Tissue Engineering
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Lecture - 06
Scaffolds: Synthetic Polymers

Today, we will talk about Synthetic Polymers. So, this will again be a reasonably brief talk. I will only focus on things which have been studied extensively.

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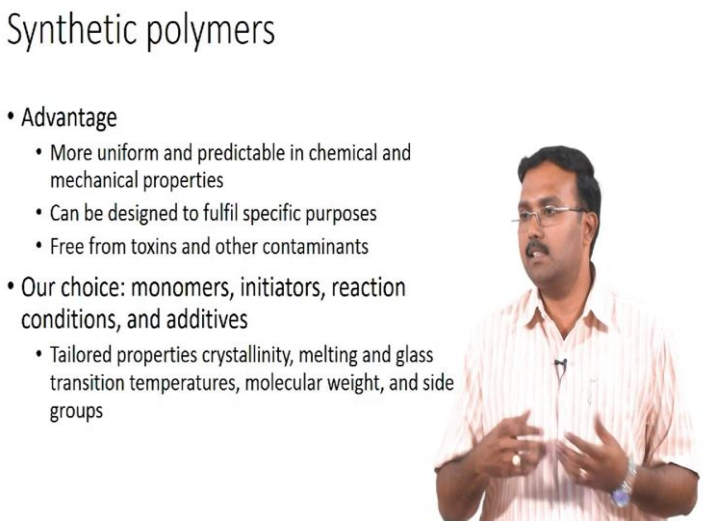

Obviously, we can look at any synthetic polymer as long as it is biocompatible; however, we will focus on some things where people have demonstrated reasonable success with respect to tissue engineering applications.

So, why do you want to use synthetic polymers? As long as natural polymers do the work, you would not have to look at synthetic polymers right. But we already discussed natural polymers have some serious limitations; source could be a problem because it can cause immunogenicity and contamination issues, batch to batch variations, your physicochemical properties are not tailorable. These are some problems which have made people look at synthetic polymers.

The advantages; synthetic polymers are more uniform, and they have predictable chemical and mechanical properties. You can design it for specific purposes; you can

make it free from toxins and contaminants because you are going to be synthesizing it in a lab. You will have a lot of control over the quality of the material you are producing.

You have a choice over the monomers, the initiators, reaction conditions and any additives you want to add to the polymer and so on. This way, you can tailor many of the properties like crystallinity, melting and glass transition temperatures, molecular weights, and side groups. So, these will give you desired properties which will be suitable for appropriate applications.

Student: What is the major difference between bioimplants and scaffold?

Ok. So if I am gone a say an implant, an implant is generally not biologically active. In a sense that it is just there to provide some kind of a replacement, it does not help in regeneration of the natural tissue. A hip and joint replacement is an implant; so, you basically use a titanium alloy, and you put it in the body that is not going to help in your bone growing back whereas, scaffold tissue-engineered scaffold is supposed to help the bone to grow back.

That is why if you were to use a hydroxyapatite ceramic, that would help in bone ingrowth. It will help in the cells for attaching and formation of a new matrix, and it will integrate itself with the existing bone. So, that would be a scaffold from a tissue engineering perspective.

Student: Sir, most of the times, it will be inactive; that implant.

The implants will usually be inert, yes.

Student: But there are some degradable bio-implants which can be helpful for the bone growth right.

So, you have degradable implants, yes. Degradable sutures, all those things are there. Because it is not permanent, does not make it bioactive. I am talking about the activity. So, if you are going to use something where you are delivering molecules, then it automatically becomes active that would make it more related to a delivery mechanism. So, it is a signal-based approach, which you are talking about right. Here we are looking at the material itself which can help in cell adhesion and so on. Does that answer your question?

Student: Yeah.

So what was the material you are talking about when you are saying material degrades?

Student: Like magnesium degradable implants are there, of course, the reaction it will be made to prolong. So, immediate degradation will lead to bone growth.

Yeah. So, I have not read a lot about magnesium, and it is bioactivity. I know professor Sampath Kumar does that. So, I do not know exactly the biology behind it, but if magnesium by degradation can stimulate it, then it would be a bioactive material. So, like hydroxyapatite.

Right, so it just depends on the biology behind it, and I am not very confident about the biology of magnesium.

Student: Because nowadays all the implants are coming in hydroxyapatite coating only. So, the reaction will be more.

Yeah.

Student: They are active, bioactive in nature.

So, that is for host integration. If you have hydroxyapatite or some ceramic coating, it makes sure that the cells can attach and your chances of dislocation and loosening come down because it now integrates with the bone alright. So, that is the idea. Host integration is another aspect of implantation. That is another major concern when it comes to even tissue-engineered products. How you integrate it with your body is going to be a question.

