Tissue Engineering
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Lecture - 12
3D Bioprinting

Today, we are going to talk about 3D Bioprinting. This is one of the more advanced technologies which is out there where people are trying to fabricate scaffolds using this. This gives a lot of advantages; you can get a more precise microarchitecture if you were to use 3D printing appropriately.

In this lecture, we will talk specifically about 3D bioprinting. 3D bioprinting is slightly different from 3D printing. So, in 3D bioprinting, you will be using cells along with the ink, whereas in 3D printing, you would just print with regular ink and then you will seed cells on it. So, we will talk specifically about 3D bioprinting today.

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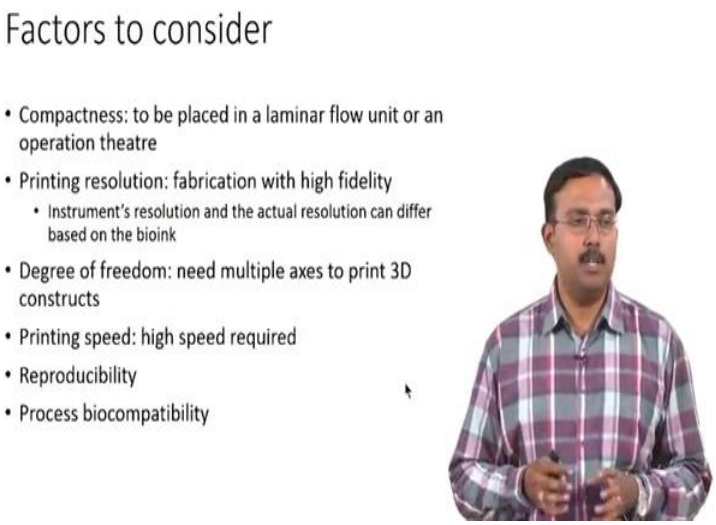

When you are talking about designing scaffolds and tissue engineering constructs, there are two approaches to it. One approach is that top-down approach where you prepare materials that resemble the shape of a tissue, and then you seed cells on top of this and then let the cells grow and culture them for a period of time, and then you implant this tissue-engineered construct into the body.

The problem with this is, you do not have control over how the cells are distributed, and the ECM microenvironment may not be created when you do this. If you were to culture the cells along with the ECM being formed, then there might be remodeling of the ECM, which might not be present in a top-down approach. Whereas, bottom-up approach is where micro or nano size blocks of cells and the biomaterials are used to build the tissue. So, what you have is very small structures that contain cells themselves, and you combine these in a way to create the ECM, which will act as a scaffold.

This can provide better control over cell distribution and also the ECM microenvironment. There are many techniques to do this. Some of the techniques are soft lithography, self-assembly, and 3D bioprinting. Today, we will talk about 3D bioprinting. In tomorrow's lecture, we will be covering self-assembly, ok. The other techniques which we looked at earlier were all the top-down approach, where we were talking about freeze-drying or solvent casting and salt leaching.

All these techniques are top-down approaches where you create the scaffold independently without using the cells, ok. Self-assembly can be both ways. Just like how 3D printing can also have a top-down approach and a bottom-up approach, self-assembly can also be top-down or bottom-up.

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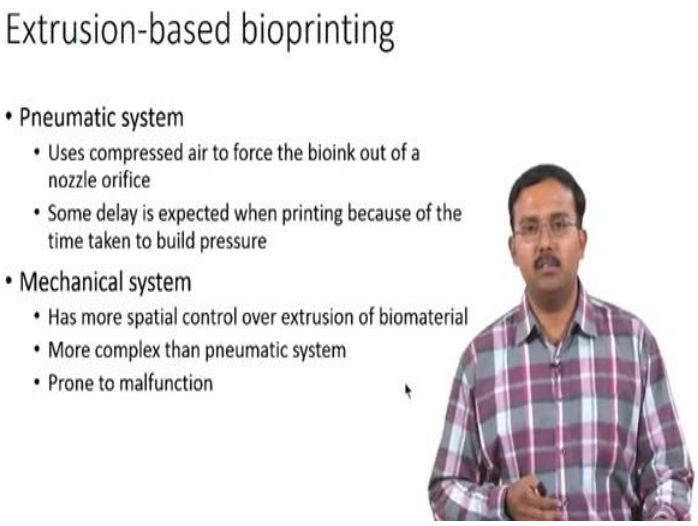

We will talk about 3D bioprinting today. This is an additive manufacturing or 3D printing technique to form complex functional living tissues. This would include a bio-

compatible material that is used along with the cells, and you might even add supporting components. For example, growth factors could be blended along with the material and the cells, and you could print it along with that. So, it is possible to do that; people have tried to do that.

Conventional 3D printing is used to create cell-free scaffolds. So, that is what the 3D printing club here is, right. There is a 3D printing club where people just print 3D structures. And those are just cell-free materials. You can print it in any shape you want, and you can actually print it in the shape of an organ if you choose to. But in bioprinting, you would want to print it along with the cells; you would want to load the cells and then print it.

The complexities which are compared to nonbiological tissues are much higher. We need to understand some of the challenges associated with 3D bioprinting so that we can adopt 3D printing technology to print biological tissues. So, we need to know what materials can be chosen, what cell types you are planning to use, whether you are planning to use growth factors, and differentiation factors. So, all these things will regulate what type of printing technology you can use and what would be the conditions you can use for printing and so on.

The sensitivity to living cells should also be taken into consideration. Mammalian cells are sensitive to shear stresses, so you would want to use something which will not cause destruction of the cell. And you felt ultimately need to understand the construction of the tissue is going to be much more intricate than a macrostructure which you would try to print. Printing a pen or a gun is not the same as printing a tissue, right. The microarchitecture is very different. So, that makes it quite complex to actually develop 3D printing technologies for biological tissues. Currently, people have tried to print different tissues using 3D printing.

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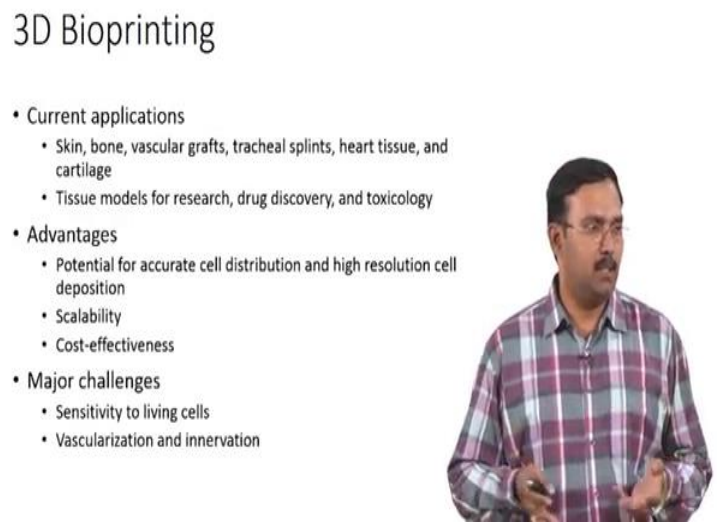

Some of these have been reported in the literature. You can look up the literature, and you will be able to find all the applications which I have listed here; skin, bone, vascular grafts, tracheal splints, heart tissues, cartilage have all been printed using 3D printing. The tissue models for research and drug discovery and toxicology have also been printed. These are just models where you would do studies or research on it, rather than to take it up for implants.

The advantage of this technology is that there is a potential for accurate cell distribution and high-resolution cell deposition. And it could be a very scalable process depending on the type of 3D printing technology you use. And it could also be cost-effective, again based on the technology you use. But the major challenges are sensitivity to living cells and vascularization and innervation.

