Tissue Engineering
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Lecture - 07
Hydrogels - Part 1

Today I will talk about Hydrogels. I hope you are familiar with what hydrogels are, but we will discuss them with a context of tissue engineering. We will first look at the fundamentals associated with hydrogels, and we will discuss as to how we can prepare these hydrogels and what materials are used for these and so on. So, what are hydrogels first?

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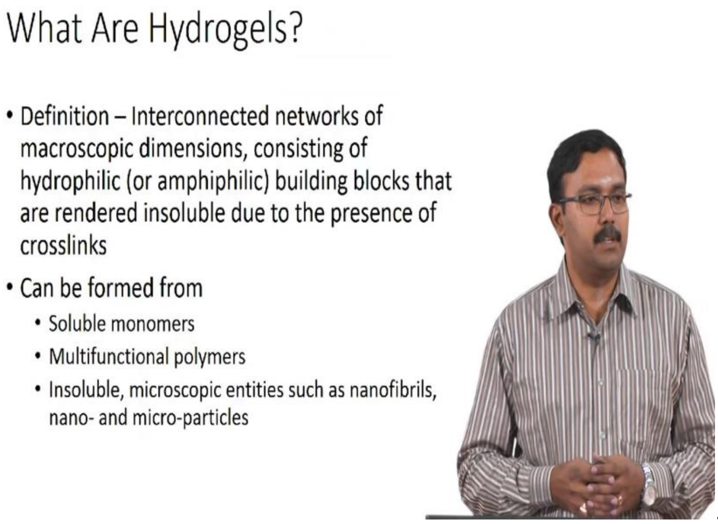

Student: Substance that retains water, moisture.

Yes, as long as it can absorb and retain the water, it is a hydrogel. That is the basic requirement for something to be a hydrogel. It is properly defined as interconnected networks of macroscopic dimensions, which consists of hydrophilic or amphiphilic building blocks that are rendered insoluble due to the presence of crosslinks. The initial monomers or the building blocks which are used are soluble in water. But you create crosslinks, and as the degree of crosslinking increases, at some point, this macroscopic material becomes insoluble in water, and that is called a hydrogel.

This kind of network can absorb and retain a lot of water. These can be formed from soluble monomers or multifunctional polymers. You can also use insoluble microscopic entities such as nanofibrils or nano and microparticles. Which would have initially been formed using hydrophilic molecules and you have these nanostructures or microstructures which can then be fused or interacted together to form hydrogels.

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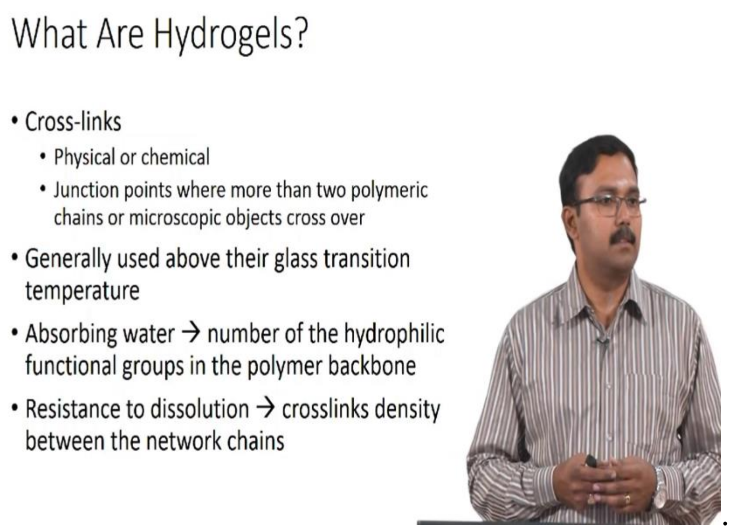

Student: With what does it form crosslinks with?

We will discuss that. So, crosslinking is one of the major aspects when it comes to creating hydrogels. It depends on the polymer you are using, and there are different types of crosslinking. We will talk about that in this lecture. Crosslinks are physical or chemical; these are technically the junction points where two polymeric chains or microscopic objects crossover.

The hydrogels are generally used at a temperature higher than the glass transition temperature. So, what is the glass transition temperature? Polymer chemistry, you would have studied glass transition temperature or even basic material science, material technology courses. Ok, use English to define what it could be, glass transition temperature? Ok above this temperature, the material will behave like a rubber whereas, below it, it will be solid; it will be like glass.

From glassy stature, it will move to a rubbery stature. That transition happens at a temperature called as a glass transition temperature. Hydrogels will be used at temperatures higher than the glass transition temperature so that it will have a rubbery texture. If it is below the glass transition temperature, it would be glassy, and it cannot be used in a biomedical application.

They can absorb a lot of water because there are many hydrophilic functional groups on the polymer backbone, which is used for preparing these hydrogels. And the resistance to dissolution itself happens because of the crosslink density between the network chains.

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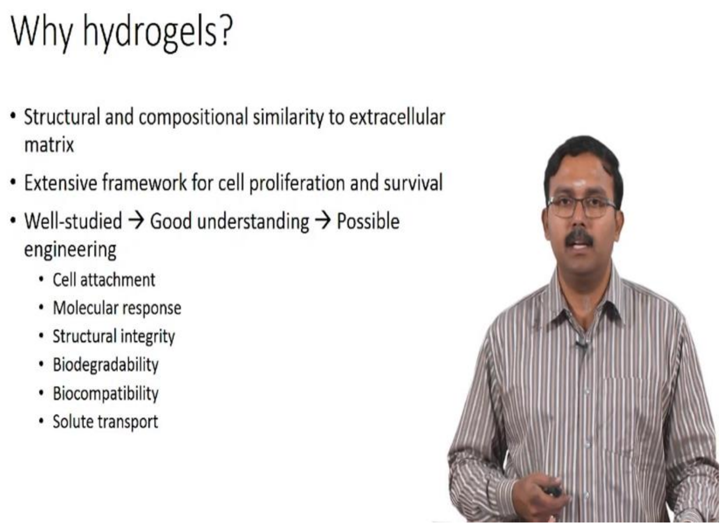

So, you have many crosslinks as I was saying. As the crosslinking increases, you create an insoluble hydrogel which can withstand the environment. So, why should we use hydrogels? We have talked about a few scaffold fabrication techniques. I specifically am discussing hydrogels. Why do you think hydrogels are important? This is one of the most well-studied systems when it comes to tissue engineering scaffolds. Why has this attracted so much attention?

