Tissue Engineering
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Lecture – 10
Scaffolds in TE: Fabrication

Good morning everybody, today we will be talking about Fabricating Scaffolds for Tissue Engineering Applications. We have looked at some of the materials which can be used; we have looked at the natural polymers and synthetic polymers. We will also be talking about other materials; however, today, we are going to talk about how scaffolds can be fabricated. As you all know from the previous lectures, we want to prepare a scaffold that can mimic the extracellular matrix.

So, the extracellular matrix support cells to adhere, grow, and it also provides cues and signals which help in the cells to perform the way they do. So, this helps in making sure that the cells integrate to form a tissue. When we are engineering this tissue, it is crucial that we prepare a scaffold that can mimic the functionalities of the extracellular matrix. Let us try and recollect what are all the properties that we look for; the first thing is it needs to be biocompatible.

That is decided based on the material you choose. So, that would not be a part of the fabrication strategy itself. If you are looking at what parameters have to be brought into fabrication, then we have to see what else are there. One of the crucial things is the surface area to volume ratio. If you have a high surface area to volume ratio, you can load more cells, which means there will be higher cell density, and there is a higher chance of the tissue functioning as it should. So, creating tissues that have this kind of a high surface area to volume ratio would be very effective.

Another factor is the pore interconnectivity. When you create pores in a scaffold, you need to have them connected to each other. Why is this crucial? When you have good pore interconnectivity, you will have better cell infiltration, which means the cells can go inside the pores and populate the tissue to form living tissue. Creating an interconnected pore would be advantageous; this can also help in the formation of blood vessels. This way, you would have sustained nutrient supply and toxin removal. This is an important factor that needs to be considered, as well.

Other than this, you also need to make sure that the material is fabricated into the shape and properties which fit the tissue which you are looking for. Considering all these, you have to design strategies that can help in fabricating scaffolds. Today, we will talk about some of the more commonly used strategies for fabricating tissue engineering scaffolds.

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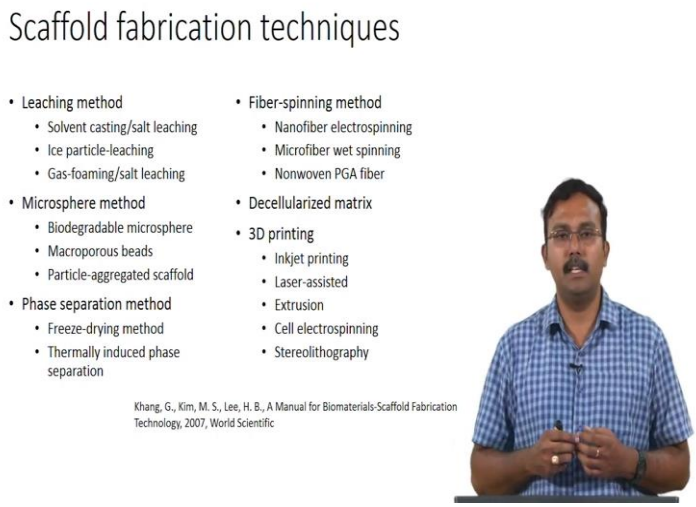

These are some of the strategies which we will be talking about today. The first one is called the leaching method. This is a broad category within which there are multiple strategies that can be used for fabricating scaffolds. The three strategies, which are commonly employed, are solvent casting/salt leaching, ice particle leaching, gas foaming/salt leaching. What is done here is, a scaffold is prepared with a gas-forming salt, which is leached out to create a porous structure.

We will see how it is done and how the scaffold would look like if this strategy is used. The next type of scaffold fabrication strategy is using microspheres. You can create microspheres using different strategies; it could be like preparing alginate beads, which you might have done as part of one of your undergraduate lab programs, or you can create macroporous beads using different strategies. And you can also have these beads aggregate to form a scaffold creating a porous structure. These are different strategies which have been employed to create a scaffold using microspheres of different polymers; it could either be synthetic or natural.

The next strategy is called the phase separation strategy. Here, you have two techniques; one is the freeze-drying method, and there is also a thermally induced phase separation method. By creating a phase separation, you can create porous structures, and this also will provide good interconnected pores. We will see more about the freeze-drying method and how exactly the scaffolds would look like and what is the principle behind the method.