The aspect of vascularization and innervation is a common problem for any aspect of tissue engineering; however, the sensitivity to living cells is more unique to 3D scaffolds, right. So, if you are going to have something like self-assembly, which is again a bottom-up approach. Self-assembly will use materials that the cells are used to, and you do not have to provide very toxic environments or like very extreme in conditions, whereas, here, you might have to provide that kind of an environment for the printing to happen. So, that makes it a unique challenge to be overcome.

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When you are talking about 3D bioprinting, these are the things that you have to look at. We will talk about pre-processing and processing. Post-processing is kind of common for all tissue-engineered constructs, and application is also the same.

So, pre-processing involves imaging and 3D modeling and slicing. You first need to understand how tissue architecture is, and you get that from images. So, you can get images from X-rays, MRI, CT scans, ultrasounds, optical microscopes whatever is at your disposal; you can use them to create images that you can process to get the 3D structure. And this 3D structure would have to be then modeled. So, that it can be fed to a computer to be printed, right. So, that is the pre-processing aspect.

And during the processing aspect, which is where you are trying to print the tissue, there are two major things that you need to work on; one is the bioink. So, what would be the ink you use. That is the material on which the cells are going to be cultured. So, identifying the bioink is a major challenge, and then that printing technology itself which printing technology would you want to use. The bioink preparation will also depend on printing technology. Hence, depending on the technology you use, you would have to design a bioink which will be suitable for that printing technology.

Post-processing is common, so anytime you create a tissue-engineered construct, you are not going to have enough cells on it, right. You are probably just going to have cells seeded on it, so you might want to expand the cells by cell culture. In cases of using stem

cells, you might want to differentiate them to form the specific cell functionalities and cell phenotypes. You also might want maturation of tissue and organ, which would require vascularization and so on, which might happen in vivo. These are post-processing things that will be done for any tissue-engineered construct. So, it will also be done for a 3D printed scaffold.

Finally, you take it to the application, whatever the application could be. Here they have just listed two applications, one is being transplanted to the patient, and the other is being used just as a model system for studying in vitro cultures and so on. So, this is the general idea of 3D printing. This is the process in which 3D printing is taken up. So, let us go through some of the steps and see what exactly can be done.

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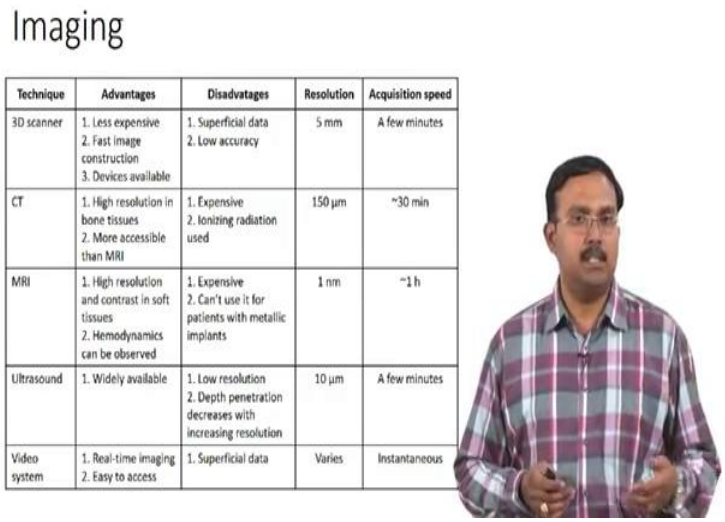

The first thing is imaging. There are different ways you can image a tissue. We can use a CT scan, MRI, ultrasounds, X-rays for bone tissues, and so on. And you can use simple video systems that can also be used, and then you can process these things. All of these have their own advantages and disadvantages. A 3D scanner is something that can be used, and it is quite simple to use. It is less expensive to use this process; it is very fast to create this image; it can scan something very quickly, and you would be able to reconstruct the image very quickly. Devices are commercially available. You can buy that and use it.

But the disadvantage would be you will get only superficial data. You will not get the intricacies of that tissue. For creating that, you might want to create multiple cross-sections, then process them together, which will add to the complexity of the process itself.

Student: Sir, is a 3D scanner is the same as an ultrasound scanner.

No, it is not. So, the 3D scanner is, I do not know where you would have seen something. It is something like what you see in an airport. The airport has a scanner that is used to detect what is in, it is more like an X-ray scanner, but this is a 3D scanner that would look like that. So, it just scans tissue or an organ all over and comes up with the 3D structure.

Student: Actually, I have seen it; it is a portable device you hold on hand. So, now, let us say an object. So, you, it is like program such a way like you move it, you just scan it through all the direction it will build an image accordingly.

Right. So, that.

Student: And there are also scanners where you put an object inside. So, it will scan accordingly from straightway.

It is like a regular scanner that scans a surface; here, it just does it for 3D structure. So, you can have it in any size and shape. And the problem with this is its very low accuracy. It will give only 5 mm accuracy, but you are getting such fast processing, so you will have to compromise on the scanning ability. So, if you are going to use a 3D scanner for a tissue microarchitecture, you are probably not going to get any information.

Like 5 mm is a too large resolution to use it for biological tissues, but the acquisition speed is very high. So, this can be used primarily for non-living like structures. So, if you are going to have only macrostructures that are there, then this kind of scanner might work.

CT scanner has high resolution in bone tissues, and it is more accessible than MRI. So, it is commonly used for scanning. MRI is quite expensive, and it is not very easily accessible. But the problem is, this is also expensive, it is not that it is very cheap to perform a CT scan. And ionizing radiation would be used while performing a CT scan.

So, the resolution is better; it has about 150 μm resolution, and it can take about 30 minutes to acquire the image, and you would be able to get enough information for organ constructions. If you are looking at smaller tissues, even the 150 μm might not be sufficient for getting all the information about the microarchitecture.

You can do an MRI or ultrasound. MRI is a very high-resolution technique and takes images using a contrasting agent in soft tissues, and it can help you in studying the hemodynamics as well. You can identify where the vascular network is and try to emulate that while you are trying to prepare the scaffold.

However, it is expensive, and it cannot be used in patients, who have a metal implant, so that would restrict the usage. But it gives a very high resolution. So, to about 1 nanometre. And it can take about an hour to do this. And not everybody is also comfortable going through an MRI.

Has anybody gone through an MRI? Yeah, you have done, ok. If you have claustrophobic, you would not want to do an MRI. You will get really scared, you have to be put in the tube, and you are trapped in there for like an hour, and you are expected not to move. It is quite a daunting thing for a claustrophobic person.

Ultrasound is a more widely used technique; it is quite easy to use. You just use a small scanner, and you can scan the person, and you will be able to see the tissue. It has a low resolution. Depth perception can decrease if you increase the resolution. It has about 10 μm resolution and can take in a few minutes, but it can again give outer structures as inner structures, can become very difficult because the resolution will get poorer as you do that.

So, video systems have also been tried to be used, so real-time video and images are processed. You take the superficial data, and you process it and try to build a 3D structure out of it. The image processing aspect of it plays a major role in constructing the 3D structure.

