Medical Bio Materials
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Lecture – 36
Experiments

Hello everyone. Today we are going to talk about how to carry out few experiments in the lab. Polymeric biomaterials are prepared with different morphologies, some of them are made as beads, some of them are made as films, some of them as nanoparticles, and also as electro spin fibers.

So, I am going to show you how to make each one of them. For example, if you take beads they are used quite a lot in bone implants, when there are bone defects, filing of bones beads are used. You can have beads filled with antibiotics or any antibacterial material. If you are going to make films, films are used in as diaphragm patches, heart patches, wound dressing and so on. If you are looking at electro spin fibers it is used as scaffolds it is again used as wound dressing material. If you are looking at nanoparticle they are used quite a lot in drug delivery systems.

So, these polymers can be prepared different morphologies and they find applications in different areas and the biomaterial field. So, we will look at each one of this how it is prepared using a simple standard polymer system.

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We will look at how to make beads of calcium sulphate. For example if you look at this, these are 4 mm calcium sulphate beads, and it is used in bone filling, bone defects after an orthopedic surgery. Calcium sulphate as you know is biocompatible, it also prevents biofilm formation and so on. We can also incorporate drugs antibiotics in to it so that the drug is slowly released and it prevents biofilm formation.

So, you need around 700 microlitre of water for about 1 gram of calcium sulphate. We need to use ultrapure water here. So, I have taken 5 times; that means I have taken 5 grams of calcium sulphate and 3.5 ml of ultrapure water.

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Because, look at these moulds here we can make 4 mm beads, here we can make 5 mm beads. So, if you want to cover as much area then you need to correspondingly take the same ratio. So, 1 gram of calcium sulphate we need 700 micro litre of ultrapure water. So, we need to mix it up well. Here I have taken 5 grams of calcium sulphate, I weight it and I take it in a mixing that is a magnetic stirrer inside. So, I pour them all together inside.

And then, I take the ultrapure water this is your pipette. So, we mix them here. As you can see slowly it starts getting mixed. This called a calcium sulphate hemiacetal. So, it got half H 2 O, it is also called Plaster of Paris, you must have all used it long time back if you have fracture; so it easily such very fast. And if you want you can add antibiotic, whatever type of antibiotic you want to use so that it can be used as a drug delivery system also. Especially if there are any initial implant related infection that can be completely avoided. So, we can see its getting well mixed or we can see the calcium sulphate is completely dissolved. We can stop the mixing.

Now we can pour it. So, as I said we can take more quantity depending upon how many beads you want to make. Here I am going to show you very small region. We just have to pour it on top of this. And then we have this applicator, so we need to tap it little bit so that the liquid flows nicely inside. As you can see here its filing up the holes and we need to tap it. So, it is done (Refer Time: 06:42) done. So, we need to leave for about 15 minutes, so that the water evaporates and get solid material in this. For convenience we have done it before, so I can show you that. So, this is made up of rubber; so it is quite flexible, it is available in the market. So, we can just buy it with different diameter sizes.

So, once it is completely dried as you can see here, we have made beads here. So, quite we can take it out here by inverting it. See, we can see. This is how the beads will come out. As you can see here, these you get beads of about 4 millimeter in diameter and these are beads which are used quite a lot in orthopedic implant surgery, bone filing, bone defects filing. After a surgery joint filing, with or without antibiotics, because it is biocompatible it will not cause any adverse reaction to the human. And we can, as I said do it with the antibiotic and without we can even think of making beads of hydroxyapatite and so on.

This experiment is going to be a demonstration of making a PVA film. PVA is a polyvinyl alcohol, it is a FDA approved polymer, it find application in wound dressing because it is got a gelling properties so it can be made in to a hydrogel. It is also used in drug delivery. So, this experiment will tell how to make a polyvinyl alcohol film. And we can make this type of morphology with different polymers. We can use water in this case PVA dissolves in water, but we can also use solvents whichever polymer dissolves in solvents then we use and then we can make films out of it. There we can also incorporate antibiotics; we can even put a nanoparticles, silver nanoparticles; for example which has got antibacterial properties.

Polyvinyl alcohol is a polymer; this particular polymer has got a molecular weight of 140000 dalton. So, it is soluble both in hot conditions as well as in cold condition. If the molecular weight goes up and up you may need to heat it up to actually dissolve it, but this polymer can also go in cold conditions. So, what we do is, we take 5 grams of PVA powder; as you can see here 5 grams of PVA, and then we need 100 ml of ultrapure water.

