Regeneration Biology
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W10L48_Different steps in tissue engineering

Hello, everyone. Welcome back to another class on regenerative biology. And today we will learn about the different steps in tissue engineering. And so far, we have seen the importance of tissue engineering, how it is done, its pros and cons, its ethical implications, etc. So today we will learn about what the steps are. Steps do not exactly mean the methodology.

We will also touch on some methodological angles, but we will try to understand the overview. Tissue engineering is a multi-step process that includes cell isolation, scaffold creation, cell seeding, and tissue growth stimulation, ultimately leading to the creation of functional tissue for transplantation or other applications. The theme of tissue engineering, or the flowchart, or what you call it, is the workflow of tissue engineering, as mentioned. A commonly applied definition of tissue engineering is provided by Langer and Vacanti, the pioneers in the field.

It is an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain, or improve the biological tissue function or a whole organism. So, this is the definition by Langer and Vacanti. And in addition, the same researchers, Langer and Vacanti, also state that there are three main types of tissue engineering. One is cells, tissue-inducing substances, and a cells plus matrix approach often referred to as the scaffold, and tissue engineering has been defined as understanding the principles of tissue growth and applying this procedure to the functional replacement of tissue for clinical use, so you need to have cells to start with, and then that cell has to become a tissue so that a function can be delivered, or you should have the cells with a scaffold on which the cells can be forced to grow and then allow them to differentiate so that you can transplant this artificially made tissue. You can either make it on-site or make it somewhere else and bring it to the site.

This is a simple approach; another description. The underlying supposition of tissue engineering is that the employment of the natural biology of the system will allow for greater success in developing therapeutic strategies aimed at the replacement, repair, maintenance, or enhancement of tissue function; another approach or definition of tissue engineering. So you may wonder why different definitions are important because the whole concept of tissue engineering itself will be unclear unless the definition or the approach is well defined. It is very vulnerable to getting diverted into an obscure or insignificant area, and it can get diluted. Many such disciplines have gotten into that trouble.

That's why defining a proper disciplinary area is very important. So developments in the multidisciplinary field of tissue engineering have yielded a novel set of tissue replacement parts and implementation strategies. Scientific advances in biomaterials involve the use of a scaffold. and also stem cells. Stem cells mean stem cells are stem cells, but understanding how to tweak them to differentiate in a given direction is important.

So these are all the understandings; understanding stem cells means not learning their biology but learning how we can convert them into a multi-cell type tissue derived from a given stem cell. Growth and differentiation factors and biomimetic environments have created unique opportunities to fabricate or improve existing tissues in the laboratory from combinations of engineered extracellular matrices, cells, and biologically active molecules. So sometimes this newly formed tissue has a future if it is assembled properly. Otherwise, it will get disassembled and fall apart. So it is very important to follow up on how well you are going to retain the stem cell properties with all their biological qualities so that you can push them into a tissue.

In a defined environment, not that I wanted to make cardiac tissue, and this time it became liver tissue, or I wanted to make liver tissue, and this time it became kidney tissue, you don't want that to happen. Among the major challenges now facing tissue engineering is the need for more complex functionality, biochemical stability, and vascularization in laboratory-grown tissues destined for transplantation. I have discussed many times that if the newly formed tissue doesn't have a future. Now it has a future because you are providing the means. But once it is put into the organism, the organism is supplying blood, but the blood and oxygen are not reaching the newly formed tissue.

How can it reach? Because no tissues will be stable if they are exposed to blood directly, since we have a closed circulatory system. It should gel well with the blood circulatory system; the angiogenesis of the newly formed tissue is very, very important, as important as the tissue itself. If someone asks if the tissue is important or if blood vasculature is important, I would say blood vasculature is important, simply because tissue has no fate if the blood vasculature is not properly made. So, the basic procedure of artificial tissue preparation is as follows. Tissue engineering is an interdisciplinary field that applies biology and engineering principles to develop biologically functional replacements.

We have seen this, and it is used exclusively in clinical applications. It offers the possibility of helping regenerate damaged tissues caused by trauma or disease. You can damage a given tissue by multiple means; it can be an infection, mechanical damage, trauma, etc., or it can be a genetic disease, also creating new tissues and replacing them. or of the failed tissue or failing tissue or malfunctioning organ, so that is the overall theme of tissue engineering.

Tissue engineering includes prosthetic or artificial replacement of the tissue, and it can be the replacement of a part of the tissue or an entire organ itself via organ transplantation. Maybe a selected area of that organ can also be transplanted. Say, for example, if you're doing a liver transplantation, you don't need to make an entire 1.5-kilo-sized liver. You can make a 10% solution of around 100 grams of tissue, and you can put it in.

