Regeneration Biology
Rajesh Ramachandran
Department of Biological Sciences
IISER Mohali
Week: 10
Lecture: 47

W10L47_History of tissue engineering

Hello everyone, welcome back to another class on regenerative biology. In today's lecture, we will learn about the history of tissue engineering and also a little bit more detail about how it is applied and what the methodology that has been used along with this history is. The history of tissue engineering dates back to as early as the 1980s; I'm not going prehistoric, but we will see that as we go on. Tissue engineering is a field focused on developing biological substitutes to replace or regenerate damaged tissues that emerged in the 1980s. With a key milestone that includes a 1988 National Science Foundation workshop where the term was officially coined in 1990, seeing the rise of stem cell and tissue engineering concepts, the terminology of tissue engineering was established in the late 80s and early 90s. But if you look into the history of tissue engineering, specifically the historic origin of tissue engineering in 1000 BC, the Indian scripture Sushruta Samhita states that Sushruta performed nose transplants on soldiers.

In the 16th century, Taliakosi was used as a forearm flap for nose reconstruction, which occurred several centuries after Sushruta; in the 17th century, tooth transplantation became possible, in the 18th century skin and corneal transplantation became a reality, and in the 19th century, the advent of sterile techniques and anesthesia emerged. They precipitated the emergence of reconstructive surgery because anesthesia is needed if you want to undergo surgery. Injury in the sense of operation requires you to create an injury. So, anesthesia is a must.

And in the 20th century, the proliferation of modern-day transplantation surgery and the utilization of synthetic materials for tissue repair came into existence.. In the late 20th century, shifting the scientific focus towards cell-based reconstructive therapies and using various biological components, the building blocks that can create the necessary elements to perform tissue regeneration. In the post-1950s, kidney, heart, lung, and bone marrow transplantation became a reality. And in 1985, the concept of tissue engineering was articulated in detail with its fully fledged principles, etc.

In 1988, the first symposium on tissue engineering was held. So if you look further into the details of tissue engineering, the early foundations happened between the 1960s and 1980s. The seeds of tissue engineering were sown in the 1960s itself with researchers combining materials, science, and cell biology. Leading to the exploration of tissue replacement, can we use tissue as a biological material or a non-biological material and use that as a material to make them more humanized or more organic? That is the whole concept. At that time, in the 1970s, Boston pediatrician Dr.

Green manipulated chondrocytes to attempt cartilage regeneration. Marking an early step in the field, bones were one of the earlier tissues on which people worked a lot to fix the damage because many people suffer from fractures, and they were one of the earlier tissue types to get attention. The term tissue engineering was loosely used in literature until the mid-1980s. Referring to surgical manipulations, or the use of prosthetics and biomaterials. So they are in the biomedical engineering field rather than using a tissue engineering field.

The term tissue engineering, as we know it today, was introduced in medicine in 1987. So tissue engineering is not just a term that has implications or applications in the treatment field. Yuan Chengfeng, a researcher, physiologist, and bioengineer, proposed combining tissue and engineering in 1985, with the term officially adopted in 1987. Although it may sound like a simple word now, the origin of that word itself is not that simple. The formalization and growth of the field from the 1960s to the 1980s was the concept's conception time, and the 1980s to the 1990s was a significant period, highlighted by the 1988 National Science Foundation workshop.

Granlibakken officially coined the term "tissue engineering" at a conference that was established. The proceedings of this workshop defined tissue engineering as the application of principles and methods of engineering and life sciences towards a fundamental understanding of structure-function relationships in normal and pathological mammalian tissues. The development of biological substitutes to restore, maintain, or improve tissue function. So this is the definition of tissue engineering accepted at the 1988 National Science Foundation workshop. Succeeding the symposia in 1990 and 1992 further solidified the concept, and the scientific literature started building up in tissue engineering; by the early 1990s, the concept of applying engineering to biological tissue repair led to the rapid growth of tissue engineering as an interdisciplinary field.