You also have fiber-spinning strategies. This is where you have different textile-based fabrication methods that are employed in tissue engineering. Nanofiber electrospinning, microfiber wet spinning, and nonwoven polymer fibers are all some of the commonly used methods for preparing fibrous scaffolds. In many of the extracellular matrix, you have a fibrous structure. So, creating a fibrous structure using these kinds of spinning techniques can mimic ECM very effectively. In the case of electrospinning, where you create nanofibers, you would also have a significantly large surface area to volume ratio, which can help in cell adhesion and proliferation. We will talk about the electrospinning strategy and look at how those scaffolds will look, as well.

The next strategy is called the decellularized matrix. This is one of the growing strategies which is being looked at, where you take the extracellular matrix from the native tissue. This could be either from a human donor or also from other species. So, what you do here is you remove the cells and all the cell-based components. This is done through different methods; it could be using some chemical treatment or physical treatment followed by complete removal of all the toxins. This way, you create a matrix that resembles the native tissue. Here, the major challenge is to remove the cells and their components without damaging the integrity of the extracellular matrix.

So, if you can maintain the mechanical properties, physical, chemical, and biological properties, then you would have an extracellular matrix that probably has all the desired functionality, and thereby, it will be able to provide all the biological cues as well. So, this would be an ideal strategy to look at; however, it is a major challenge to make sure that the properties of the scaffold are not lost during the decellularization process.

We will not be talking about this today, but you can read about this on many of the papers which are readily available. The last and the up and coming strategy, which people are looking at is 3D printing. 3D printing is a novel technology where people are

looking at how scaffolds can be prepared by bioprinting the scaffolds. Here, a special ink is used. The ink will basically contain the material which you want the scaffold to be formed with, and this can be used for fabricating scaffolds based on the design we need, using auto CAD and other CAD-based software.

Here, you can also load cells along with them and print the 3D structure for the tissues. So, we will be talking about this in a future lecture. There is also another strategy which is called self-assembly, so that we will also be discussed in detail in a later lecture.

Now let us look at some of the more traditional strategies, which are the leaching methods where you will be talking about the solvent casting/salt leaching and also the gas foaming/salt leaching method. So, we will first start discussing this.

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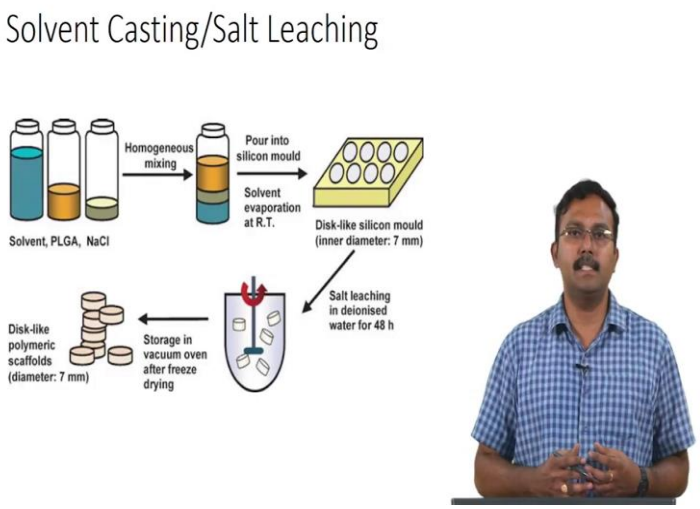

Let us first talk about the solvent casting and salt leaching method. So, this is an example of how you would use this method to prepare a scaffold. In the protocol given, you have three things; first is the solvent, which can dissolve the polymer and the salt which you are using for this method.

The polymer chosen here is PLGA, and the salt which is used is NaCl or sodium chloride. So, these three are poured into one vessel, and they are mixed to create a homogeneous mixture. This homogeneous mixture is then poured into a silicon mould where you keep it and evaporate the solvent. Once the solvent is evaporated, you now

have a solid structure, which will have the shape as the silicon mould. Here, they have shown a disc-like mould. So, you would end up with disc-like scaffolds.

Now, this scaffold contains the polymer and the salt. The salt crystals would have formed in different parts of the polymer scaffold because it was a homogeneous mixture of salt in solution, the salt crystals would have formed in different parts of the polymer scaffold. The next step is to leach the salt. This is done using deionized (DI) water. So, you place the salt polymer scaffold into the DI water and mix it for 48 hours. By doing this, the salt will get dissolved out, and you will end up with a disc-shaped scaffold, which is only the polymer.