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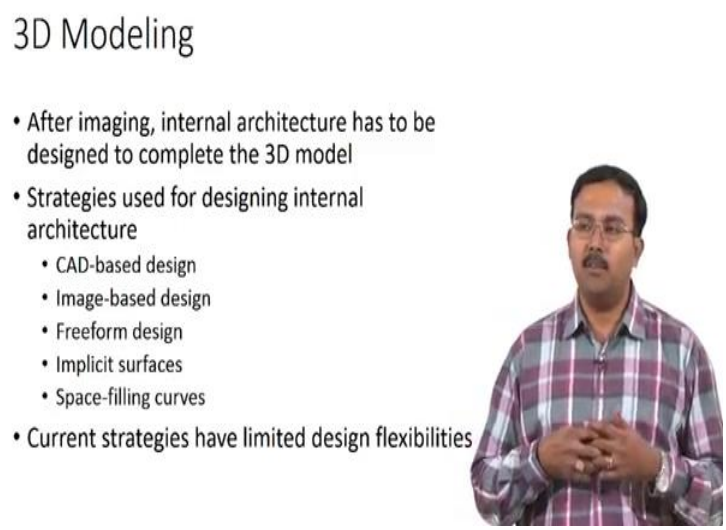

So, these are the different imaging techniques that are used. But after you get the imaging, you still have to come up with the internal architecture. You might not have all the details of the internal architecture with just the imaging that we have done. You would still have to fill the voids using the architectures for which building the 3D model is done through different approaches. So, some of the strategies which are used in designing the internal architecture are CAD-based designs, image-based designs, free form design, using implicit surfaces, and the space-filling curves.

I will not go into details of all of these. I have given a couple of references at the end of this lecture. So, these are two very good review articles that were very recently published. I will try to upload them to moodle as well. You can go through them if you are interested in getting the details of all these processes.

The current strategies which are being used have limited design flexibilities. It is not like you can get an exact microarchitecture. So, that is the problem. Although 3D printing theoretically can give you the exact microarchitecture, you still have to draw that microarchitecture onto a computer. That has to be some way to do that, and those aspects are still not very accurate. People are trying to work on it; people are trying to use different things to get something that would be similar.

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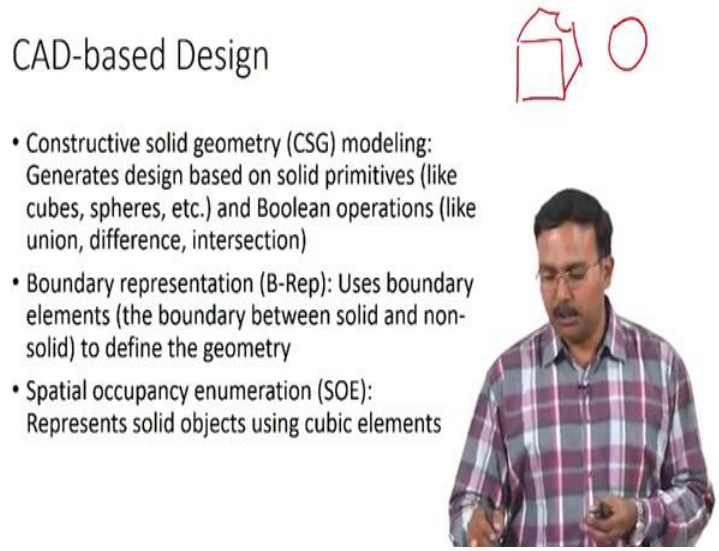

CAD-based design; there are three major approaches which are used in CAD-based designs. This is one of the more commonly used strategies for designing internal architecture.

Constructive solid geometry modeling is one where you use solid primitive structures and Boolean operations to build the actual structure. If you were to take two structures, let us say you have a cube and a sphere. Now you can combine these two in different ways to get different structures. You can add these, and you would get something that has a sphere here, or you could delete this, and you will end up having something like this where you have this as the structure, right. It is possible to do either of these. And you can also have an intersection which will only be a portion of this sphere and so on. So, this is just for the sphere and cube. You could have other solid primitives which you can bring combinations thereof using Boolean operations to get different internal architectures, which can closely resemble what you would see in real tissue.

Another strategy that is used is a boundary representation. Here, the boundary elements are used. It is basically the line between the solid and the non-solid is taken as the boundary, and this is used to define the geometry.

Spatial occupancy enumeration is another strategy that is used. This represents solid objects into cubic elements. So, you know pixels, right. So, what are pixels? The tiny

squares on a 2D surface, right. If you take an image, you look at how many pixels are there. Your screen resolution is usually given in terms of pixels per square inches, right.

Similar to that, for 3D structures, there is something called voxels. So, these are 3D cubes that are used. And you can organize these 3D structures to get the desired shape. If your voxel is very, very small, then you can get very nice 3D structures. So, these are just some of the strategies which are used for designing the scaffolds using 3D printing.

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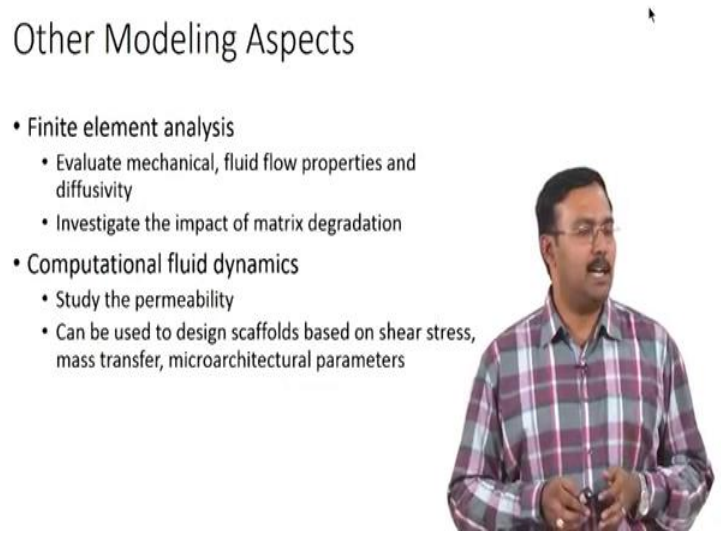

There are also other modeling aspects that can be incorporated into 3D printing. It is not just for bringing in the internal structure; you can apply the finite element analysis method to evaluate the mechanical and fluid flow properties and diffusivity of a scaffold that you would be printing. You can also investigate the impact of matrix degradation, just looking at how the scaffold will change due to this.

Computational fluid dynamics can also be used to study the permeability, and it can also be used to design scaffolds based on the shear stress, mass transfer and the other microarchitectural parameters which should be there because of the fluid flow into the system, ok. These are some of the modeling aspects which people have tried to incorporate as part of the 3D printing technology.

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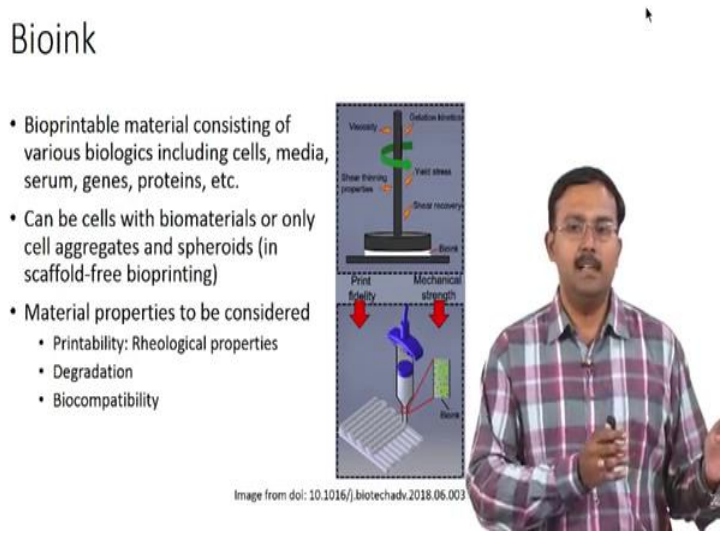

Whatever we discussed until now are the aspects where you would need expertise in image processing and computational techniques and so on. The aspect which we are covering here is where you would need people with more chemistry and materials background. So, you need to design a bioink. Whatever your printing basically has to be some kind of ink which you print it out, right. So, this bioink is nothing but a printable material, which will consist of various biologics.