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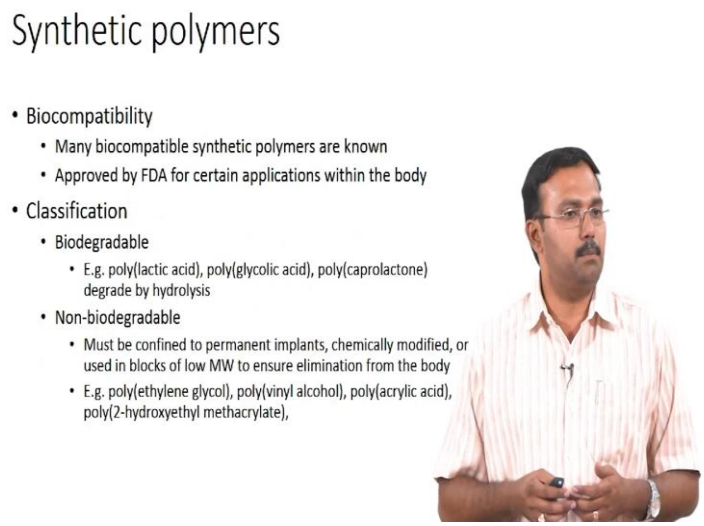

With synthetic polymers, you have certain advantages. People have tried different synthetic polymers, and they always stuck to things which have already been proven to be biocompatible. There are a lot of biocompatible synthetic polymers that are known.

Many of them have been approved by the FDA for specific applications. It might not be specifically for tissue engineering, but you would usually have it approved for various applications. If it is shown to be compatible, then you would want to try to see whether it is effective as a tissue engineering scaffold.

Broadly, these materials are classified as biodegradable and non-degradable. Biodegradable are the ones which degrade in your body in vivo, non-biodegradable do not degrade inside your body.

Examples for the biodegradable would be PLA, PGA, PCL and so on. These usually degrade based on hydrolysis. Simple hydrolysis reaction which will just degrade these into products that can be excreted out; whereas, non-biodegradable polymers cannot be hydrolyzed, there are no enzymes which can actually break it down.

Because of this, they have to be confined for specific types of implant. If it is going to be a permanent implant, then you might want to use a non-biodegradable material or you would try to chemically modify these non-biodegradable materials to make it

biodegradable. And you can also use it in blocks of low molecular weight. So that, they are eliminated from the body.

For example, polyethylene glycol is not biodegradable, but below a certain molecular weight, it can be excreted through your kidney. So, if you use it in that molecular weight, then it would not be a problem, it can be removed from your body. PVA, polyacrylic acid, poly HEMA are some of the examples of non-biodegradable materials, which have been tried out for tissue engineering applications.

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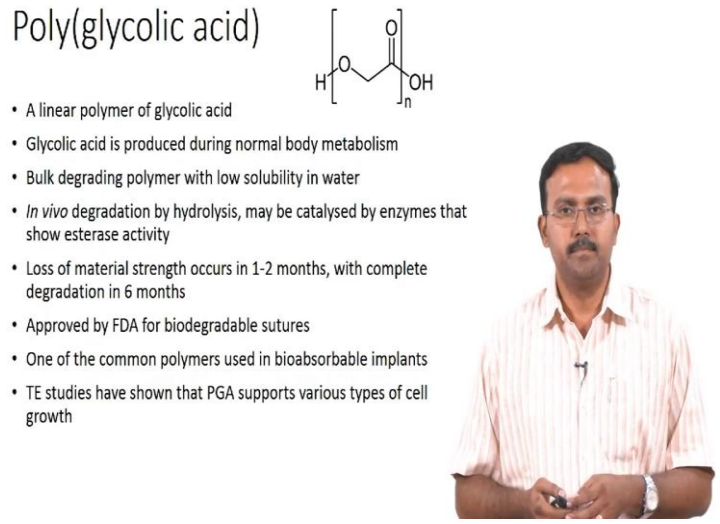

We will go through some of these in reasonable detail. So, this is PGA, poly glycolic acid. This is the linear polymer of glycolic acid. Glycolic acid is actually produced in your body during normal metabolism, which means the degradation products of poly glycolic acid are not going to have any toxic effect on your body; however, this material itself goes through bulk degradation. It is not just surface degradation; surface etching is not what happened. Bulk degradation occurs, which means it can get degraded reasonably quickly.

In vivo degradation happens only through hydrolysis, this process can also be catalyzed further by molecules which have esterase activities. The enzymes that have esterase activity can rapidly hydrolyze PGA. The in vivo degradations studies have shown that it usually loses its strength within the first 2 months; 1 to 2 months it loses it is mechanical strength, and complete degradation happens within 6 months.

It is approved by the FDA for biodegradable sutures. You would have come across these in surgeries; people now use biodegradable sutures rather than the regular sutures so, you do not have to go back again for removing the suture. So, these will be absorbed by your body. This is one of the most commonly used polymers which are bioabsorbable. So, it has been extensively studied. There are commercial products out there which use PGA.

Student: It is only glycolic acid produced in the body or even PGAs.

No glycolic acid is produced in your body, not PGA; poly glycolic acid is not produced. You can prepare poly glycolic acid which just gets hydrolyzed to glycolic acid. Tissue engineering studies of PGA have been done. People have shown that different types of cells can actually adhere and grow on top of this polymer.

