Regeneration Biology
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W5L21_Organ regeneration: Basics with examples

Hello, everyone. Welcome back to another session of regenerative biology. And in today's class, what we will study is organ regeneration, which has its basics explored along with some examples. What we should learn in this class is how and why some organisms are able to regenerate properly, why some tissues are able to regenerate better than others in the same species, and whether there are hindrances occurring to regeneration in some species or if bypassing those hindrances can remove some stumbling blocks. favoring a proper regenerative response. So that is what we are trying to learn.

So, organ repair and regeneration is an overview. So some questions can be asked easily. Will we ever be able to regenerate human organs and parts the way that a newt or an axolotl can? Some very naive and interesting questions. Are stem cells able to reconstruct a whole damaged tissue or organ? While the answer at this point is premature because we haven't reached the stage from which we can make an organ from any species.

The news, as we know, makes the future seem quite uncertain. Promising, good, when all possible strategies are put to use. So, that is what we can anticipate. What is, for example, the relationship between stem cells as we know them in mammals and the de-differentiating nude cells? We know that mammals have stem cells. We cultivate them.

But the differentiated cells from newt, zebrafish, or axolotl, are they similar in terms of their characteristics that create the source of regenerating tissue? Because in newts, axolotls, or zebrafish, the existing tissue has to generate the so-called stem cells. Are there similarities? If so, can we learn from them how and why they were able to create or restore a damaged part of their body? Indeed, newt cells do express factors that characterize them or give them the status of stem cells. Can we learn how the cells create these progenitor cells and guide them via scaffolds? Scaffold is a technical term used to describe the extracellular matrix. Or, in other words, you are making a multi-storied building. The first thing you make is pillars and beams, and then you fill the gap with bricks.

That is how you make rooms and houses. The same logic applies. So the pillars and

beams are nothing but scaffolding for a building. And we have to understand that the scaffolds are essential, and these tissues only secrete the scaffold. Also, we can build tissues, and organ scaffolds are essential.

Can we artificially make some scaffolds? Can we use one of the best scaffolds possible so that the stem cells will be forced to make a damaged organ? These are all some questions that remain. There are three concepts. What we know is classical animal regeneration, stem cell-mediated regeneration, and tissue engineering. So classical animal regeneration means a damaged organ is regenerating by itself, not without too much trouble or something, and then stem cell-based can we use the stem cell and inoculate it into a damaged area; can they give rise to, can they restore the damaged portion? That is another question. And third is tissue engineering, where you are saying, "Okay, I don't need any support from the organism; I have stem cells, I have a scaffold, I will make this organ on a petri dish, and I will just transplant it into the host.

" So these are all some dimensions which you should think of. So, these three concepts can provide the necessary raw materials to materialize the final goals of regenerative biology and medicine. So when regenerative biology is used for a therapeutic purpose, we call it regenerative medicine. That is the word we use. So if you look into mammalian fetal organ regeneration, every animal shows that we know mammals don't regenerate every organ.

But whether it is a mouse, human, rat, or any mammal you can think of, in the fetal stage, they have tremendous capacity for regeneration. They do this without the formation of any scar. That is called scarless regeneration. Something that you can see in the model organism, axolotl, or the newt larvae, etc. They do not leave any scars.

Scar means we have seen it before. It is the over-deposition of fibroblasts and the ECM. That is called fibrosis. Fibrosis is what leads to scarring. So if you have a surgical wound in your body, it can still be seen even after 10 or 20 years, which means that there is a scar.

Scar lasts a lifetime. And it is nothing but the fibroblasts and the ECM deposition that create the scar. So in the embryonic stage, that is the fetal stage, mammalian fetuses can regenerate skin and other tissues during early development. But this ability is lost as they mature. Once they mature, they lose this ability.

You have several examples. A classic example is skin regeneration. In the early fetal stage, fetal skin can heal rapidly and without scarring after injury. That is important. This process restores the skin's strength, function, and extracellular matrix architecture.

That is why there is no scar formation. If there is damage, you can't find out where the scar is. Even if you notice, small kids, such as one-year-old children, are not in a fetal state. They will celebrate their first year only after birth. If they get a wound or injury on their skin surface, after one or two years it will disappear.