It could be just the cells, or cells and media with serum, genes, proteins, growth factors, whatever. So, that would be the bioink which you have to use. This can be cells with biomaterials or only cell aggregates as well. You have scaffold-free bioprinting where people have tried to use only cells which are suspended to be printed, but the problem with that is usually the mechanical strength is not very good. You would not be able to get a strong tissue that you can use for replacement; however, you would have very high cell density. So, it is a trade-off between the two.

Material properties that you need to consider while you are designing a bioink are printability. Whether the ink is printable, whether you will be able to load it, and whether its rheological properties will help you in printing the tissue. Then, you have to look at the degradation factor. How well this material is going to degrade and whether it will degrade during the process, so then and so on, and compatibility. So, these three aspects have to be chosen carefully to identify the appropriate bioink for 3D printing technology.

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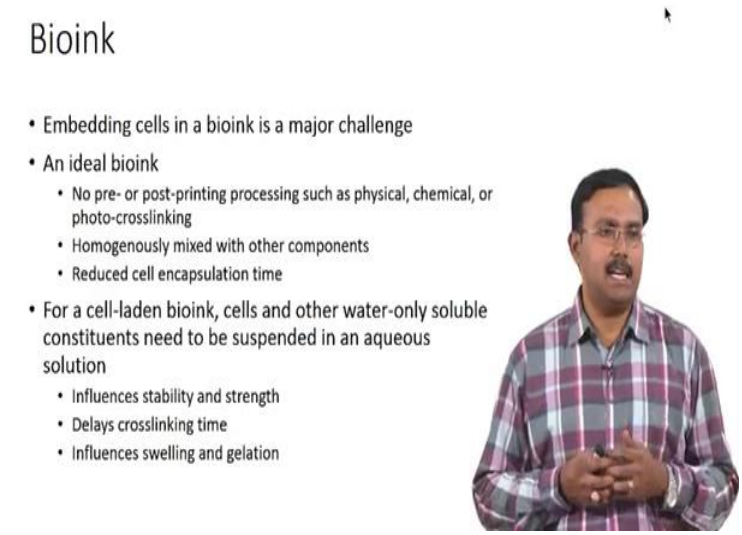

In designing a bioink, the major challenge is embedding cells into the bioink. You need the cells basically encapsulated by the bioink when it is being printed, and that can be a challenge. An ideal bioink would require no pre or post-processing after printing; you should not have to treat it with chemical agents to crosslink or provide some physical stresses for it to crosslink to form a solid structure. It should be able to form this kind of a solid structure while it is being printed. So, that is going to be a requirement for getting an appropriate 3D printed construct.

You need to have a material on which everything can be homogeneously mixed because you might end up with something viscous. These inks are not going to be like aqueous solutions; these are going to be viscous solutions. So, which means you need to have an ability to disperse things homogenously, otherwise if you are going to have them loaded in one part, and it is not going to print uniformly or print the way you expect them to print.

You also should have reduced cell encapsulation time; in the sense that it should not take a long time for the cells to be engulfed or dispersed in this, and if that is going to be the case, then the cells are probably going to die. So, you would want it to have a short encapsulation time.

For creating a cell-laden bioink, cells and other water-soluble components are usually suspended in an aqueous solution, and their concentration in which it is being dispersed

and other components that are loaded can have a serious effect on the stability and the strength of the final construct which is printed. It can also delay the crosslinking time; it will obviously, affect the swelling and gelation properties. So, while you are preparing these inks, preparing the appropriate aqueous media, aqueous solution also plays a role. It is not just about the polymer you use.

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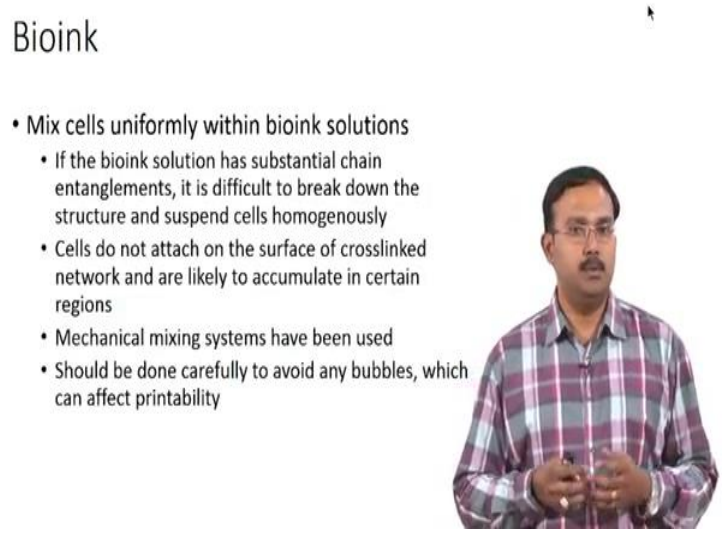

When you are trying to use a bioink, you are trying to mix cells uniformly within the bioink solutions most of the time. When you try to do this, you need to understand that if the bioink solution has many chain entanglements, it will be difficult to break this down and suspend cells uniformly. You want the bioink to crosslink as soon as it is coming out of the printer, right. So, only then you will have a solid structure.

So, you would want it to have some entanglement initially, you cannot expect all the entanglements to happen, right after being printed. If you are going to have these entanglements initially, it needs to be in an optimized way, so that cells can be uniformly dispersed; it cannot completely affect the cell distribution. Cell distribution cannot be just in pockets, that will be a problem.

Student: Even if any, initially, cell distribution is pocketed or fragmented, but later on when cells are proliferated, it will be uniform later on, right?

It depends on how the crosslinking has happened, right. So, if the entanglement is very strong and you have a lot of crosslinking, cells will not be able to infiltrate.

Student: Ok.

So, that is the challenge.

Cells do not attach on the surface of the crosslinked network and are likely to accumulate in certain regions. It will not be dispersed if you have too much crosslinking. For getting a uniform distribution, people have even tried mechanical mixing systems. While you are mixing it using mechanical mixing systems, you need to make sure that the shear stress which the cells encounter is lower because the cells are shear sensitive, and you would not want just to keep rotating them in a flask for a long time.

In addition, it should also be done very carefully so that you do not have any bubbles. If you have bubbles, it affects the printability. While the ink is being printed, you should not have any bubbles; it should be a uniform flow of the ink. So, these are things that need to be taken care of while you are preparing a bioink.

