Regeneration Biology
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W11L51_Tissue 3D printing and organ culture

Hello, everyone. Welcome back to another class on regenerative biology. In today's class, we will learn about tissue 3D printing and organ culture in detail. So far, we have seen the prospects of 3D printing and the making of different matrices, etc. For the inoculation of the cells. Now we will see how you do 3D printing and organ culture in practice.

What are the types of printers used? We will study. So if you look at an overview of 3D printing, this cartoon explains to you in a nutshell what 3D printing is all about. 3D bioprinting is a state-of-the-art approach to the design and processing aspects of organ culture. The major applications in the biomedical area, including the repair of bone, soft tissues, and organs, are very prevalent.

The current limitations and the latest evolutions in bioprinting-based strategies also need to be examined. The existing results and new horizons are discussed to provide a picture that is useful for both senior and early-stage researchers. As you can see here, this is the bioprinting and tissue engineering system. And you can see for cardiovascular issues, skin, bone, cartilage, liver, urinary system, cornea, reproductive system, muscles, glands, dental issues, respiratory system, neural system, tendons, etc. Due to the limitation of time, we will not address each tissue.

We will pick a few of them and try to understand how it is implemented and how it is done. So this is another schematic illustration of the steps that are required to generate bioprinted tissue structures. So, the bioprinting process involves fabricating functional tissues and organs by integrating and assembling various biomaterials and bioactive molecules in 3D. So, when you say 3D is about the X, Y, and Z axes, that is the meaning of 3D. So bioprinting techniques provide reasonable control over both cellular and cell-laden constructs by mimicking a specified configuration and surface and structural properties, leading to steering their cell activity.

So the workflow of bioprinting techniques commonly starts from medical image datasets such as magnetic resonance imaging or computed tomography (CT) scans. Which provides macrostructure information of organs and tissues, then the architecture of the 3D

structures with high fidelity is achieved through computer-aided design. You may have heard about mechanical engineers; people use this CAD short form; it is called computer-aided design software. So, in a nutshell, you can say. Different organs are present in the human body, and you can see how a 3D CAD model of a kidney looks and what the manufacturing strategy is, and then proceed to bioprinting, followed by maturation and culture of that organ before implantation.

So it starts with the image acquisition in the patient, and then you try to map the entire organ, come up with a 3D printing strategy, and implement it. So 3D bioprinting is a successful use of bioprinting technology. It is attributed to the functionality of the resulting structure, which is defined based on the components' structural components, as well as the bioprinting device used, and cell interaction depends on all these three factors. For example, if you are putting a very solid metal structure on a plastic substrate and support device, then that metal structure's strength is not going to be of much use because it is attached to plastic. Wall or a plastic material.

So if your structure is metal, it should be at least anchored to a metal or equivalent; it cannot be weaker than that. So this is the idea you should have. Several techniques have been developed for the accurate and controlled deposition of different biomaterials, creating intricate structures that mimic native tissues and organs. Based on the principle of operation, three commonly used 3D bioprinting techniques exist as of now: inkjet-based, laser-based, and extrusion-based bioprinting. These are all three approaches that are available as of now.

3D printers are inkjet-based. Let us understand how and why they function. Inkjet-based bioprinting operates on the same principle as traditional inkjet printing. But instead of using ink, it uses bio-ink containing living cells. This technique involves precisely ejecting bio ink droplets from a print head onto a substrate to form specific 3D structures.

It has to be because you have a scanned image available with the printer already, which you got from a scanner. Inkjet-based bioprinting is considered a non-contact technique that creates discrete droplets under pressure and precisely deposits them onto a substrate at desired locations, where interactions between the droplets and substrates are created. The interactions have to be created between the droplets and the substrate so that they will gel together. Pressure pulses impact the fluid chamber by overcoming the surface tension of the bio ink, triggering the droplet ejection according to various actuator mechanisms; whether it is thermal, piezoelectric, electrostatic, etc., different actuations can be applied, causing the droplet to eject.

You have ink ready, but how, with what force, and in which direction you will push

them is what needs to be taken care of. Inkjet print heads are temporarily deformed either thermally or piezoelectrically to eject droplets of various sizes during printing, depending on what you are going to print at a given location. The thermal inkjet printer uses a heating pulse from a thermal actuator to eject vapor bubbles and ink droplets from the nozzle. This is another approach to using what should be the actuation method. However, it may have limitations regarding printing viscous bioinks and retaining cellular viability during the printing process.

