Regeneration Biology
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W12L57_Adhesion, migration and aggregation of stem cells

Hello everyone. Welcome back another class of regeneration biology. In today's class, we will be learning more in detail about the different interactions the cells have on the scaffold. And to some extent, we have seen it in the previous class, that is addition, migration and aggregation of stem cells. We will revisit very quickly and we'll move on to its implications in organ culture. So, if you look at an overview, the stem cell addition, its migration and aggregation are crucial for the development, function and therapeutic applications.

In yesterday's or previous class, we have seen how the adhesion is measured, how their interaction with the environment is determined and how you evaluate the migration and aggregation and settling down efficiency for creating an environment in which the cells are able to form an organoid. So in today's class, we will learn how this has been put into reality and put into practice. So the addition allows the cells to attach to other cells and also to the extracellular matrix. And this facilitates the interconnections between the cells and also favors the cellular signaling events.

So the migration involves the stem cells moving throughout the body, which occurs in a real time scenario. And it is often guided by various signals to reach specific locations. Mainly, they are called chemokine signaling, where the cells will migrate from location A to location B. And this is very prevalent in locations like bone marrow or injured tissues. The dynamics or the movement of the cells are very high in these tissues.

aggregation on the other hand refers to stem cells assembling into three-dimensional structures which can influence their biological properties and behavior so if a cell is not able to form aggregate then it is less likely a suitable cell type or a isolate for an organoid organoid or organ culture so addition let us quickly visit cell ecm interaction is often referred to as addition stem cells are specialized addition molecules like integrins and selectins which is present on the cells and bind to the components of the ecm that's why the ecm is different in different tissue types Although molecular level they may be more or less the same, but there will be some unique proteins present in individual cell type. Similarly, if you are using an artificial material as a scaffold for organoid culture, we need to ensure the cells which you are planning to culture has got a given set of

interacting proteins like integrins and selectins. So my scaffold should allow interaction with those molecules. And ECM can have specific proteins such as fibronectin, collagen, and laminin. These are some of the ECM proteins.

And cell-cell interaction, that is also part of adhesion. Adhesion molecules also mediate cell-cell interactions, allowing stem cells to interact with other cells and form tissues. So this interaction is a must without which no cell is going to multiply. Then comes the signaling event. Just interaction is not good enough, signaling should kick start.

Addition events can trigger intracellular signaling pathways affecting stem cell survival, proliferation and differentiation itself. so what is the importance of all this in a nutshell you can say proper addition is essential for stem cell survival localization and function in different tissues and different environments so now look into the the next aspect that is the migration so migration has got one unique feature that is called homing homing basically mean the stem cells particularly hematopoietic stem cells actively migrate to specific locations a process known as homing often guided by chemokines and other signaling molecules without which a given stem cell will not be able to distinguish themselves from the rest of the stem cell. They often need to distinguish themselves simply because not that every stem cell is going to give rise to Only one type of cell, say in bone marrow, we know we have myeloid lineage and lymphoid lineage, but they are coming from the same parent cell. So who tells one cell to become myeloid lineage? Who tells the same cell to become a lymphoid lineage? So this kind of variations are possible because of their homing behavior. And mechanotransduction.

Stem cells can sense and respond to physical and mechanical environmental cues influencing the migration. Say a stem cell is taken and you bend it. So it is stretching the cytoskeletal and ECM interaction in a given way that itself can trigger a proliferative response. So the mechanotransduction is very, very important for the behavior of the stem cell. Then comes the chemotaxis.

Chemokines like SDF1 one of the chemokine attract stem cells towards specific locations such as bone marrow or to the injury sites and what is their importance is the migration allow stem cells to reach tissues which needed repair because that is damaged tissue if you have an injury of course there is a damage so you need cells so if no cells are coming there naturally that wound will never get healed and also this is this allows these chemokines are specific for wounded tissues or a cell undergoing apoptosis this will allow the stem cells to reach tissues that need repair regeneration or development so now let us see the aggregation three-dimensional structures are basically resulted from cellular aggregation stem cells can self-assemble into 3d aggregates 3d means three-dimensional aggregates mimicking a tissue architecture and enhancing their biological properties a

tissue can perform its task Not when it is in 2D, two dimensional. Often it need a three dimensional structure for performing the task optimally. So influence on its function. So aggregation can affect stem cell proliferation, differentiation and secretion of growth factors, potentially improving their therapeutic efficiency. So that is why aggregation is so important because it can influence the function of the stem cell itself and the techniques used are different methods can induce a stem cell aggregation including gentle steering or controlled detachment from solid surface etc whenever you give a altered scenario or an altered environment to the stem cell they tend to aggregate So the importance of aggregation is it can improve stem cell delivery, retention and functional activation in vivo, leading to a better therapeutic outcome.