It will grow back and become a normal liver. So that is the overall theme of the transplantation of artificially made tissues. Artificial tissues include the artificial pancreas, artificial bladder, skin, and bone marrow. Oral mucosa can all be made by tissue engineering, and the list continues; these are not the only tissues used extensively for various clinical applications. So let us look into the detailed breakdown of these steps: cell sources and isolation, identifying the appropriate cell type.

The first step is to determine which cells are needed to regenerate the desired tissue. Say you want to go for a.. vacation, so the first thing you need to know is what my destination is; then everything else comes into the picture: how I will go there, do I have the money, who will go, how many days I will stay—everything can be worked out if you know the destination. So, cells have to be identified, cell source and from where I will get it, and then comes the cell isolation.

Cells are obtained from the patient; they are basically autologous cells, a donor. Which won't be autologous. But what if this patient doesn't have cells to donate? Say that someone's pancreas is failing. Where will I get pancreatic cells from him? Of course, I can make stem cells from him or her and then convert them into a pancreas. But it would be nice if I could get the pancreatic cell itself so that I can make the organ without too much of a problem.

It can also be derived from the stem cells, as I mentioned just now, and can be from the iPSC cells from the patient. But these all add to the cost of production. Every step you take when going back adds to the cost. Cell expansion and differentiation: the isolated cells are cultured and, if necessary, induced to differentiate into the specific cell type required for engineered tissue, so you should be able to convert x into y or a stem cell into that y tissue, and scaffold design and fabrication. The next part is, okay, I have isolated the cells.

I know how to differentiate. Everything is there. But now I want a particular-shaped cell because that is what I'm using for transplanting. Then you need to have a particularly shaped scaffold on which you will colonize these cells. Scaffold material selection is very important.

A suitable scaffold material is chosen based on the type of tissue being engineered, considering factors like biocompatibility, biodegradability, and mechanical properties. All these things come into picture. And scaffold fabrication. The scaffold is fabricated using various techniques, including 3D printing, electrospinning, or other methods to create a porous three-dimensional structure. So why is it porous? Because only then can cells move across.

Then only can the medium go between the pores. Everything is important. Then comes seeding and tissue growth. Seeding basically means inoculation. You may have seen how your mother or anyone in the family takes a curd and puts it into the milk, and that is nothing but inoculation.

Like that, you have to do the seeding. Cell seeding. The isolated cells are seeded onto the prepared scaffolds. You are putting it into the scaffold and then culturing and stimulating. Culturing means they should obtain the medium.

The scaffold cell construct is cultured in a controlled environment, often using the so-called bioreactors to promote cell growth, differentiation, and tissue promotion. So you are stimulating the cultured cells on a scaffold now to differentiate again by controlling the medium. You changed the contents of the medium. Say C2C12 cells, myoblast cells; if you put in 2% horse serum, they can become more differentiated, and they can even start twitching in a petri dish. They can become contracting, contractile muscles.

So growth factors and signaling molecules contribute to their maintenance, survival, and progression. Growth factors and other signaling molecules may be added to the culture medium to stimulate cell proliferation, differentiation, and tissue organization. Like C2C12 cells, they are not used for any organ transplant, but they are a muscle cell line. You can make them differentiate into muscle fibers just by putting horse serum instead of bovine serum albumin. If you put horse serum, it will differentiate.

There is one example I am telling you, so in tissue engineering and its implantation, say you made differentiated tissue on the scaffold. Everything has been done now; the tissue formation, the cells that proliferate on the scaffold, and interact with the scaffold. They form a new tissue structure. That the scaffold shape is the tissue's shape.

So then, the implantation comes. The engineered tissue construct is then implanted into the patient's body, integrating with the surrounding tissues and performing its intended function. Then you can tell that this is completed or that one circle is completed, starting from the cell into transplantation. Until that stage, it has been done. Now let us see the steps to prepare artificial tissue, which is the scientific methodology of this artificial tissue. The tissue engineering procedure involves several steps.

which starts from cell selection, cell isolation, and culturing of the primary progenitor or stem cells, and inducing their differentiation to certain phenotypes, seeding and cultivation, design of adequate scaffolds including selection of proper materials and routes to process, porosity of the scaffold, interconnectivity, and surface characteristics, Everything matters because the newly formed tissue is only as good as the scaffold or whatever you started with; that quality will only be there. It's like there is a popular saying: if you put dirty water on your head, when it comes to your feet, it is not going to become pure; it is going to become dirtier. So it is very important that you are starting off. Taking which scaffold, what is the shape of the scaffold, and what cell am I using for seeding? Everything contributes to the final success of tissue production.

