Regeneration Biology
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W10L50_Major challenges in tissue engineering in practice

Hello, everyone. Welcome back to another session of regenerative biology. In today's class, we will learn about the major challenges in tissue engineering when it is applied as a practice. So this class may look a little bit technical, and we will try to take it as lightly as possible, but understand that this is also very important when you try to understand how to apply tissue engineering and organ culture, et cetera, into practice, simply because in reality, When you try to implement something, the problems you face may have nothing to do with the actual science. For example, in reality, you can make an ideal footwear that is very comfortable, very smooth, and friendly to your feet. But when you try to manufacture it, if the manufacturing cost becomes one lakh rupees, then we know it is not going to be sustainable.

Or, in other words. It is cheap. You are trying to implement it. For walking on the road, if it is not made of the right material, it is cheap, but it will not stand the rough terrain on the floor.

A simple example I gave is shoes. What you have to understand is that cost has to be taken care of, ruggedness has to be taken care of, and friendliness to the feet also has to be taken care of. Tissue engineering is also something like that, so let us see some of the challenges. This is basically a picture that shows how the tissue engineering setup is arranged. So one immediate point to remember is the microenvironment.

The proper reconstitution of the microenvironment for the development of basic tissue functions and properties is an important point to consider. Cells communicate through the local microenvironment, and if the environment is not friendly, as you can see in this model, there is a 100-micrometer tissue microenvironment. So we should know that 1000 microns or 1000 micrometers is 1 millimeter. So 100 micrometers means 1 by 10th of a millimeter. So there are many cells, such as progenitor cells, hematopoietic accessory cells, etc.

They are all adhered to a substratum. Let it be natural or artificial; it doesn't matter. Decellularized or artificial, it doesn't matter. It has a matrix that needs to be placed into a bioreactor, which is now assembled from multiple pieces into an automated cell culture

system ranging from around 10 to 100 centimeters. And these automated cell culture systems will be clubbed into 1 to 10 meters in height, length, and width.

And it's a cell manufacturing facility where you will house them in large rooms. So this is a typical way of arranging things. And then the neighboring cells, what are the cells that are present, like we have seen when you culture the stem cells, they need to have a feeder layer, etc. The interaction with the ECM, signaling molecules, cell geometry, dynamics of respiration, supply of nutrients, and removal of metabolic waste products, etc., everything has an implication in posing a threat to the cell culture.

And this mimics the microenvironment; it mimics the dynamic chemical and geometric variables that are normally seen in the actual tissue of the organism. Now the question is, once you set up the microenvironment, how nicely can we scale it up? We have to generate enough numerically, or it should be viable enough or scalable enough. A properly functioning microenvironment has to be established. Say, for example, you have one group of a small bioreactor, and now you have assembled it into 100. Now, it can't happen that three of them are not receiving nutrition.

Then whatever organ you are making may be futile. So each unit has to have its complete responsibility for the distribution of nutrients. And then only will you end up getting a clinically meaningful tissue outcome. And then comes microcirculation. How are you circulating the nutrients that contain glucose, amino acids, oxygen, etc.

? It connects the microenvironments to all tissues. And metabolically active cells are located within a few hundred micrometers of the capillary. Capillaries provide the nutrition. And capillaries connect every cell to a source and a sink; there is an input of nutrition and an exit of nutrition. Then comes the challenge of automating the system: how nicely you are automating it in the sense that it should be viable.

It cannot be, like I gave an example, that you don't want a shoe to be made for an ordinary use of one lakh rupees. The automation system should have its own cost-effectiveness so that it can work operationally at a clinically meaningful scale to which it can be pushed. So these are all some challenges, and let us see some other challenges when you are looking into, you know, printing an organ or making an organ—so different challenges and possible solutions. Let us see, due to the complex biological properties and 3D ultrastructure of the native ECM, we have seen in the previous class how decellularization is important to get the 3D structure of the native ECM. In the same way, the 3D structure of the native ECM is as important as the organ culture itself.

It has to replicate or mimic the use of traditional manufacturing methods to that of

making a matrix artificially, which mimics the endogenous or normal ECM of an organ. So this is the question. So traditional manufacturing methods and biomaterials can often become challenging; ideally, everything works out, but when you put it into practice, you find that it is not strong enough. It's just like if you are making a car: you want the car to be very light for fuel efficiency and cost-effectiveness, but nobody makes a car out of plastic because it will not be strong enough. It may be very light, but by the time you close the door a couple of times, it will break apart.