Now, this can be kept in a vacuum oven after freeze-drying to remove all the leftover water. Thereby, you now have a scaffold that is prepared through solvent casting and salt leaching. So, how does this create a porous matrix? The salt which was present would have crystallized and formed salt crystals in the polymer blend. So, this salt, when it leaches out, the volume occupied by the salt crystals, will now become pores. So, these porous structures are used for cell attachment and culture.

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These kinds of scaffolds would look like this under a scanning electron microscope. So, what you see here are images of salt leached scaffolds. You can see nice structures, which are the regions where the salt crystals were occupying. So, when you look at these SEM images, you would also see that there are some areas that are dark black, while

some are grey, indicating that there is a 3D structure, and there are crystals that have been formed all over, creating such pores. However, one of the limitations you would see is in many of these pores; there is very poor interconnectivity.

For example, you look at this particular pore. So, this pore is fully covered, you have a region where cells can go and attach, but they cannot penetrate the scaffold if they go into this pore. This would mean the interconnectivity might not be excellent when you are talking about the solvent casting and salt leaching method.

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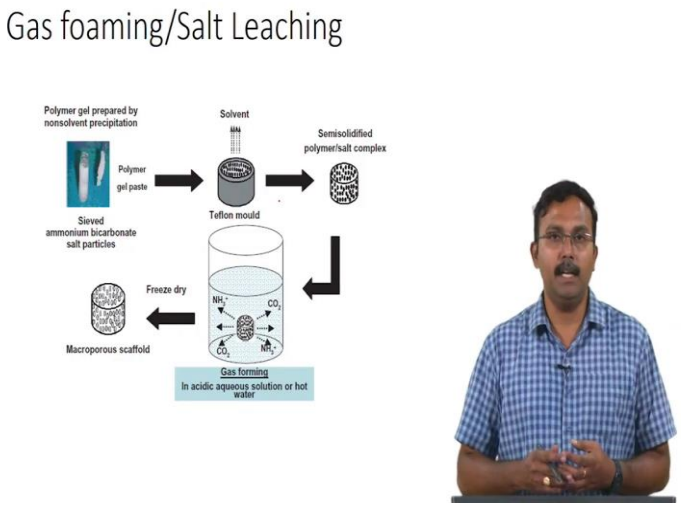

To overcome this limitation, people have looked at the gas foaming and salt leaching method. This also uses very similar principle; however, instead of using a simple NaCl kind of salt, you use salt, which can generate gases. Here is an example where they have prepared a polymer gel using a non-solvent precipitation method, and the polymer gel paste along with the ammonium bicarbonate salt particles is mixed, and you pour it into a Teflon mould to create a semi solidified polymer salt complex.

This semi solidified polymer salt complex is then added to the acidic aqueous solution or hot water. So, what happens when you do this is the ammonia and carbon dioxide gets generated and starts effervescing, and releasing into the water. From the salt polymer blend, you now have the gas leaving leads to the formation of a porous scaffold. So, you end up with the macroporous scaffold, which can then be freeze-dried to be stored for further use.

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The scaffold which you have prepared using gas foaming and salt leaching method would look something like this. So, what you see here are pores that have good interconnectivity. Because the gas is pumped through the pores, you would have nice interconnectivity for the pores.

So, you can see that there are lots of pores in the SEM image. This is the pore, and these are some of the pores. And as you see, most of these pores are very deep, and even in this particular pore, you would see that are smaller pores which are much darker in color, indicating that they are much deeper and they are probably interconnected to some other pore.

This kind of highly interconnected pore can be very useful when you are talking about tissue engineering applications. As I had already mentioned, cells will be able to infiltrate; you would also be able to get blood vessels forming, and also, it can help in good diffusion. In the early stages of any tissue being implanted, nutrient supply has to come through diffusion. So, if you have such highly interconnected porous structures, the diffusion will be effective; thereby, it would not be a limiting factor when you are talking about tissue formation.