Having said that, every tissue has its own viscosity and its own tissue density. Accordingly, the ink is nothing but the cells themselves. Quality or viscosity also has to change. Laser-based, another printing approach, is ink-based. Now, laser-based bioprinting uses a mechanism similar to that of inkjet printers, but it uses laser pulses to deposit cells and biomaterials onto substrates precisely.

Remember, these so-called inks should have both the substrate as well as the cells, not just the cells alone, not just the substrate alone. This technique creates a laser-induced pressure wave that propels cell-containing droplets from the donor slide to a receiving substrate, forming the desired pattern. 3D pattern, not a 2D pattern. A pulsed laser beam is directed onto the interface between the target substrate and the absorbing layer. So this causes thermal volatilization and the formation of microbubbles, which is part of the integral part of the 3D structure itself.

The expansion of the microbubbles ejects. Bio-ink droplets. Initial bioprinting systems using laser-based processes were also called stereolithography or SLA. So the SLA bioprinting techniques utilize visible light or ultraviolet light to layer by layer photopolymerize a photosensitive solution in specific areas to generate a suitable structure. So this is the step-by-step mechanism of a laser bioprinter.

The laser beam's small size allows the SLA to produce cell patterns with intricate structures and achieve a high level of resolution at the submicron level. Submicron means below one micron; it can reach this size because a cell's diameter is roughly around four to five microns. So, definitely not more than 10 microns. So submicron is a great resolution to have. Digital light processing, also known as DLP, and SLA techniques use photosensitive materials, but their implementation or the actual procedures can differ slightly.

During the DLP process, a digital micrometer device is used to target light onto a region that crosslinks all points related to each layer. So this is the overall concept of using a laser printer. So, when you think about 3D printers and the extrusion method, that is the third method. Extrusion-based bioprinting is one of the most common and versatile

techniques used in bioprinting. It is very well used because it has many advantages and benefits when you implement it.

It involves the continuous deposition of biomaterial ink through a nozzle using mechanical force or pressure driven by a delivery system. It can be a gas pressure or any other type of vacuum pressure. Such as air, a piston, or a screw. Some pressure is needed.

Simple approaches. The bioink is carefully extruded layer by layer to print a desired 3D structure. The pneumatic extrusion-based bioprinting drive system is simple and primarily dependent on the pneumatic pressure, while the mechanical drive mechanism provides better spatial control. So both have to work hand in hand in order to achieve a proper resolution. More than that, the mechanical techniques regulate the bioink more directly than pneumatic systems, which rely on the delayed response of compressed gas in pneumatic systems. Nonetheless, we should understand that pneumatic systems offer advantages when applying various types of viscosity.

Like earlier, we discussed that the viscosity of the ink can be a detrimental factor, but here the extrusion method, because you are applying pressure, is no longer subjected to the ink's viscosity, etc. This allows for different types of viscosities of bio inks by adjusting the valve gate time and pressure, so you can adjust the valve. For a given pressure, you can decide based on its viscosity how much volume has to be pushed in. So both pneumatic and mechanical techniques can print highly viscous bioinks through mechanical systems that might offer superior spatial control. So as long as the organ is going to be resolving at an adequate resolution, then the extrusion-based, which is a pressure-based approach, is more handy.

So the resolution of extrusion-based bioprinting is typically influenced by pressure, nozzle diameter, size, deposition rate, and material type. But still, one thing you need to know is that the achieved printing resolution is relatively low. That only laser printer can provide submicron level resolution. But this is relatively low. That is ranging from 200 to 1000 micron.

The laser printer is giving sub-micron level, 200 microns to 1 millimeter (1000 microns), which means that this is a limitation compared to other bioprinting techniques. This technique offers high resolution and includes multiple types of growth factors in the bio ink because its resolution is low, but it allows for better or higher viscous bio inks; it can also tolerate. Moreover, its case of use, scalability, and wide range of applicable biomaterials make it a preferred technique for printing biomimetic 3D tissue constructs. Despite its low resolution, people still use the extrusion method. However, passing bioink through the nozzle diameter may raise shear stress because it's a viscous solution.

You have to push it through the nozzle. You have to apply more pressure, which leads to decreased cell viability. Ink is there, but ink is nothing but a cell. You apply too much pressure. Ink may not or the cell may not stay healthy.

This is a challenging point that needs optimization and affects the printing speed and size of cell aggregates that can be printed. The most widely used technique for biomaterial extrusion in 3D bioprinting is fused deposition modeling, also known as FDM. It's a type of extrusion-based technique. So the integration of 3D bioprinting with other approaches is necessary. The 3D printing offers a versatile approach to fabricating customized biomaterial scaffolds with interconnected pore networks that facilitate the transport of proteins, oxygen, and other nutrients compared to traditional methods or techniques.