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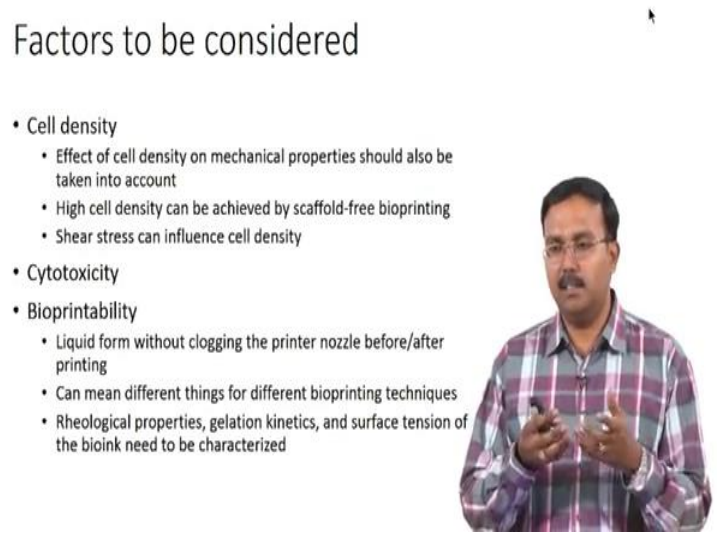

The factors which you need to consider while you are preparing a bioink is cell density. The effect of cell density on the mechanical property should be taken into consideration. If you have very high cell density, then your mechanical strength is probably going to be low. Because the more the polymer, you will have better mechanical strength, right. If

you are going to have a lot of cells, you cannot have a lot of polymers too; so, your mechanical property will come down when you have very, a lot of cells.

Student: Sir, do they use any reactors or anything for the bioink preparation?

I do not think they use it for bioink preparation. The reactors are used for post-processing. Once you have the cell layered in construct, then you can use a reactor to provide signals, physical cues, and other signals for the tissue to be developed into a mature tissue; but for preparing inks, they do not use any reactors. High cell density can be achieved by scaffold-free bioprinting. You need to understand that shear stress can affect cell density. So, depending on the 3D printing technique used, the cell density will also be affected.

Cytotoxicity of the ink has to be taken into consideration when you are using it. It is not just the polymer you are using; you might have a solvent; you might have other additives that are present along with the polymer. So, you would have to take all of that into account while you are printing. So, the cytotoxicity of all the components has to be looked at.

Bioprintability is an important aspect, whether it is actually a printable thing. The liquid form should be able to come out without clogging the nozzle. We will talk about the types of printers later. But, in some types of printers, you have a nozzle, which means the ink actually comes out of this nozzle. So, this should not clog the nozzle. If it clogs the nozzle, then whatever the ink which comes out is not going to come out at the same flow rate, so the printing is going to be very different, and as it clogs more and more, it will completely stop the flow. So, the ink should not clog the nozzle.

Another thing is, this printability itself can mean different things for the different techniques which are being used. While we talk about the techniques, we will talk about what exactly would be a printability for that specific technique, right. You have a question.

Student: Yeah. Regarding the printer nozzle, is it possible to engineer the nozzle instead of just the liquid so that, I am not sure that I do not know anything about it, maybe the material or something or maybe the shape, so that it does not stick in; is not that

possible? Because it will be on a small scale. So, it will be dealing with those kinds of problems.

Yeah. So, see nozzle, you can design nozzles in different ways you can look at using different materials for nozzles and things. But the problem usually is, if you want a very high resolution of printing, the nozzle is going to be very small, right. So, then clogging is a problem which you will face anyways. If you are looking at the larger diameter tube, then it is not a problem. If you are looking at micrometers kind of diameters, then it can be a problem.

Student: What kind of scale are they printing at these days?

So, to about a few micrometers, the resolution of a few micrometers people have been able to print.

Student: Are they able to place single cells?

Yes. With laser technology, people are able to place single cells. So, each droplet contains a single cell. But laser printing has its own disadvantages. Some of the properties which govern the printability are the rheological properties, gelation kinetics, and the surface tension of the bioink. So, all these things have to be very well characterized, and we need to understand how this will affect the process before we take it for printing.

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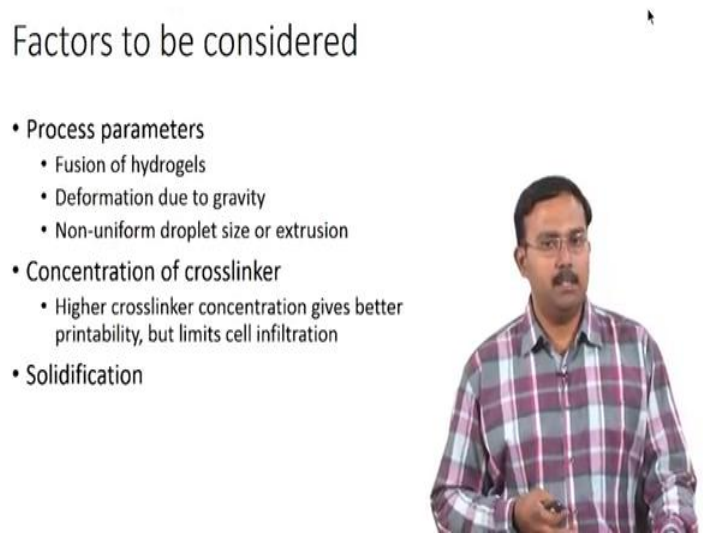

Some of the process parameters that need to be considered are the fusion of the hydrogels while it is being printed and deformation due to gravity. If you are going to print a 3D structure, it should just not collapse, right. Because it is quite possible that may happen due to gravity. Non-uniform droplet size or extrusion can happen because of how the process is being taken care of. The concentration of crosslinker will also play a role in some of these properties. For example, gelation will depend on the concentration of crosslinking.

If you have high crosslinker concentration, you will have better printability because it will form like very stable structures after printed, but it will limit cell infiltration. Another thing is the final solidification of this construct after it is being printed. So, all these factors have to be looked at while you are printing a 3D structure.

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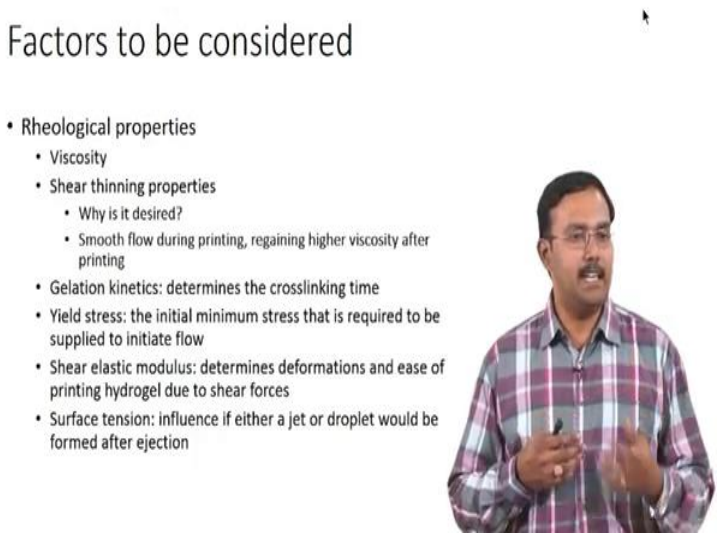

When you are looking at the rheological property; viscosity plays a role when you are printing it. Another important property you look for is the shear thinning property. So, why do you want shear thinning property? So, what is a shear-thinning property?

Student: For non-Newtonian fluids shear force, there is the viscosity reduces.

Ok. So, it is a non-Newtonian fluid where when you give a shear, the viscosity reduces. Newtonian fluids and non-Newtonian fluids are two types of fluids. Newtonian fluid;

viscosity is constant. Non-Newtonian; viscosity is not constant. There are different types of non-Newtonian. So, the shear-thinning property is desired for 3D printing. Why?

Student: Because you will be forcing the fluid through the nozzles, so we will be producing shear, and it should not increase the viscosity also.

Student: It should have low viscosity.

Yeah. It should not increase viscosity. You cannot have a shear thickening liquid; then, it will just clog the nozzle. If you have shear thinning, the advantage is when you press it through the nozzle it is going to have lesser viscosity than its actual viscosity. So, it will come out like a fluid, in a much better fluid flow, and once it comes out, the shear is gone. So, it's viscosity goes up, and it actually can form its original shape more easily. So, the viscosity can be regained after the printing. That is why people look for shear-thinning liquids for printing.