It's a slow process, but it will not stay. But if the same kid gets an injury when they are 10 or 15 years old, that is going to stay for the rest of their life. So, what do you understand? One year old kid versus a 10 year old kid, something has changed. So late in the fetal stage. Fetal skin heals with scar formation in the late fetal stage, similar to adult skin.

So what you should understand is the scar formation, and you should also understand one more thing: even if a scar is formed in this late fetus, over the future several days or months, the intensity of the scar can decrease. It may not be null, but it can come down, and it may reach a stage where it is not very conspicuous. So mammals fail to regenerate organs when wound contraction drives scar formation. Now you are learning a new terminology that is called wound contraction. So, what is wound contraction? You know, you have created, say, a one square centimeter area.

You have made an open wound. So the cells or the organism are trying to close that wound as early as possible. And for this, the migration of cells comes into the picture. And then that locality.

The ECM. And also, the cytoskeletal protein. The myofibroblast comes into the picture. They close in. Because of this close-in. The wound contraction occurs.

Like you may have seen. In a rat. The rat has got two teeth. Top two teeth. Bottom two teeth. Which roughly grows about 3 to 4 inches in a year.

And it can penetrate through its brain. To avoid that. They will keep it. Grinding stuff will keep eroding the teeth, so they will stay at a peculiar length. If you see a rat's teeth, the top two front teeth and the bottom two front teeth will be the longest, despite gnawing; that's why they keep grinding stuff.

Not to eat, but just to sharpen their teeth. Why does this have to be done? Because the bottom of these teeth is not contracting unlike the rest of their teeth. Your teeth also, if you look at them very clearly, the base, the part that is joining the gum, is contracted. That is why your teeth are not growing longer.

Elephant tusks. It's nothing but teeth. It's continuously growing because there is no contraction. The reason I gave this example is that contraction doesn't mean there is no blood supply, no neurons, etc. It is there, but this contraction is good enough to prevent persistent growth. This means the nutrition supply is limited.

So what you understand is that when you have this contraction, the area is now getting into a sealing mode rather than going into a regeneration mode. Because regeneration is always slow. It needs some open wounds. If the wound remains open, and of course you don't want any infection to happen, the regeneration can proceed. But the moment the wound is contracted, it completely stops the propensity for the formation of a neat wound closure or a neat wound.

you know, repairing mechanism that leads to scar formation. So can we think of preventing wound closure? That is what researchers have thought. Mammals in their early fetal stages have been shown to regenerate skin following severe injury. Early stage, no matter which mammal you are talking about. Investigators have been impressed by the fetal wound healing observed in several mammalian species and have occasionally referred to it as flawless.

Flawless means there is no scar formation. It looks perfect. While others have described the process as resembling regeneration, This scarless regeneration, even though it is skin, often mimics that of regeneration or scarless wound healing. So we can technically call it a resembling regeneration scenario.

Skin is a less complex tissue than others. It has stem cells and also serves for routine repair. But if there is too much fibroblast deposit, then it will lead to scar formation. However, at about two-thirds of gestation, depending on which species you are talking about, two-thirds of the time has passed. That is called the late fetal stage. The mammalian fetus experiences a transition from scarless skin wound healing to healing that leads to scar formation.

Scar formation is a result of fibrosis. In sharp contrast to the late mammalian fetus or adult mammals, several amphibians, adult mammals, and late fetuses have wound healing through scar formation. But several amphibians, such as newts and axolotls, can regenerate entire limbs spontaneously throughout their lifetime. Forget about scar. Forget about wound healing.

It generates the entire limb. We will learn about this much later in the upcoming classes. But keep this idea in mind. Investigators have tried for several years to understand how the early mammalian fetus heals without a scar to apply knowledge towards functional

restoration in a diseased organ. Say, there may be many people who are suffering because they don't have a hand, limb, finger, or palm. Can they be restored? That is an important question However, the reason behind the loss of regenerative ability during gestation remains unknown.