This is the growth of the field of tissue engineering. and it had further maturation event Joseph Vacanti a surgeon very famous figure in the field of tissue engineering and Robert Langer another professor at MIT are credited with seminal work in this field you can find lots of literature from these two people Vacanti approached Langer with the idea of designing scaffolds for cell delivery as opposed To rely on naturally occurring scaffolds, such as using extracellular matrix from some animals, can we use artificially made biomaterials? This was the whole idea: Vacandy designed and implemented studies to generate functional tissue equivalents using synthetic biocompatible biodegradable polymers; they are biocompatible. Plus, biodegradable and biocompatible basically means it doesn't elicit an immune response. Biodegradable polymers mean those that can eventually be degraded as the ECM produced by the cells replaces them. And these polymers acted as scaffolds that were seeded with viable cells.

If you provide viable cells seeded on them, they will eventually get biodegraded. The first tissue-engineered product that Vacanti was involved in was the skin equivalents because one of the simplest tissues in your body is skin, and they are the ones; skin equivalents were the first ones tried. The first FDA approval for an engineered tissue product without a living cellular component occurred with the pre-market application, also known as PMA approval, of Integra TM artificial skin in March 1996, so this is another angle of tissue engineering: you are fixing a tissue without any biological origin material. These days, people are also making cornea substitutes; you don't need a cornea from a living organism. One option is to culture the cornea from limbal cells.

Another option is to use artificial material. So, there are multiple definitions of tissue engineering. Few are listed here because today's class is about historical aspects and the methodology. An interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organism itself. This is by Langer and Vacanti, the pioneers in the so-called tissue engineering field.

The use of a combination of cell engineering, materials and methods, and suitable biochemical and physicochemical factors to improve or replace biological functions was expected, and more definitions have emerged regarding the application of principles and methods of engineering. And life sciences towards the fundamental understanding of structure-function relationships in normal and pathological mammalian tissue and the development of biological substitutes to restore, maintain, or improve tissue function. This proposal and this definition have come from Y.C. Fung, the application of biological, chemical, and engineering principles towards repair.

Restoration and/or regeneration of living tissues using biomaterials, cells, and factors alone or in combination came from C.T. Laurencin. So many different scientists, including famous ones, have proposed their own definitions. But in a nutshell, you understand what it means.

so let us look what is the preliminary steps or what is the outline steps one has to follow up for doing this so-called tissue engineering step one getting the tissue sample so you need a tissue if you want to do any tissue engineering so it can be patients on cells The tissue of interest is taken, and the researchers should pick the tissue they want to use for tissue engineering. Then, the researchers break the tissue into pieces; we will see how it is done in a short while. It has to be made into single cells, and in step two, the cells are grown into the new tissues that are needed. After breaking the tissue into smaller units, which are the cells, the cells need a structure, nutrients, and oxygen. Here, we are referring to a supporting material.

The above requirements of the cell are fulfilled by the scaffold, which means the scaffold can provide structural support; if the medium is put onto the scaffold, it gets the nutrients, and the oxygenation can come from artificial oxygenation or from atmospheric oxygen itself. The scaffold helps in the regeneration process of the cells because healthy cells do not climb on each other. They need a scaffold for their movement and support. What is a scaffold? Scaffolds are materials that have been engineered to cause desirable cellular interactions to contribute to the formation of new functional tissues for medical purposes. So, this is the definition of a scaffold, as you can see here in this picture.

I don't think it is so big; big, big holes are there, but this white part you are seeing has a very microscopic structure at the ultra-structural level. They are like, you can imagine, something like that of cotton candy. Everybody would have seen cotton candy. What is the shape of cotton candy? If you look closely, it is a meshwork; something like that is. And even cotton candy itself, people are using as scaffolding.