Gelation kinetics determines the time of crosslinking. So, that is a rheological property that has to be studied. Yield stress is the initial minimum stress that is required for initiating flow. You might have a highly viscous fluid, and some amount of stress is needed for the viscous fluid to flow, right. Viscosity is nothing but resistance to flow. So, to overcome that resistance, you need something called yield stress. So, that will create the flow.

And shear elastic modulus determines the deformations and ease of printing of the hydrogel, due to the shear forces. And you also need to look at surface tension because these influences, if either of a drop or a jet, would be formed after ejection. Depending on the surface tension properties, you would either have a small drop, or it could just be a jet a fluid coming out.

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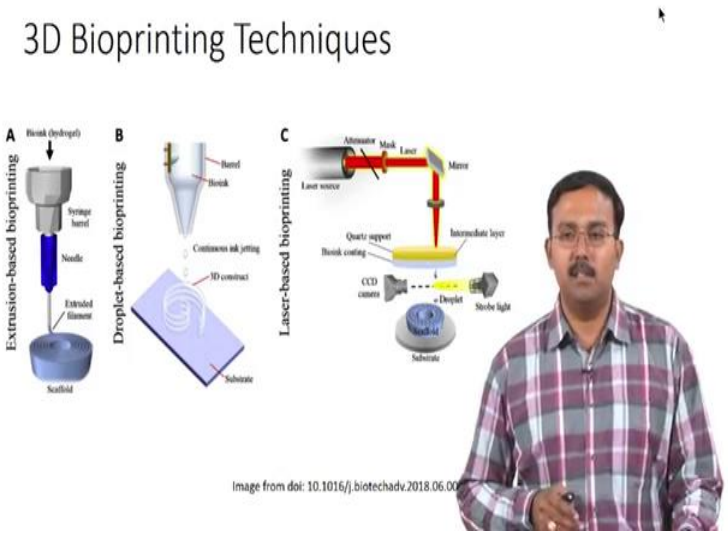

These are the three major 3D bioprinting techniques, the classifications; extrusion-based, a droplet-based, and laser-based. So, I will talk about them in the opposite direction. I will first talk about laser-based, then droplet-based, and then I will talk about extrusion-based.

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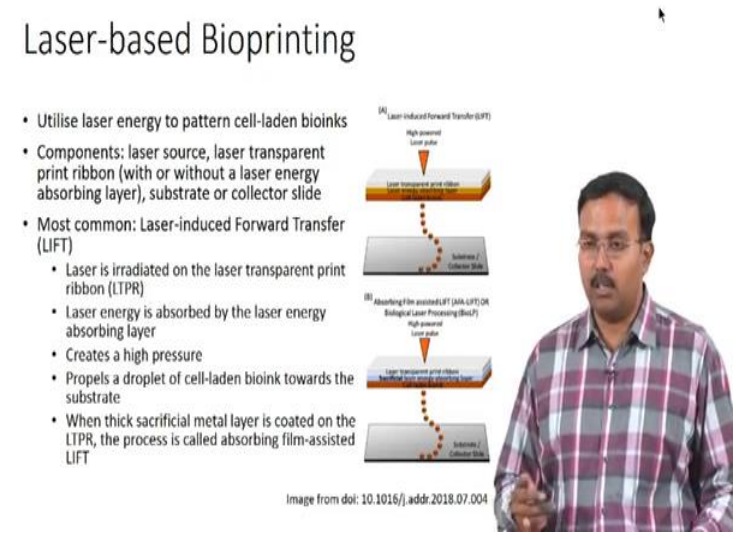

Laser-based uses laser energy to pattern cell-laden bioinks. This technology has three major components. So, you have a laser source; you have a laser transparent print ribbon. So, this print ribbon can either have a laser energy-absorbing layer or not. And finally,

you have a collector or a substrate. So, you see this here, this would be the laser source, and you have the ribbon on which the ink is laid, and then you have the substrate on which the printed ink will fall. This is what you see in a laser-based bioprinter.

The most common technique which is used is in laser-based bioprinting is the laser-induced forward transfer or the LIFT. Here what happens is laser is irradiated on this laser transparent print ribbon. So, this is the lift technique. You have the laser which falls on the transparent ribbon here. And you have a laser energy absorbing layer which absorbs the laser energy, and below which is the cell-laden bioink. So, this creates a lot of pressure on the bioink and propels a droplet of the bioink. And these droplets are collected on the surface of the substrate, and that results in the formation of the 3D structure. So, this is the LIFT technique.

Student: Sir, the droplet is that means, the in the case in a semi-solid state. Is that what you are saying?

Highly viscous fluid.

Student: It sacrifices and then again for the ready case.

It is a highly viscous fluid that is loaded on the surface of this ribbon.

Student: Ok.

So, it is a highly viscous liquid. So, it is not just going to flow freely. Think of something like honey, right, as pure honey; it is not going to just fall off quickly.

Student: Yes.

It is going to depend on the processing types. So, if you have something which is even more viscous than that, then it will work this way, right. So, it depends on the viscosity of the material.

Here you could have this laser energy-absorbing layer, or you could have something which is the sacrificial laser energy absorbing layer. So, instead of having just a layer that is not sacrificed, you could have a sacrificial metal material, which will absorb more of the energy, and that type is called as the absorbing film assisted LIFT or the AFA-LIFT. This is what is commonly used for biological laser processing because you want to

have the cell-laden bioink to be protected from the laser energy to the maximum extent possible.

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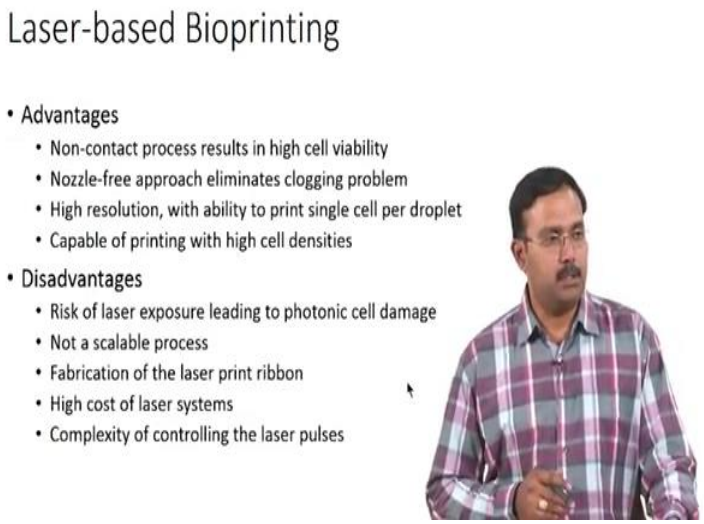

Advantages; it is a non-contact process, which means there can be very high cell viability. When you have a shear process, like when you have a nozzle, cells have to go through shear, which can cause cell destruction. Here you do not have that kind of shear stress which is happening. Nozzle free approach eliminates clogging problems as well. So, you can use material which is very highly viscous. That could be an advantage because once it's printed, it could form a gel very quickly.

It has very high-resolution; people have been able to print a single cell per droplet. So, it shows that high-resolution. It is capable of printing with high cell density, so if each droplet has a cell and so on, then you can create a very highly dense cell-laden ink and structure. But the disadvantage is you always have the risk of laser exposure to the cells leading to photonic cell damage. And this is not a scalable process; it is really expensive for having something like a laser construction for even a small system.