You don't want that. Such a car is not necessary just because you want to reduce the weight. So there is a trade-off that works behind this. In the cultivation and application of organoids, a critical challenge lies in developing mature organoids that possess the functional and physiological properties of the native organs essential for tissue repair and biological function reconstruction. You want to have an extra room, like you want to add a patch to your existing house, but if you forget to put on the roof, then that room is useless; or if you forget to put in the floor, it doesn't work out, although you tried making it.

It did not work out, but still, all the walls and roof are there, while the floor is not, so everything makes a difference when it comes to organs. Therefore, every organoid you are making must be a miniature replica of the organ itself. To minimize the batch-to-batch variations due to different donors, some researchers are focusing on using stable cell lines to produce this extracellular matrix, thereby offering new insight into these challenges. Like you are telling the cells to release the matrix protein so that it will be dense enough, both quantitatively and qualitatively, to mimic an endogenous organ.

That is some approach. So we have heard about the dECM, which is decellularized ECM materials, and let us see how it is working out for renal organoids in an engineered organ. Some examples we will see so that we will know more about the challenges renal means, you know it is about the. The urinary tract or kidney, or based on which organism you are talking about or which part of the excretory system is affected, there is production of, or there is the possibility of transplanting those organs; so, kidney transplantation is an effective treatment for end-stage kidney diseases but faces a chronic shortage of kidney donors, which we know is a. A challenge in every organ demanding patients, kidney organoids represent a potential tool for producing transplantable kidneys. The decellularization and re-cellularization of kidneys into kidney organoids provide a solid foundation to start with kidney development.

Preserves the complex structure, composition, and microenvironment of natural kidney tissue, supporting the self-organization and differentiation of stem cells into specific kidney cell types, such as nephrons, collecting ducts, and stromal cells, closely

resembling. The cellular diversity found in natural kidneys is what is demanded, but are we getting that in reality? That is what we should focus on, especially considering the challenges different decellularization protocols can yield materials with varying histological properties and human cell risk of endothelialization degrees. Varying degrees of preserved microvascular integrity and functionality thereby influence the differentiation of various embryonic stem cells into renal lineages. This is what is an implementable thing. This offers new insights into the importance of optimizing decellularization protocols.

Only your organoid is as good as the decellularized matrix you started with, so looking further into the kidney organoids, kidney transplantation is an effective treatment. We know that kidney organoids represent a potential tool; the decellularization and recellularization must be done effectively with the kidney decellularized matrix. It has the ability to be taken from a pig or from any other related organism; all you care about is the 3D structure. Now, you should put this dECM into a chamber that we call a bioreactor, where you will seed the dECM with the cells, and the cells you are putting in will be closely related to the patient or from the patient himself or herself. But now the question is, should these cells accept that dECM as their own native matrix? If it did not, for some reason it rejected, then they would not colonize.

So this has also posed several challenges. So let us see the application of dECM materials in renal organoids in engineered organ cultures. So this is a schematic illustration of the conventional and dECM-derived bio-inspired scaffold for renal tissue regeneration, which highlights their biological and chemical mechanisms. You can see here and in panels A and B, which basically shows that you are using a scaffold. It can be artificial, it can be natural, but in either case, you need to seed them with the cells.

In panel C, what you are seeing here is that once you seed the cells, they undergo inflammation, and if it is not a proper scaffold, you can get too much fibrosis. If it is the right way of ECM performance, then you end up getting proper tissue that will be generated. Here, what is used is PLGA (polylactic-co-glycolic acid), which is the scaffold that is used. Different scaffolds with varying chemical natures have been used; we will not go into that as of now. And in panel C, the decellularization and gelation processes of the porcine kidney.

Porcine means pig's kidney. They have taken and removed the kidney from the host, and then they decellularize it. And they lyophilize it, killing all the cells just to get the dECM.

And which is ready for... colonization of the host-derived cell. And then you can see in panel D the characterization of these cells, whatever they got, whether they are good or

bad, are they colonizing, etc. From this decellularized and engineered renal organoids cultured on kidney decellularized matrix, they exhibit more mature tubular structures. Tubular structure means that the internal anatomy of that kidney has to be restored. Otherwise, you cannot make urine, or that kidney will not be able to filter urine out of it.