Ok yeah, It can be used for drug delivery. I am asking about in tissue engineering, why do you think hydrogels are important?

Student: Ok in wound healing, the moist environment provides.

Ok, so the application of wound healing, it will provide a moist environment which can help in the healing process. But that is not the only application where hydrogels are used; it is used in other tissue engineering applications as well. Can you think of reasons why? So, the idea is the anytime we use a material, the goal is to emulate what is there in nature right. The hydrogel has a structural and compositional similarity to ECM. So, that is why hydrogels are preferred. The ECM actually behaves like a hydrogel it looks and has the properties similar to that of a hydrogel.

It also has an extensive framework for cell proliferation and survival. Any time you use a scaffold, it should be able to support cell adhesion and cell proliferation. So, hydrogels usually have good ability to do that. They are also well studied; there is a good understanding. There is potential for engineering this so that we can improve cell attachment, molecular response, structural integrity, biodegradability, biocompatibility, and solute transport.

These are crucial factors because for example, with respect to solute transport; if you have a scaffold which is a 3D structure, even without vascularization; there has to be some amount of solute transport, only then the cells can survive at the initial phase.

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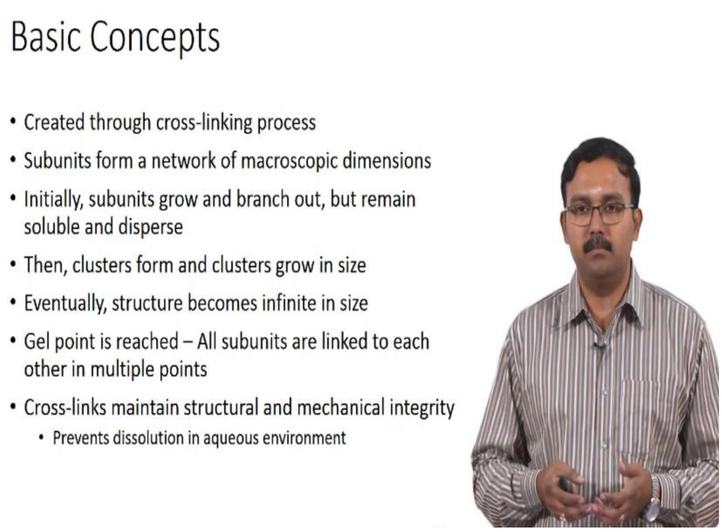

Assuming that vascularization eventually forms, you still need to have efficient solute transport initially. For that reason, hydrogel would be a good material where you would have very rapid solute transport. So, that helps the cells which are seeded. As I said, hydrogels are created through the crosslinking procedure. So, what happens is the subunits form a network of macroscopic dimensions. Initially, the subunits start growing and branch out but remain soluble, and they are dispersed in the solution. But as the number of crosslinks increases, you are going to have clusters which are forming and these clusters are going to grow in size.

Eventually, these clusters become infinite in size; in the sense that they start interacting enough that they will form something like a gel. At this point, gelation happens. So, this is called the gelling point. This gelation could happen due to different reasons; it could be because of the polymer concentration itself or the crosslinker concentration, temperature, or pH. There are many factors which can actually trigger gelation. The factor which triggers gelation probably depends on the type of polymer you are using and the type of crosslinking you are going to be performing.

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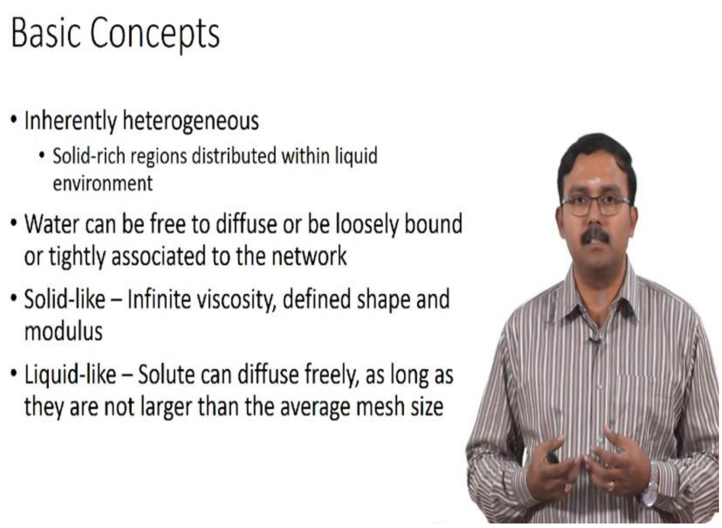

All subunits are linked to each other at multiple points so that there is a strong interaction between these subunits, which have been used for preparing these hydrogels. The crosslinks ensure that they maintain the structural and mechanical integrity and prevents dissolution in an aqueous environment. The hydrogel is inherently heterogeneous. When you put it in a water medium, it is going to have solid-rich regions which are distributed within a liquid environment. Because when you have a hydrogel what happens is, it will actually swell significantly when you put it in water.

There are going to be regions where the polymer is present, which should be the solid-rich regions, and the gaps are going to be filled by the water or buffer or media, which you are using. So, that is going to be the liquid environment in which this solid is present.