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So, what we do is this is again got magnetic stirrer as you can see at the bottom, and then we add them together. And then we add 100 ml of water. We have to here both do both agitation and heating around 60, 70, 80 degrees is enough, lower the molecular weight faster will be the solubalization here. As you can see mixing is taking place, we need to heat it up also here. And approximately around 15 minutes this polymer should completely solubilize in water. As you can see PVA is completely dissolved in water. Once it is done we need a watch glass in to which we are going to pour the solution. The diameter can be bigger or smaller depending upon the size of film you want to have, and of course you need corresponding amount of the PVA solution also.

So, you need to place it and pour it uniformly inside. So, we can pour it inside uniformly so that it spreads. And then we need to leave it like that. So, the solution completely covers the entire surface uniformly, and then we need to leave it for 24 hours so that the water completely evaporates and we can get the film which can be peeled out. After 24 hours the solvent would have evaporated or in this case water, and we will be able to peel out the film very nicely as you can see.

You can see the PVA film here, it got a gelling property; that means it will swell when you add water and it is used as a wound dressing. And if it is coated with antibiotic it will also have antibacterial property and so on. So, similar approach can be adopted for making films of several other polymers, like PLA, PLJ, but you may have to use solvents in those cases also.

This is a nano fiber electro spinning unit, we can make polymers or polymer blends as a fiber form in nanoscale.

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Typically, if you look at nano fiber matte of a polymer it look like this it is very useful in wound healing applications as well as for scaffold design. And nowadays lot of interest is shown on electro spinning, because they are a nanoscale so there will be more surface area to volume. We can even incorporate drugs; we can even incorporate growth factors so that cells grow very well.

So, what is the principle of this? We take a polymer solution here and solution comes out through the needle, there is a voltage applied at the needle tip. The solution hits the collector where there is another voltage. So, because of the positive and negative the solution travels and hits the collector. As you can see there is an aluminum foil collector. So, we start collecting the fibers and we adjust the various parameters to get nanoscale fibers in the collector.

So, there are many parameters we need to control here. The voltage you are applying here, the distance between the needle tip and the collector plate, and the flow rate of the polymer that is coming out, we can adjust the temperature of this chamber. So, all these are parameters which will needs to adjust. And of course, the polymer solution itself is there we need to adjust the composition of that solution so that we end up getting nanoscale fiber uniform fibers.

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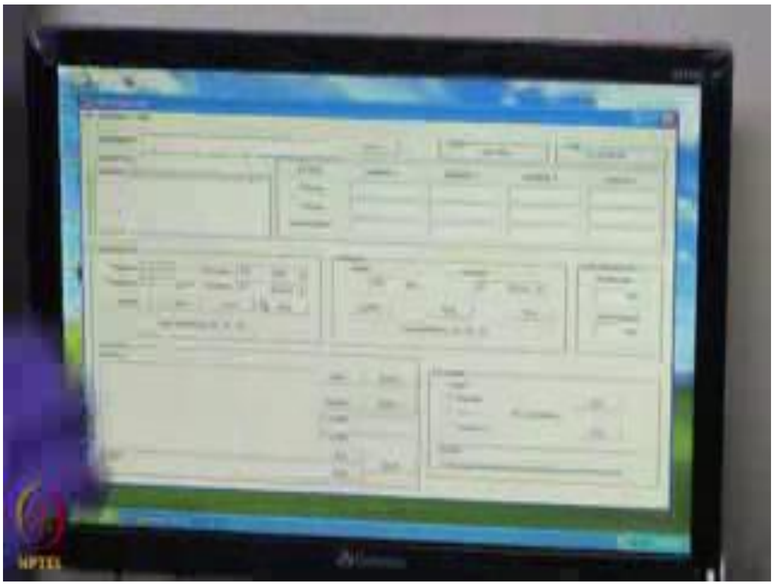

There is control software here which controls electro spinning unit, several parameters can be controlled here. For example, the volume of the liquid polymer solution you have taken is given here, the flow rate at which the polymer solution is coming out of the needle is adjusted here, the diameter of the syringe, the duration how long you want to run it. So, this is a flat plate. In case you have a plate which is rotating it is called a rotating mandrel we can set the speed of the rotating mandrel also here and also we can tell how long we want to run this electro spinning unit also by setting here.