If you are going to have a very large scalable material, that is not going work. If you want to look at it from a commercial perspective, which is eventually where you want to go, this is probably not going to be viable. And it is also a very high cost for laser systems, so many of the lasers which would be used for such biological processes are quite expensive. So, even some of the microscopes which are available in biotech you

which use laser-like confocal and things like that, the laser would be the major expense. So, it becomes very expensive to set up something like this.

And there is also difficulty with respect to controlling the laser pulses, and fabrication of the laser print ribbon is also tricky. All these make it one of the least preferred technologies for bioprinting.

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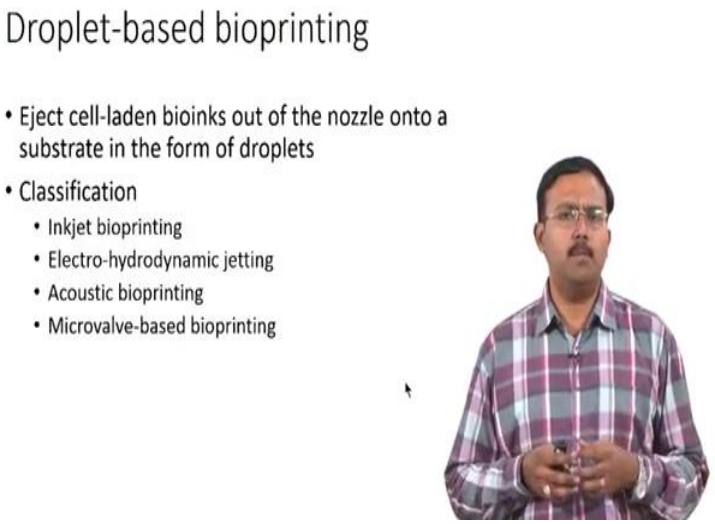

Droplet-based bioprinting is more popular, and this is where 3D printing kind of started. Inkjet bioprinting is where 3D printing became commonplace, right. People were able to modify commercial inkjet printers to print 3D structures. Here what happens is cell-laden inks are ejected out of a nozzle onto a substrate in the form of droplets. Small droplets come out of this nozzle and form 3D structures. These are classified as inkjet bioprinting, electro-hydrodynamic jetting, acoustic bioprinting, and microvalve-based bioprinting. Yeah.

Student: Sir, what guides this bioink to form a particular shape?

That depends on the images you have taken, right. You would have taken images and built a CAD model, and this printer is now connected to a computer that sends signals on what should be printed. Just like how your printer in itself does not know what to print, right. You have to have a word document that is sent to the printer to be printed or a pdf,

which is a sent to that. Similarly here, you would have something called an STL file. That STL file will be printed. So, whatever you have in that STL file will get printed.

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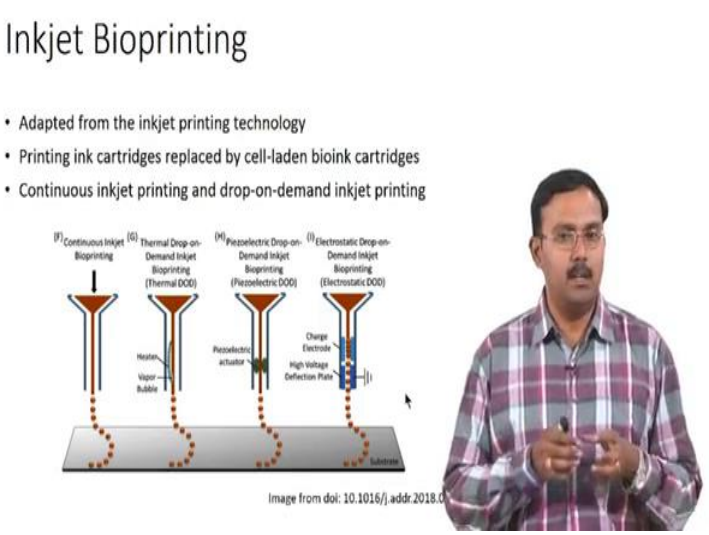

This is a common inkjet bioprinting structure; it is adapted from the inkjet printing technology itself. The printing ink cartridges are replaced with cell-laden bioink cartridges. Here the only thing is you need multiple axes to be printed, not just a single axis. These are some of the common inkjet printing technologies. This can be classified as two things, one is a continuous inkjet printing, and the other is drop on demand inkjet printing.

In continuous inkjet printing, drops will be continuously formed, whereas, in the drop on demand, depending on the stimuli which are being given, you will have drops being formed. So, DOD printers will have better control over the microarchitecture if you need to use them.

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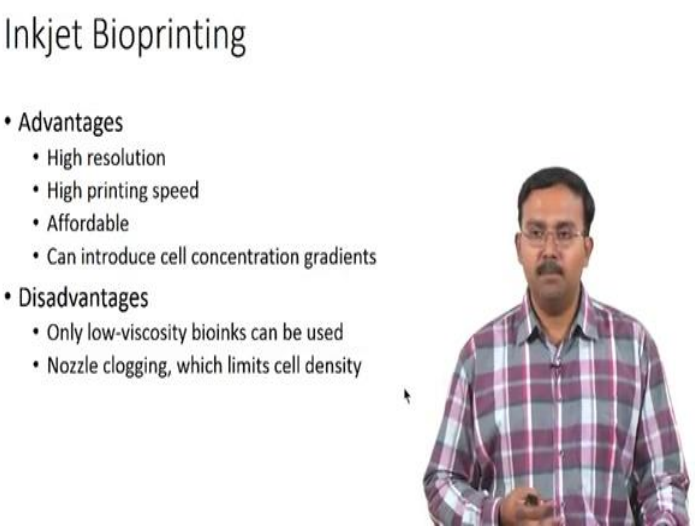

The advantage of inkjet bioprinting is it has very high resolution and high printing speed. It is also very affordable. It can introduce cell concentration gradients by drop-on-demand techniques.

The disadvantage is it can only use low viscous bioinks. If you use highly viscous bioinks it is going to clog the nozzle, and clogging of the nozzle can also limit the cell density which you can use.

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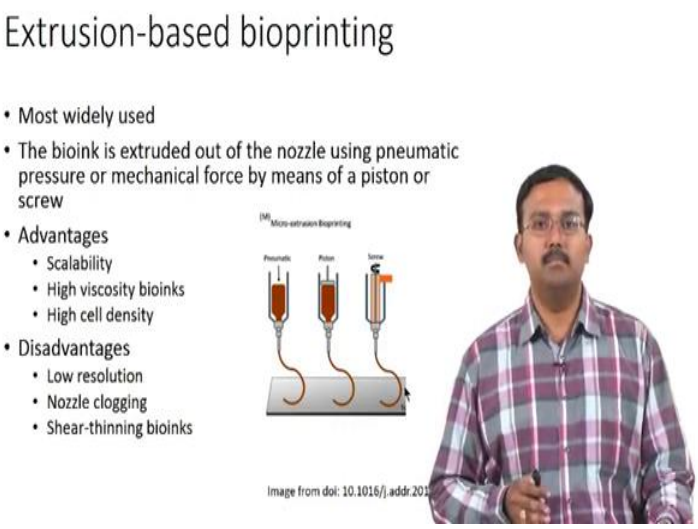

Extrusion-based bioprinting is the most widely used method, and the bioink here is extruded out of a nozzle either using a pneumatic pressure or a mechanical force. The mechanical force could be either a piston or a screw, which is used to push out the ink. The advantage is, it is a very scalable process. So, that is why it has become widely used, and it is one of the more commonly used technologies in commercial 3D bioprinters. This can use highly viscous bioinks, which means you can use high cell densities.