Water can freely diffuse or be loosely bound or tightly associated to the network depending on where it is present. If it is present close to the points where it can form hydrogen bonds, then it will be very strongly bound. It could just be loosely bound like what is being adhered to the surface, which can just be blotted off with the paper. It could be freely diffusing through the pores which are present in the hydrogel.

This actually behaves like a solid because it has infinite viscosity. It would not flow; so, the hydrogel will not flow. It would have an infinite viscosity, and it also has defined shape and modulus. So, this makes it a solid, but it also has liquid-like properties because solute can diffuse freely, and this helps in solute transport. The only condition is the solute size has to be smaller than the average mesh size.

Mesh size would be the size between these crosslinks, not exactly the pore size, but like size between the crosslinks, ok. You might have a nonporous hydrogel as well. It is possible to prepare non-porous hydrogels. Hydrogels can have different porosity. So, mesh size is not purely dependent on porosity; it is dependent on the crosslinks.

Student: Given that it is heterogeneous, both the mesh size varies from place to place.

Yes, it will. That is why I said average mesh size. It will be different, but you can prepare it with reasonable distributions.

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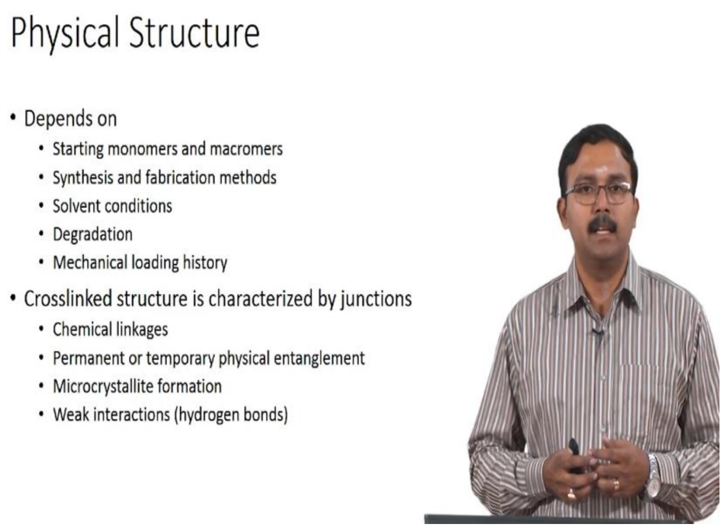

If you are going to have chemical crosslinking. For example, depending on the functional group's density; you are going to have a similar crosslinking. So, you can alter crosslinker concentration to get uniform crosslinking and so on.

The physical structure itself depends on the starting monomer or the macromer which you are using. It also will be defined by the synthesis procedure and the fabrication methods that you are going to be using. The solvent conditions in which these hydrogels are prepared will also influence the structure of this hydrogel.

After your hydrogel is prepared while you are using, there is going to be some amount of degradation and there is going to be mechanical loading depending on where you are using it. Even for cartilage, people use hydrogels. So, people use different kinds of hydrogels for cartilage repair. There you are going to have significant mechanical loading. This is going to cause damage to the tissue and the hydrogels. So, that could actually change the physical structure of the hydrogel.

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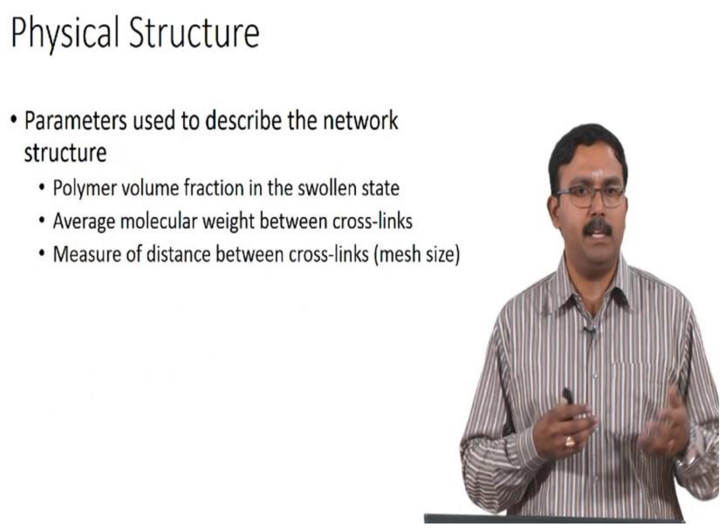

The crosslinked structure is characterized by the junctions; chemical linkages or permanent or temporary physical entanglements or micro crystallite formations or weak interactions like hydrogen bonding. These are all the different things which can be present as the crosslinking structure.

When you are talking about the hydrogel network; there are different parameters which are usually described. The three parameters which are very commonly described;

The polymer volume fraction in a swollen state. So, when you take a hydrogel and immerse it in PBS or water, it is going to swell till it reaches equilibrium. At the point of equilibrium, you are going to have some fraction which is the polymer, and the rest is going to be water. Depending on the water fraction and the polymer fraction, you will know what the swelling of the material is. So, this is one parameter which is used.

Average molecular weight between the crosslinks is another parameter which is used to define the density of crosslinking, and the other aspect is the measure of the distance between the crosslinks, which is the mesh size. This will help us in understanding what solutes can pass through the hydrogel and what cannot. These are the parameters which are used.

While I was describing this, I said that swelling reaches in equilibrium, right?. Why do you think it reaches equilibrium? Why not just keep swelling? When a hydrogel is placed in water; let us say, there is an excess amount of water. Let us say, I take 10 grams of a hydrogel and place it in 500 kilograms of water. There is a lot of water it can absorb. Why does not it keep absorbing it? What is actually preventing it?

Student: It has limits.

Yeah.

Student: Into wide water-saturated.