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We have to fill the polymer solution in this syringe which also has a needle. This is not like a doctor’s needle because it is blunt so that the solution can come out when there is an applied voltage. Here again we are planning to make electro spin fibers of polyvinyl alcohol, that is PVA. We take 5 grams of polyvinyl alcohol of about 140 kilo dalton molecular weight in 100 ml water. If we heat it slightly with about 10 to 15 minutes it completely dissolves. So, I am going to take this polymer solution in to this syringe.

This syringe is filled with PVA solution, we place it here. After placing the needle in the holder and tightening the screws this is the positive electrode to which the needle is placed. This is the collector plate on which we have aluminum foil for easy removal, then we place it in the collector, and then tighten the screws. And we clamp it with the negative.

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Let me explain these controllers here: this is the heater in case you want to heat the chamber to higher temperature. The temperature inside the chamber is displayed here. This switch is for the lamp here, when we are doing electro spinning we will switch off this and we will use the halogen lamp which is shown there, because you can see the fibers coming out only through the halogen lamp. And then this switch is for the controller, this is for the exhaust, because when you are using solvents we need to remove the solvent vapour. And this one is for the setting up of the high voltage system.

So, here it displays the voltage and here it displays the current. We set the voltage and current so that we get very fine nanoscale size fibers and also the fibers are loose sothat it does not sort of bind to each other.

Now, we switch off this lamp and switch on the halogen lamp, shut the door. And we have to initiate the run through the computer so that the polymer solution starts coming out, then we adjust the voltage and the current (Refer Time: 19:49). We increase the current little bit and then start increasing the voltage, as you can see the voltage is increasing.

Student: Now we use the current (Refer Time: 20:41).

Once we have collected enough fibers we switch off this machine, we remove the voltage (Refer Time: 21:28). And then we remove the collector slowly, and we can see the deposit of the polymer. We can see the deposit of the polymer, nano fibers on this aluminum foil. We can nicely peel it off as you can see here from the aluminum foil; and these.

Student: (Refer Time: 21:57).

This is a nano fiber of a polyvinyl alcohol. If we look under a scanning electron microscope you should be able to know the diameter of the fiber, it should be in a nano region. So, this product can be used for wound healing purposes, burn, injuries and it can also be used as a scaffold material for growing cells of various types.

I am going to demonstrate how to make beta glucan nano particle. Glucan is also known as a curdlan, its beta glucan and it is produced by bacteria, will also find it in some of the food products also. Glucan is approved by FDA to be used as a gelling agent and it also has immunomodulatory properties, so it is well approved. And when we make nanoparticles with beta glucan we can have drugs encapsulated, sometimes we can have flavoring agents', colourance and so on.

So, when we make these nanoparticles they will be in order of less than 100 nanometers. We have to add polyvinyl alcohol to stabilize these nanoparticles, otherwise these nanoparticles may agglomerate together and it may form bigger particle size. So, I am going to show how to make this beta glucan nanoparticle.

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This is not water soluble it is soluble in alkali; it is also soluble in acids like formic acids. So, we are going to dissolve this in formic acid. So, I have taken 40 milligrams of curdlan or beta glucan, I have taken 2 ml of formic acid. And we are going to mix them at room temperature using magnetic stirrer.

As you can see this is the magnetic stirrer, put it inside, and then I stir it. So, we stir it for about 5 to 10 minutes so that it completely dissolves in to it as I said beta glucan is not soluble in water, so it is only soluble in alkali or even mild formic acid. So, we do this for about 5 minutes. We need to make a PVA solution, well PVA is polyvinyl alcohol I am using 140 kilo Dalton. PVA will try to stabilize these beta glucan nanoparticles.

So, PVA I have it here as you can see its fine powder, and then 0.5 grams of PVA I take, and I take 50 ml of water. I again stir it, but here I have to heat it 250 degree centigrade. And again stir it here and I have to heat it 250 degree centigrade. So, for another 5 to 10 minutes I set the temperature, because PVA is not soluble in cold water so we are heating it 250 degree centigrade. Now I transfer this PVA solution, now I need to add the beta glucan formic acid drop wise using a syringe in to this. So, I have previously prepared beta glucan in formic acid, as you can see here beta glucan in formic acid.

