Experimental Nanobiotechnology

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Lecture 16: 3D Bioprinting

Hello everyone, today we are going to learn about 3D bioprinting. In today's lecture, we are going to learn about what is bioprinting, what are the various types of bioprinter and what are the challenges of bioprinting. At the end of the lecture, we are also going to learn about this bioprinting technique in more detail through a practical demonstration. Let us see what 3D bioprinting is.

The 3D bioprinting process involves using bioinks that contain living cells to 3D print natural tissue-like structures. So that means we are going to use the bioink, and we are going to print an artificial organ or artificial tissue using the 3D bioprinter. And this bioink is made up of natural or artificial biomaterials that are blended with the living cells, and we can print artificial organs or tissues that will allow the researchers

to study human body functions in vitro. So, this is the conceptual bioprinter. So, we can print artificial organs or artificial tissues, and that will be very useful to understand human body functions in lab conditions. And as I told in the previous lecture also when compared to this two-dimensional cell culture studies,

we can go for this kind of three-dimensional printed tissues. So, which will offer more biological relevance whenever we discover some new drugs or whenever we make new nanomaterials, we can use this kind of 3D printed tissues and we can understand the toxicity of these nanomaterials or we can also understand the therapeutic efficiency of the drugs. Let us see the difference between 3D printing and 3D bioprinting.

In 3D printing, we will be making the physical organ model which will replicate the shape and structure and mimic the mechanical properties but there is no biological activity whereas in case of 3D bioprinting so we'll be making the bioactive tissue model it will simulate like a original tissue microenvironment and imitating the original tissue structure and it also has the biological activity that means we'll be making the tissue

which will be mimic like our original tissue so that is the 3D bioprinting and here we'll be using the bioink

which contains the cells. If you see the application of 3D printing, it is mainly useful for intraoperative navigation and also for medical education and device testing. Whereas, the 3D bioprinting will be useful for understanding the efficiency of the drug by screening the various drugs and we can also make these artificial tissue for tissue regeneration and organ transplantation.

From this slide, you can clearly understand the difference between 3D printing and 3D bioprinting. In 3D printing, the materials commonly used are mainly thermoplastics like PLA and ABS. We can also use metal 3D printers to print metal scaffolds, which can be useful for hip or joint replacement. Ceramic printers can print ceramic structures for bone repair and replacement. And if you see the 3D bioprinting, as I told earlier, we will be using these bioinks which is composed of living cells, hydrogels and other biomaterials.

These create biological structures that mimic your original tissue or organ. In terms of applications, 3D-printed scaffolds are mainly useful in manufacturing for rapid prototyping, as well as for creating complex parts, and they are particularly useful in the automotive, as well as aerospace and other industries like electronics. In contrast, 3D bioprinting is mainly focused on medical applications, including

creating tissue models for drug testing, as I mentioned earlier. We can use 3D-printed tissues for screening various drugs and also for developing complex organ structures as well as tissues for transplantation and regenerative medicine. In 3D printing, the technology is already optimized, and a variety of machines are available for different types of 3D printing. In contrast, 3D bioprinting is more complex and an emerging field because it requires sophisticated technology.

Handling of living cells and also precise control of biological environments to 3D print the tissues. Let us learn about bioinks in more detail. Bioinks are used as base materials for bioprinting tissue, organ, or bone structures. Selecting the right bioink composition and density plays a very important role in cell viability and density. So, these bioinks are prepared by mixing the cells with biocompatible materials.

For example, we can use this hydrogel, and selecting the suitable hydrogel or the particular biomaterial is based on the organ you are planning to print. For example, if you want to print the bone, you can use the collagen because the collagen is widely used for

bone printing. These are the various other bioink materials. We can use collagen, alginate, or fibrin.

These are widely used bioink materials because they have excellent biocompatibility and homogeneously incorporate the cells and growth factors. They are very easy to process under mild conditions. And also very easy to modify by simple chemical reactions. So, and also it has the sol-gel transition.