After crossing two-thirds of gestation, what stops, blocks, or prevents the ability of that embryo to regenerate properly? And its regeneration is an intrinsic property or intrinsic pathway of mammals that cannot be changed later in life. If it has, it will stay. If it is not there, it is very difficult to rekindle it. So this is a property that some organisms have, whereas others don't.

And even mammals have them. In the early phases of their embryonic development during the fetal stage. Later, it was lost. So this poses a conundrum. That is why researchers are very curious about what is so different in the post-delivery or late fetal stage. What is it that has gone wrong? Scar formation is a key outcome of wound contraction.

Like I told you, wound contraction is an adaptive response of the organism. You don't want any infection. You want to finish the job as quickly as you can. You may have seen on the National Geographic channel when a lion.

.. For some leopards, they hunt immediately to try to store the food in a tree, or they will try to eat as fast as they can because scavengers like hyenas or larger predators can come and snatch away their food. The same logic applies to wound healing; it happens as fast as possible, and infection can be avoided. If you have an open wound, it is a welcome signal for a lot of pathogens to come and infest, which can lead to septicemia, and the organism may die. You may wonder it may not be a serious thing in the modern world where you go to the hospital and get it fixed, but in olden times as a hunter and gatherer, or when you were uncivilized as a species, a wound could give you a death sentence. Organisms with the fastest wound closure and their wound contraction, even though there is scar formation, that is not an issue.

In contracting adult skin wood, many MFBs; what is MFB? Myofibroblasts are held together in assemblies by intercellular junctions. Many myofibroblasts come into the picture, and individual cells are connected through, just like, you know, 10 people standing when they are crossing a river; they are holding hands so that they act like a massive organism; hence, they will not fly away. The organization of such myofibroblast assemblies, particularly the spatial orientation of individual MFB axes, appears to align with the physiological macroscopic wound contraction forces that ultimately reduce the wound size. The take-home message is that when individual cells keep coming and

establish a connection, because of this connection, they become myofibroblasts, which have the contractile property, so they will come much faster. If I want to give an example, say you put 10 nails in a circle and place a rubber band around them; as the rubber band gets tighter and tighter, it will try to bend the nails, and the rubber band is trying to come into the center.

So the nails, the vertical nails, will now slant, and the rubber band is dragging them. Some logic of that nature applies. Myofibroblasts will try to stretch it, although cells are needed. So this stretching will create a lot of fibrosis and scar formation. The spatial distribution of MFB assemblies and their resulting mechanisms will create a stress field.

The stress field generated in the injury site due to the contractile forces applied by the MFB or myofibroblasts depends on the anatomical shape of the injured organ and the local geometry of the wound undergoing healing. If it is a circle, it will come in a contractile manner. If it is an oval-shaped cut or a square-shaped cut, accordingly, the wound closure will also happen. Not that it always has to be circular.

This picture explains everything very well. Wound contraction and associated wound healing responses in three adult injury models occur in the absence of spontaneous healing and in the presence of a dermal regeneration template. That's called a DRT graft. In a simplistic sense regarding DRT graft, I can tell you that contraction is prevented. That means the wound is trying to contract, and you're putting a DRT graft in there.

It is prevented. It is not letting this wound contraction happen. Rather, in other words, you are delaying the healing process. In other words, if you can explain it. So you can see here, the blocking of wound contraction by DRT has been done in different tissues.

It has been done on skin. It has been done in the peripheral nerve. It is also done on the conjunctiva. That is the boundary between your cornea and the sclera.

Cornea and the... What do you call the adjacent white part of your eye that is conjunctiva? It has also been tried in multiple places, so what is the simplest form? It is a simple and direct test of the hypothesis that wound contraction—you are asking the question—is wound contraction. If you prevent it somehow, are we getting better regeneration? That is the question.

So we are thinking of a scenario: can we put a... Dermal regeneration template, which is a structure that does not allow wound contraction, means it will retain the open wound as an open wound. If you have a wound, doctors will put a ligature or a suture, which means they are bringing the two wounds closer together; now you are retaining the wound as

open as possible. That is the scenario. And this dermal regeneration template also has a scaffold, which means it is providing the ECM, extracellular matrix. Wound contraction is associated with scar formation and consists of using an efficient contraction blocker, which is the DRT, and observing if such use of the DRT prevents scar formation.