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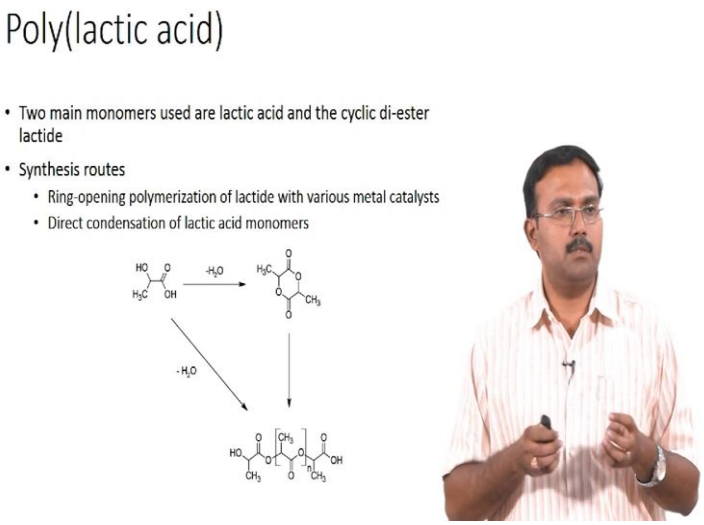

Another set of polymers which have been studied extensively is PLA or poly lactic acid. Here these are made from two different monomers. One option is to use lactic acid and prepare the polymer. Another option is to use the cyclic di-ester, which is lactide and then prepare this. It is the direct condensation of lactic acid monomers to form PLA done at milder temperatures. So, only then you would prevent the formation of cyclic di-esters.

If cyclic di-esters form, then you through the ring-opening polymerization of the lactide, which can be catalyzed by the different metal catalyst. Using this, you actually prepare PLA.

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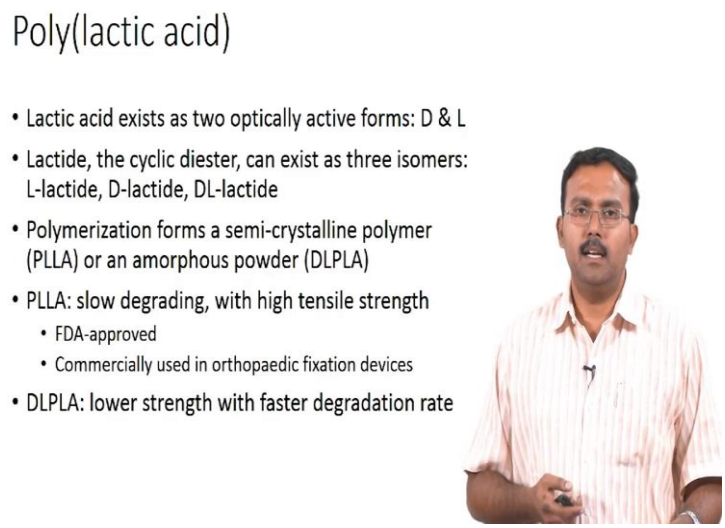

And lactic acid itself exists in two optically active forms which are D and L. Lactide, which is a cyclic di-ester can exist in three isomers; it could be L-lactide, D-lactide or DL-lactide. And the polymerization can form a semi-crystalline polymer or amorphous powder, depending on which stereoisomer is used for the preparation of PLA.

If PLLA is formed, then it is semi-crystalline. DL-PLA which is both D and L together that forms an amorphous powder. The advantage is that you can tailor the strength and the degradation rate based on this. PLLA has a slow degrading rate with very high tensile properties because of this; it has been studied for many bone applications.

Commercially, it has been approved for orthopedic fixation devices. Like bone screws and things like that, so they have used PLA. And the D and L combination has lower strength and a faster degradation rate. So, we can try to combine these two to get the desired properties. PLA is also one of the commonly used inks in 3D printing. Most of these 3D printers would use PLA.

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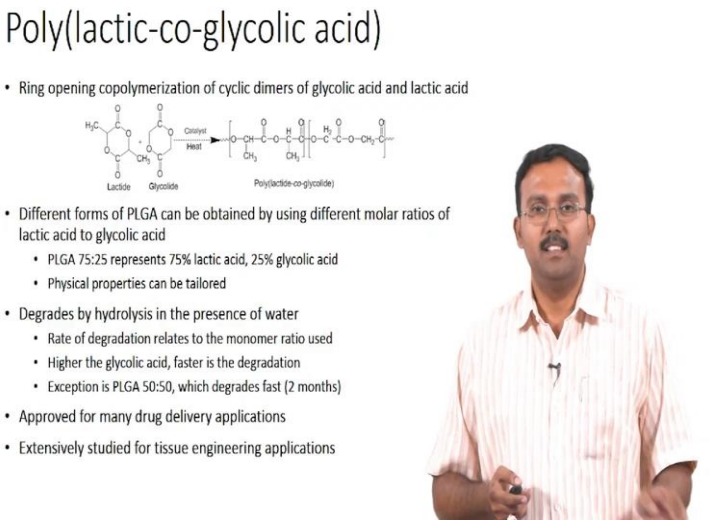

PLGA is a copolymer of these two; this is to try and use both the advantages. So, ring-opening copolymerization of the cyclic dimers of glycolic acid and lactic acid is done to get this PLGA.

What people have shown is, there are different forms of PLGA can be formed based on the initial molar ratios of lactic acid to glycolic acid. They are usually written as PLGA x:y; where x is the percentage of lactic acid and y is the percentage of glycolic acid; 75:25 means 75 percent lactic acid 25 percent glycolic acid.

The advantage of doing this is the physical properties themselves can be tailored. This also degrades by hydrolysis, in the presence of water. The rate of degradation can be related to the monomer ratio. If you have very high glycolic acid, then the rate of degradation is faster because PGA we saw that had a faster degradation compared to PLA. So, it would have faster degradation.

However, this correlation is not linear. In some points, it does not fit that; one example is 50:50 PLGA. PLGA 50:50 actually shows much faster degradation, which is actually faster than both PGA and PLA. So, I do not know the chemistry behind it, but in general, this has been observed.

