Regeneration Biology
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W11L55_Influence of niches and scaffolds on stem cells: An organ culture perspective

Hello, everyone. Welcome back to another class on regenerative biology. In today's class, we will learn about the influence of niches and scaffolds on stem cells from an organ culture perspective. So in today's class, what we would focus on is mainly two sections: how and why a niche and scaffold have to play a role or have any influence on the cells. And what are the ways in which the cells interact with the scaffold and which can be analyzed and assayed when you are doing an organ culture? So let us see the influence of niches and scaffolds on stem cells at first. So, stem cell niches and scaffolds regulate stem cell behavior, influencing their self-renewal, differentiation, and migration.

The scaffolds and stem cells have a strong interconnection, and the niches, as you know, are the immediate neighborhood environment of a given cell called a niche. Niches are specialized microenvironments that provide physical and biochemical signals to guide stem cell fate decisions. So stem cell fate basically means that if a cell divides into two, one of them will differentiate, and one of the two newly formed cells will either contribute to a proliferating group of stem cells or it will go back to the kitty of the existing cell. So, depending on the demand, the stem cells or the number of stem cells present in a group determine how many of them have to proliferate; not that if there is a group of 100 cells, all 100 have to divide—maybe only five will divide.

Everything depends on the niche. At the same time, scaffolds act as artificial extracellular matrices that can mimic the natural niche environment that supports stem cell growth and differentiation, so matrix and scaffold are one and the same. The word scaffold is used if it is artificially made, and the word matrix is used if it is naturally present. So let us look into the stem cell niches. By definition, niches are specialized microenvironments within the tissues that contain stem cells and the cells that support them.

Sometimes in this niche, it may not be just a matrix. Sometimes the niche will contain other cells as well, which are just like a feeder layer in a stem cell culture. providing these cells that support in the niche. They provide signals that regulate stem cell activity. And if you look into the components, the niches consist of various components that include niche cells, cells that directly interact with the stem cells, providing signals for their support.

Support can be of two types: maintenance support and proliferation support. Then comes the ECM, extracellular matrix. It provides structural support and can influence stem cell

adhesion and migration. So stem cells adhere when needed and migrate when a cell has to expand into a new zone. Secreted factors are also present in the niche, which are molecules that influence stem cell behavior, such as growth factors and cytokines.

These are all the broad groups of secreted factors that influence the stem cells through the niche. The physical factors include tissue stiffness and shear stress, which can affect stem cell behavior; sometimes, a bending force, a pushing force, or a change in the cytoskeletal protein dimension can influence stem cell behavior, determining whether they continue to remain in a quiescent state or divide. Everything is also decided by these physical factors. So now let us see stem cells at home, which is the stem cell niche. Let us watch some cartoons.

So niche we have seen is a microenvironment where the stem cells provide support and interact with the to-and-fro interaction. So you can see the niche and the stem cells, which are given a blue color, and they have mutual communication; stem cells influence the niche, and the niche will influence the stem cells; that is this bidirectional arrow. The direct contact is one influence that stem cells receive; it is physically in contact with the niche, which can be either niche cells or the matrix protein. The interaction with the niche is through soluble factors, which can be growth factors or cytokines. As you can see, this mainly involves growth factors and cytokines as soluble factors.

And then there can also be some intermediate cells. Sometimes you know when there is damage, and the stem cells have to come into the picture to contribute to fixing the damaged tissue through regeneration. Sometimes, because of this damage, you will have some intermediate cells that can come into that site, and these intermediate cells help in multiple ways. One thing is that the communication between the stem cell and this niche will become more effective and more productive. Sometimes, these intermediate cells will be able to release some of the chemokines, cytokines, etc.

, that will stimulate both the niche cells and the stem cells. So sometimes it's just like, you know, friend A and friend B have a rift. Then friend C comes and patches up the rift, unhappiness, or argument between A and B. So the presence of C can facilitate this whole process. So this is like a catalyst in a chemical reaction.

So this intermediate cell sometimes becomes very influential. They are not part of the niche. That's what we should understand. Many a time, they will come from elsewhere and participate in this interaction of the stem cell with the niche. So now let us see the regulation of stem cell fate.