In summary, addition, migration and aggregation are interconnected processes that play crucial roles in stem cell biology and therapeutic applications. So understanding these processes can help us in designing stem cell based strategies or therapeutic strategies and for various diseases and injuries. So that is why understanding these three pathways is a very important addition: migration and aggregation. So now let us see from an organ culture perspective. In an artificial organ culture using stem cells for regeneration, addition, migration, and aggregation are crucial for tissue formation and functionality.

This we have already seen and discussed. Stem cells must adhere to a substrate and migrate to the appropriate location. Why? Because then it can only signal to another cell, plus it can differentiate into a given organ. Trajectory aggregates to form functional tissue structures, and then it should be able to aggregate so that the tissue can have a multicellular structure, which has uniqueness in each and every cell. For example, if a tissue has a thousand cells, not all thousand are equal; there may be 10 different types of cells, with an average of 100 individuals each.

10 into 100,000. In this way, the organ can perform better. So you don't want all cells in an organ to behave or perform the same way. That is not how organs are designed in the body of an organism. Engineering these processes in a controlled manner is key to creating functional artificial organs. So how are these processes controlled? Let us see; this is influenced by different conditions.

One is the properties of biomaterials. What scaffold have you used for making this stem cell culture environment? The type of biomaterial used in the artificial organ culture, including its stiffness, topography, and chemical composition, can influence cell adhesion, migration, and aggregation. So the growth factors and signaling molecules can also have a big say in controlling these processes. The molecules can guide stem cell differentiation and migration within the artificial organ, ensuring the formation of desired tissue structures. So, that is why the growth factors and signaling molecules are

important.

So, three-dimensional culture. Culturing stem cells in a three-dimensional environment such as hydrogel or a spheroid can promote aggregation and mimic the natural tissue organization that is seen in vivo. So you don't want an organ that is unique? I made a unique kidney or a unique liver, which are very different from what is seen in humans. Of course, it's a good idea. However, that liver has to work in conjunction with the rest of the organs present in our body.

So hence it is always good that the liver organoid you make or a kidney organoid you make has to be similar to or as close to the real tissue as possible rather than making something in a shortcut or a unique manner. Here are some examples of its application. One is bond regeneration. Stem cells can regenerate bone tissue by promoting bone cell addition, migration, and differentiation within a bone-like scaffold. So you make a bond-like scaffold.

First, inoculate them with stem cells. Allow them the appropriate guidance cues. Then it will differentiate into bony plates, and you will end up getting bone and cartilage regeneration. Stem cells can repair damaged cartilage by promoting chondrocyte proliferation and differentiation within a three-dimensional cartilage scaffold. You can design based on what should be the ultimate organ or ultimate tissue that you are desiring.

So, organ regeneration stem cell-based approaches are being explored to regenerate various organs, including the liver, heart, and kidneys, by inducing cells. Stem cell differentiation and migration occur within the biocompatible scaffold. So these are actively emerging areas, and some examples will show how they are being implemented in human patients. Let us see the 3D stem cell spheroid and organoid systems for tissue repair and regeneration. This picture is an overview of 3D stem cell culture systems that mimic the in vivo environment and enable basic research about various tissues and organs.

So the therapeutic applications of stem cell-based spheroids and organoids, including engineering techniques and tissue repair and regeneration, have completely changed or revolutionized regenerative medicine as a whole. So the three-dimensional stem cell culture systems have attracted considerable attention as a way to better mimic the complex interactions between individual cells and the extracellular matrix that occur in vivo. That is what I told you already: it should mimic exactly how a natural organ or organism functions, and those interactions should be incorporated. Moreover, the 3D cell culture systems have very unique properties that help guide specific functions, growth,

and processes of stem cells. For example, what is seen during embryogenesis, morphogenesis, and organogenesis? So this has to be simulated.

These embryonic properties, or a little bit later when you have morphogenesis, or much later when you have organogenesis, should be copied into organ culture. As you can see here, you take stem cells, and you can apply different forces to adhere and form organoid structures. One is a hanging drop, and another approach is in a micro well, so you can use different matrices. Different microfluidic approaches sometimes use magnetic force, and you can spin them to churn the cells so that they will form aggregates. Once you have the spheroid that is formed, it can automatically give rise to a stem cell organoid, which is ready for transplanting into the damaged organ or part of a patient.