That is also something that some people have used. So cells are often seeded into these structures that are capable of supporting three-dimensional tissue formation. That is the overall idea. Cells normally form a monolayer. If you want a three-dimensional structure that consists of healthy, non-cancerous cells, then you need a scaffold.

Scaffolds mimic the extracellular matrix of the native tissue, recapitulating the in vivo milieu. Milieu means immediate surroundings. I am sitting here. My milieu consists of my table and chair. So that's the idea you should get.

In vivo milieu is mimicked by the scaffold, allowing the cells to influence their own microenvironment. First, to start with, they depend on the scaffold; later, they replace the scaffold with their own ECM secreted by these cells. So the function of scaffolds allows cell attachment and migration, delivers and retains cells and biochemical factors, enables diffusion of vital cell nutrients and expressed products, and exerts certain mechanical and biological influences to modify the behavior of the cell phase. Scaffolds provide mechanical and shape stability to the tissue defects. So they can provide structural support and not just superficial support.

They also provide some nutrients. They can be made in such a way that these growing cells find a home. They will find these scaffolds to be a stimulating environment. It's just like you may have noticed: if you are buying a cake and you see a cake that is, you know, the color of dirt or a color that is not normal for food, like a blue cake, you may not buy it. Normally, a cake will be orange, brown, or white.

You usually won't find a blue-colored cake like this. So, normal food is not connected with the blue color. So there are many friendly colors you can use for food coloring, which mimic those of fruits. In the same way, the mat scaffold should also be very friendly to the cells so that they can easily climb on it and grow. So these are all the factors that should be taken into account.

So, some pictures are here showing different types of scaffolds just for understanding. Don't worry about the full form of this scaffold: PLA, what is PLA, what is PCL, what is UBM, what is PMMA scaffold? No, don't worry about it. I am showing this just to get an idea of how it looks. This has a peculiar shape, and this has another peculiar shape that looks like a hairstock.

And this looks like a paper. And this looks like a three-dimensional structure. Looks like a sponge. The ultrastructural shape of a sponge. These are all chemical matrices.

They can act like scaffolds. We will revisit the exact chemical nature of the scaffolds in a later class. Not right now. and implanting new tissues on them. This is the final step in tissue engineering. The scaffolds that are seeded are implanted into the body, and once the tissue becomes compatible with the surrounding body, the scaffolds dissolve completely as the tissue grows normally.

So they have to disappear. You don't want them to be there. Even if it is there, it is okay, but it should not stay forever. Eventually, it should disappear. Sometimes, ex-vivo monitoring is also done if possible. So, what is the fate of these cells? One picture has been provided.

This is a scaffold, and they can be different, including biological and non-biological types. Collagen type 1 is a biological material and hydroxyapatite. PLA, PGA, and PCL are all chemicals used for seeding cells. Primary osteoblasts, MSCs, mesenchymal stem cells, and osteoclast co-culture; endothelial cell co-culture, etc. are done, and they are all now colonizing the scaffold.

You can see it, and they are put into a bioreactor where chemical, perfusion, mechanical, and electrical factors influence the healthy growth of the cells on the scaffold. And then, once you make it the final product, what you made was a piece of bone you wanted to replace, and you see it is implanted down to the middle of the missing part of the bone. Clinical trials are all done by various scientists, and ideally, this tissue-made bone structure can encapsulate or get embedded into the bony structure of the patient. So applications of tissue engineering can be used in a variety of scenarios: one is breast reconstruction, cardiovascular system improvement, cardiac tissue engineering, blood vessels, heart valve, musculoskeletal system improvement, bone regeneration, bone and cartilage reconstruction, and production of artificial skin. The artificial production of organs such as ear, cup, kidney, liver, etc.