Let us see the step-by-step procedure. First, you start with the biopsy. A biopsy; all of you would have heard about it. That is one way of accessing cells from a tissue to test for cancer. So a biopsy is basically donor tissue extraction. Either from fluid tissue like blood using centrifugation or apheresis, which is an easier process to concentrate the cells, or from solid tissue that involves more steps, like needle biopsy, people take with a needle, solid tissue is minced, then enzymes like trypsin or collagenase are used to remove the extracellular matrix; finally, the cells are free-floating.

It was extracted using centrifugation or apheresis. So you first dislodge the cells by trypsinization or any other protease treatments. Trypsin selectively cleaves the matrix protein, resulting in an individual cell suspension; then you spin it, concentrate it, and obtain the cells. So lately the trend in donor tissue extraction is to emphasize more on a non-invasive method, so if the organ is very vital, I want some cardiac tissue; I don't want to make a pinhole in my heart, which can invite more trouble. Much easier methods are being developed these days.

Cell isolation and cultivation manipulation of cells. It is safest to use autologous cells, which are primary cells extracted from the same person's healthy tissue to which artificial tissue will be transplanted. Recently, there has been a trend to use mesenchymal stem cells from bone marrow that can differentiate into various tissue types. Other types of cells that can be used are allogenic, which is a donor from the same species, like person A donating to person B. Both are Homo sapiens, which are allogenic, and heterologous, which is homo.

Or xenogenic, that means the donor is from a different species; the donor can come from a different species. In those cases, rejection by the host's immune system and possible disease transmission are concerns. However, when you are not able to find a donor, xenogenic becomes attractive, and the risks must be considered because every. Every topic, every situation has its own pros and cons, so if you look at scaffolds and seeding and the cultivation or implantation of cells into an artificial structure that can support 3D tissue formation and resembles the extracellular matrix, this is one approach that is extensively used. And there are certain requirements that scaffolds need to meet.

What are they? Biocompatibility that is acceptable for cells, high porosity, and adequate pore size. These biocompatibility is an important criterion. Then comes biodegradability. Biocompatibility means it should not raise an immune response in the host, and it should also degrade. That later it will be replaced by the extracellular matrix released by the cells grown on the scaffold and the non-brittle nature means that if it is brittle, this scaffold or this tissue will break easily, so you don't want them to be brittle.

It is preferable for the scaffold to be absorbed by the surrounding tissue, vanishing into thin air, which is what is most expected. It can be functionalized with biomolecules, and they can function as nutrition for cells. Sometimes, you know, think about the situation: while the scaffold is degrading, it acts as nutrition for the cells. So such approaches are also being considered. All these adequate properties must be present in this scaffold so that the formed tissue will be ideal.

And implantation, which is implantation into the living body and then detection. That is basically called property analysis. That means I put in a tissue. Okay, it is staying there.

Is it doing the job? We have to see the end product. as the function of that organ. Say, if you made liver tissue, it should produce liver enzymes; then it has meaning. If you look closely at an overview of tissue engineering, you can see there are novel cell sources and tissue architecture techniques, and IPSCs are present, as well as reprogrammed cells. Cell culture in vitro can be made into a bioreactor, and you can make them into whatever tissue you want using growth factors and transcription factors. And you can give different types of stimuli to these cells so that they can be made into a proper organ part on those various engineered materials.

This engineered tissue, when it is ready, can be anything; sometimes people use decellularized organs as well, like taking an organ, say a heart, and getting rid of all the cells. For example, I took a pig's heart or a buffalo's heart or any other animal's heart and removed all the cells, but I'm using the matrix of that organ. Now it allows the cell to colonize. I don't need to make any difficulties or face any difficulties because the shape is already there in matrix form. The cells will colonize, and it will look like a heart, particularly a heart-shaped structure.

And people also use 3D bioprinting these days so that you can scan the dimensions of an organ and use cells to print a proper organ for final implantation and validation of the organ's function. Now tissue culture is also used for some interesting projects, so this is called a tissue culture art project. While it is called art, it has a lot of artistic viewpoints. It's an ongoing research and development project that was initiated in 1996 to explore the use of tissue technologies as a medium for artistic expression. Some of their projects include a semi-living worry doll, victimless leather, a prototype of a stitchless jacket grown in a techno-scientific body, and a semi-living stick 2000.

So let us see some examples. So this is a semi-living worry doll. So it was created in 2001 by Oron Katz, Einat Zur, and Guy Ben Eri. So these three scientists created it. The semi-living worry doll is a project that reinterprets the story of the Guatemalan worry doll to deal with contemporary anxieties. If you have this doll with you, you will not deal with anxiety, and it's made of living meat, as you can see in this picture in a petri dish.

It's a doll, and people find it—this is the artistic view. Another example is victimless leather; if you are wearing a leather jacket, you have a feeling, "Oh, this is the skin of some animal." And I killed it, or you know, I am wearing some leftover from a dead body—those kinds of feelings. So these days, you may have seen on mobile phones people say, "Oh, we are using vegan leather." Vegan leather means it's an artificial leather, but no animals are hurt or harmed.