That is the question. Dermis regeneration template is a highly porous collagen-based scaffold that is called a DRT. Its original material is ECM protein, which is collagen. It effectively blocks contraction in skin wounds and peripheral nerve wounds, and it is also done in the conjunctiva after trauma damage; if any damage happens to your cornea, you can go blind, but if there is damage to the conjunctiva, it can heal. DRT induced downregulation of the inflammatory response. So another important thing you should understand is that the DRT, when present, reduces the inflammatory response because the absence of proper collagen can invite an inflammatory response.

But if you put in a DRT, the inflammatory response is compromised. This is something you should keep in mind because this subject is going to come up after a few slides in this class. And it can reduce the downregulation of the inflammatory response. The inflammatory response is carried out by the immune system. And the inflammation means that if there is a mosquito bite or you have a pimple, the surrounding area will be reddish in color.

That is a simple term that means inflammation. And this DRT reduces it, including cytokines. Required for MFP differentiation, such as TGF-beta-1, could account for this healing benefit. So the presence of low inflammation, the presence of a proper required scaffold, and the absence of wound contraction favor slow healing but scar-free healing. So this is what has been shown in these different tissues, like skin: you can see that in the no DRT natural scenario, there is a scar, whereas with the DRT, the scar is minimal due to less ECM deposition. In the case of no DRT, you can see what happens in the peripheral nerve, whereas with the DRT, you see proper nerve formation here.

And here, there is no DRT in the conjunctiva; you end up getting too much deposition of the matrix or collagen. Whereas with DRT, you see much milder tissue or wound healing in the conjunctiva as well. So, emerging rules for inducing organ regeneration are something very interesting. In regenerative medicine, no widely accepted paradigm is currently available to guide the formulation of new theories on regeneration mechanisms in adults.

No prevalent or accepted universal theories exist. Three structural features of scaffolds that are required for regenerative efficiency. As we saw, DRT is acting as a scaffold and preventing wound closure. So an active scaffold emerges as a temporarily insoluble

collagen surface. That is what usually happens when equipped with sufficient ligands.

So this scaffold should also provide ligands. Ligands mean that they can favor the migration of cells. You don't want some meshwork to be deposited. You want them to be friendly to the cells that are migrating, and they should have sufficient ligands for the integrins of the contractile cells, which are able to migrate over it.

That inhibits wound contraction. So that is what is needed. Scaffold, DRT scaffold should be there, and they should also have ligands that do not allow this contraction to happen while also serving as a topographic template for new trauma synthesis because cells are blind. They don't know where to go, how to go, and which place to settle down. So they depend on this scaffold or this so-called ECM. So once you artificially place it, they are not under tension from randomly coming together or randomly closing the wound, and there is no need for what you call contraction of the wound because of the presence of this artificially placed scaffold, which helps in healing without scar formation.

And decellularized matrices. So many times what people do is take a tissue, remove the cells, kill the cells, and let the protein stay behind. That's called a decellularized matrix. And it will look like cotton candy. All of you would have seen what cotton candy is, which is made of sugar, sucrose.

But it has holes. That is why it looks puffy. So if you decellularize, the liver will look like a big cotton candy, but it will be light in weight.

No cells are there, but only... The ECM proteins are there. Matrices have been used during the past few years to regenerate the whole or part of the urethra, abdominal wall, Achilles tendon, bladder, trachea, and other organs in several animal models and occasionally in humans. People have artificially created urinary bladders using these decellularized methods and transplanted them into humans as well. So they have all been successfully done because the urinary bladder is one of the easier structures for organ repair and regeneration. Several organs have an intrinsic ability to regenerate, a distinctive feature that varies among organisms.

Organ regeneration is a process that is not fully understood at present. However, when its underlying mechanisms are unraveled, it holds tremendous therapeutic potential for humans. That is why people look into all possible options. Can we tweak the regeneration? Can we tweak the wound healing process? Can we think of making an organ, etc.? The repair and regenerative potential of various organs and organ systems have been explored, such as the thymus, adrenal gland, thyroid gland, intestine, lungs,

heart, liver, blood vessels, germ cells, nervous system, eye tissues, and hair cells. Kidney and bladder; bladder means urinary bladder; skin, hair follicles, pancreas, bone, and cartilage.