So, it has limits for the points where it can actually adhere to water. Also, there are forces which act against swelling. The number of crosslinks is going to prevent the hydrogel from swelling.

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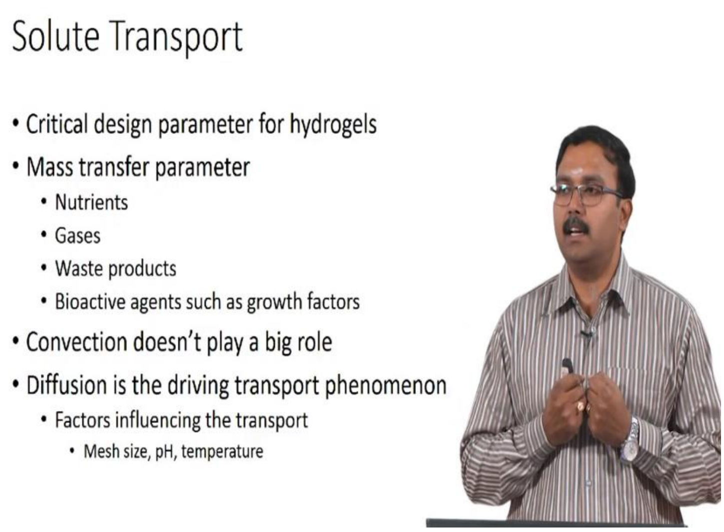

You are going to have water which is coming in, which is forcing the hydrogel to swell and the crosslinks which are trying to hold the hydrogel together. These two are counteracting forces, and at some point, these forces are going to reach equilibrium. That is also going to play a role in how much the hydrogel can swell.

Solute transport is one of the most critical parameters which is used for designing a hydrogel. This defines the mass transfer parameters for nutrients, gases, waste products, bioactive agents, and other molecules which should be involved during cell culture and cell growth. In a solute transport convection usually does not play a role because when you keep this in a physiological environment, you are not going to have flow in this direction, right. So, convection will not play a major role unless if your pore size is very large. If your pore sizes are very large, then there can be convective forces within these pores, even when you are in a static environment. So, diffusion is the driving transport phenomenon.

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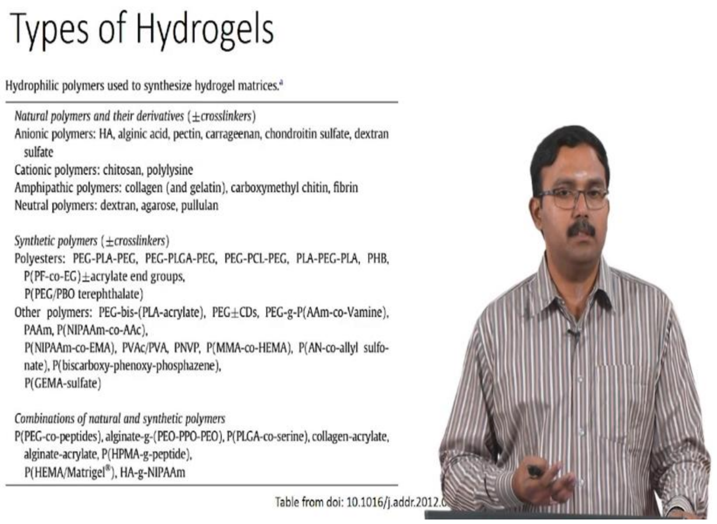

And there are different factors which can influence diffusion; mesh size, pH, and temperature right. So, all these can affect the diffusion parameters, which helps the solute transport. This talks about the types of hydrogels, and we will look at some of the polymers which are used for preparing natural and synthetic hydrogels.

This is not a comprehensive list. You can prepare hydrogels using many many types of polymers as long it is hydrophilic, and it can absorb and retain water. The only thing would be the optimization of the crosslinking procedure to form the stable hydrogel.

What I have listed here is just a brief introduction for what are all the materials that are used. Basically, you can classify them as two things; the natural polymers and the synthetic polymers that are used. Natural polymers such as hyaluronic acid, alginate, pectin, carrageenan, chitosan, polylysine, collagen carboxymethyl chitin, carboxymethyl cellulose, dextran, agarose, pullulan; all these have been studied for preparing hydrogels. For different applications, people have tested these. People have also used these along with the synthetic polymers to get desirable mechanical properties and degradation properties.

Amongst these, they have classified as anionic, cationic, amphipathic and neutral polymers depending on the charge of it. The anionic polymers would be hyaluronic acid and alginic acid or alginate and so on. And you can have cationic polymers like chitosan which also can be used for preparing hydrogels.

These charges play a role when it comes to cell attachment and cellular interactions. You can design hydrogels using these. You also have neutral polymers like dextran, agarose, and pullulan, which have also been used for preparing hydrogels. When it comes to synthetic polymers again, the list is endless. So, whatever is shown here is a very small fraction of what you would find in literature, you can use many many things.

Polyethylene glycol is one of the more well-studied hydrogel systems; so, is poly HEMA. These are materials which have been studied extensively for different applications, and people have shown the properties of hydrogels using these materials. Combinations of natural and synthetic have also been studied and here are just a few which have been studied and which you can find in literature. In natural polymers, the other way to look at the categorization would be to talk about what type of a molecule it is. You have protein-based molecules and polysaccharide-based molecules which can be used. Whatever they have listed here some are polymers, but collagen is a protein, polylysine is a polypeptide and so on.