However, we should understand one more thing. Many existing 3D printing methods lack the resolution to produce filaments suitable for scaffolds in various tissues. When the resolution is low, you cannot expect the ECM to be made as it should be because the cells are around 5-6 microns. So ECM will also be around that range. Additionally, the pore size of the 3D printed scaffolds is often larger than the size of the cells, adversely affecting cell seeding efficiency and tissue formation.

Instead of a thick normal cell, it will look porous; it will look like a sponge. You don't want to look like a brick, but now the tissue is looking like a sponge because the matrix is larger and cells are attaching; the middle is living empty. So, this is the issue. Among these techniques, electrospinning stands out as a robust and straightforward method that employs high voltage to generate nanoscale fibers with a large specific surface area. So this is taken into consideration when you are using 3D bioprinting with other approaches that you are catching on to.

Combining 3D printing and electrospinning techniques proves advantageous in overcoming several limitations. This integration of these two techniques, 3D printing and electrospinning, allows for the creation of materials with controlled shapes. Highly porous interconnected structures, sufficient support strength, and the ability to incorporate nanopatterning, which are very friendly for a matrix to be fabricated. It also provides ECM-like niches and bioactive cues to cells. Depending on the specific application, 3D printed scaffolds and electrospun fibers can be combined in various ways, such as electrospinning onto 3D printed scaffolds, 3D printing onto electrospun fibers, and alternating the use of 3D printing and electrospinning.

This means you can do this permutation combination using electrospun fibers as inks for 3D printing and decorating or infusing the 3D printed scaffolds with electrospun

nanofiber segments, or fabricating the electrospun scaffolds onto 3D printed collectors or templates. Basically, mismatch either of them just like you can jumble them up so that you end up getting a structure that is quite friendly and usable. Now let us see some example 3D printing approaches to engineer skin tissue. It's a schematic illustration of the design strategy and applications of a 3D bioprinted photo-crosslinked hydrogel of SF gelatin for full-thickness wound repair or skin wound healing.

That is what you are seeing in panel A. In panel B, there are illustrations of the hydrogel-treated incisional skin wounds at a specific time. Panel C shows the stimulation, the simulation of the corresponding wound. You are creating a similar wound because you are going to heal it. These are all done in rat or rodent models. Panel D is a quantitative analysis of the wound site progression rates in different groups.

How it is successfully staying in different model rodels in an in-situ bioprinted scenario. And E is the workflow. As you can see here, panel E is the workflow of an adaptive multi-degree of freedom in in situ bioprinting. As you can see, this is the workflow. And F is an example of the generation of hair follicles.

Generation of hair follicles occurs within four weeks following the robotic bioprinting and manual implantation process. And in G, here is the total number of hair shafts found in each group and wound. And what you are seeing is the skin generated inside and outside. So this is the overall implementation of a skin that is generated through a bioprinting approach. Then, 3D bioprinting approaches are used to engineer cardiac tissue.

It is not that easy. The heart is the first functional organ that forms during embryogenesis. Although the tissue is neuronal tissue, the full-fledged organ is the heart. And it has a stylized four-chambered muscular anatomy and is responsible for continuous blood circulation throughout our bodies, which we all know. A complex hierarchy of cellular diversity consisting of cardiomyocytes, endothelial cells, fibroblasts, connective tissues, smooth muscle cells, and other specialized cells poses great challenges to the manufacturing of functional heart tissue. Although 3D bioprinting cardiac tissue is still in the early stages of exploration because it's a tough tissue, recent research has indicated the possibility of 3D bioprinting functional heart tissue, particularly customized cardiac valves and patches.

However, such approaches have limitations regarding full vascularization and synchronized contractile activities. Although the tissue is fine, it should contract perfectly. Settner et al., a researcher, introduced a new generation of anatomically precise 3D functional patient-derived models at various stages that can be used as a high-fidelity

and robust platform to study various cellular microenvironment interactions. comprising the geometry, ECM composition, flow thermodynamics, flow hemodynamics, and biomechanics under both dynamic and static flow conditions of the cardiac tissue.

So models of the human embryonic heart tube and fetal left ventricle were bioprinted using hydrogel based on seeded endothelial cells and analyzed experimentally and computationally. Based on the cardiac geometry and flow conditions, there was a great variation in endothelial cell proliferation and endothelialization in the bioprinted constructs. So these results show that a similar and precise flow hemodynamic pattern in the 3D space and the bioprinted constructs exists. By focusing on optimizing bioprinted cardiac patches, Roche and colleagues developed alginate-gelatin-based heart patches for myocardial regeneration. The results showed the presence of EC networks, which formed a durable structure and exhibited the contractile function, the most important function of the heart.