That is what this cartoon is depicting. So if you look at the historical perspective, it's always important to remember the history from where we have come, and this picture is depicting exactly that. It started in 1907; the year it has started, and we stand now in 2025. So you can see all the steps that have happened, and those who are interested can read the reference I have given here.

A few of them are listed here. 3D cell culture has a history of about 100 years. In 1907, Wilson et al. Described the first attempt at reorganizing an organism by demonstrating the ability of dissociated sponge cells to self-organize into a whole organism. We have also seen this in Hydra.

We call it morpholaxis. Just take 100 cells, put them in a petri dish, and you will end up with a miniature hydra that will grow to adult size. That same year, the hanging drop method was established by Ross and Harrison. While exploring ways to culture and maintain frog embryo nerve fibers in vitro. This is another approach taken by Ross Harrison.

Thomson et al. Later, successfully isolated cultured human embryonic stem cells derived from human blastocysts in 1998. Later, the Yamanaka group established iPSCs by reprogramming mouse and human fibroblasts, significantly influencing stem cell and organoid research from 2006 to 2007. Hiraku and colleagues generated self-organized and polarized 3D cerebral cortical tissues from mouse and human embryonic stem cells. In 2009, Sato et al. It was reported that 3D intestinal organoids were formed by adult mouse intestinal stem cells isolated from primary intestinal tissue.

So much research is going on. A few of them are listed here. A lot of epic research has gone into making regeneration biology the stage it is at now. The future prospect, if you look in the near future, of applying microengineering and nanoengineering-based

platforms such as particle fibers, structural cues, fluidic chips, and 3D bioprinting at the micro and nano scale may facilitate increased production, improved reproducibility, and the development of a highly mature organoid system, which is very likely to happen. Let us quickly visit what 2D cultured stem cells are used for and how they are applied.

So far we discussed 3D. Of course, 3D is good for making organoids, etc. Let us quickly visit how beneficial or how useful the 2D culture is. Transplantation of stem cells can be utilized for the treatment of diseases such as the regeneration of tissues and organs instead of using complex surgical procedures or tissue and organ transplantation. In recent decades, tissue engineered using a variety of stem cells has been applied to epithelial surfaces. Two-dimensional structures such as skin, cornea, mucosal membranes, and heart tissues.

Examples include bone, tooth, enamel, dentin, and cementum, which need only a partial repair. You don't need to have the so-called organoid culture. Stem cell-based therapeutic applications for tissue regeneration involve transplanting either stem cells with controlled functions or engineered scaffolds combined with stem cells. So it's a combination of these two stem cells and the scaffold is specially made for a specific therapeutic application. Various engineering approaches are used to improve adhesion, proliferation, differentiation, migration, and paracrine factor secretion from the stem cells used to regenerate various tissues such as bone, cartilage, tendon, muscle, nerve, tooth, and skin.

Micro and nano-engineered platforms and biomaterials have been utilized to modulate the behaviors of stem cells and can enhance their function by providing them with a specific microenvironment. So the 2D cultured stem cells also have tremendous potential when it comes to regenerative biology. So let us see what the approaches and tissues are that are done using 2D approach examples. Bone tissue regeneration, cartilage tissue regeneration, tendon and muscle tissue regeneration, nerve tissue regeneration, dental tissue regeneration, and skin tissue regeneration. They can all be manipulated more easily by a 2D approach.

So let us see what the future of 2D culture is when you want to use it in a therapeutic scenario. Currently, the most commonly proposed stem cell therapy in clinical trials is performing in vitro expansion of a sufficient number of autologous stem cells; autologous means from your own body, isolated from the patient, and then injecting them into the target site. That is a simple approach. Uh, workflow; however, stem cells transplanted into the target site have critical limitations, such as low efficiency; that is, cells can be easily washed out at the target sites due to various reasons, because they were not able to establish, or they may not be able to adhere to the existing tissue, or the immune system attacked. Even though they are autologous, maybe some uneasiness or strangeness may

be felt.

From these stem cells, the immune cells fail to maintain their viability and function, and because they have to self-renew, sometimes self-renewal may be affected. Multilineage differentiation occurs; you wanted X tissue, but it is not making X tissue; it is making Y tissue because the stem cells have the potential. So, what do you need? You may not get it. It's almost like when you are in a boat, you want to go in the next direction. You are most likely going with the flow of the water.