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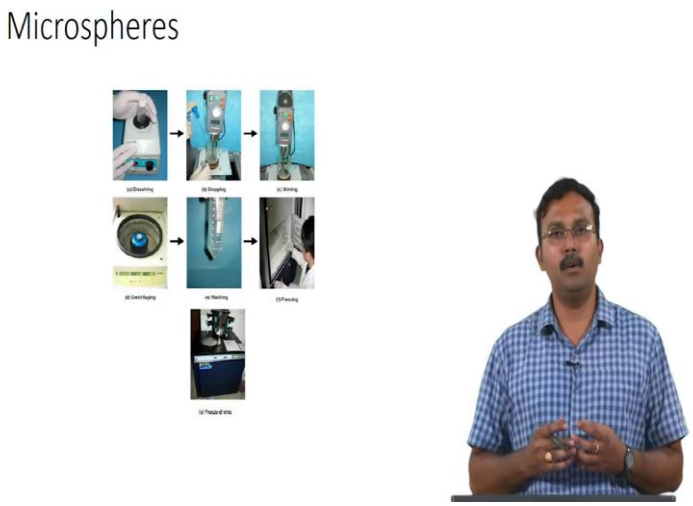

Let us move on to the next strategy. One of the strategies which I mentioned is the formation of microspheres. Here, we will be talking about one of the three or four different strategies which have been used, where microspheres have been used to prepare scaffolds. First, the polymer is dissolved in a solution, and then it is dropped through a small pore using a maybe a syringe pump or something into another solution. And then it is being mixed; it is stirred, and then it is centrifuged to remove these beads, these microspheres which are washed and then freeze-dried.

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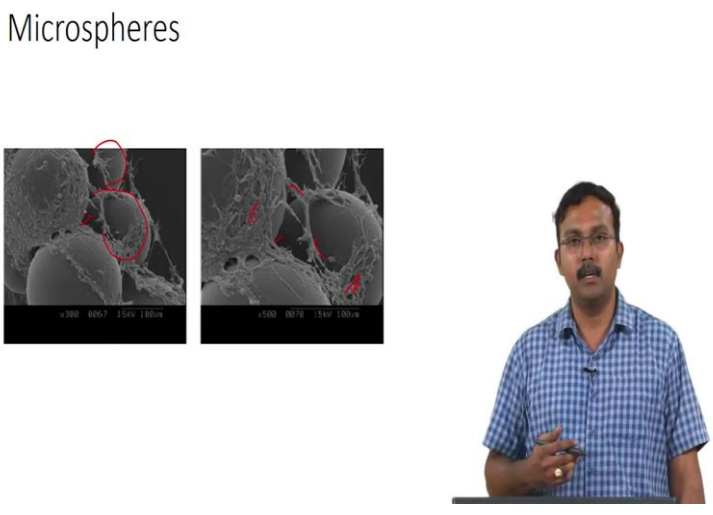

These freeze-dried microspheres can form structures like this. As you see, these are nice spheres which are present, and you see the cells which have adhered to this as well. These images, which you see on the scaffold, are cells that have been adhered to the scaffolds. Because you have these microspheres which have assembled to form the scaffold, you also have a porous structure that is present in between these spheres. So, this can help in the infiltration of nutrients and also hopefully, cell infiltration. So, this is what is done for preparing microspheres.

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Moving onto the next topic, you have freeze-drying. Freeze-drying is one of the most commonly used methods for creating a highly porous scaffold. What you do here is, you create the polymer solution, and you pour it in a mould, and this polymer blend is then frozen and kept for freeze-drying. Freeze-drying is also called lyophilization. After freeze-drying, it is washed and dried. You can also re-lyophilize it to make sure that you have a completely functional scaffold.

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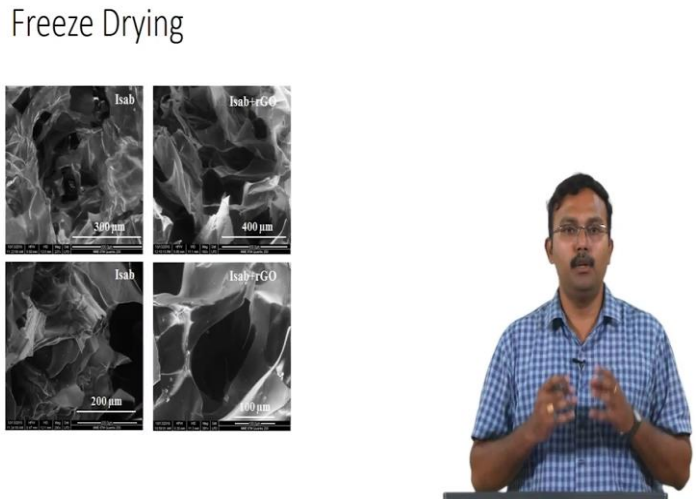

So, when you observe these scaffolds under a scanning electron microscope, it would look something like this. These are scaffolds which we were prepared in my lab. It is made up of isabgol, one of the carbohydrate polymers, which was lyophilized to form scaffolds. As you can see, it is a highly porous structure, and you have huge pores that are very nicely interconnected as well. This kind of a highly interconnected structure, which you can see in the magnified images can help in cell infiltration and also with nutrient diffusion.

