

**Regeneration Biology**  
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**Week: 10**  
**Lecture: 46**

W10L46\_Tissue engineering-Why and how?

Hello everyone, welcome back to another lecture on regenerative biology, and in today's lecture, we will learn about tissue engineering: why and how. By now, we have learned about how regeneration takes place and how regeneration allows certain organisms to survive more effectively than the rest. Now we are stepping into the next stage of creating organs for human benefit. So the introduction of tissue engineering is important because we need to know where it sits or where its strategic position is. So, tissue engineering is an emerging interdisciplinary field that applies the principles of biology and engineering for the development of viable substitutes that restore, maintain, or improve the function of human tissues. There are plenty of definitions, one of them being this: it is a Venn diagram where this is biology principles, this has engineering principles, and this has medicine principles, and all three overlap in a The triple point is where tissue engineering lies; in other words, we can say tissue engineering requires all three subject areas: biology, engineering, and medicine.

Tissue engineering is a field that combines biology and engineering and is rapidly evolving these days, offering the potential for regenerative medicine and organ replacement. Current advancements include 3D bioprinting, stem cell therapies, and advanced biomaterials, with future scope focusing on personalized medicine and organ regeneration. So this is the place where you either stitch a dress for your fitting, buy shoes that fit your legs, or make an ornament that fits you. The same logic is getting into tissue engineering as well.

Tissue engineering uses a combination of cell engineering materials, methods, and suitable biochemical and physicochemical factors to improve or replace biological tissues, so we should also understand that when you use the term tissue engineering, it need not necessarily involve creating a full-fledged organ; it can also refer to replacing a heart valve, for example. Actually, it is a flap, so it need not necessarily be a full-fledged organ, but it is part of an organ. In other words, you are fusing the middle of a broken bone. If a bone is supposed to be one foot long, a 10-centimeter piece is lost. Now you have the option of placing a metal rod versus the option of inserting that 10-centimeter portion artificially, which is a biological material, into the middle.

And now the one foot of the bone is restored. So you should keep in mind that it uses a different strategy based on what tissue or organ you are trying to fix. It need not be a full-fledged organ. Tissue engineering involves using tissue scaffolds to form new viable tissue for medical purposes. We will see more in detail about scaffolds, but for the time being, you should understand that a scaffold is an extracellular matrix.

All of you know by now because we have discussed it a lot; it is something similar to that of the ECM. Or you can think about the pillars and beams of a building; you make a building not only on the walls. Many buildings these days, say a 30-floor building or a 50-floor building, are not made solely on the walls. You create the skeleton of it and then fill the gaps with the walls. The same logic applies: the beams and the pillars are the scaffold, and the walls and the bricks are the cells.

So this is the idea you should have: using the right scaffold holds a lot of potential during artificial tissue or organ production. While it was once categorized as a subfield of biomaterials, the scaffold has grown in scope and importance; it can now be considered a field on its own, as there are many journals that have the name biomaterials, and some of them are exclusively dealing with scaffolding or the scaffolding properties of various biomaterials or artificially produced chemical or physical materials. Using fibroblasts in skin replacement or repair. Simple tissues. Say you have a huge tumor on your face.

The doctor removed it, and you don't have a cheek at all. Your whole mouth, interior and everything, is seen. But they're filling the gap with the natural-looking appearance of your own skin. It makes a hell of a difference, so one may think, "Oh, skin is not a major organ; how does that matter?" It depends on which part of the body you are discussing. In some parts, it becomes extremely important.

The same way, take the tip of your nose; it won't look proper at all if your nose is broken and you don't have a proper nose. So at that time, that simple flap of tissue makes a huge difference in the appearance of the person. Cartilage is repaired by living chondrocytes. These are all simple tissues; there is no complexity at all. If you can make it from the same person and you put it in, that makes a lot of difference in the life of that affected person.