And let us see a few more challenges with this approach. Kidney dECM is one of the most promising biomaterials for constructing renal organoids and bioartificial kidneys. However, insufficient recellularization of scaffolds, as I already told you, is the issue; the scaffold is fine, decellularization is fine, but the inoculated cells or seeded cells are not finding it a comfortable place. So this inefficient recellularization can be a problem in vivo, still challenging the application of kidney dECM. Sometimes it can happen that there is nothing wrong with the dECM itself.

It is something wrong with the cell that you have seeded. If the kidney of the patient was perfect, or if this organ's body was perfect, then their kidney would not have gone bad, so what you are trying to mimic is not so optimal or not so perfect. This can also pose a challenge, but can we call it a challenge? That is the question, because we cannot tell a patient that their organ is failing; so if it is successful, they will get another life; otherwise, you know. I don't care about your life.

That is not possible. Even though the cell is bad, we should be able to fix it. Only then does it become the right approach. Generating complex organ-like kidneys through 3D bioprinting presents several other challenges as well. The first challenge is that the kidney structure is complex.

This we know. Composed of macrostructures such as renal arteries, veins, and ureters. Every organ has to have arteries. We have already discussed angiogenesis because without it, that organ is a waste. At least it will not remain in the patient's body. But the kidney has much more complex internal structures.

They have renal arteries, veins, ureters, and other microstructures like nephrons, and this trauma occupies the space between them. Everything has to be perfect because kidney urine filtration depends on the proximity, intracellular and extracellular environments, and the movement of ions so that a concentration gradient is created, allowing the lost ions and fluid to be absorbed. It does lots of functions, everything based on its structure. Secondly, selecting the correct positioning of the cells is challenging as the kidney has over 20 different cell types. Although it's a small organ, there are 20 different cell types.

Not that you have 20 cell types put together randomly. They have to be arranged in the place where they should be. Moreover, choosing suitable biomaterials to preserve the

printed kidney's structure and function warrants careful consideration. Let us see a few more challenges. In recent years, progress has also been made in kidney fabrication through bioprinting.

Combining 3D bioprinting with renal organoid technology allows for the rapid, high-throughput generation of renal organoids with reproducible cell numbers and cell type ratios, which means that the printing specifies where each cell should be, as you are already making a 3D scan of the organ and printing precisely, just like creating a color print with a blue flower and a red flower. And green leaves, it will never happen that you know a red-colored flower now looking green in color, and the blue color is looking white; it never happens. It will be blue, will be blue, green will be green, red will be red in that particular place, not randomly somewhere. The same logic applies; however, generating complex organs has to depend on 3D bioprinting. Choosing suitable biomaterials is also essential for preserving the printed kidney structure and function, which is necessary for maintaining its future.

Otherwise, you can make a kidney now, but it should be standing or staying in the patient. dECM materials for hepatic organoids and engineered organs. Another organ that is very vital. The liver is a vital organ for metabolism and homeostasis in the body, as we all know. The high mortality rate associated with end-stage liver disease, ESLD, is called.

It has posed a significant global public health challenge because the liver is one of the most affected organs, or the most hardworking organ we can say in your body, because it is trying to safeguard you every day, as every food you consume can have some toxin or another. So the liver's job is to detoxify it. Data from the World Health Organization show that liver diseases rank as the 12th most common cause of death worldwide. That is a large number considering it is neither heart nor brain, etc.

Still, liver damage is more prevalent. That is what you should understand. Not only is it prevalent, but it can also lead to death. Currently, orthopedic liver transplantation is the most effective therapeutic option to enhance the survival and quality of life of ESLD patients. Whole organ bioengineering and regenerative medicine represent promising new technologies that could alleviate the liver shortage by increasing the number of organs available for transplantation. Means the organs available for transplantation are much lower than the number of people who are waiting in queue.

So, dECM materials are used to make hepatic organoids, as you can see in this schematic. Application of dECM materials in hepatic organoids and engineered organ cultures. In panel A, you can see the preparation process of DLS. How do you make a native liver and decellularize it? This is the liver with the blood tissue and everything,

and finally, you end up getting a decellularized matrix. The morphology of the intrahepatic bile duct tree is what you see in panel B, and in panel C, the vascularization.

The process of the DLS, what you are seeing here, is the vascularization process, and in the microscopic images, as you can see here in panel D, there are my ultra-structural images of the matrix and scanning electron microscope (SEM) images of the DLS that matrix you created. And the vascularized liver scaffold, VLS. As you can see, A, B, C, and D are the vascularized images. And in F, panel F is a photograph of the perfusion culture apparatus placed in the CO2 incubator used for recellularization of bile ducts in DLS. So how can you make a proper bile duct? Because a bile duct defect is also very serious.