The niches provide cues that influence stem cell self-renewal, differentiation, and migration. Self-renewal is making a copy of itself. Differentiation is a demand-based concept. Whether there is a need for differentiation, it will do that.

Then migration. If it differentiates and sits there itself, the damaged area will not get fixed.

So the cells have to be produced from their source. That is where stem cells are located, and they should migrate to the damaged area so that it can be repaired. And self-renewal basically means the initials can provide signals that can promote stem cell renewal, allowing them to maintain their identity and generate more stem cells. They have nothing to do with the regeneration, nothing to do with the damage fixing, nothing to do with the differentiation.

They are simply making copies of themselves. But remember, stem cell populations can be kept in a fixed scenario or fixed state provided you have a healthy population of or a healthy number of stem cells, which can be possible only because of self-renewal. Then comes the differentiation. Niches can also direct stem cells to differentiate into specific cell types, such as muscle cells and bone cells. So this begs the question.

Isn't it true that stem cells are always interacting with their niche cells? And then why is stem cell not differentiating every time? So the answer is that the niche will not send the same set of chemical cues at any given time. Depending on whether the need is to self-renew, such chemical cues will be produced. If the need is to differentiate, such a chemical cue will be produced by the niche. So, the chemical cues will vary.

And then, the migration. The niches can guide stem cell migration, ensuring that they are positioned correctly within the tissue. Say a cell is formed, and it is not in the correct place where it is supposed to be. So these cells have to migrate properly, and then they will give rise to a well-set morphology in the damaged organ. So now, what is the impact on tissue homeostasis? How will the niches contribute to or influence homeostasis? Homeostasis means managing day-to-day affairs, like when a person gets up in the morning, brushes their teeth, has breakfast, and then takes lunch later and dinner before finishing the day. This is a day-to-day event, but.

.. One day they may go watch a movie. That is a change. One day they may go to attend a conference. That day may change.

That is not homeostasis. That is different. One day, they decided to take a long trip. But if they are traveling 50 km daily, that is homeostasis for them. But if they decide not to travel that day, that is a difference. So this is what you should understand: what homeostasis means.

Niches play a crucial role in maintaining tissue homeostasis by regulating stem cell activity. And then comes the stem cell activity, which mainly contributes to regeneration and also addresses aging and disease. The niches can be essential for tissue regeneration, providing stem cells for repairing damaged tissue. This is the main purpose of stem cells and their dynamic interaction with the niche, which is to regenerate, fixing a damaged area or damaged cell type.

Aging and disease. Alterations in niche function can contribute to aging and disease development. Many times, what happens, as we have discussed, is that older people face wrinkles. Why do they have wrinkles there? Because there is too much wear and tear in certain parts of that tissue which is getting fixed, but there are not adequate stem cells available, or even if the adequate stem cells are there, they are not powerful enough or the quantity is not sufficient to fix the damaged area; hence, it is not a wound. Bleeding or an injury site, but the filling doesn't happen; the cellular filling doesn't occur as efficiently as possible, and on top of that, wherever there are wrinkles, the wear and tear continue. If wear and tear is stopped completely, maybe at some time it will get fixed.

A simple example of wrinkles is... It's not that the stem cells are less present there. Stem cells are still present. But because of old age, you are not able to supply enough stem cells to fix the damaged area. So this is the simple logic. So aging is sometimes like a disease.

Some diseases also develop because the tissue is not being fixed properly. The way it should have been at a young age. So, age-related diseases affect various organs. Again, credit goes to the inadequate interaction of stem cells with their niche.

Not every disease is caused by that. But if you have stem cells, as we have seen, sometimes the pregnant mother receiving stem cells from the fetus into the bloodstream can have her damaged organs fixed without any medical intervention. We saw it when we studied ethics. So, it is because you know there is a surplus of stem cells available and that the damaged organ was easily able to be fixed. So that logic should be connected here with the abundance of stem cells, especially when you see it in aging and disease conditions. So scaffolds are defined as artificial extracellular matrices.