We will see more examples. Regenerating a human ear using a scaffold is extensively done due to some accident or something. You lost this external hood, or the pinna is lost. So it will look very odd, so you can replace it; although it's a simple structure, of course, it helps in listening properly without echo, etc. These foldings, flappings, everything has got a biological role.

It's not that you have your ear formed with, you know, it's not smooth; it's not looking like an umbrella. You should not think like that because this plays a major role in your listening ability. In vitro meat can be produced when there aren't enough animals to sacrifice, or when someone believes that killing animals is wrong but still wants to eat meat; thus, in vitro meat is possible through tissue engineering. The bio-artificial liver device, artificial pancreas, and artificial urinary bladder mean that lab-grown tissues, such as cartilage, have been successfully used to repair knee cartilage. The knee is a very sensitive joint, and knee cartilage is very, very important to replace upon injury or damage.

Artificial skin is constructed from human cells embedded in a hydrogel, such as in the case of bioprinted constructs for battlefield burn repairs. Many soldiers who get severely injured on the battlefield require a lot of such artificial skin for repair. Artificial skin does not mean just any artificial material. It is only the skin that is made artificially. Artificial bone marrow, artificial bone, oral mucosa, tissue engineering, and foreskin.

So these are all parts of the body that are needed in different scenarios. So this is a picture of an artificial ear, an artificial trachea, an artificial bladder, a urinary bladder, a tissue-engineered heart valve, and lab-grown mice kidneys. There are many examples. These are some of the pictures to know about. These are all meant for transplantation into the human body, different parts.

If you look at the different steps of tissue engineering, step one is to get the tissue sample from the body, which is usually done via biopsy. We will see these steps in much detail. Now we are seeing the outline and the principles. Step two, after you have collected the cells from the person or the patient, is to grow the cells into new tissues. Cell isolation, cultivation, scaffold, and seeding are involved in that.

So you have to grow these biopsy cells and identify a given type of cell that you will be proceeding with to see whether this cell can grow well. Are they healthy enough? Then you identify a scaffold where you want to populate it. And then colonize them, providing them the medium so that the cells will grow over the scaffold. Normal cells cannot climb over each other because they follow the rules; only cancerous cells can climb on each other. Normal cells, if they want to grow, then they should have a scaffold; only then do they grow.

Two cells cannot climb on each other because they have something called contact inhibition. And then comes the implantation of new tissue, which is implantation and detection. Implantation means delivery into the recipient or the patient, followed by the detection of its normal function and stability. So the basic process of tissue engineering

can be summarized in a nutshell: you can put it here. You harvest the cells and proliferate them, and you also choose the correct scaffold and then seed the cells onto the scaffold, which is called cell seeding.

And then you put this scaffold with the seeded cells into a bioreactor in which the culture medium will be pumped, and the cells will proliferate. This newly growing cells will produce their own extracellular matrix (ECM), and eventually, this scaffold will disappear; these scaffolds are no longer necessary. So that is why the selection of the scaffold becomes so important: you don't want that scaffold to hang around just like that. You may have heard about absorbable sutures used in some surgeries. If you have an external wound and an internal wound, doctors will use absorbable sutures for internal suturing so that those sutures will be absorbed into the body, and you won't have to remove them.

For external sutures, after a few days, usually around 10 days, doctors will say, "Okay, come in, and I will remove those sutures," so that your injury can heal. These sutures should eventually disappear. That is, the scaffold should disappear and be replaced by the matrix; then only can you claim, "Oh, it is a normal cell," and the outlet of the used medium will go as waste and be expelled, resulting in an engineered construct, which means constructed tissue material. It can be nasal septal quadrilateral cartilage; this one, so these are all vulnerable to damage in road accidents, on the battlefield, etc.