It's as good as no liver function. If your bile duct is damaged, it's one of the complex structures in your body. If that is damaged, it's as good as a liver not functioning. So then, panel G reconstruction of biliary tree-like structures was achieved through the recellularization of bile ducts using liver ductal organoids. And these are the microscopic images of their functionality.

Those who are interested can read this article. So, what are the other challenges and limitations of 3D printing? Although we are saying that 3D printing can be done to solve the problems of organ shortages, 3D bioprinting has several other limitations and has the potential to revolutionize tissue engineering (TE stands for tissue engineering and regeneration). But it plays an important role in personalized medicine by enabling the design, prototyping, and fabrication of 3D tissue structures for various therapies. Means you can make, like I gave you an example, person-specific shoes, person-specific dresses, like that. You can make a person's specific organ because of 3D printing. Although 3D printing has progressed considerably, the entire biofabrication platform must be standardized and integrated from software design to tissue processing after printing.

It's like I told you earlier: designing or conceiving an idea is one thing. Implementing it in reality is another thing. So both many times don't go well. So we use the software designed for this 3D printing approach to develop next-generation bioengineered tissues. It is essential to address the drawbacks of biofabrication platforms regarding speed, complexity, material selection, printing resolution, and cell processing.

This must be integrated effectively. The choice of bioink. So far, we have been talking about printing, printing, printing, but what ink will be used? So we use the terminology that bio ink is one of the most important aspects of bioprinting. And it helps to circumvent some of these challenges. We will see more about these things in the bioprinting area, but for the time being, get familiarized with the terminology of bio ink.

You use something called ink—bio ink. Bio inks mimic the complex structure and composition of different organs and tissues, which means you are printing an organ, so the ink should be of such good quality that after printing, the organ looks like an organ; you don't want an organ to look like a wooden piece or a plastic piece.

They act as a medium to protect cells during the printing process and provide a suitable environment for developing microtissues to mature after printing. So this printing should happen. The cells will find a familiar place to live in a printed organ. So the bioink should have a viscosity suitable for cell growth and differentiation, as well as for printing. However, when you try to apply it in practice, it should have the suitable viscosity for bioprinting to support cell viability.

Like I mentioned at the beginning of the class, when you make a shoe, the cost is one thing, and here, what you are telling me about the viscosity when you use it for bioprinting may be ideal for printing, but it is not ideal for the cells to live in. Then you try to make it suitable for cells to grow, but it is not suitable for printing, so this is the trade-off one has to focus on. On the other hand, shear stress can affect cell viability and bioink stability. Once you make everything perfect, then the ink will not print; it will not be able to.

So you should think about strategizing. how the ink will suit the cell's familiarity. Another consideration is cellular sources for constructing 3D-bioprinted tissues. Primary cells are mostly harvested in vitro before heterogeneous tissue constructs are bioprinted. Primary cells for transplantation are removed from the patients to avoid any immune reaction.

So this is the approach that is usually taken for any bioprinting methods. So what should we... Try to understand that, in conclusion, the decm-based organ culture is designed to remove immunogenic cells while preserving the original tissue architecture and composition. It's like harvesting something from the farm; you take the whole plant, such as wheat or rice, but then you get rid of the hay, etc.

In this way, you make use of every beneficial part while discarding those parts that can be immunogenic. Not so friendly for the recellularizing cellular components. Due to its inherent structure, enhanced bioactivity, reduced immunogenicity, and favorable biodegradability, the dECM has garnered widespread attention in tissue engineering and biomedical applications. While 3D printing remains an active option, the decellularized extracellular matrix and recellularization of it have always remained an attractive proposition for tissue engineers. Despite the challenges in exploring DECM for organoids and engineered organ fabrication, ongoing research and technological advancements

bring us closer to creating viable transplantable tissues and organs.

We are reaching step by step towards a very sustainable and suitable approach; whether you should go for organoid production via 3D printing or production of an organoid via a decellularized matrix depends on the approach you are going to take. Take for fixing a damaged part of a patient. These developments also include optimizing the cellularization process, refining organoid construction methods, scaling up production, and enhancing the matrix biological cues to guide cell behavior; another approach is that the continuous improvements of these technologies hold great promise for the future. Organoid engineering and engineered organ fabrication. So we should understand these technologies has to be implemented wisely and effectively in a patient dependent manner.

Then it will become a very suitable approach for humanity. We will learn more about 3D printing and organ culture in the next class. Thank you.