Extracellular matrix means a natural substance that can be obtained artificially. We have seen some examples in the previous classes; many chemical names were listed there. Those who have doubts can go back and refer to that. Artificial extracellular matrices provide a three-dimensional structure for stem cells to grow and differentiate. So this is a simple definition of scaffold, and the materials can vary from a lot, starting from natural scaffolds to materials that occur naturally, but that are not ECMs.

For example, cellulose is not an ECM protein, but you can use it as a matrix or scaffold. Protein scaffolds can be made from various materials, including natural polymers such as collagen, which is an ECM protein, and synthetic polymers like polylactic co-glycolic acid. Several examples we have discussed and the functions the scaffolds can perform for a stem cell include supporting stem cell adhesion and proliferation, allowing the cells to attach and enabling them to proliferate. We have seen that sometimes synthetic polymers need to be modified with some amino acid derivatives so that the cells can find it comfortable to bind to them.

We have already discussed that. So, the structure and composition of the scaffold can

influence stem cell adhesion and growth. They can. Their main purpose is to allow adhesion and proliferation. They can mimic the niche microenvironment. So scaffolds can act as if the cells are at home.

The cell does not feel like I am in a strange place. It is making you or the cell feel homely. So they mimic the niche; microenvironment scaffolds can be designed to mimic the physical and chemical cues present in a stem cell niche, influencing the stem cells' behavior. That means it should be able to make a copy of themselves through autologous renewal.

It should be able to differentiate on demand. They can promote differentiation. Scaffolds can be engineered to promote stem cell differentiation into specific cell types. If scaffolds are non-permissive, then whatever cues you provide, they will not be differentiated. So you don't want such things to exist. And what are the applications? Those scaffolds are used in tissue engineering to create new tissues and organs for transplantation.

Simply put, it's very simple: you want new tissues and organs for transplantation, and this can be of two types. One is wound healing; simple wound healing scaffolds can promote tissue regeneration and wound healing, like if you have a severe wound. The dermis got damaged by a burn wound, so you can put the scaffolds on the skin so that the newly formed stem cells can migrate with the help of the scaffold, and the skin healing will become faster, preventing excessive scarring; otherwise, deep wounds will lead to excessive fibrosis and scar formation. And then the disease treatment comes. Scaffolds can be used to deliver stem cells for the treatment of several diseases, such as myocardial infarction.

If an infarct is nothing but a fibrotic area, what is present? So if you can remove them with some enzymatic treatment and allow some scaffold to be placed in that area where the fibroid or the collagen fibers are removed, and if the newly formed cells can migrate there, then you have fixed the damaged area. Like that heart I gave as an example, it can be the liver, kidney, or many other tissues that can have a similar kind of example. So now let us see how stem cells are used in tissue engineering. I guess this slide we discussed earlier as well, but I'm bringing it up once again simply to refresh your memory.

Stem cells need to be isolated. They need to be characterized. They need to be expanded. They need to be differentiated. And for all these steps, except for isolation and characterization, expansion and differentiation, you need a scaffold. So, isolation ensures that we can isolate individual stem cell populations, ensure that the cells retain their functionality and potential to differentiate, characterize, and track the stem cells from the different populations, and ensure that the cells are healthy and functioning.

Powerful enough to be transplanted for expansion onto any scaffold and to culture the stem cell line in a stable multi- or pluripotent state, free from any mutations and of sufficient quantity and quality, and to enable economical expansion to make cell therapy a reality. If the expansion involves one crore rupees, then people can't afford it. So it should be the

choice of the scaffold, and the strategy should be friendly enough for people to accept or adopt that technique. And then differentiate, control, and activate the stem cell differentiation to the desired lineage. Functionally active differentiated cells can be used in the recipient.

So what are the challenges in stem cell research? It is uncertain whether the human embryonic stem cells in vitro can give rise to all the different types of cells in the adult body. Because sometimes, although on paper it can say yes, in reality it may not reflect a given tissue that you made, which has three types of cells: A, B, and C. Stem cells can give rise to all A, B, and C. Say if A and C are 25 percent and B is 50 percent, then what if, because of your differentiation, B is now only 10 and A and C are more? Then it is not the real tissue.