So this is something called victimless leather, made in 2004. A prototype of a stitchless jacket. The good thing is that if you can culture cells and make them, then you will not have any stitches because normally, any leather jacket you make consists of pieces that you stitch together. You don't have to stitch it. It will be stitchless leather. Uh, and grown from cell culture into a layer of tissue supported by a coat-shaped polymer layer on top of that, with the scaffold in the shape of a coat, and in that, you are allowing the cells to grow.

It is grown inside a bioreactor that acts as a surrogate body. As you can see here, medium is being given. Every condition is maintained, and you end up getting this jacket.

As you can see here, the artistically grown garment is intended to confront people with the moral implications of wearing parts of dead animals for protective and aesthetic reasons, which confront notions of relationships with the manipulated living system, may lead some people to wonder why they should carry a part from the dead body of another animal. These are all thoughts that people can have, and they can be overcome by this kind of approach. So, another option you can see is Semi-living steak; so here, what has been done is that in 2000, the first steaks measuring around one centimeter in diameter were composed of prenatal skeletal muscle cells.

What they did was take a small piece of steak, which people eat as food, that was around one centimeter in diameter. Prenatal skeletal muscle cells. So from an embryo, you took the skeletal muscle cells from an unborn sheep. Sheep skeletal muscle cells are taken, which are then seeded on a degradable matrix. So skeletal muscles are growing onto that matrix, which is basically a scaffold.

And the semi-living stick was the outcome of a residency at the Tissue Engineering and Organ Fabrication Laboratory at Harvard Medical School in 2000. So the semi-living steak is the first known proof of concept for using tissue culture exclusively for food. We should understand that in the future, it is very likely that people will run short of natural resources to satisfy the need for natural resources as food material. If the number of people is up to a certain threshold, you will be able to provide for them from nature itself. Like there is a saying, isn't there? There is enough in nature to satisfy the need, but there is not enough to satisfy the greed.

In the same way, when the number of people consuming food is too high, the food will fall under the category of greed. Although it is a need, at that time you might have to come up with strategies to make those who are non-vegetarians, or even probably, for that matter, vegetarians, come up with artificially made options; one is a plant tissue, another is an animal tissue. That is the difference between vegetarian and non-vegetarian. But without killing an animal, if you want to have the feeling of eating non-vegetarian food, people are also exploring the possibilities of.

.. I think it's already out there. People are putting a collagen gene, which is an extracellular matrix protein, in potatoes. And you end up having a... Non-vegetarian potato, so vegetarians cannot have that because it's a non-vegetarian potato, but it is not from an animal; it is just a gene that is produced in the plant itself, so we are now kind of making the boundary lines thinner.

But it has applications. Why is this being discussed? Like coming back to this semi-living worry doll, you may wonder what the implications are in tissue engineering. Of course, it is tissue engineering. The only thing is you did not use tissue engineering to save the life of a patient. That is the only difference.

But in tissue engineering, you created a doll that is made entirely of meat. Exactly made of, you know, flesh, so that is what it's basically. You are satisfying the, you know, you are, you know, this is basically Guatemalan belief of worry dolls. You can read about it; that is used for dealing with contemporary anxiety disorders and victimless leather. Also, we discussed that victimless leather; also, people want to have all the qualities and features of leather. They love leather; that is why, you know, I discussed the vegan leathers that have come into the picture.

So it is not for what you call it; it is not that people don't like something, but the... property of that material or the means through which you have created a leather that worries you.

Oh, this is from a leftover of a dead body. So this worries people. That's why some people don't use it. So once you know that no animal is there and there is no connection with this animal, then your problem is solved. This is what you should understand: although it is costly, there are people out there who can deal with it, who can take it, and there is no issue whatsoever with that. That is why proof of concept and proof of principle can be done, and it also gives you an idea. Until where science can go, we should understand that while using technology to do something, we should also recognize that it has other markets as well.

For example, when Corning Gorilla Glass was discovered, people never knew what it would become, as it was discovered in the 1950s, if I am not wrong. At that time, people did not know that the new glass would not break if you dropped it, but it never took off because.

.. People said it was very costly. Why should I use? If the glass breaks, I will buy a new one. But now mobile phones are more expensive. Hence, if you drop your mobile phone, you don't want the screen to be broken. So that is why, after several years, the discovery was ahead of its time.

In the same way, these are victimless leathers. In the future, it may reach a situation where it can become a fashion statement; it may be costly to start with, but it can become cheaper eventually when it becomes popular, the same way the semi-living stick, which we discussed, can. Although it may sound very trivial, especially when there is plenty of food available out there. But it won't be trivial when that is the only way of getting food. So we will learn more about regenerative biology in our next class. Thank you.