So, these are the advantages of these bioink. Let us see the difference between bioink and biomaterial ink. In bioink, as I told earlier, the cells are the mandatory component. So, this is a cell plus biomaterial. And by using the 3D bioprinter, we can print the particular three-dimensional tissue.

Whereas in case of biomaterial ink, we are going to use the biomaterial as the ink and we are not going to add any cells at the time of 3D printing. So once we 3D print the particular scaffold, by layer by layer we are making this porous scaffold. So once you make this 3D printed scaffold, then you add the cells on the scaffold and grow the cells on the scaffold and make the final tissue

so that is the difference between the bioink and biomaterial ink let us see the advantages of 3D bioprinting the 3D bioprinting allows mimicking the real structure of desired tissue or organ that means we can use the 3D bioprinter and we can print the artificial organ or artificial tissue in the lab environment and that can be useful for studying the efficiency of drugs more accurately and again that minimizes the animal testing.

So we can study the efficiency of drugs using this 3D-printed tissue, and that reduces the use of animals and also, this is highly biocompatible, and it's an automated process, so fewer human errors and again, by using this 3D bioprinting technology, we can create patient-specific and organ-specific treatment. For example, some patients have a tumor Right.

So we can isolate the tumor cells and grow them in the lab environment. We can make a 3D-printed tumor model. So once we have this 3D-printed tumor model, we can study the efficiency of different anti-cancer drugs. For example, you have anti-cancer drugs like two types, A and B. and we can study the efficiency of these anti-cancer drugs on this three-dimensional tumor model and

suppose if the anti-cancer drug A is efficient, then we can give this particular drug to the particular patient so in this way, we can improve the efficiency of the cancer therapy we

got the overall idea about the bioink now let us learn about the 3D bioprinters; these 3D bioprinters are designed to handle sensitive living cell-containing materials with minimal damage.

And these 3D bioprinters can be broadly divided into three types: inkjet-based, extrusion-based, and laser-assisted. Each has its own advantages and disadvantages, which I will discuss in the subsequent slides. The compatibility between the bioprinter and bioink is very important to obtain the proper 3D printed tissue. So, these are the various types of 3D bioprinters. Extrusion bioprinting, inkjet bioprinting, and laser-assisted bioprinting.

So, let us learn one by one in detail. The first one is inkjet-based bioprinting. So, the principle of inkjet-based bioprinting is that it uses thermal or piezoelectric actuation to print cells suspended in a liquid medium. In thermal inkjet printing, an electric current pulse creates bubbles that propel ink droplets onto a surface.

In the case of piezoelectric printing, it uses acoustic waves to eject the bioink. The size, velocity, and morphology of the droplets can be controlled by actuation pulses. Inkjet bioprinting is widely used for both non-biological and biological applications. It is similar to your standard 2D ink-based printing. So most of you might have seen this inkjet printer where you will be using this ink as well as the paper for printing.

So, it is similar to that here. Instead of the ink, we are going to use the bioink, and instead of the normal paper, we are going to use the biopaper. This bioink is basically, as I told earlier, a spherical cell aggregate. It is a clump of cells, and the biopaper is the supporting biocompatible gel. So, these droplets will be printed on this biopaper, and then the next layer of this biopaper will be kept, and again it will be printed.

It will be a kind of layer-by-layer printing, and once it is printed, then we can remove this biopaper. So, the biopaper is basically made up of thermosensitive as well as pH-sensitive gels. So, by simply changing the pH or by simply changing the temperature, we can remove this biopaper and allow the cells to grow until you get the final living tissue. Nowadays, we have modern inkjet bioprinters, which are designed for high precision, speed, and resolution.

And one of the limitations of this inkjet-based bioprinting is the biomaterials that is, the bioink should be in liquid form for droplet formation. So, the next one is extrusion-based bioprinting. So, the principle is it uses a nozzle and pressure system to deposit bioink to

create these structures. And this microextrusion bioprinter, again, can be broadly divided into three types based on pneumatic, piston, or screw.