It has everything, but it's not real tissue. So this logic is to be taken seriously. It is unknown if stem cells cultured in vitro, apart from the embryo itself, will function as the cells do when they are part of the developing embryo. You made it. You made kidney tissue, heart tissue, or liver tissue. But will they do as they have been doing? In an embryo or developing embryo, stem cell development or proliferation must be controlled once placed in patients.

You don't want the stem cells to continue proliferating and become a tumor or an enlarged organ. The possibility of rejection of stem cell transplants as foreign tissues is very high; although they are stem cells, your immune system may suddenly detect a large number of tissues and may start attacking them, which can give rise to autoimmune cells, and contamination by viruses, bacteria, fungi, and mycoplasma is also possible. So, to summarize, in short, both niches and scaffolds are essential for regulating stem cell behavior, influencing their self-renewal, differentiation, and migration. They are essential. Understanding these interactions between the stem cells and their niches is very important for developing effective stem cell therapies and regenerative medicine strategies.

Now let us see what the ways are in which this cell scaffold interacts in reality; in practice, how do they interact? Let us see what the types of interaction are. Scaffold influences cell viability, growth, function, and... The types of cellular interactions under the influence of the scaffold are three types: addition, migration, and aggregation.

So these are all three outcomes of cell scaffold interaction. First, in addition, most tissue-derived cells require attachment to a solid surface for viability and growth. We know cancer cells can climb on each other, whereas normal cells need a substratum such as ECM or a scaffold. Cell addition to a surface is critical because it is followed by other important phenomena, such as cell spreading, migration, and differentiated cell function. You also know that when you find a place to stand, you will think about sitting.

If your leg is hurting, you will not think clearly without standing. You will not think of sitting down. You need a place to stand; then you sit. After sitting, you must think about stretching your leg. The same logic applies to the cells. It needs a scaffold; then it will think

of spreading, proliferating, and spreading.

And the phenomena include cell attachment, cell spreading, and focal adhesion. So they are all three main ways in which adhesion can take place. The techniques used to determine cell adhesion are sedimentation detachment assay, centrifugation assay, and fluid flow chambers. So cell attachment, the cells attached to the surface of the scaffold and formed a monolayer on the scaffold.

This is what happens. And then the cell spreading comes. Surface-attached cells divide and proliferate to cover the surface of the scaffold. The cells also penetrate the interconnected pores of the scaffold. That's why you have pores in the scaffold. So that it can form intact tissue.

Remember, later the scaffold will get absorbed. It will be replaced by the ECM. And then the focal adhesion comes. Focal adhesions are large dynamic protein complexes through which the cytoskeleton, which is the protein present in the cell, outside the cytoplasm, including integrin, actin, and myosin, acts because the cell has a skeleton, and at certain points, the proteins will come out of the cell membrane and interact with the outside. See, just like the pillars of a building rest on the walls, the pillars do not end there.

They are going into the basement and underground. Same logic. So if the pillars are only touching the ground, the building will fall down if a strong wind comes. Integrin, actin, and myosin are types of proteins, and the cytoskeleton of a cell connects to the extracellular matrix; in this case, it is called a scaffold. This is a picture of different types of cells, their different dimensions, and how they attach to the scaffold, migrate, and proliferate. You can see some green fluorescent protein expression, etc. So the techniques to determine cell adhesion, sedimentation, and detachment assays.

So what they do here is the sedimentation of cells onto a surface. You take a group of cells in a liquid medium, allow them to settle down, and incubate the sedimented cells in the culture medium for some period of time to give them time to attach. Detachment of loosely adherent cells occurs through the removal of the culture medium by repeated washing. Just decant it. Whatever is not attached will go off.

Whatever is healthy will attach and stay there. And the extent of addition is determined by the number of cells that remain associated with the surface or the number of cells that were extracted with the washer. Say you have 100 cells you put into a tube. Only 10 cells attached; 90 did not. Then you can tell 10 of the cells are adherent. You can know that this is what you put in here, allowed them to settle down, and then washed.

You see, you put so many cells, and here you can see only six cells attached out of this many cells, so the rest will wash away. And techniques to determine cell adhesion. Another approach is a centrifugation assay. The seeding of the cells onto the scaffold surface is

complete.

