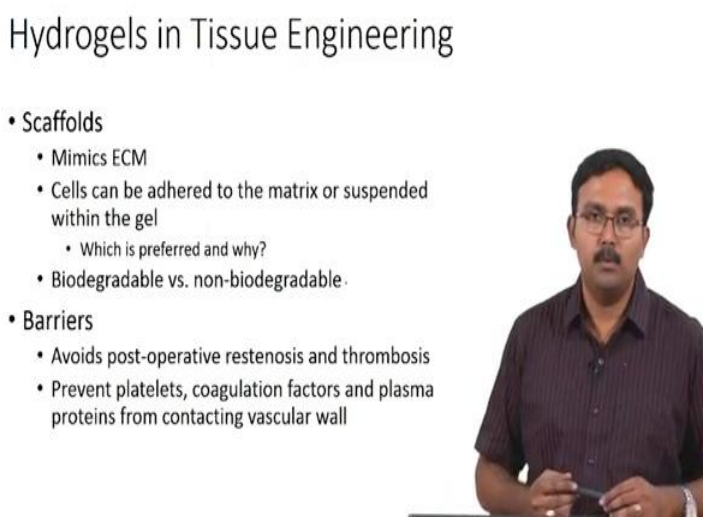

When you are talking about hydrogels in tissue engineering, they are used primarily as scaffolds because they mimic ECM. Cells can adhere to the matrix, or they can also be suspended within the gel. So, that is why you can have injectable gels in which cells are suspended.

For example, if I am going to inject cartilage, I can have an injectable hydrogel, which is in liquid, and cells are suspended in this liquid. And, when I inject it, this hydrogel will form into a gel upon injection in your body when the temperature is higher than your LCST in your body. This will now have cells seeded to it. So, I can have it suspended within the gel as well. So, which one would you prefer if I were to give you the option which do you think you would prefer and why?

Student: Suspension will have uniform gel distribution of system, cells, especially for proliferation involved.

So, the suspension would probably have more uniform cell distribution fine. So, what would be the advantage of cells adhering to the matrix?

Student: Better cell proliferation.

Ok. Why do you think better cell proliferation?

Student: Because of better cell adhesion.

Better cell proliferation is possible, but not because of better cell adhesion, but primarily because when you are seeding a cell on top of it, the cells can get the nutrients more easily. And, they are also nicely adhered and spread on the surface. Whereas, when you are suspending it within the gel, there is no guarantee that they will have the nutrients, and they might not have got the morphology which they need for the proliferation and migration, ok. What would be the disadvantage of seeding it on the matrix other than non-uniform?

Student: It can get crowded.

What do you mean it can get?

Student: So, cell proliferates so much there in one place the nutrients will not be enough for it to proliferate more.

Ok.

So, you mean overcrowded in that region, because of proliferation. Ok, usually, cells do not divide that rapidly. And especially, in vivo, the cells will not divide that rapidly when you have other factors coming in. But the problem usually with adhered thing is cells can leach out; cells can migrate out of the matrix ok. So, when you are placing it in vivo, the cells can migrate out of the matrix, whereas in suspended, it is not going to leach out very easily. But, yeah, the disadvantage would be it may not get the nutrients depending on the size of the hydrogel and how the hydrogel properties are, yeah.

You can have hydrogels as both biodegradable and non-biodegradable hydrogels. Depending on how these hydrogels are fabricated you can have them both ways. And, they are also used as barriers where it is used for preventing postoperative restenosis and thrombosis. When there is blood coagulation, these non-ionic hydrogels can prevent this blood coagulation, and this prevents the platelets and coagulation factors from contacting the vascular wall.

The ruptured vascular wall will trigger the coagulation cascade. By using these as barriers, you can prevent the platelets from contacting the ruptured wall, and these being non-thrombogenic will not trigger any blood coagulation cascades. So, that is one way it can be used.

Student: Is the stent itself made up hydrogel or just by adding the hydrogel with the stent.

Restenosis is not only for the stent, ok so, even with post-operative restenosis can happen with any other.

Student: Surgery.

Yeah. So, any surgery.