, and they can be replaced. Let us see what the clinical applications are: bone and cartilage regeneration tissue engineering techniques are being used to repair bone and cartilage defects. Skin grafting and engineered skin substitutes are used to treat burns and wounds. And oral and maxillofacial rehabilitation tissue engineering offers viable alternatives to traditional bone healing techniques because bone healing or bone grafting techniques are very slow and less efficient; that is why tissue engineering gives you adequate opportunities to. What you call to make whatever shape you want to give, like if you are expecting the bone to heal in a natural way, it may not grow the way you want because you cannot tweak the system in a living organism. But if you already made it, if you allow my nose to grow naturally, it may not get the proper shape, so it is already properly shaped when you put it.

It's a question of just fusing, so that is why making them properly shaped structures is very, very important. Gastrointestinal tract tissue engineering holds promise for treating GI defects, avoiding complications associated with current therapies. If you have to remove or cut the colon off, then.

.. you know they have to run with a bag for you know their to hold their stools etc but if

you can put back an intestine with an artificially made intestine then their life will become great. Heart tissue regeneration. Novel biomaterials and technologies are being explored to improve heart tissue repair after a myocardial infarction. We kind of discussed this in the previous classes as well. The infarct, the fibroid tissue that is deposited, will come in between. It's almost like, you know, when you are happily eating some food, I put a stone between your teeth; then you can't bite down on the food, so that stone should be removed.

Something like that, this scar will come between the contraction, and it should be removed so that the heart's efficiency is restored. The challenges are plenty, just like any other system has. It is the scalability and cost-effectiveness. In the previous class, we discussed scalability, which basically means that although the idea is perfect, you can do anything and everything you want, but it will give you only one microgram of tissue or one milligram of tissue.

You want 10 grams. then it becomes a hypothetical scenario. So tissue availability and scalability are very, very important. And the cost. While scalability is a concern, cost cannot go proportionally to that; it's like you may have seen in the market: if you buy 100 grams of material, the cost may be, say, 50 rupees. The same thing applies if you buy one kilo; it may come for 400 rupees.

Technically, it should have been 500 rupees for 100 grams, so when you buy more, it is normally cheaper. Whenever you say other things, mention that you are buying one meter of fabric or cloth. If you buy 10 meters, they will not give any discount. They will say one meter, multiply it by 10, and then say it. So that logic may not work in the case of tissue because sometimes when you need a large tissue, you should find a way in which it can be made cheaper so that more and more people will benefit from it.

So, scaling up tissue engineering technologies for clinical use can be challenging and costly. This is associated with that. So one should find the ways through which one can reduce the cost. Immunogenicity. Ensuring that the engineered tissues do not trigger an immune response in the recipient is very crucial.

It will be almost like saying the operation was successful, but the patient is dead. So you made a beautiful tissue. Everything is a transplant. Everything is done. Within one month, your body rejected it in spite of being given immunosuppressants.

So one has to make sure that the so-called immunological targets are not overabundantly represented in these engineered tissues; therefore, one has to take care of that. That's why we use the term "humanized pig tissues" or "humanized organs." One angle is

humanized, and the other angle is that even between my tissue and your tissue, they may not be compatible, although you are also human and I am also human. So that's called HLA typing, and that also has to match. So vascularization, creating a functional vascular network with engineered tissue, is a major hurdle.

You made a tissue; you nicely made a tissue; everything is fine. But how will you maintain it? So far, you have been maintaining the medium. You are providing culture medium, and it is receiving oxygen, nutrition, and everything else. But in your tissue, who will pour the medium on top of the cells? Blood circulation should improve. So when you make it, you have to make sure that the blood vasculature is also there in that tissue.

And it should interconnect with your existing circulatory system. Only then is there a future for that tissue. And regulatory hurdles. Obtaining regulatory approval for tissue engineering therapies can be complex and time-consuming. Because in the previous class we discussed the ethics and morality of tissue culture research and stem cell research.