That is the incubation of the cells in culture medium for some period of time. And the plate is inverted and subjected to a controlled detachment force through centrifugation. Whatever we did in the previous step has been done. And then you invert it. The extent of cell attachment is then quantified.

So you made it, allowed them to attach, then inverted it and spun a little bit. But the attached cell will not fly away. Loosely bound or unbound will fly away. So, techniques to determine cell adhesion also include fluid flow chambers. So here is a simple technique. So fluid mechanical forces are utilized to produce cell detachment in a well-controlled and quantifiable manner.

So the cell suspension is injected into the chamber, and the cells are permitted to settle down on the surface of the scaffold and adhere, as you did in the previous two techniques. But first technique, you just washed it. The second technique is to invert and spin it. Here you don't do both. What you do is, after incubation, the fluid is forced between the two parallel plates, and non-adherent cells are removed with the flow of the fluid.

That means you are not decanting, you are not spinning, but you are laterally flowing the medium, and the unbound one will wash away; the attached one will stay back while the adherent cells remain on the surface, which can be quantified. So now comes the migration: the migration of individual cells within a tissue is critical for the formation of the architecture of the organ. Now we know we assess, okay, ten percent of the cells are attaching or five percent of the cells are attaching, but are they attaching forever? Are they migrating? We need to have an assay. In tissue engineering, the ability of the cells to move in association with the scaffold surface or through other cells will be an essential part of new tissue formation or regeneration.

If they move alone, they can give rise to proper tissue. So the techniques used to determine cell migration are Agarose gel assay, filter assay, and direct visualization. So, the techniques to determine cell migration, the first one is the Agarose test. A cell suspension is placed in a well of the semisolid agarose, which you cast to run the gel, etc. Many of you would have done it. As you can see here, you put it, and the motile cells will crawl on the solid substrate underneath the agarose.

There is a small gap. You put the cells here. If they are non-migrating, then they will stay here only for some time. If you watch it, maybe it will be after an hour. But if they are migrating, they will go underneath. As you can see, they will move.

Motile cells will grow underneath the agarose wherever there is space. Then comes a filter assay. Cell suspension is placed on a filter with small pores. As you can see here, there are tiny pores.

You put it in here. The motile cells will crawl through the pores of the filter. They can move the material to the other side. They can crawl to the other side where they get detected. Just like you have done this osmosis through a semipermeable membrane, as you can see here. The direct visualization assay allows the paths of movement of many individual cells to be directly observed for cells migrating on the surface within solid gels, where individual images are taken multiple times every 10 minutes; then, you superimpose them, and you will see the moving, non-moving, and dead cells, which, although attached, will stay in place.

So then the aggregation comes. It is important for tissue development. So it's migrating now, attaching, migrating. But do they aggregate? That is the question. So it is an important step in tissue development. It correlates cell-to-cell interaction during cell differentiation. The viability and migration for subsequent tissue formation are very important for the aggregation property.

Aggregate morphology allows reestablishment of cell-cell contact in tissues. Thus, cell function and survival rate are enhanced in aggregate culture. If the cell is moving but it's not forming aggregates, it doesn't help much in forming an organ. The formation of the aggregates involves incubating cells in suspension and adding serum proteins to promote cell aggregation. This is one approach. So the techniques used to determine aggregation are direct visualization, electronic particle counters, and aggregometers.

So, these are all the approaches used in measuring the aggregation properties. So the direct visualization is monitoring the aggregate size to determine the extent of aggregation. So just to monitor them, watch how big it is becoming with an electronic particle counter. So that which is invented by Moscona determines the kinetics of aggregation by measuring the aggregate size distribution over time. So this is the approach done using an electronic particle counter. This procedure utilizes computer image analysis to track the disappearance of a single cell within a given time.

You can use electronic devices to measure that. Aggregometers are simple devices. Small angle light scattering through the rotating sample qubits is used to produce a continuous record of the aggregate growth, just like how you measure bacterial growth through a qubit. So you can, aggregometers can be used whether they are joined together; it will reflect in their absorbent spectrum. So we will study more about regenerative biology in the next class. Thank you.