PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SlIDE

Lecture Number: 13

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Cellular Repair & Regeneration

In the previous lecture we discussed the role of cell proliferation in cellular repair following injury. This lecture will be divided into two parts: the first part of the lecture will discuss the role of the extracellular matrix (ECM) and its components in cell repair, and the second part will be focused on the regenerative capacity of different cells, and how it can aid in repairing injuries. For referral purposes, this lecture covers pages **63-65** of ***Robbins Basic Pathology***.

Part 1: the ECM and its role in cell repair

To start with, the Extracellular Matrix (ECM) is everything that can be found outside of our cells. It is what provides your shape. Even bone tissue is comprised of calcified extracellular matrix which holds onto your minerals (calcium, phosphate..etc) and constitutes your entire skeleton. So without the ECM, the parenchymal organs of the body would be nothing but a 'blob' of cells.

This ECM holds onto many different elements including:

1. Water, which provides turgor and forms a gel-like matrix which the cells can attach and adhere to.
2. Growth factors, which are concentrated in this tissue and are used in special situations like during wound healing processes.
3. Minerals, giving rigidity to bone

Furthermore, the ECM is the tissue which the epithelial cells are bound to and provides polarity to the epithelial cells. So for example without the basement membrane, epithelial cells wouldn't know what way to point towards. In other words, the ECM 'polarizes' our epithelium, giving them both an apical and a basal pole.

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Another important feature worth noting is the fact that the ECM is important for neutrophils and other leukocytes as it provides a fluid medium in which they can move to the place they have to get to, since leukocytes don't have pseudopodia or cilia for their movement.

Since the ECM contains growth factors, it is also important in proliferation, which in turn is one of the main processes of repair. Without an intact basement membrane or without an intact ECM you can't properly repair a wound.

The extracellular matrix (ECM) is generally divided into two major types:

1. Interstitial Matrix
2. Basement Membranes

The interstitial matrix is presented in the spaces between cells in the connective tissue ,and between epithelium and supportive vascular and smooth muscle structure , it's more of an amorphous gel, containing both fibrillar and non-fibrillar collagen, elastin, proteoglycans and hyaluronans (hyaluronic acid), and throughout this lecture we are going to discuss each one of these components in detail.

The basement membrane on the other hand has type IV collagen (non-fibrillar), and laminin which binds to the cells and the type IV collagen forming a plate (basement membrane) that surrounds blood vessels and smooth muscles. Laminin is what holds your cells together in a very organized manner. There are also adhesive molecules which attach the basement membrane to the cells, and one of these types of adhesive molecules are called **integrins**.

Note: we discussed integrins in the inflammation lectures when we talked about the way in which leukocytes/lymphocytes attach to the endothelium of blood vessels during the process of diapedesis, this attachment is mediated by integrins and other adhesive molecules.

The major extracellular components are as follows:

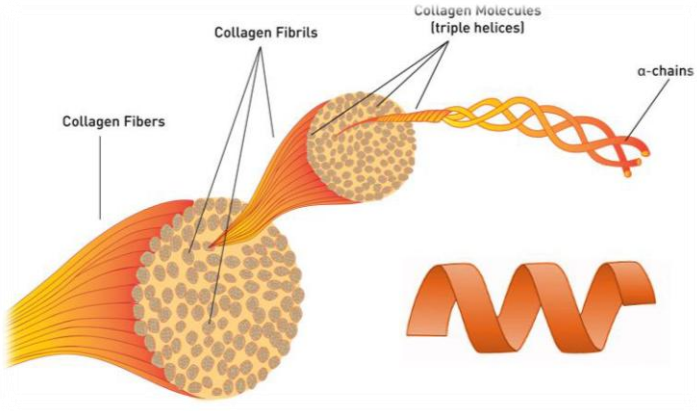
1. Fibrous structural proteins; including collagen (for tensile strength) and elastin (for recoil)
2. Water hydrating gels; which provide turgor for your tissues and lubrication in your joints for example. These include proteoglycans and hyaluronic acid (hyaluronan).

3. Adhesive glycoproteins – a big group however we are going to focus on fibronectin and laminin. These are important in matrix-matrix, and cell-matrix adhesions. They are also bound to cell adhesion molecules like integrins, however integrins are not a component of ECM, they're a component of the cell membrane.

Now that we've discussed the general features of the ECM, we're going to look into each individual component in more details.

1. **Collagen:**

Collagen is made of alpha helical chains. These alpha helical chains are then bound around each other to form what we call a triple helix (جدلة). These triple helices in turn bind to one another through cross linking mechanisms to give collagen fibrils. And finally, the individual collagen fibrils cross link to form a super-structural collagen fibre.