PLGA has been approved for many drug delivery applications and has also been extensively studied for tissue engineering applications. People have shown many

advantages to using this. There are some disadvantages as well because of the acidic nature of this material, the PH in the localized regions could become lower, which could cause cell death, and it could cause some damage. Some studies have shown that. So, trying to use this has its own advantages and disadvantages.

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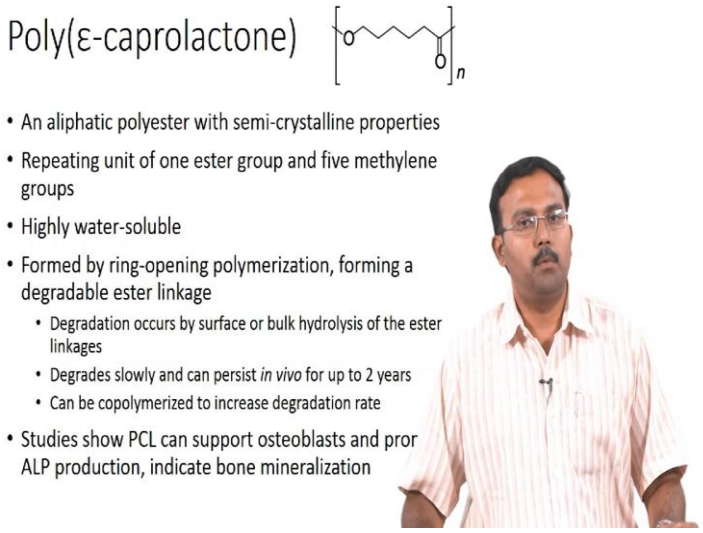

Poly caprolactone is an aliphatic polyester with semi-crystalline properties. The repeating units are 1 ester group and 5 methylene groups. The structure you can see there, it is a highly water-soluble polymer, and this is formed by ring-opening polymerization to form degradable ester linkages.

The degradation occurs by surface or bulk hydrolysis of these ester linkages. Degradation is very slow, and it can be present in your body for up to 2 years. So, it is usually used for processes where the regeneration is going to be very slow. So, PCL has been used in bone tissue engineering applications. They have shown that osteoblasts can adhere to this PCL and produce ALP. ALP is Alkaline Phosphatase, which is a marker for biomineralization.

When ALP production is increased, then you know that there is mineralization of bone. People have shown that when osteoblasts are cultured with PCL, it shows an increased ALP production. To optimize the rate of degradation, many studies have tried to use PCL copolymerized with other materials like PEG and PVA, and so on.

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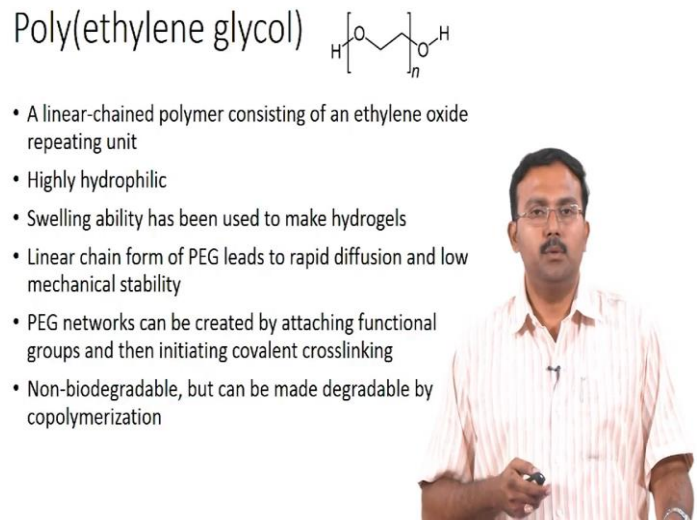

The once which we looked at till now PGA, PLA, PLGA, and PCL are all biodegradable so, the once which we are looking from here on out are non-biodegradable. PEG is a non-biodegradable material. However, it is very highly hydrophilic; it has very good swelling ability. So, because of this reason, it is used extensively in the formation of hydrogels. We will look at what hydrogels are, and we will go into details of hydrogels later, but can you tell me what you understand with the term hydrogels? Have you come across the term hydrogels?

Student: Yes simple definition; it is a matrix which holds water.

Ok.

Student: The water.

So, it can absorb water right, ok. So, that would be one, sorry?.

Student: Highly hydrophilic.

Ok, highly hydrophilic; so, if you were to take a sponge right, and you dip it in water that also holds a lot of water right. So, does that make it a hydrogel?

Student: It should interact with water, should hold water.

It should hold water; so, it is not just that it absorbs water, it should also hold water. The sponge if you squeeze the water is going to come out; whereas, the hydrogel if you squeeze the water will not come out. So, PEG is used because it can actually form hydrogels which are very hydrophilic and it can absorb a lot of water.

The advantage of that is, it will have very limited diffusion problems. If it can swell very nicely, then the network can swell very nicely, nutrient transport is going to be very effective, but the other side of it is the linear chain leads to the rapid diffusion of the material outside of it, which means it just dissolves away, and it has very low mechanical stability.

For this reason, people try to create PEG networks by attaching functional groups to PEG and try to crosslink it using covalent or other types of crosslinking. This is non-biodegradable but can be made to be degradable by copolymerization with other degradable polymers. And as I already mentioned, below a molecular weight threshold, it can be excreted by your kidneys. So, if you were to use it at that molecular weight, then it is usually not a problem.