, can be easily done using tissue engineering. This approach can be done very effectively; then what are the advantages that help to cure diseases? We have seen it earlier, also, in diseases related to bone marrow. If the bone marrow is misbehaving in some way or another, you can completely get rid of the bone marrow and replace it with fresh bone marrow from a blood relative. So the patient's blood relative means that having tissue compatibility allows the patients not to wait for a donor; that is the beauty of tissue engineering. Because your own tissue has been taken and expanded, you are getting your own help. It's just like when you scratch your hand; you are not waiting in a queue for someone to come and scratch your hand.

You are using your other hand to scratch your itching hand, so a permanent solution is obtained because of this, as you are making what you need. So, if you are eating and someone gives you a chapati, you don't eat the whole chapati; you make it into pieces based on your mouth's size or chewable size, and take it. Every two people will not make the same piece based on their palatability and liking. They make a piece, so this is the beauty of tissue engineering, and the success rate is high because it uses your own cells. A distinctive feature of tissue engineering is to regenerate patients' tissues and organs that are entirely free of poor biocompatibility issues that can come from a donor if the tissue has been taken, as well as low bio-functionality and severe immune rejection; these are all associated if it is from a donor.

If it is not from a native, or if it is from a donor or other person. What are the disadvantages? It is a time-consuming process. If I say tissue, you cannot order like fast food. Oh, I need this one kilogram of tissue delivered tomorrow.

It is not possible. An expensive practice to engage in. A lot of money is involved. This technique is not well developed. That is the limitation of science.

And there are ethical issues. Oh, this person with this damage is supposed to die. Why are you fixing him? Now you are allowing him to compete with the rest of the world.

Actually, he is not a natural.

He or she is not natural. The doctor has... contributed to his success. So, these are all ethical issues. It requires a lot of knowledge about the organs to practice on complex organisms. And it's an irreversible process. Once you fix it and something goes wrong, you have to live the rest of your life with that because money is involved, and sometimes you have to undergo new surgery.

And the risk of commercialization. This is the highest ethical problem that people may start using anything and everything for commercial purposes, and it will create a scenario in which people can be reckless in their lives. People say, "Oh, if my nose is gone, it's okay." I can buy another nose and put it on. It's like you don't care too much about your shoes. You don't care too much about your, you know, your shirt or something because you can buy it, but you care a lot about your nose because a nose is not available.

The shirt is available to purchase. So ethical problems also involve the role of a cell bank. The privacy of a donor may be at risk in some situations. Organ trafficking can become an issue. So the use of human embryonic cells is unethical to use in human embryos. But what about using an aborted fetus? And like I mentioned earlier, people may end up using fetuses for the cells, and people may forcefully abort just to get that tissue.

Let us see what the current status is. The current status of tissue engineering is technological advancement. Where do we stand? That is the 3D bioprinting that enables the creation of complex functional tissues and organs. As of now, what scientists are doing is that instead of using ink in a printer, they can use cells that act like ink or a cartridge in a printer, and they will print, allowing you to scan the internal organ. So what actually happens is if one kidney of the organisms is ready for transplantation, but what if the interior of that person’s belly is not perfect enough for this surgery because some organ may be bulging? Then can you create an organ that allows for the shape and size of that locality in this way? You can create a beautiful organ if you look inside. So what they do is open up the organ or the organism's internal structure, then scan it in 3D and print the organ at that spot.

And printing the organ has another beauty because while you are printing the organ using cells as the cartridge, you can appreciate the internal three-dimensional structure. So the inside part can also be met. You can read about 3D printing, which is popular because people use it to create 3D printed photographs that resemble a person's sculpture. These days, people print houses and buildings using large robots; you can print a house, and it is faster than constructing one. Large house printing means I'm not talking about toy houses; you can happily print them.

The 3D structure has been developed, and that is the beauty of 3D printing, which is one of the emerging fields of science. And it enables the creation of complex and functional tissues and organs. Like in the previous class, we discussed that newly formed tissue must have proper blood vasculature. Say blood vasculature has a complex tissue. An array of distribution, you know, by scanning—oh, these are all the places in the blood vasculature.