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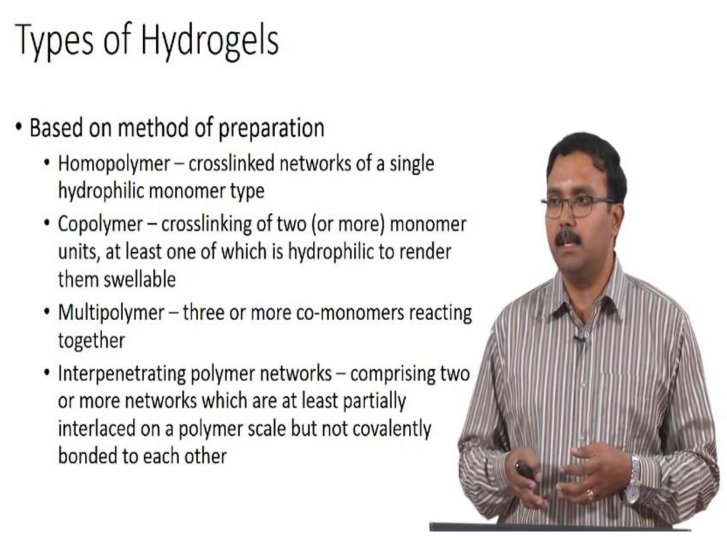

You could prepare hydrogels using protein-based materials or polysaccharide-based materials. And all these have very good water retention properties. Based on the method of preparation, hydrogels can be classified as homopolymers, copolymers, multipolymer, or interpenetrating polymer network hydrogels. Homopolymers are the ones where you have a single type of hydrophilic monomer which is used, and it is crosslinked to prepare the hydrogel. The copolymer is two or more monomeric units are used to prepare the hydrogels, and multipolymer at least uses three or more co-monomers reacting with each other to form the hydrogels.

Interpenetrating polymer networks are slightly different from multipolymers. Here also, there are more than one polymers which are used; however, what is done is the first polymer network is formed initially, and the second network is formed following the initial network. The first network and the second network are not covalently attached to each other. They just are interacting with each other. This is why it is called as an interpenetrating polymer network. There are also things like double network hydrogels and many other types of hydrogels. So, people have tried to do these to alter the mechanical properties and degradation properties of the hydrogels.

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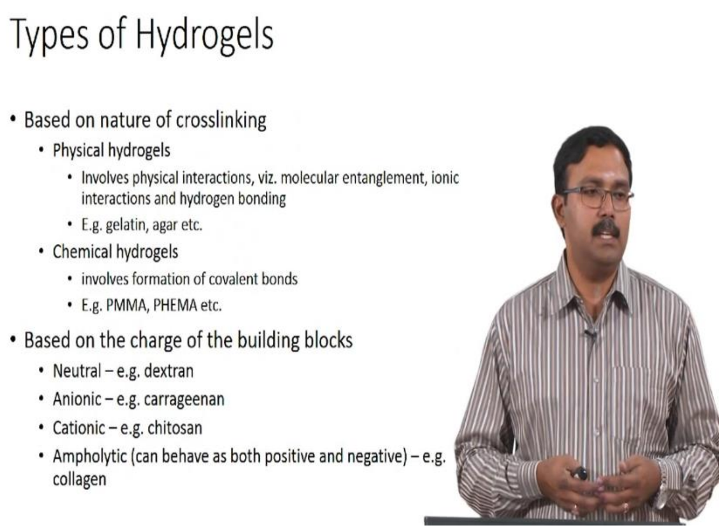

Based on the nature of crosslinking, you can classify them as physical hydrogels and chemical hydrogels. Physical hydrogels involve physical interactions like molecular entanglement, ionic interactions, or hydrogen bonding. Whereas, chemical hydrogels have crosslinkers which form covalent bonds. And examples for physical hydrogel would be gelatin or agar, and chemical hydrogels would be PMMA, poly HEMA and so on.

Based on the charge of the building block, hydrogels can be classified as neutral, anionic, cationic, or ampholytic. All these have their own advantages depending on the application; people try to use different things.

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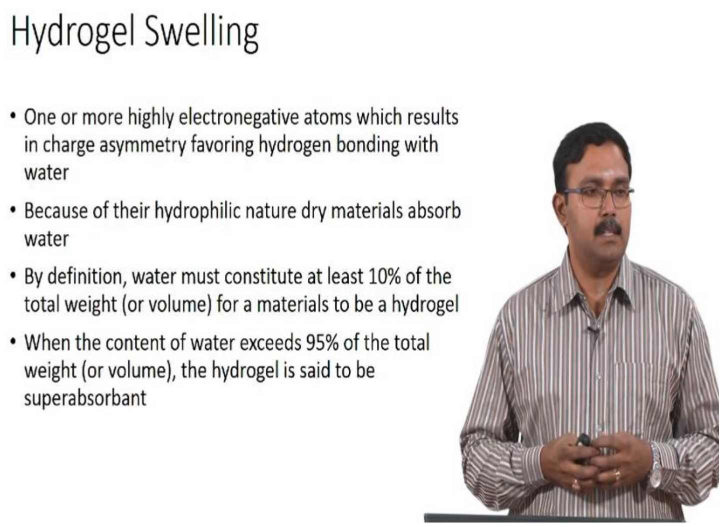

When we talk about hydrogels, swelling is the most important parameter we are looking for. Because it can absorb a lot of water and that is one of the most desirable properties of a hydrogel. So, what does this mean? So, when one or more highly electronegative atoms are present, what happens is there is charge asymmetry which promotes hydrogen bonding with water.

Thereby you have a lot of water which is retained by this hydrogel. This hydrophilic material can absorb a lot of water when it is completely dry. A dry hydrogel is called as a xerogel, and when you place it in water, it will start swelling and absorbing a lot of water. By definition, the water must constitute at least 10 percent of the total weight of a material, for it to be called a hydrogel.

That is actually a very small number; most hydrogels absorb a lot more than 10 percent. In some cases, the water content can exceed more than 95 percent of the total weight or volume, and these hydrogels are considered to be superabsorbent. The hydrogels that we prepare in our lab can absorb anywhere between 2 to 10 times its weight. Depending on the application, we can actually prepare different kinds of hydrogels.