This is one of the common strategies which is used; We will look at the principle, which causes this kind of porous structure. In the earlier cases, where we looked at salt leaching or gas foaming, it was quite simple. We had the salt crystals, which are present, which got washed away, creating the pore. You had the gas-forming salt, which then releases the gas, which could be ammonia and carbon dioxide in the example we showed; so, this causes the pores. However, here where are the pores coming from? We only did lyophilization. For that reason, you need to understand what the process of lyophilization does.

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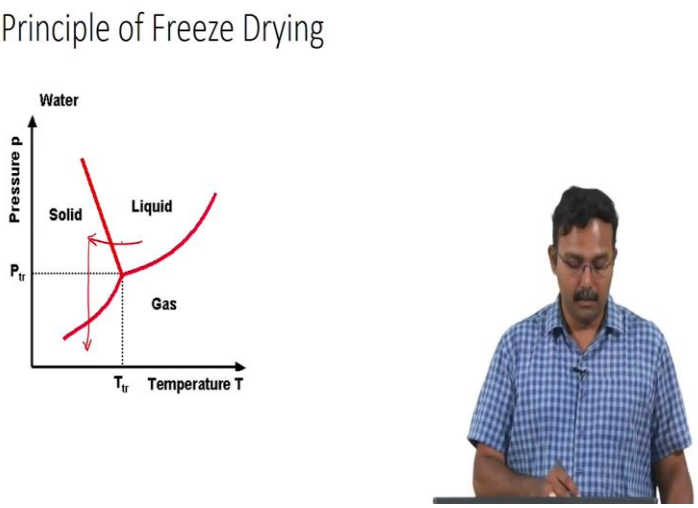

Let us look at the phase diagram for water. This is a simple phase diagram plot between pressure versus temperature for water. You have three phases, you have the solid phase, you have the liquid phase, and you have the gas phase.

These three phases are existing in the given temperature and pressure conditions. This point is the triple point; so, the triple point is where you would have all three phases existing in equilibrium. You also have these curves, which are the vapor-liquid equilibrium. This would be the gas-liquid equilibrium curve, and you would have the solid-liquid equilibrium curve here and the gas-solid equilibrium curve here. So, these are the curves that you have in this phase diagram.

Now, when we are talking about lyophilization, what do we exactly do? See when we prepare the polymer blend, and we pour it into a mould; you have liquid water. This liquid water, if it is evaporated, would be the process where you have liquid under some condition moving to gas. So, this is the process of evaporation. However, when you are talking about freeze-drying, what you are doing is not evaporation, you are removing the water at very low pressures.

What you do is, you freeze the water to form ice crystals, and these ice crystals are directly sublimated instead of being evaporated. It does not actually melt to form water and then gets evaporated; instead, these ice crystals directly sublimate to form water vapor.

So, how is this accomplished? This is done because at very low pressures in this region, where you see the gas-solid equilibrium curve. Here, solid can move to gas under this condition and pressures; then, you would end up with sublimation instead of evaporation.

What is done here is, you cool the water, So that it solidifies, and then you reduce the pressure to such a large extent that this solid ice crystal becomes a gas. By creating a vacuum and reducing the pressure significantly, you can convert solid ice crystals into gas. This way, you are creating what you would see with salt leaching or gas foaming techniques.

So, what you have done here is, you have created ice crystals within the polymer blend, and this ice crystal is then sublimated; So, causing something like a gas-forming effect. This results in the formation of highly porous structures. This is one of the most commonly used methods for fabricating scaffolds.