The disadvantage is, it has a very low resolution. If you go with very thin nozzles, then your clogging is going to become very rampant. So, you would want to use something of certain diameter to avoid nozzle clogging. Otherwise, nozzle clogging becomes a problem. And you need the ink to be shear thinning because when you are using highly viscous things, and you are trying to push it, you would need a lot of energy to push it out, which will cause a lot of shear to the cells. So, if the ink is shear-thinning, then the stress will not affect the cells as much.

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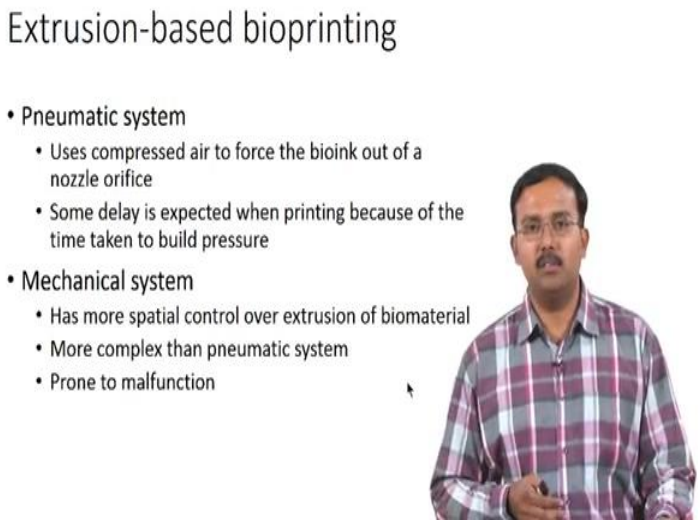

The pneumatic system uses compressed air to force the bioink out of the nozzle orifice. And there is some delay expected when printing happens because it takes time to build the pressure, so the yield stress would have to be taken care of before it starts printing. In the mechanical system, there is better special control over extrusion. It is more complex than a pneumatic system, and it is prone to malfunction because of that. So, those are the challenges with extrusion bioprinting.

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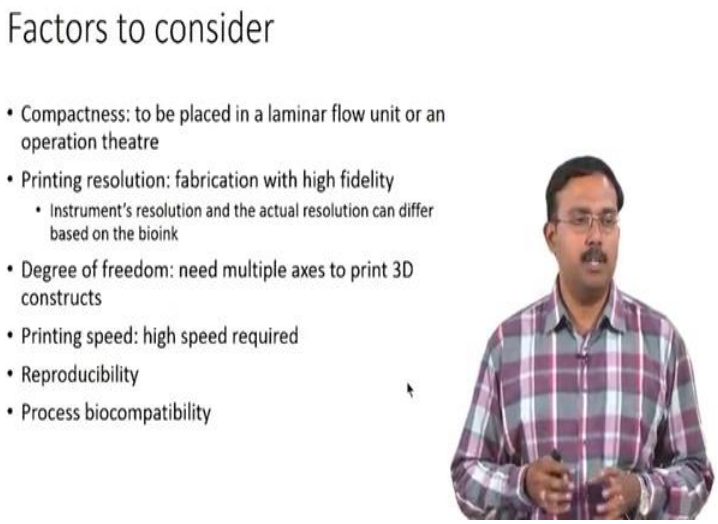

Factors you need to consider when you are printing the thing is; the printer needs to be compact because you might have to place it inside a LAF (Laminar airflow), or you might want to keep it in an operation theatre. So, you would not want something too large. That is one aspect that needs to be looked at when you are taking a printer. And the printing resolution would have to be very good so that you can fabricate with high fidelity. The instrument's resolution alone does not determine the printing resolution; the actual resolution can be based on the bioink you use as well. So, you need to consider both while you are talking about the resolution of the printed scaffold.

The degree of freedom needs to be looked at because multiple axes might be needed for printing 3D structures. Just a single axis might not give you the desired 3D structures. Printing speed should be high so that you can actually print complex structures in a faster way, and it should not take too long. So, it needs to print in a faster way, so that it can be used for medical applications quickly. It also needs to be reproducible, so just because you are able to print it once does not mean it is effective; the same STL file should give you the same printed structure every single time for that ink. So, that reproducibility needs to be there. And the process biocompatibility should also be taken care of. The biocompatibility of the ink is one thing; the process itself can kill the cells, right. For example, shear or temperature. So, inkjet, if you are going to use a thermal response in the DOD type, then there can be high temperatures used, which could kill the cells.

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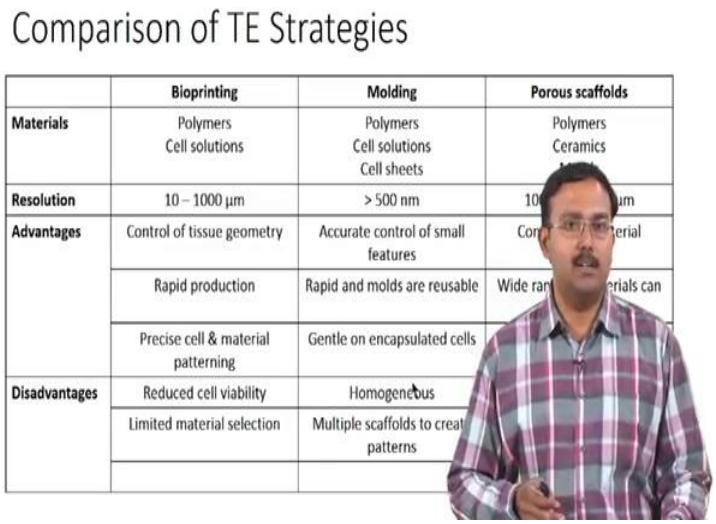

The comparison of these tissue engineering strategies is given here. I will not go into the details. So, you can look up this while when I upload the slides. These are some of the techniques which are commonly used and gives you how the resolutions are, and the advantages and disadvantages of these techniques are.

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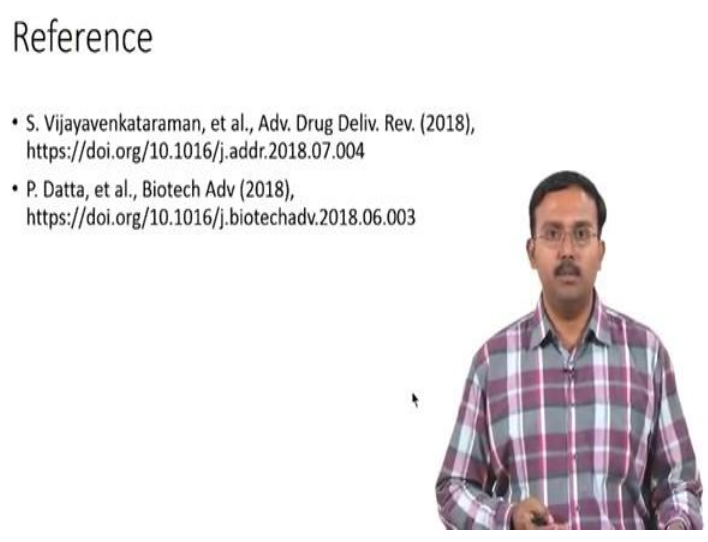

And these are the two references which I have used. So, they are very new references. This gives you all the information. I have covered only about one-third of these two review articles. There is much other information which is provided in addition to that

example of where the applications have been. Please do read this review article when you get time. And we will take it from there, ok.