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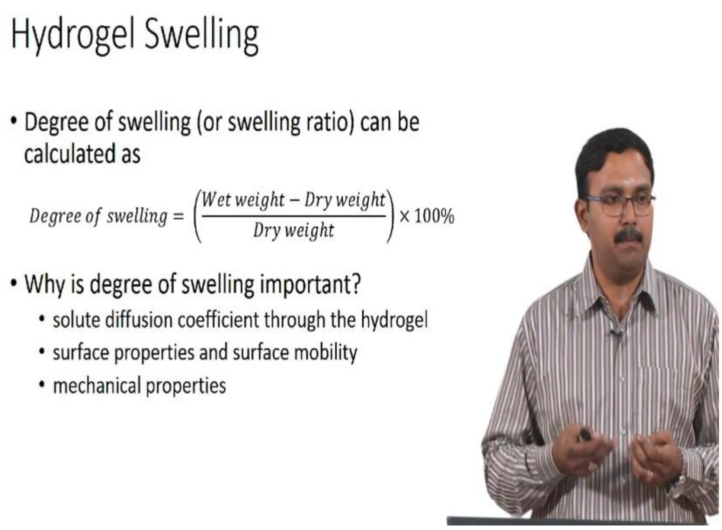

To understand and quantify hydrogel swelling, a term called the degree of swelling or swelling ratio is used. This is calculated as

$$Degree\ of\ Swelling = \left(\frac{Wet\ weight - Dry\ Weight}{Dry\ Weight}\right) \times 100\%$$

There are different ways you can do it; you can look at swelling kinetics which is basically taking the dry hydrogel, putting it in buffer or water and measuring its weight after certain time intervals to see how the swelling progresses or you can look at only the equilibrium swelling. So, you take it and place it in water, leave it for like 2 hours and then take it out and see what is the final state in which it is present.

In most cases, people want hydrogels that can swell very rapidly. There can also be specific needs where hydrogels do not swell at certain conditions, but the hydrogels which are generally prepared swell very quickly and within an hour they reach equilibrium. So, why is this factor, degree of swelling important? Why do we need to measure this as a part of characterization?

Student: So, how much weight it can retain.

Ok, it will tell us how much water it can retain, but more than that from an application standpoint, it helps us understand the solute diffusion through the hydrogel. And also, the surface properties and the surface mobility on the material and also the mechanical property because you are going to be using this material in its swollen state. So, you need to understand how the mechanical properties can be altered while the swelling happens. So, those factors can be identified when with this value.

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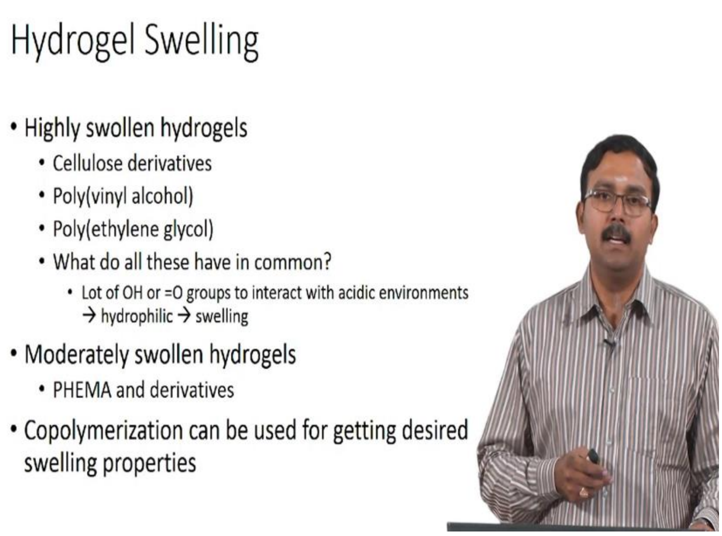

There are many hydrogels which are very highly swollen. Some of the hydrogels are listed here; cellulose, polyvinyl alcohol, polyethylene glycol. So, what is common between these molecules?

Student: They are all polymers like, huge polymers.

No, they are all polymers fine, but why does that mean they have to be highly swollen hydrogels? So, I am asking you what property of all these three makes them form highly swollen hydrogels.

Student: Hydrogen bonding with water.

Hydrogen bonding with water is possible, but what is their structural feature, which causes the hydrogen bonding with water.

Student: OH groups, Hydroxyl groups.

Hydroxyl groups. So, they have a lot of -OH groups. So, that is what makes them highly hydrophilic, and they can actually swell a lot and absorb a lot of water, ok. There are also moderately swollen hydrogels like poly HEMA and copolymerization can be done to optimize the swelling ratios to get desired swelling ratios using two different materials.

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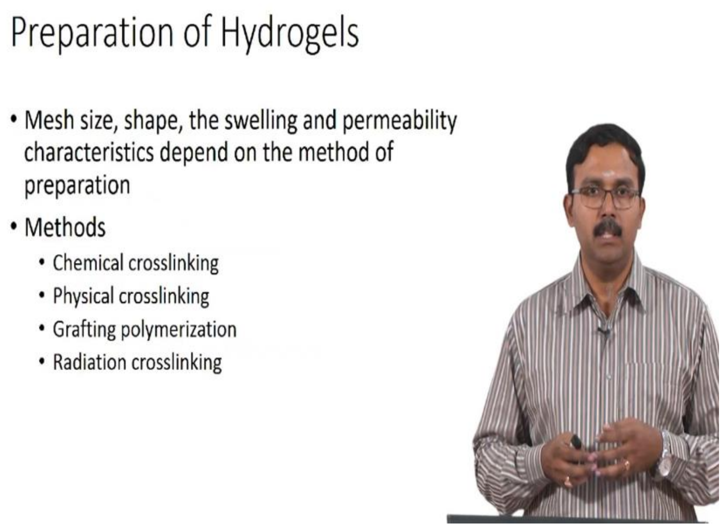

While you are preparing hydrogels; the mesh size, shape, swelling, permeability characters, all will depend on the method of preparation itself. The method of preparations can be based on chemical or physical crosslinking. You can also have graft polymerization and radiation crosslinking, which can cause either chemical crosslinking or physical crosslinking depending on the polymer which is being used.