In our bodies, we have more than 30 different types of collagens; fibrillar and non-fibrillar. The fibrillar collagen includes types I, II, III, and V. The important part about collagen is the covalent bonds which are formed between the alpha helices, triple helices and fibrils; these eventually leave us with what we're familiar with: collagen fibres. So without these covalent bonds, the tensile strength of collagen will be abolished.

There are two important enzymes required for the cross-linking mechanism: lysyl oxidase, and lysyl hydroxylase. The doctor mentioned that while the book mentions that lysyl oxidase is Vitamin C dependant, this is actually a faulty and vague statement. The fact of the matter is; lysyl oxidase is **Copper dependent**. Lysyl hyroxylase on the other hand, is the enzyme that is Vitamin C dependent. Lysyl hydroxylase basically hydroxylates Lysine in order to make it a much better substrate for cross-linking than the normal lysine residue.

Note: lysyl oxidase can still cross-link normal lysine residues, however hydroxylysine is a much more suitable substrate for the enzyme.

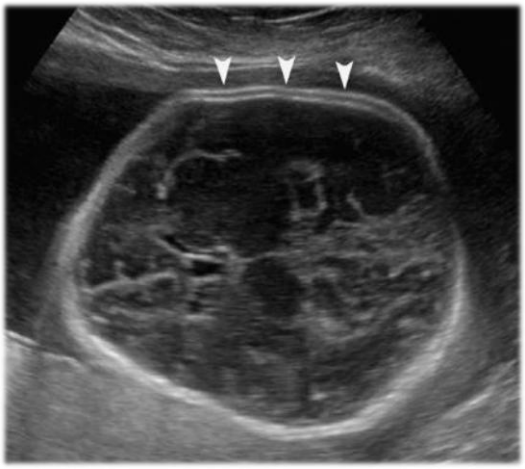
So if someone has Vitamin C deficiency, **lysyl hydroxylase** is the one that cannot function. The common disease for Vitamin C deficiency is **Scurvy**. Historically speaking sailors were known for experiencing Scurvy because they didn't have a very good diet and didn't have adequate sources of Vitamin C (fruits).

Symptoms of scurvy include: bleeding gums, fragile vessels, loss of teeth.

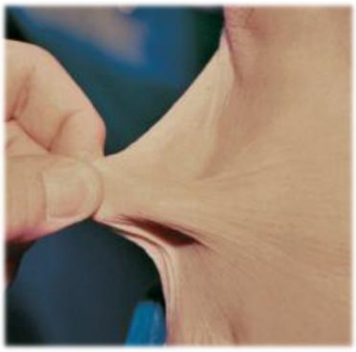
Scurvy patients experience these symptoms because collagen is essentially losing its tensile strength, and so vessels bleed more easily, and generally the tissue isn't as intact as it usually is and so it's easier to lose teeth.

Genetic defects in the various types of collagen, for example the fibrillar types of collagen (1, 2, 3, 5) can lead to a variety of different diseases. Two broadly mentioned diseases are:

1. **Osteogenesis imperfecta:** also called brittle bone disease, which is typically due to a collagen type 1 deficiency it's characterized by bones that are prone to fracture .There are a wide variety of differing brittle bone diseases depending on which type of mutation and how severe the mutation is. It could be anywhere from the only manifestation being Blue Sclera, to a very severe type 2 A in which there is even no ossification in the skull! The image to the right shows the skull of a baby with type 2A osteogenesis imperfecta in which even minimal pressure from a sonogram can cause lethal damage to the child's brain. Since we said ECM holds on to water and minerals, a lack of collagen type I prevents this binding, and so there will be no ossification of the bones.