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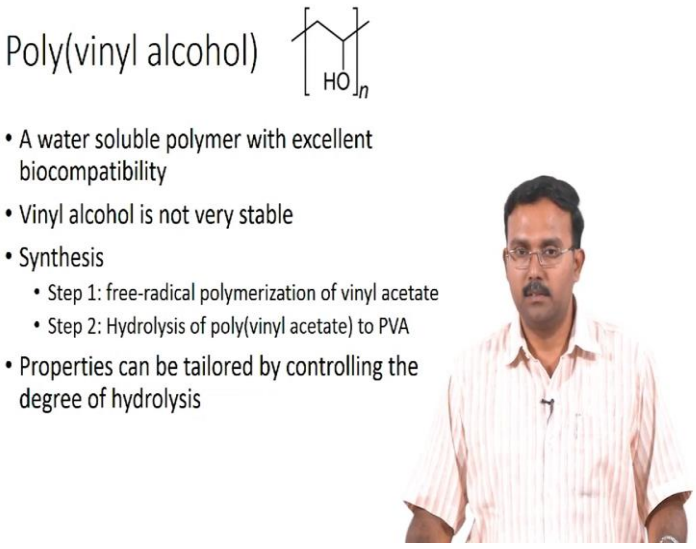

Student: Sir, the activated charcoal would be a hydrogel?

No. Why do you think it would be a hydrogel?

Student: It is solid absorber.

It does not absorb water right. It just absorbs other things, Ok.

Polyvinyl alcohol is another commonly used material. This is the water-soluble polymer with excellent biocompatibility. A lot of studies have worked with PVA and shown that it is very useful for biomedical applications, and vinyl alcohol itself is not very stable so, it is synthesized using a two-step process.

It's not just a direct polymerization of vinyl alcohol which is done. So, what people do is, the first step is the free radical polymerization of vinyl acetate, and the second step is the hydrolysis of the polyvinyl acetate to form PVA, polyvinyl alcohol.

So, this hydrolysis can be controlled. You can have varying degrees of hydrolysis. Just like how you have varying degrees of deacetylation for chitosan; you can have varying degrees for hydrolysis for PVA, and based on the degree of hydrolysis, the mechanical and the physical properties can be different.

It is used in many applications. There are review articles which talk about PVA for tissue engineering applications you can go and read it up; it is done for many different things. The only limitation with PVA is, it is so hydrophilic that it does not support cell addition very effectively. In some cases, you need to maintain your balance when it comes to hydrophilicity and hydrophobicity. If it is going to be very highly hydrophilic, it does not support the cells as much as it should.

Because of this reason, people usually use it along with the other natural polymers. Various types of natural polymers have been used along with PVA; cellulose and cellulose derivatives, hyaluronic acid, collagen all these have been blended with PVA to improve it is cell addition properties.

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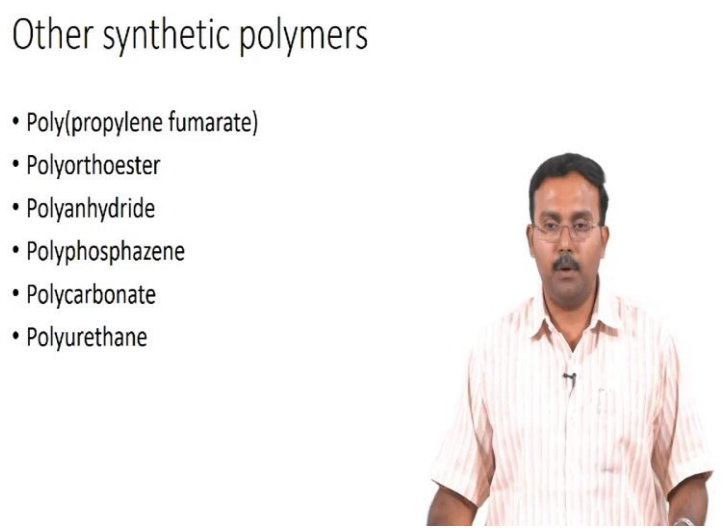

This is just a bunch of other synthetic polymers which have been studied. So, you have polypropylene fumarate, polyorthoester, polyanhydrides, polyphophazene, polycarbonate and polyurethanes which all been studied for different applications. These are all approved for biomedical use, and they have been shown to be biocompatible. So, people have just tried it for tissue engineering applications. You will be able to find papers which talk about other synthetic polymers as well, but these are some of the more commonly used synthetic polymers.

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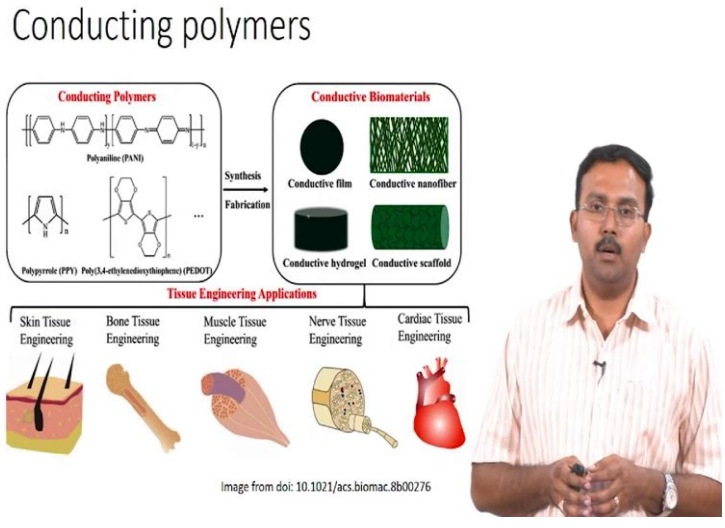

Another class of synthetic polymers would be the conducting polymers. Polymers, in general, do not conduct electricity right; they are very poor conductors of electricity. There is a special class of polymers which are called conducting polymers. This is actually a Nobel Prize-winning discovery. This discovery actually won the Nobel prize in 2000, I think for chemistry.