It should start thicker and, towards the end, it should be thinner. These are very fine details, but when you make new tissue, it should already have the blood vessels; later, it may regenerate. By you know, your body may fix it, but to start with, it should have a perfect dimension of blood vessels. This will be ensured because the 3D scanning, just like many of you would have seen in hospitals, how MRI scanning or CT scans are done, gives you a view of the three-dimensional structure. Like that, you can get a three-dimensional structure of the organ so that not just the muscle mass or not just a cell mass, even the angiogenesis or the blood vasculature can be.

Fixed perfectly in the organ because of 3D printing. Then comes the biomaterials: the development of biocompatible and biodegradable scaffolds that support cell growth and tissue regeneration. This is an area that is very explosive in nature; it is growing rapidly. The use of biomaterials has huge potential, and it has progressed to the extent that you can have the biomaterial implanted in the damaged area, and the surrounding cells will automatically migrate onto it. It is just like, you know, you want to explore an unknown city; how helpful Google Maps or any other mapping service is! All you need to know is how to drive your vehicle, but everything else—the three-dimensional or two-dimensional roads of that city—is clearly available in your hand.

Like that, if you can provide a matrix. Which is made of a compatible biomaterial; on that site, the cells will automatically colonize, so it is a great advantage to have. Then comes the stem cell therapies, which have been discussed multiple times. However, stem cell therapies also have some drawbacks that we will address. Utilizing stem cells for tissue repair and regeneration, along with advancements in isolating and differentiating stem cells for specific tissue types, raises the question of what happens with the stem cells. Many times, the stem cells have too much expression of MHC class 1; normal differentiated tissues will express MHC class 2 proteins, while class 1 is very minimal.

Although class 1 and class 2 both are... Not targets of your own immune system normally, but the stem cells, because they have this class one protein present on them, your immune system all of a sudden will get alerted to why this cell has too much class one, and there is a possibility. That they can be attacked by your own immune system and stem cells may not stay, but the qualitative nature of stem cells demands lots of MHC class 1 major histocompatibility complex class 1 and class 2 proteins present, so this can be one of the reasons why stem cells, when you put them, are affected. Although ideally they should differentiate, sometimes if you put in 100 cells, 100 out of 100 may not because they are vulnerable to attack by your own immune cells. So these are all the angles one should explore.

Then comes vascular engineering. They focus on creating functional vascular networks within the engineered tissues. To ensure adequate oxygen and nutrient supply, although it can be easily discussed, in reality, it is not that easy. Why? Because the vascular nature of individual tissues varies drastically. Individual tissues do not have the same vascular nature; for example, the vascular nature in your liver does not mean that you have the same vascular nature in your kidney. Depending upon the organ you are talking about, it will have a different vasculature.

Therefore, understanding the vasculature in detail is needed for proper vascular engineering when you enter the field of tissue engineering. Then comes the organoids that are creating the 3D mini organs, so organs and organoids are related terms. Organoids are basically derived from tissue-engineered cells, where they are helpful to study a disease or how the therapy is responding, etc. If you have an organ. Alone, taken out from the organism, it may or may not be very suitable for your research study, but if you have made an organoid, what happens is its behavior at the cellular level will be the same as that of an organ, and this organoid, being produced in an external environment, will be more resilient than the other tissue types or other organs you take from the organism.

From this organism itself, that is why the organoid concept is also very, very new, and it has many advantages in understanding disease progression. Like I told you, tissue culture, in general, is one of the most basic aspects of organ or tissue engineering. Tissue culture comes in every phase. What differs from an ordinary tissue culture is how you are allowing these cells to grow in an organ. Or getting the shape into an organ is what is more important, so understanding tissue engineering is also a detailed way of understanding the tissue culture itself.

With this, we will stop today's lecture on tissue engineering. But I would request you to read the related literature in detail as well, and we will see more about tissue regeneration in the next class. Thank you.