And this microextrusion bioprinter, some of the microextrusion bioprinter have multiple print heads to disperse several materials simultaneously. And some bioprinter includes a fiber optic light source for photo initiative activation. For example, if you want to do the cross linking and based on the light, so that can be possible by using this fiber optic light. The third one is a laser assisted bioprinting. Here we will be using this laser to transfer the biomaterials onto a solid surface.

So you can see here we are going to use these laser pulses and cells are loaded here. So with respect to the laser pulses the cells are deposited on the substrate. So the substrate may be made up of biopolymers or it can be cell culture medium. And the substrate supports the cellular attachment and promotes the growth of the particular tissue. So, from this slide, you can understand the difference between the various 3D bioprinters.

And we have to select the right bioprinter and right bioink depends on our final application. For example, if we select this inkjet, you can see that cell viability is up to 85 percentage and the printer cost is low. Whereas in case of microextrusion printer you can see the cell viability is between 40 to 80 percentage and this cost of the printer is medium. And in case of laser assisted 3D bioprinter you can see that cell viability is more than 95 percentage and the printer cost is high. So, depends on your final application and cell type and bioink you have to select the right 3D bioprinter.

Let us learn about ideal material properties for bioprinting. The first one is printability. So, the material should be printable. That depends on the viscosity, gelation methods, and rheological properties of the material. The material should be biocompatible in nature.

That means it should not induce any adverse effects in the body. So, it should be compatible with the biological system. The next one is degradation kinetics and byproducts. That means it should be biodegradable. So,

The degradation rate should match the ability of the cells to produce their own ECM. For example, you have this 3D-printed scaffold, and if it degrades before the cells can produce their own ECM, the cells should make their own extracellular matrix and be able to grow and form original tissue. When the biomaterial degrades, the degraded products should not induce any toxic effects. So, the degraded products should be non-toxic.

So that is why we have to check the particular material whether it is biocompatible and also it is biodegradable before we are using it for 3D printing application. The fourth one is structural and mechanical properties of the material. So material should be selected based on the required mechanical properties. Depends on the organ or tissue which you want to print it, you have to select the correct material.

For example, we can select the thermoplastic polymer fibers if you want to have a strong material and we can use the soft hydrogel if you want to make the soft tissue. So, the soft hydrogels will provide better cell compatibility. So, it depends on your final application, you have to select the correct material with the correct mechanical properties. So, the last one is the material biomimicry.

So, the main aim of this 3D bioprinting is to create artificial tissues or organ which will mimic like your original tissues or organ. So we have to select the material which biomimic, which mimic like your original tissue or organ. So we have to select the particular biomaterials. In that case, the success rate of the final product is very high. Let us see the overview of 3D bioprinting process.

So the first step is pre-bioprinting. In this step, we will be selecting the right bioink, depends on your final application. And the second step is bioprinting. the processing. So, here we will be selecting the suitable 3D bioprinter and print the particular tissue and

third one is once you printed the tissue in the third step that is the post bioprinting where you will be stabilizing the bioprinted construct with cross linking and we can also do the further modification like we can remove the sacrificial inks and feed with the cells. So, the sacrificial ink means it is mainly used for creating the channels open channels which is essential for nutrient transport and cell migration and in some cases it can be useful for vascular network design and this sacrificial inks can be removed simply by dissolving the water

or it can be degraded at specific temperature by simply changing the temperature we can remove the sacrificial ink let us learn about what are the challenges we face when we do the 3D bioprinting and how to overcome those challenges So the first problem is poor bioink formulation. The reason may be due to the polymeric solution flows freely. So that can be overcome by measuring the viscosity before loading the cells to ensure the injectability.

And the second reason may be that the cells are not viable in the matrix. So in this case, we have to check the biopolymer cytotoxicity before we load it into the 3D bioprinter. And the third reason may be difficulty in the post-bioprinting process. So that can be overcome by using proper cross-linking agents and an appropriate bioprinter. So the next problem is polymeric clogs in the bioprinter.