Polonchuk and colleagues successfully constructed functional cardiac spheroids composed of ECs, endothelial cells, fibroblasts, and human cardiomyocytes blended into an optimal alginate-gelatin hydrogel with viscoelastic properties comparable to native heart tissue. As a result of spontaneous contraction and stimulation of bioprinted cardiac tissues, it was possible to record electrical signals and contractile properties of cardiac spheroids on the microelectrode plates, so these results indicated that the bioprinted cardiac spheroid has great potential to reconstitute human cardiac tissue for long-term in vitro studies because much progress has been made. So now, if you look into the corneal tissue, which we all know, when you say that donating the eye means you are basically donating the cornea. The cornea is a highly organized, dense, avascular, and transparent tissue that protects the eyes from the external environment and is responsible for incident light transmission and refraction.

Not just a window, but it also refracts light. It has a specific arrangement of collagen fibers forming 200 to 250 lamellae, which means layers and layers, and which gives strength and a spherical shape. The tissue's collagen lamellae and anterior part are intertwined and aligned with the middle and posterior regions. So the transmission and refraction of light, which then focus on the retina, are caused by the particular alignment, the way in which they are arranged, of different lamellae. They contribute to the function of the cornea.

An important contributor to global blindness is corneal disease. Currently, corneal transplantation is the most common treatment for corneal blindness. We have to wait for a donor, which is normally from cadavers. However, donations are the main source of corneal transplants, which are far from meeting the demand. A critical shortage of donor

corneas has prompted research into effective corneal replacement. So, nonetheless, many questions remain that the current research cannot resolve, including the restoration of optical function and reconstruction of the original geometry of the eye.

3D printing allows the deposition of materials on digital command to construct results with sophisticated geometric patterns that can be created because of 3D printing. Advances in 3D bioprinting technology have been expected to allow precise control of corneal curvature and thickness based on specific refractive requirements of individual patients. Corneas' properties change from patient to patient, overcoming the drawback of traditional tissue engineering. So the two major challenges for conventional corneal materials are rapid epithelialization and stable epithelial processes, because you don't want the cornea to become opaque due to the epithelial or vasculature growing on it. These processes are attributed to the corneal curvature, which determines the epithelial response to corneal damage and the corneal healing processes.

On a lighter note, we know that we don't want blood vasculature to form. So, for other tissues, we want angiogenesis. But in the cornea, angiogenesis is a menace. It will make the cornea opaque. In a study by Xu and colleagues, GelMA and collagen were utilized to generate smooth 3D-printed convex corneal implants.

These implants exhibited curved structures that could regulate cell organization and adhesion. So this is a 3D printing approach to engineer corneal tissue. Cell proliferation: live and dead. You can see a schematic of the PEG-A GelMA hydrogel formation by a bioprinted procedure in panel A. In panel B, there is a cell proliferation, live and dead image that shows the changes in the transparency of rCEC-containing hydrogels, which you can see in this panel, and panel C is done in the rodent model.

In vivo evaluation of the effectiveness of the corneal scaffold in a rabbit model was conducted. It's a rabbit model, not a rodent model, and a graduated reduction in the size of the corneal epithelial defect is represented by the green area, as you can see mentioned here. The green area, which is the center of the cornea, was observed due to the migration of the epithelium to the hydrogel and the defect. So in panel D, what you can see here is the gene expression and the histological analysis shown by red arrows and the rectangles that represent unfinished regions of the epithelium and the stroma. In panel E, the thickness of the entire epithelial layer, the trauma of the cornea, and the relative gene expression of different genes, ALDH and AQP1, are all specific to the cornea.

In the cornea, that confirms that the cornea is being made in a healthy manner. So considering this progress, like the values reflect the fold chain, as you can see here, these approaches give lots of promise, and we can conclude in some way that the powerful

capability of 3D bioprinting will make it possible and practicable. To streamline manufacturing processes in the future, because, like I told you, due to the limitation of time, I am covering only a few of the organs, like the kidney, heart, or cornea, there are many other tissues that can also be worked on: advances in technological processes, computational modeling, artificial intelligence, and machine learning, as well as a more comprehensive understanding of the. Optimal interactions between the biomaterials and the cells will significantly improve the biomimetic capabilities of bioprinted tissues in the coming years. Bioprinting can be applied to create complex biomimetic tissues, allowing for the inclusion of vascular, lymphatic, and neural networks.

So what we have learned so far is the promises that 3D printing is offering to fix damaged organs in patients. We will learn more about regeneration biology in the next class. Thank you.