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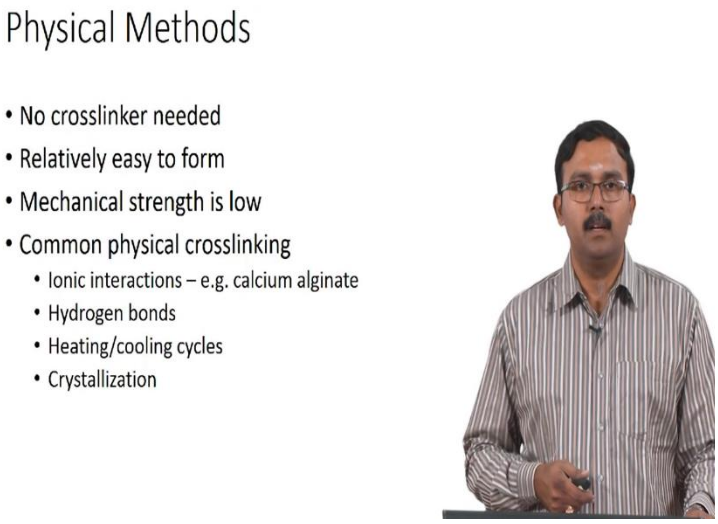

The advantage of using physical crosslinking is no crosslinker is needed. Any time you add additional chemicals, it can cause problems, right?. The crosslinker could be toxic, although your material might not be toxic, or there could be excess crosslinker which could have other adverse effects. Even if it is not toxic, it could have other biological effects, which are unintended. So, you would not want that.

So, physical crosslinking does not require this crosslinker, makes it very safe that way. You know for sure that the material you are using is the only thing which you are putting inside the body. It is also relatively easy to form; however, the mechanical strength is low for most of the physical crosslinking because these are just weak interactions.

The common physical crosslinking which are used are ionic interactions and hydrogen bonds. It can also be created using heating and cooling cycles and crystallization. See, ionic interactions and hydrogen bonds; you would understand what it is. Heating and cooling cycles in the crystallization of polymers are done using this kind of process.

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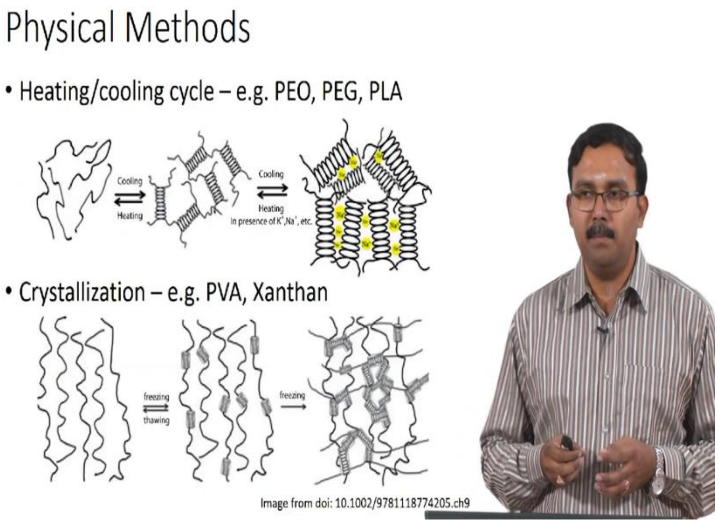

In the heating and cooling cycle, you end up forming these kinds of coils, when you heat and cool. You keep doing this cycle multiple times these coils are going to be formed. And finally, in the second step, you do the same heating and cooling along with ions like sodium ions or potassium ions. So, these are then stabilized and thereby forming physical crosslinking, which forms the hydrogels. This is usually done for PEG, PLA, PEO, and so on.

Crystallization is another common method which is used for crosslinking PVA and Xanthan. So, what is done here is freezing and thawing. The polymer solution is taken, and it is frozen and then thawed, and this is repeated multiple times. So that these coils are formed and finally, you freeze it so that these coils interact with each other and form a very strong hydrogel.

Crystallization is a technique which can provide reasonably good mechanical strength. In many cases, crystallization can even give mechanical strength better than your chemical crosslinking.

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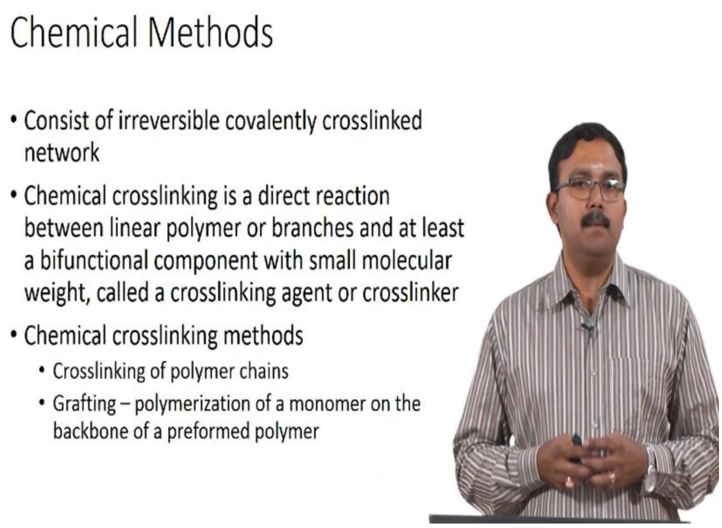

Chemical methods consist of forming irreversible covalent crosslinking. The chemical crosslinking is a direct reaction between the polymer or its branches with a bifunctional component with small molecular weight, which is the crosslinking agent or the crosslinker.