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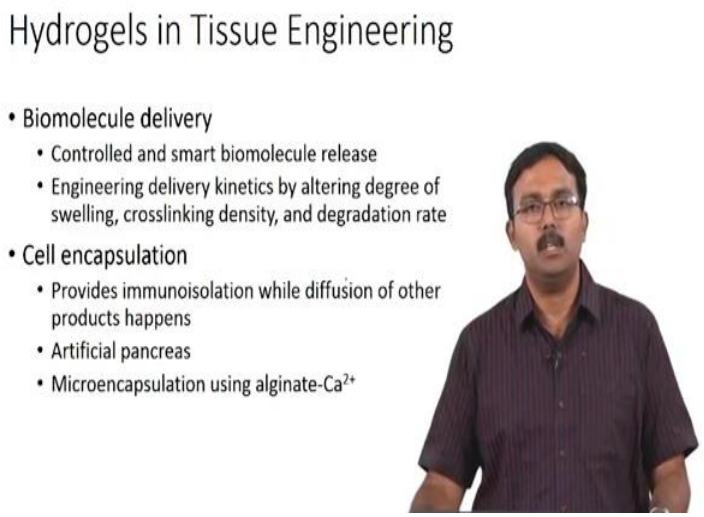

In tissue engineering, it is also used for biomolecule delivery. Drug delivery is one aspect, but concepts of drug delivery are used in tissue engineering with respect to the delivery of biomolecules. Different biomolecules which have different specific applications like growth factors and other proteins can be loaded to these hydrogels so that it can be released to provide desired signals while the hydrogel is being prepared.

Thee rate at which it is delivered or when it gets delivered, or the environment in which it gets delivered can all be controlled when you design the hydrogel appropriately. Cell encapsulation is something that I talked about where you can actually suspend the cells, and you can encapsulate the cells.

The advantage here is, it will provide immunoisolation while diffusion can still happen. One application where this is used, this would be useful is in an artificial pancreas. People have tried simple microencapsulation using calcium alginate beads where they

have just suspended Islets in calcium alginate beads and shown that it can be reasonably effective; however, it still not completely successful. That is one of the things which people are working on.

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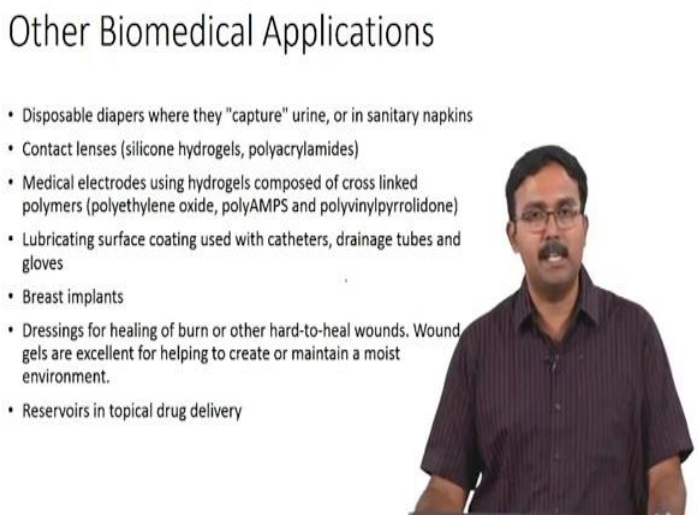

These are a bunch of other biomedical applications in which hydrogels are used. Disposable diapers where they capture the urine is one of the common things. So, you would see a used diaper quite thick compared to the original diaper that is because it can hold a lot of the water. Contact lenses are made of hydrogels. And medical electrodes, you have other coatings for lubricating the surfaces and your breast implants, wound healing materials, these are all hydrogels, and they are also used as reservoirs for tropical drug delivery and so on.

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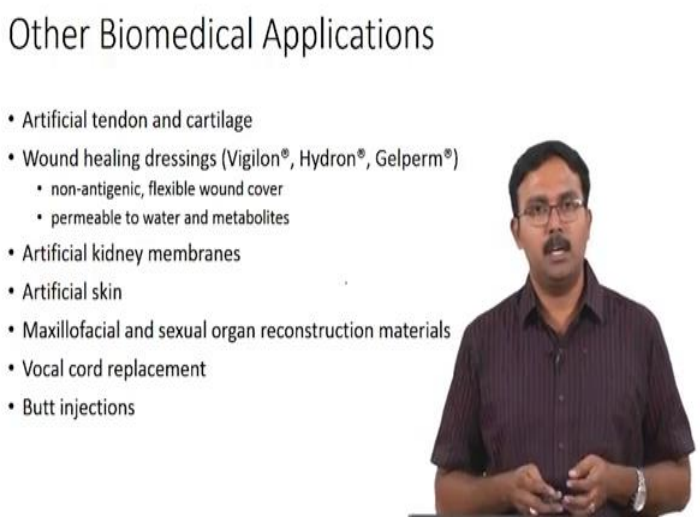

Currently, there are some hydrogels that are commercially available for biomedical applications. With respect to wound dressing materials, there are quite a few which are hydrogels that are commercially available Vigilon, Hydron, and gel Gelperm are some of the more popular ones.

And, people have been exploring these hydrogels for many many applications like kidney membranes, artificial skin, reconstruction of plastic surgery kind of things and even for vocal cord replacements and just like how you can have breast implants other implants and injections these are all different types of hydrogels which have been tried out, ok. So, even these Botox injections, all these things are kinds of hydrogels which have been tried out.