Handling the regulatory hurdles in the same way is another challenge, so what is the futuristic scope of personalized medicine tailoring tissue engineering therapies to individual patients' needs? Their own cells and biomaterials can be used, so it will become like, you know, I have lost one finger, so I took tissue from my body and made a new finger and fixed it. This is the idealistic scenario we are looking for in the future. Organ regeneration is developing technologies to regenerate entire organs, addressing the shortage of donor organs, and you don't need to worry about the vasculature. Also, I lost one finger, and I am tweaking some cocktail here so that my finger is growing. I won't mind whether it takes six months or one year, as long as I get my finger back; I am happy.

So I don't need to culture it, and automatically my body is making blood vasculature into it. These are all the future promises of regeneration or tissue engineering for regenerative medicine, disease modeling using engineered tissues and organoids to study diseases and test new therapies. This is another vista of exploration. Gene editing utilizing CRISPR Cas9 technology to enhance tissue integration and function sometimes results in a situation where the tissue I created has a specific HLA type that is present and may be rejected by the recipient. What I can do is knock down or mutate that gene if it is not an important gene for the recipient.

Survival of the tissue; I can just get rid of that, which will be targeted by the recipient's immune system. So that host tissue, I can tweak it using CRISPR-Cas9 technology. I will not go into the details of CRISPR-Cas9 because it's a completely different field. But

those who are interested can read about it and understand what I mean.

Advanced bioprinting. Developing more sophisticated bioprinting techniques to create complex, functional tissues and organs. So this is bioprinting, where you make something like a 3D print. You can print the organ at will in a given shape and size. The transplant and integration of artificial intelligence these days is a time of AI everywhere, using AI to analyze data and optimize tissue engineering processes because, while making a tissue, we should understand whether that particular tissue is going to impose an additional burden on that patient. Say a liver has failed; now I am asking what pressure that organ is going to exert on the blood pressure or any other internal bulk pressure on that person, and will it withstand everything? This needs to be monitored and calculated; that is why AI comes into the picture.

It won't rely solely on measuring the dimensions; it has to be considered as multi-parametric. That is why AI comes into the picture. And microfluidics, which is based on various physiological platforms, creates microfluidic devices to mimic physiological environments and study tissue function. This means you can use the microfluidic system to support these tissues so that they will benefit from the recipient organism. So tissue engineering, if you look further, you can see what the scopes are and their status as of now.

The term tissue engineering was created to represent a new concept that focuses on regenerating tissue. It is a spelling mistake: NEW, new tissues. From cells that have the support of biomaterials and growth factors, we should understand that it is an integration of biomaterials and the live tissues they come together, and there is also a new research field called biomedical engineering, which is a new area of research abroad in foreign countries at many institutes. This interdisciplinary engineering has attracted much attention as a new therapeutic means that may overcome the drawbacks involved in the current artificial organs and organ transplantation, which have also aimed at replacing lost or severely damaged tissues or organs. That is the essence of tissue engineering; however, the tissues regenerated by tissue engineering and widely applied to patients are minimal, including skin, bone, cartilage, capillary, and periodontal tissues.

They have not been applied to every organ; they have been applied to some. Frequent and easily doable. They are restricted to this easily doable minimal list of tissues. What are the reasons for such slow advances in the clinical application of tissue engineering? Obviously, one can anticipate biological limitations and financial limitations, both of which come together. The fundamentals of tissue engineering involve cell sources, scaffolds for cell expansion and differentiation, and carriers for growth factors, but for some tissue and organ differentiation, we are still learning.

We cannot just say, "Okay, I have a stem cell; you make a liver. I have a stem cell; make a kidney." We haven't reached that stage of tweaking in every aspect. Aspects of organ formation, animal, and human trials are major parts of these applications; based on these results, some critical problems need to be addressed and resolved for the advancement of tissue engineering, which is being approached from the engineering point of view these days, emphasizing the close collaboration between medical doctors and biomaterial scientists, so the current clinical status includes. Patients are mainly treated surgically by grafting methods in three ways.

That includes autograft, allograft, and xenograft. What is an autograft? Autograft to move tissue from one side of the body to the other side. Say I want some skin on my cheeks. I took the skin from my thigh and cut open and I pasted here, sutured here.