The conducting polymers which are shown here are polyaniline, polypyrrole, and PEDOT, which is poly ethylene dioxy thiophene. So, these are some of the common conducting polymers. These as polymers themselves have conducting properties. However, they have some limitations because they do not have the desired mechanical properties. They are brittle when they are fabricated into scaffolds and other materials. So, they are blended with other polymers to prepare conducting polymer composites.

They have been fabricated into multiple things and have been used for different tissue engineering applications where electrical properties of the scaffold play a role. People do try to prepare these materials in different ways, and we will look at how to fabricate these scaffolds.

Fabricating a scaffold is a crucial aspect right. Having a polymer is one thing. From the polymer to actually make it resemble the ECM, is a serious thing which we need to look at. So, the fabrication strategies have to be optimized. There are many different strategies. These are just a bunch of them which I have shown here, and you can look at many other things as well.

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Leaching methods where you have a solvent casting and salt leaching, ice particle-leaching, gas-foaming, and salt leaching. These are all some of the leaching methods. And you have microsphere formation where you can have biodegradable microspheres or macroporous beads or particle-aggregated scaffold being formed. Phase separation methods like freeze-drying or thermally induced phase separation could also be tried. Fiber-spinning methodologies to form nanofibers, microfibers, nonwoven fibers, and so on.

Injectable gels can also be prepared. 3D printing is one of the latest technologies where people are trying to print many of these things. So, these are some of the scaffold fabrication strategies. We will discuss some of them here and 3D printing I will discuss in greater length in the next lecture.

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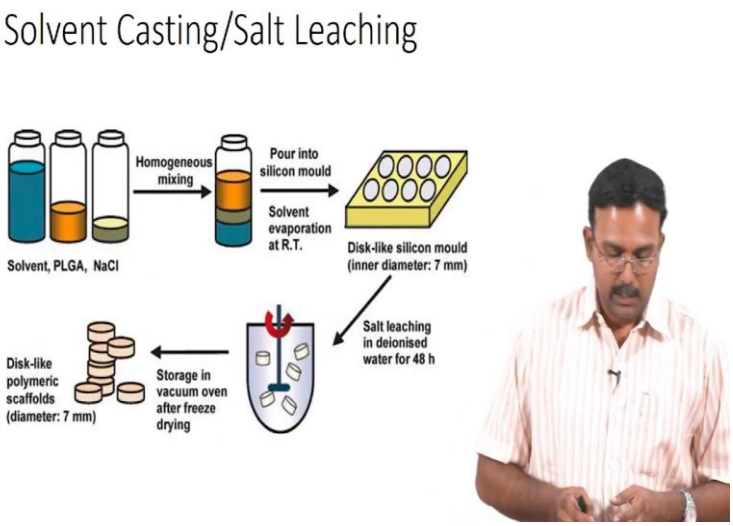

This is solvent casting and salt leaching. So, this is a very simple technique. All you do is, you take three things; the solvent, polymer, and salt. So, the salt her is NaCl. You could also use sugar or whatever right. It is something which can form a crystal.

You mix all of these and now what happens is, the salt has dissolved along with the polymer, and you pour it in the mold, whatever the mold could be. So, here they have just shown a disk-like mold, you pour it in the mold, and once it is dried out. You keep it in room temperature or in a vacuum to evaporate the solvent. So, now what you have is polymer disk in which the salt is dispersed all over right.

So, you put it in water and wash it. What will happen is you would have dissolved all the salt away. Now, the positions in which the salt was present have these pores. So, that is a salt leaching technique, and then you can freeze-dry it to eliminate all the remaining solvents and so on.

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So, this is an SEM image of the solvent cast/ salt leached material. You can see nice cube kind of pores; this is where the salt crystals were present. Those got washed away, and you got these pores. So, what you think could be the advantage of this technique? Any porous material will give you more surface area. So, this specifically has some advantage also.

Student: Semi-permeable property. These are all interconnected pores.

Ok, that is actually not correct. We will get to it in the next thing but.

Student: Easy fabrication process becomes.

So, it is a very easy process that is one thing. What else?.

Student: There is no heating involved.

Ok so, milder conditions for processing, all that is fine.

Student: Regular matrix of pores quite easily because the salt will be dispersed everywhere.

Ok, so you are able to get regular pores. Ok, I will rephrase it slightly to fit what I want to say. So, you can actually control the porosity. Based on the salt concentration and the

type of salt you use, that pore size and pore distribution can be controlled. So, that is the advantage here.

As far as your claim of it being interconnected pores it is actually not true. If you were to look at these points. These are actually deep pores right. So, the ones where you see the dark black are deep pores, whereas if you look at these parts which are more greyish or even whitish, they are not deep. So, you are saying the polymers surface itself right, but these are pores which have been formed.