So that may be due to improper bioink formulation. So in this case, we have to check the polymeric properties like mechanical strength and rheology before we use it for 3D bioprinting. The other reason may be due to misaligned bioprinter axis and parameters. In this case, we have to contact the technical scientist for calibration, and the speed may be too low or too fast during the process.

So, we have to optimize the parameters and standardize the bioink before we use it for 3D bioprinting. So, the next problem is deformation post-printing. So, the reason may be due to low cross-linking density. So, we have to optimize the curing conditions or cross-linking parameters. And another reason is high ambient temperature or humidity.

So, we have to optimize the temperature and we have to use the dehumidifier. And cells are not viable after 3D printing. So, the reason may be due to the cross linker toxicity. So, we have to check the toxicity of cross linker by using the viability assays. Or it may be due to the improper process.

So, analyze the porosity, nutrient absorption and metabolite release study whether the cells are viable after optimizing all these parameters. Let us see the role of nanomaterials in bioprinting. The traditional bioink has some limitations. For example, so the traditional bioink space challenges in rheological, mechanical, physiochemical and biological properties.

So, this traditional bioink can be improved by use of nanomaterials and when we are using this nanomaterials and it can improve the efficiency of bioink and it can mimic like your natural tissue features. So, in this way we can improve the bioink and with the enhanced properties for the 3D bioprinting application. So, when compared to the traditional bioink, this nano bioink has lot of advantages. So, it has the nanomaterial, it has the cells as well as it has the biomaterial.

So, this nanobioink can be useful for various applications. For example, if you are using this magnetic nanoparticle in the bioink, then we can with the help of external magnetic field, we can allow the drug to release in a slow and sustained way. And we can also have

photoresponsive materials, we can add it. With the help of light, we can control the release of growth factors.

And we can also have the pH responsive materials as well as we can also have some of the nanofibers as well as some of the nanosilicates. So that can improve the mechanical strength of the particular bioink. Let us see the role of nanomaterials in 3D printing. So here we can use the nanomaterials like silver nanoparticle which has the antibacterial property

and we can also use these nano liposomes which can be loaded with the anti-cancer drug. And these nanometers can be loaded into the 3D printed scaffold. And once you remove this bone tumor and there will be a defect. So that defect can be repaired by using this scaffold. This scaffold is already loaded with the anti-cancer drug.

So, it will kill the residual cancer cells. At the same time, it can also support bone regeneration. Let us see another example to understand the role of nanomaterials in bioprinting. So, whenever we print cardiac tissues, we have to make sure that the printed tissue is able to sustain electrically excitable cardiomyocytes and

exhibit robust and synchronized contraction for functional 3D cardiac structures. But the challenge with the current bioink is that most cardiovascular bioink is made up of polysaccharide or protein polymers, and it lacks conductivity. So, which hinders the electrical coupling with the host myocardium.

So, to overcome that, we have to incorporate conductive nano-biomaterials like gold nanorods into the bioink which will enhance the electrical impulse transmission and improve cardiac cell adhesion, organization, as well as provide synchronized contraction. as i told earlier we can also use the 3D printed matrix model for assessment of nanoparticle toxicity So, when compared to the two-dimensional cell culture plate, we can use this.

3D printed three-dimensional tissue for understanding the toxicity of nanomaterials. Because this three-dimensional bioprinted matrix model closely mimics the real tissue environment, it will provide a more realistic assessment of nanoparticle toxicity. Let us briefly learn about higher-dimensional cell culture models. The first one is a four-dimensional scaffold.

This four-dimensional scaffold responds to external stimuli. Once we do the 3D printing, and load it with some kind of magnetic nanoparticle with the help of an external magnetic

field, the particle scaffold can shrink or expand. If you add some nanoparticles that can respond to light,

the scaffold can shrink or expand in response to light. This means the four-dimensional scaffold is basically a 3D scaffold that responds to external stimuli. It responds to external stimuli, which is why it is called a four-dimensional scaffold. The next one is a five-dimensional scaffold. This five-dimensional scaffold involves the rotational motion of the printing head at defined angles, especially for curved layers.