That is an autograft. One approach. The patient is already suffering from a disease. Thus, grafting the same body will be very painful. It might lead to graft failure. Because they are not perfect.

I got into trouble here not because I am healthy. I am having trouble. And making another wound in my body and trying to expect that wound to join can be problematic. So that they are the drawbacks in elderly patients, the grafted cells and tissues might not regenerate or might not adhere after harvest because they are in their downward journey. Allograft means from another person of the same species, with Person A donating to Person B. This method also has problems and suffers from issues like donor scarcity and immune rejection. Like I told you, HLA typing mismatch—you may have heard about it—kidney donor, father gives to son or mother gives to daughter, or that will be more authentic, or closer, or sibling donation is closer than a stranger.

Husbands give to wives; although people do it, they don't have any genetic identity, so they have to be under severe immunosuppression. Additionally, the donor should be disease-free; otherwise, if the donor has a disease and donates the organ, the recipient may also contract that disease. This presents another problem: diseases such as infectious viral infections and those kinds of things. Xenografts include tissues and organs taken from other species, such as pigs. Problems such as biocompatibility and immune rejection are common in this method.

Moreover, animal tissues are more prone to contamination than human tissue. So, after grafting, the chances of infection still persist. So these are all some challenges of which you should be aware. Now let us understand what the main angles are in which tissue engineering is the only option. Why do we need to go for tissue engineering? Some I

already explained to you, like a bone of one foot that has lost 10 centimeters. You cannot say, "Okay, now from today onwards, you can have one 10-centimeter shorter bone," because the other part of the body will not.

Integrate with that so you need it; otherwise, you cannot have one leg longer and one leg shorter. Such an organism will not survive, and that will give extra stress on its pelvic girdle and backbone, causing it to be bedridden soon. So, although it is just a 10-centimeter shortage, you have to retain that original length for having an equal balance of your gait and movement. So donor tissues and organs are always in short supply. Hence, the topic of tissue engineering itself comes into the picture to minimize immune system response by growing cells or finding novel ways to protect the transplant.

Like I told you, if I'm tweaking the cells in a Petri dish, I can understand it better, and I can get rid of all the troublemakers. Oh, this protein is here.

Immune rejection will occur. Let me get rid of that. Oh, this protein is here. It may cause some trouble. It may cause some diseases. Let me get rid of it. So it gives you just like, you know, you pluck some fruits from the wild and you clean them and take only the good parts. Sometimes you remove the skin of an apple just to make sure that I don't want to eat skin or I don't want some pesticides landed onto the skin.

So multipurpose, you can clean, but nobody eats the skin of the banana. You remove it, and then you eat it. So, it's not just about the taste. It also gives a protection. So the ultimate aim of tissue engineering is to repair, regenerate, replace, and restore.

So there are four R's. First is to repair, regenerate, replace, or restore, depending upon the context. In some situations, you just need a repair. The situation is not just repair; you need to regenerate it; otherwise, the scenario will not work. Sometimes you need to replace an organ that is gone; just like a kidney, they remove the whole kidney and do not try to fix that broken or damaged kidney.

Instead, they put in a new kidney. In some situations, it is restoration, which means the organ is fine, but some connections are affected, and you make the proper connections so that it will now start functioning. So there are different scenarios in which these troubles exist. An overview of the tissue engineering process is listed here in this picture, so it doesn't matter whether this person is healthy or a patient; cells are collected by biopsy, a monolayer of cultured cells is made, and then these cells are expanded. And it expanded them by culturing them.

And then what has been done is they cultured a 3D polymeric scaffold. And this 3D

polymeric scaffold is pushed into a generated graft. And this generation graft is now pushed back into the patient. So you can see here, this is donor tissue and isolated cells. And you have made an in vitro culture, and then you put it into a scaffold and in vitro implantation.

So understand these are the flow of the regeneration process. We will learn more about regenerative biology in the next class. Thank you.