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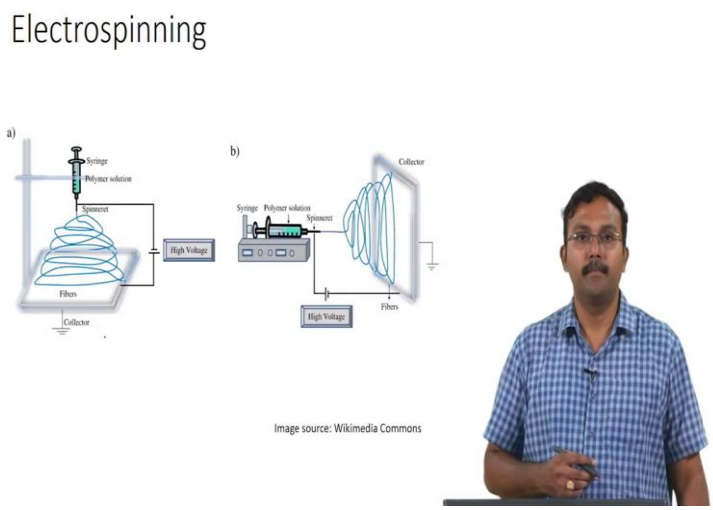

Let us move on to the next topic, which is electrospinning. As I already mentioned, some of the extracellular matrix is fibrous in nature. So, it is important to create a structure that mimics the natural ECM. For this reason, people have looked at some of the textile-based techniques, such as electrospinning, for creating scaffolds. Electrospinning creates nano-fibrous scaffolds that have been studied extensively for a variety of different applications.

Let us look at electrospinning here. So, what is electrospinning? Electrospinning is a technique where you apply a high voltage to create very thin fibers from a polymer solution. What you do is, you create a polymer solution that is loaded to a syringe, and this syringe is pumped using a syringe pump. So, this polymer solution that comes out of the needle is exposed to a high voltage, and because of this high voltage, it would form very very thin fibers. These thin fibers are collected on collectors, which could either be a flat surface or it could be a rolling drum, and so on.

Here, the parameters which can be varied are the flow rate at which the polymer solution is pumped, you can also vary the viscosity of the polymer solution, and you can vary the diameter of the needle; you could vary the voltage which is supplied. There are also parameters such as humidity, which can play a role in how these fibers are formed. Based on the distance between the collector and the spinneret, you would also have the thickness varying.

Another factor that can be looked at is the different types of collectors that are used. By using a flat collector, you would be able to get a nanofibrous mat that is not aligned. However, if you were to use a rolling drum or something, you can create the aligned fiber.

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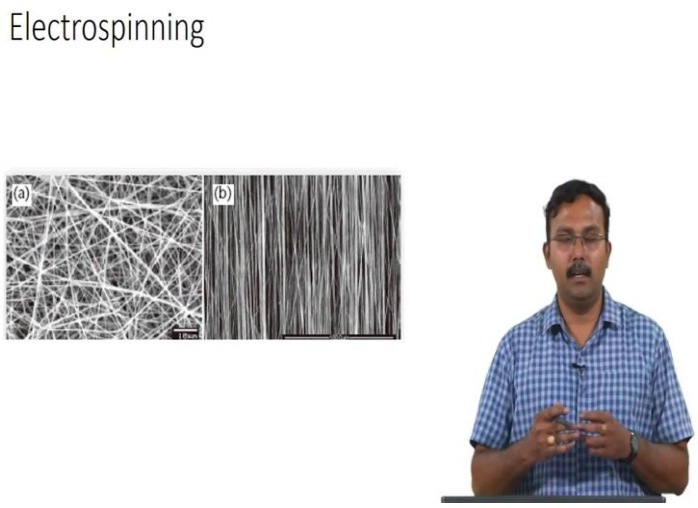

The scaffold, when you look it under scanning electron microscope, would look something like this; what you see on the left is non-aligned electrospun fibers, and what

you see on the right is the aligned electrospun fibers. These are some of the commonly used techniques which are used for scaffold fabrication.

So, we have given an overview; we have not gone into great detail of each of these techniques. Depending on the polymer which we have chosen and the application for which we are working, we have to choose fabrication strategies that will provide scaffolds that mimic the natural ECM. With this, we will move on to some of the more advanced techniques which are being currently researched for scaffold fabrication, which would be self-assembly and 3D bioprinting.

The self-assembly will we be discussed by Ramya one of my students; she is currently working on her Ph.D., she will describe how self-assembly can be used in tissue engineering. Her research is focused on self-assembly, so she would be able to give a very nice perspective. See you all in the next lecture.

Thank you.