So you can see here it can rotate in all the direction. So that is five dimensional scaffold. The next one is 6 dimensional scaffold. So it is a combination of 4D plus 5D. So it responds to the external stimuli and it also has a 5 degrees of freedom.

It can also rotate. So these are the difference between the 4D, 5D and 6D scaffold. I hope you got the overall idea about the 3D bioprinting. Let us go to the lab and learn this technique more in detail. Before proceeding with the experiment, we will sterilize the biosafety cabinet and all the required materials.

In today's experiment, we will perform 3D bioprinting using Gelma as a biopolymer, which should be synthesized by the gelatin with the methacrylic anhydride. We will begin by preparing a 10% Gelma bioink. To do this, we have already weighed 1 gram of Gelma, which will be mixed with 0.5% LAP, that is lithium phenyl (2,4,6-trimethylbenzoyl)phosphinate, a photo initiator.

Now, we will add 10 mL of this solution to the GelMA. Once the solution has been added, we will place the mixture at 37 degrees Celsius. until the biopolymer is completely dissolved in the photoinitiator solution. Then, place the solution in a dry bath at 37 degrees Celsius until it is fully dissolved. After some time, you will observe that the solution is completely dissolved.

Now, we need to load a sterile syringe with the GelMA solution. We will pour the solution into the syringe and carefully push the plunger up until a meniscus forms at the tip of the syringe. The plunger will also help control the flow and prevent dripping. There are two extruders available.

We can use either one, but the extruder we select later must be connected to UV crosslinking light. For now, we will place the syringe into extruder one. Once done, we will begin the bioprinting process. First, we need to switch on the machine so that it can

align to its ideal position. Then, open the software on the PC and connect to the machine through Wi-Fi.

Login to the software through Chrome by entering your credentials. Once you logged in, the available bioprinting machines will be displayed. Select the correct machine and press connect to establish a connection. The machine will then be ready to operate through the PC. If the machine is idle, meaning there are no ongoing projects, we can begin setting it up for bioprinting.

First upload the STL file of the design you want to print. Next specify the layer height and print speed which will define the height of each layer and the speed at which the machine will print. Then choose the build plate. In this case we will use a cell culture dish.

Next calibrate both extruders even though extruder 1 already contains a sample. Start by calibrating extruder 1. If you press the X axis calibrate button, extruder 1 will move to its origin position in the X direction. Press the Y axis calibrate button to move extruder 2 to its origin position in the Y direction. For the Z direction, we must be very careful as it defines the distance between the needle and the surface

Where the design will be printed. We need to leave the minimal distance according to our layer height and the needle type. Press the button to calibrate. Once extruder 1 is calibrated, press the up button on the screen to calibrate extruder 2. This extruder will be used for UV crosslinking, so calibrate it accordingly.

Next, we need to set the parameters for extruder one, such as temperature and pressure, which should be adjusted according to the biopolymer we are using. For UV crosslinking, the settings will vary based on the polymer's crosslinking ability. For extruder 2, its only role is UV crosslinking. So, no other settings are required for printing.

Once all the settings are configured, press print. The machine will start the bioprinting process. Extruder 1 will begin printing the design layer by layer. After each layer is completed, extruder 2 will apply UV crosslinking for 60 seconds to cure the layer. This process will alternate between printing and curing after each layer.

Once the printing process is complete, you will see the printed design on the build plate. In this demonstration, we have shown the basic steps involved in bioprinting, including the necessary parameters for using GelMA as a biopolymeric ink. These steps are

essential for successfully carrying out a 3D bioprinting process. As a summary, in today's lecture, we learned about what bioprinting is,

the various types of bioprinters, and also the challenges of bioprinting. Through a practical demonstration, we also learned this bioprinting technique in more detail. Thank you for your kind attention. I'll see you in another interesting lecture.