None of the tissues has been spared. Pretty much every organ has been actively explored, with some good success in many of these systems. So organ repair and regeneration, if you look further, you can see in this picture there are three sections: A, B, and C. In A, you are seeing local cells, either differentiated hepatocytes or tissue-specific stem cells; this is the liver as an example, and they proliferate and repopulate the injured area, providing essential growth factors.

So this is the so-called scaffold. or should provide these required growth factors. This concept can be applied to the de-differentiation of cells on the injured side, as we usually see in amphibians. And in section B, what you are seeing is an artificial scaffold. This is natural. This is an artificial scaffold.

It's seeded with cells to repopulate and deconstruct the lost part of the organ. So you have a scaffold in different spots. Random distant spots, equidistant spots; you are inoculating 111 cells, and these cells will divide and colonize. That is the whole concept. The cells from the bone marrow, this part C, can be brought via blood vessels to the injured area and contribute to the repair or regeneration.

So this is what the whole concept is about. You can learn from the liver, you can use artificial scaffolds, and you can also learn from bone marrow stem cells. So now let us see how inflammation and immunity are coming into the picture for organ regeneration. Like we discussed some time ago, inflammation and immunity can be problematic. So let us see if there is any evidence to support this. Both inflammation and its resolution are necessary for the successful regeneration of amphibian limbs and zebrafish caudal fins.

Inflammation is a must. If inflammation is not present, you are preventing the inflammation. Forget about regeneration. You can't get it. So that means inflammation is a welcome signal. Regeneration requires immigrating macrophages to remodel the extracellular matrix (ECM) and possibly resolve the inflammation.

Inflammation happens, then macrophages come into the picture and they try to deal with it. Macrophages are of different types. M1 and M2 are here. We'll see them in the future classes about those things. Numerous pro-resolution genes are expressed in the early phase of regeneration in the damaged tissue; along with this inflammation, they will also trigger the expression of some of the genes that allow resident cells to differentiate.

That is what we call resolution-specific genes. Resolution of inflammation is required for pattern-related gene expression. So you have to get rid of this inflammation. Inflammation is needed to begin with.

And then you don't want that inflammation to come back. You want the inflammation to go away. And then only you can have the regeneration-specific gene expression. So immediate amputation, you see an immediate early gene response, which is associated with inflammation. Then the inflammation should go away. Then you start getting some proper dedifferentiation-specific gene expression.

And prolonged inflammation prevents patterning and regeneration. So what you understand is that you do not want a prolonged regeneration. Say you went to a restaurant to have a meal at breakfast time, but you are not coming out of the restaurant. You are constantly eating. Is that good? So you go to a restaurant, have one plate of food, finish it, come out, and do what is needed for your job.

So that is why inflammation is a must, but you don't want inflammation to continue. It can affect the patterning and regeneration. Evidence supports the role of regulatory T cells and the suppression of autoimmune reactions in stimulating regeneration in regeneration-incompetent axon larvae. So what people have done is, if you can make an anuran larvae regeneration-incompetent normally, frogs after their metamorphosis have compromised regeneration; they are not able to regenerate. If you see a frog, the larvae can regenerate better, but the adult frog cannot regenerate effectively.

It can do wound healing, but it doesn't regenerate effectively. Compromise its immune system by blocking some cell immune responses, etc.; then you can get a better response. So in this picture, what you can see is that the expression profiles after amputation are the immune-related expression of chemokines, cytokines, and annexins, etc. The major type of leukocytes involved is being depicted here. As you can see in this graph, pro-inflammatory cytokines and then anti-inflammatory cytokines during this time, neutrophils come into the picture, macrophages come into the picture, and during this time, cellular reprogramming happens and growth and organ patterning are happening, so we should understand the immunosuppressive methods in Xenopus larvae favoring regeneration, and this de-differentiation is necessary, which is facilitated by the inflammatory response. We will learn more about regeneration in the next class. Thank you.