You would use something which is bifunctional. So that what happens is, one side of the functional group will interact with one polymer chain, and other functional group will interact with the other polymer chain; thereby, it forms the crosslinks. Glutaraldehyde is one such molecule which is very commonly used. So, you have two aldehyde groups. It can easily react with amines and other molecules. So, it will immediately form crosslinks. That is a very common crosslinker which is used.

Chemical crosslinking methods involve crosslinking of polymer chains themselves which is what I am talking about with respect to glutaraldehyde. You can also have grafting where polymerization of a monomer is performed on the backbone of a preformed polymer. You might just be forming branches using another monomeric molecule and cause this polymerization to create a hydrogel. These are some of the chemical processes which are used.

Depending on the functional group, you will choose what kind of crosslinking you can perform.

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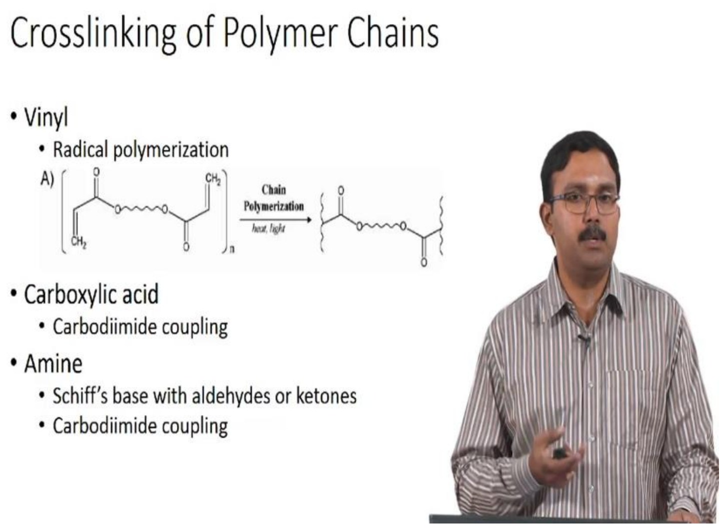

If you have a vinyl group, then you can use radical polymerization. If you have carboxylic acid, then you can do something called carbodiimide coupling. Carbodiimide coupling is where the carboxylic acid group is activated using a carbodiimide, and this activated carboxylic group can react with hydroxyl groups or amine groups. So, with amine groups, it will form amide bonds, and with hydroxyl groups, it can form ester bonds. Once you have this activated carboxyl group, it is easier to cause reactions. Amines are easy to work with using Schiff's base reactions with aldehydes, or you can use carbodiimide coupling to interact with carboxyl groups.

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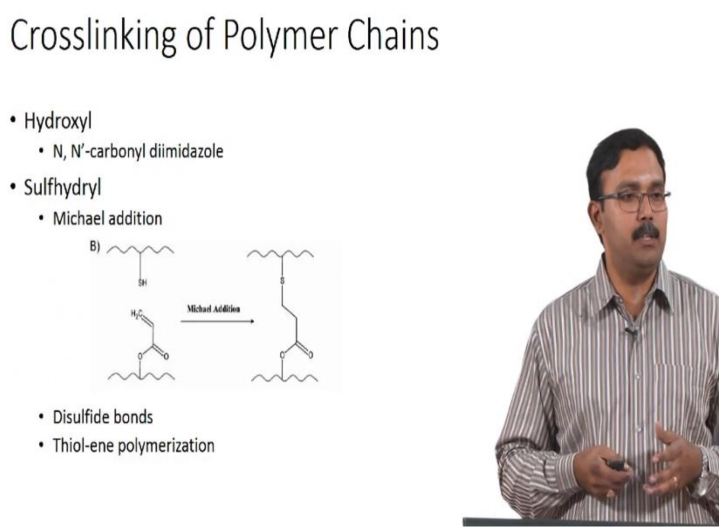

Hydroxyl groups can also be used. If you have hydroxyl groups, you can activate these hydroxyl groups and create crosslinking using carbonyl diimidazole. And sulfhydryl groups which might be present can be used for chemical crosslinking using Michael's addition or disulfide bond formations.

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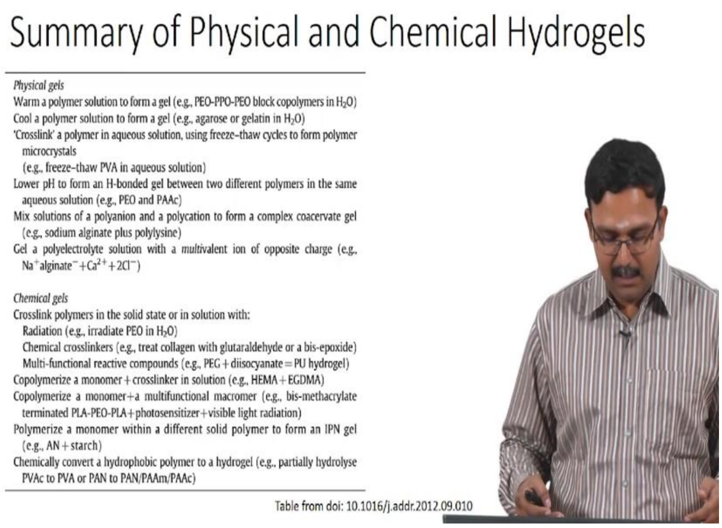

So, this basically summarizes all the physical and chemical hydrogels with the different crosslinking techniques. I am not going to go into the details of everything. I am putting it there for you to look up while I upload the slides. So, with that, we come to the end of this lecture. We will talk about stimuli-responsive hydrogels in the next class.

Thank you.