Those are places where the salt was present, and it has just been dissolved away. So, in some cases, you would have gotten interconnected pores, but in many cases, you would not have very good interconnectivity in this method.

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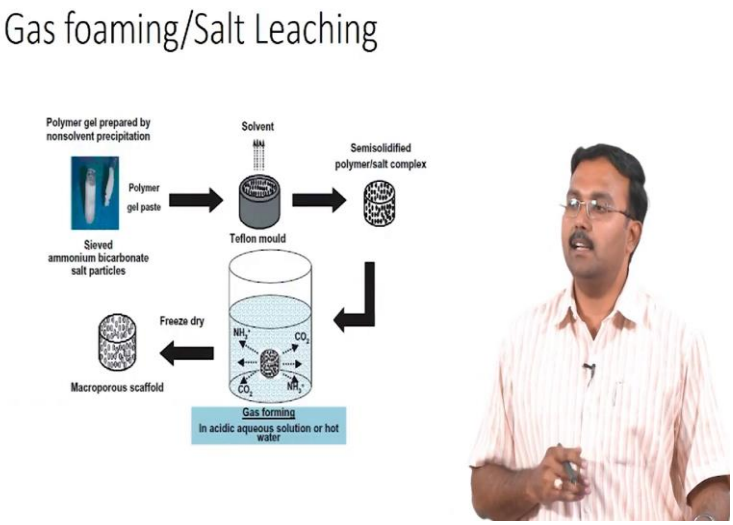

To get better interconnectivity, that is why you do gas foaming and salt leaching. What you do here is a polymer gel is prepared, and instead of just adding salt, you add a salt which can release gas. An example would be ammonium bicarbonate. You add this, and then you evaporate the solvent and put it in water or put it in a buffer, what will happen is you will have the carbon dioxide getting released from this. So, this gas is going to get released from the scaffold which you have prepared.

When the gas comes out, it is going to create these pores. So, you will end up with porous scaffold; this after drying and freeze-drying you can get a macroporous scaffold.

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The scaffold would look like this. If you look at this, even the smaller of the pores would have some of these connections where you would see that it is reasonably interconnected.

Those are the places where the gas would have escaped out and created these pores. Because of this, the scaffolds show very good interconnectivity. That is the advantage of using a gas foaming technique. These are all very simple techniques which people have been using where they have shown that you can create porous structures, but what would be the disadvantage of this compared to the salt leaching.

Student: Density would be very less yeah right.

Density would be very less for any porous material. If you have increased porosity, your mechanical strength is going to come down. As long as you keep increasing your porosity, the mechanical strength will have to come down because you have pockets of just air. Can you elaborate on what do you mean accumulation?

It is non-uniform. Whenever I ask what is the disadvantage of this compared to something else, it would usually be the advantage of the other technique. The other technique you had control over the pore size and porosity. Here you have reasonable control over the porosity, but not the pore size and so on. The distribution, pore size all those things you are not; it is not very well organized.

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Microspheres you guys would have prepared it for different applications, primarily for entrapment of enzymes and things. The same method can be used in tissue engineering applications as well, but people do not generally use just a microsphere. Although people have earlier studied it. Nowadays, people just do not use just a microsphere by itself because that does not resemble what your ECM is. People will use microspheres along with other materials, where you can use the microsphere to load molecules and so on. Microsphere shown here is quite simple. All you do is dropping it while the crosslinking media is spinning and you create a microsphere.

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This is a simple PLGA microsphere I believe on which human disk cells are cultured. This shows that cells can adhere and it depends on the material you have chosen, and you will get nice beads of uniform size. All that you need to control is the viscosity of the material, the rate at which you release the material into the media and also the diameter of the pore which is used for releasing them.

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Freeze-drying is another commonly used technique. This is one of the more popular technique which is currently being used extensively. Even more than salt leaching or gas foaming because of its simplicity. All you do is dissolve it, mold it, freeze it and dry it, that is it, as simple as that. You create pores because of this freeze-drying technique.

We in the last class or some class I discussed freeze-drying. So, freeze-drying is lyophilization technique where you have a sublimation process happening. So, can somebody draw the phase diagram for water and explain the sublimation process. How would it look?

Student: Pressure versus temperature.

Pressure versus temperature.

Student: Yes.

Something like this right.

So, which region is which?

Student: High pressure, low temperature, phase solid, yeah.

High-pressure low temperature is.

Student: Right, solid.

Solid.

Student: Then, high temperature, low pressure is gas.

What?

Student: Steam.

Which is what?

Student: Gas that.

This one, yes.

Student: Yeah.

Steam.

Ok, this is liquid. Ok, confident?

Student: Yes.

Ok. So, you guys are doing thermo now, right?

Student: Yes.

So, now that you have the phase diagram, can you tell me what is the process of lyophilization from here?

Student: Basically, at a low temperature, they reduce the pressure. So, all the water, they are actually going from solid to gas, yeah there is no liquid.

We just have to go here. See, usually solid goes to liquid and then to vapor. So, instead, you have sublimation. So, it depends on which pressure region you are in. So, if you are

in a very low pressure, that is why you create the vacuum during lyophilization. At vacuum you make sure that you can sublimate it at low temperatures rather than heat it and where you have to have a vaporization process.

So, the advantages; it creates pores as well as it does not damage the material. It is done at very low temperatures; because of this, it does not damage the material.