So, now I will take it in a syringe. As I said PVA will try to stabilize the nanoparticle. As you can see here small white that is the nanoparticle of beta glucan, can you see this? We need to use the probe sonicator to arrive at nanoparticles of beta glucan stabilized by polyvinyl alcohol. This is the probe sonicator and we have to keep our sample inside. Sonication will be done for 5 minutes, the sonication will be on for 9 seconds and 1 second off. Because lot of heat is produced, we have to keep the whole sample inside the ice buff. As you can see here, this is the control unit for the probe sonicator. So, we need to set this control, we are going to sonicate for 5 minutes, it will not be continuous there will be pulses and then stop. But every 9 seconds there will be pulse and 1 second there will be off.

We will switch on the control unit of the probe sonicator, we will set the sonication time for 5 minutes; now we will set the pulse, pulse on for 9 seconds, pulse off for 1 second enter. So, it is got pulse on for 9 seconds pulse off for 1 second, it is going to be done at cold temperature. On this once this sonicator tip is well dipped we start the control unit, and the sonication proceeds so that you get nanoparticles. For 5 minutes we sonicate the sample. Once the sonication is done we have to centrifuge, so we transfer the material in to a falcon tube to collect the nanoparticles. This goes in to a centrifuge and centrifugation is done for 10 minutes at 10000 rpm. And later on to lyophilizer where moisture is removed and we get very fine product at minus 60 degree centigrade overnight.

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Nanoparticle which we prepared using a sonication needs to be recovered from the liquid. We use a centrifuge here, we have to centrifuge at 10000 rpm for about 10 minutes. Use an adapter and place the falcon tube and we also have to balance it with another empty tube, then we can close the centrifuge. This is a lyophilizer where the wet nanoparticles can be completely dried by removing the moisture at about minus 80 degree centigrade, and this is run overnight so that all the water gets removed.

After centrifugation and lyophilization we get dried nanoparticle of beta glucan or curdlan stabilized by polyvinyl alcohol. As you can see here, these are product if you incorporate drugs then it will be drug loaded beta glucan, we can incorporate food and flavoring, even coloring agent. So, it can go in to food industries as well. And as I said originally beta glucan has immunomodulatory properties, it is got anti inflammatory properties, and it is been approved by FDA.

I am going to demonstrate how to prepare iota carrageenan and beta cyclic glucan hydrogel. Hydrogels can be used for wound healing, drug delivery, drug encapsulation.

This iota carrageenan is polysaccharide made up of sulpogalactone, and it is obtained from marine upper fibers with almost 20000 molecular weight. And this cyclic beta glucan is produced by bacteria; it is got 10 to 12 glucose in a cyclic form. So, the molecular weight will be around 1200 to 1400. Together they will act as a hydrogel, it will be very hydrophilic, it will be able to absorb and retain moisture and the beta linkage in the glucan also will act as aid for wound healing as an amino modulatory and so on actually.

So, we start preparing this carrageenan beta glucan hydrogel. You will have 75 percent carrageenan, 25 percent glucan. This beta cyclic glucan is water soluble, whereas the beta linear glucan or curdlan is not water soluble. This glucan is water soluble that is why we are using only 25 percent and the 75 percent will be the carrageenan. And later on we will be cross linking with glutaraldehyde.

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So, initially we start with 4 ml water, this is hot water bath. So, we heat this water to 50 degree centigrade, we take carrageenan 56.45 milligrams of carrageenan, we add to this hot water. Now we raise the temperature to 70 degree centigrade for about 15 minutes so that the carrageenan completely dissolves in it. We can intermittently mix this; this is a mixer. As you can see here we can mix this, and then again heat it up so that it is completely dissolved. Once that is done we add cyclic beta glucan which is 18.75

milligrams to this. Again we heat it up to 70 degree centigrade, mixing it from time to time.

We add 0.75 milliliters of glutaraldehyde; glutaraldehyde is a cross linking agent, so it will cross link carrageenan and the beta glucan; that is this is 1 percent. So, we add it to this. We can pour this in to a watch glass, and leave it for air drying for 12 hours; that is overnight. This is the dried overnight dried carrageenan beta glucan film. We need to peel this. So, we use 70 percent methanol water, spray it on top. Now it is easy for you to peel it out. As you can see this is the glutaraldehyde cross link, iota carrageenan cyclic beta glucan, and the ratio of 75 to 25.

We call this hydrogel because it will swell when we add water to this. When we add water to it, it should retain water and swell. That is why it is called hydrogel. As you can see the gel has swollen, taking up lot of water. And this property helps in designing wound healing patches with this carrageenan and beta glucan.