If you don't have an oar or a stick to regulate the movement, then whatever direction you turn, the boat or float will go in whatever direction it feels like. So this possibility exists. If you put a stem cell in a defective organ, and it is a perfectly functioning organ, you don't have to put it in. The defective organ is already underperforming. So it won't receive the right cue for the stem cells to differentiate.

So this has resulted in limited success in restoring damaged tissues and organs, especially in large-scale tissue repair or regeneration. Therefore, effective delivery systems that regulate stem cell survival, behavior, and function are required to efficiently transplant stem cells into the target sites. Recently, many studies demonstrated that 3D cultured stem cells enhanced viability, differentiation, paracrine secretion, and tissue regeneration compared to 2D cultured stem cells. Additionally, 3D cultured stem cells, spheroids, and organoids promoted the secretion of growth factors and the expression of cardiomyocyte-specific markers, and the spheroids improved. Cell engraftment and survival within the myocardium, along with enhanced neovascularization and myocardial regeneration, result in myocardial infarction recovery compared to single cells; therefore, the 3D cultured stem cells can be good alternatives to stem cell transplantation due to more realistic biochemical and biomechanical microenvironments.

2D cultured stem cells allow the spheroids formed by aggregating stem cells to result from the self-assembly behaviors of single cells in suspension due to embryogenesis, morphogenesis, and organogenesis. As we have already discussed, the formation of spheroids involves complex homogeneous and heterogeneous binding of the cell adhesion molecules. ECM proteins and integrins. Stem cell spheroid assembly occurs in multiple steps. First, the single cells are drawn closer to form loosely adhesive cell spheroids because ECM fibers and complementary binding of the peripheral cell surface to the integrins can encourage the preliminary aggregation that is what we want.

Next, the cadherin on the cell membrane surface induces tight connections between the aggregated cells because of the hemophilic homophilic interactions. Cadherin binding of the peripheral cells. So cadherin can favor aggregation in a nutshell. Finally, early cell

assemblies formed by both pathways generate contractile forces via the rearrangement of actin stress fibers, leading to the compression, as I told you, of mechanotransduction, leading to compression and the formation of mature spheroids. So, after these processes, strong, compact multicellular spheroids are formed.

Depending on their complexity, 3D cell structure systems can be classified into three major organizational and structural types: spheroids, multicellular spheroids, and organoids. Spheroids are generally considered 3D cell aggregates generated from a single or various cell types, but cannot completely mimic the complex contact of other cell types. Stem cell-derived organoids have a similar phenotype to the in vivo situation, which has higher tissue complexity than spheroids. Although the differences between spheroids and organoids are vaguely defined as of now and inconsistent among researchers, stem cell spheroids are usually close to the meaning of simple stem cell aggregates that can be utilized in a series of processes for developing organoids.

from the stem cells. So the internal developmental processes drive organoid formation. Spheroids develop primarily through cell-to-cell adhesion. Stem cell-based organoids can be formed by providing the appropriate physical and biochemical signals for differentiation and development. Although you made the organoid, it also has to differentiate eventually in the host. The organ-like phenotype or the embryoid body forms after the formation of stem cell-based aggregates and spheroids.

So, the engineering techniques for the formation of stem cell spheroids are important. So, the generation of stem cell spheroids is based on the common self-assembly principle. Cell self-organization occurs in the test tube if the cells cannot attach to the substrate surface and thus must interact with one another. Naturally, you will receive the spheroids. The tight connection, cell-cell communication, and cell ECM communication within the cell spheroids contribute to controlling stem cell behavior and functions.

This is the outcome of spheroid formation. such as viability, stimulus responsiveness, protein secretion, etc., leading to considerable differences compared to the monolayer cell culture. Spheroids can perform differently. The high-throughput nature of generating stem cell spheroids with uniform size and composition is an important factor in the fabrication of the whole assembly unit. To maximize the functionality of stem cells and cell-cell ECM connections when culturing 3D spheroids, various platforms based on commonly used spheroid formation methods such as hang drop, well plate, spinner flask, hydrogel matrix, magnet, microfluidic chip, etc.

are utilized. Have been developed. So commonly used for the formation of methods and advanced engineering, formation methods of stem cell spheroids are listed here. You can

see that the different approaches are like a hang drop method, a well plate method, etc. listed here and here. You can see that there is a magnetic particle, magnetic force application, etc.

Done to create the spheroids. So we can understand that spheroids and organoids have been developed for advanced therapeutic applications, functional 3D stem cell culture adopted for tissue regeneration and repair, and state-of-the-art organ culture. Advanced engineering technologies have been applied day by day to improve the quality of the organoids. So we will learn more about the organoids and the spheroids in the next class. Thank you.