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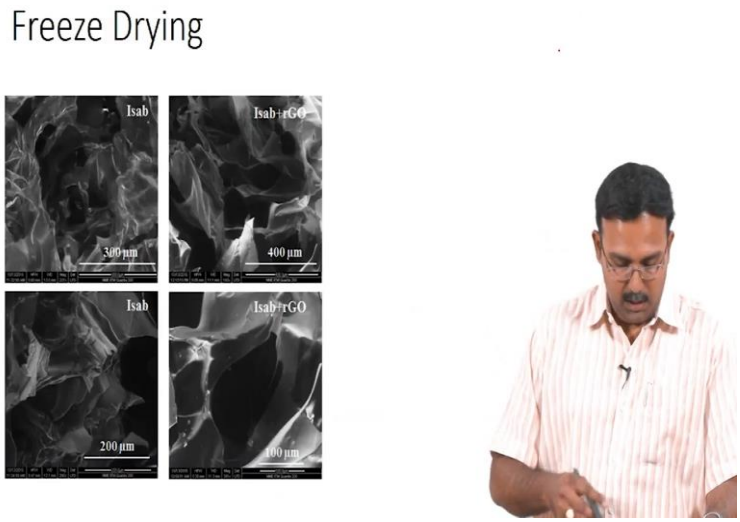

This is a scaffold which we developed in our lab. This was prepared by lyophilization technique. You can see very nice pores which are there and very nicely interconnected, while it resembles what an ECM would look like.

Student: What kind of microscope is used here?

It is a scanning electron microscope. We will discuss characterization extensively. There is a lot of things which we can use; so, this is the scanning electron microscope. There are different microscopes which can be used for such things. So, even the image which I showed, most of these images are actually SEM images only. This is also a scanning electron microscope, and this is also a scanning electron microscopic image.

For observing surface morphology, SEM is the most common method used.

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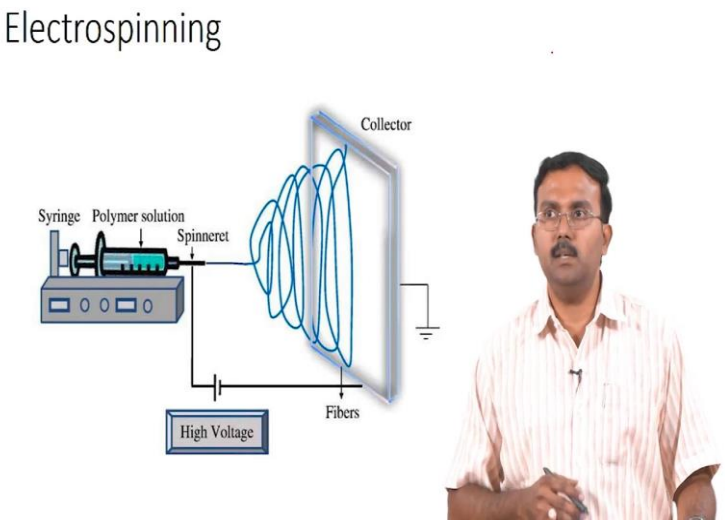

Another technique which is commonly studied is electrospinning. In this technique, what you do is, you take a syringe, and you fill it with a polymer solution, and the syringe dispenses the polymer solution which is exposed to a high voltage environment. And this basically breaks the polymer flow into thin fibers which is collected by a collector which is grounded. The collector can be a rotary drum; it can be a flat surface, and so on.

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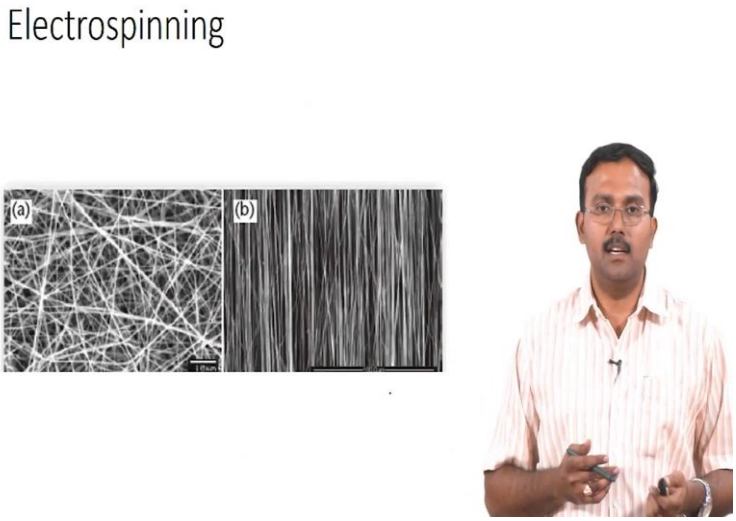

Depending on the collector surface, you can get either non-aligned or aligned fibers. So, this is electrospinning. You get very thin fibers; a few nanometers thickness is actually

possible when you get these things. This provides a very high surface to volume ratio, and it also provides a nanofibrous mat which resembles ECM in many of the tissues.

For these reasons, people work with such electrospun fibers. Right now, people are also trying to use electrospun fibers on which you actually have these freeze-dried materials on top. So, it will be more like a combination of both, which is more close to what an ECM would be. People are trying different things, and these are some of the technologies which are used for preparing the scaffolds. So, we will talk about 3D printing in the next class.