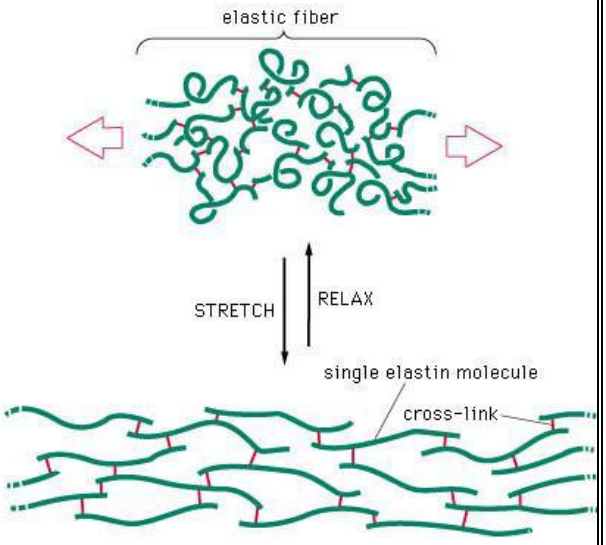
2. **Ehler Danlos syndrome:** there are between 6 and 10 different types depending on the mutation that you get. Symptoms include hyper-extendable skin, lax joints, and vascular defects. This syndrome is caused by improper formation of types 3 and 5 collagen, so in order to make up for this, elastin takes over. Elastin needs collagen in blood vessels in order to prevent the vessels from expanding too much (same case in skin), so without this collagen, things can get very elastic.



*the doctor said that there's no need to memorise anything about the above mentioned disorders, just as long as you understand what happens when we have a deficiency in collagen.

Some types of collagen are specific to specific regions of your body. For example, as we have previously mentioned, the basement membrane is composed of collagen type IV. The collagen in your intervertebral discs is mainly comprised of collagen type IX. The dermal-epidermal junction mainly contains type VII collagen. And so on..

2. **Elastin:**



Elastin is composed of little curly proteins (curls are perhaps due to different hydrophobic attractions), these curly proteins then form cross-links between one another, eventually forming bands of elastin. When stretching of these elastin molecules occur, the curly structure of the proteins distends until they become straight.

The core of elastin molecules are surrounded by a glycoprotein called fibrillin, and without this fibrillin, elastin is fragile. This fibrillin is encoded by the FBN-1 gene. If the FBN-1 gene is defected, then Marfan's syndrome arises. Marfan syndrome patients are generally very tall, their arm span is around the length of their whole body, however what we really care about in this syndrome is the aortic aneurysms. When elastin is defected, the elasticity of the blood vessels is lost, and so they are no longer able to withstand the huge volumes of blood passing through them.

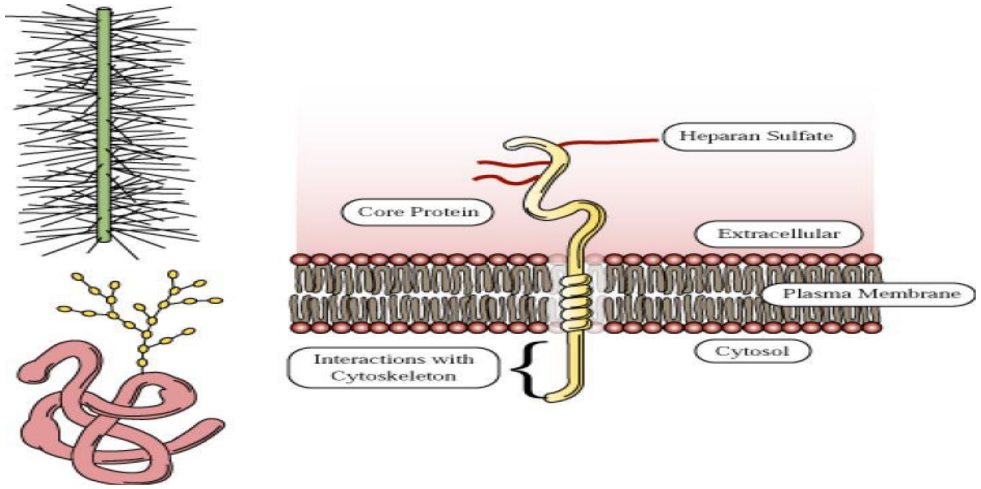
Note: an aneurysm is an abnormal dilation of a specific blood vessel due to certain defects, which will eventually lead to a rupture of the vessel. Aortic aneurysms are the most serious as you lose all your blood within a minute or so.

3. Proteoglycans:

Not to be confused with glycoproteins. Glycoproteins are regular proteins with branched monosaccharide chains. Proteoglycans are composed of long polysaccharides called glycosaminoglycans/mucopolysacchardies linked to a protein core. The difference between the glycoprotein monosaccharide chains and the proteoglycan GAG's is the fact that the monosaccharides in glycoproteins are continuous linear disaccharide repeats.

Proteoglycans provide lubrication because they have the ability to store water. It is also responsible for resilience because it is compressant – that's why it makes a gel-like structure in the matrix. Also because of this gel-like structure, it can bind to growth factors and store them when needed, or provide high concentrations of local growth factors when needed (e.g. in regeneration and wound repair).

Some other proteoglycans are actually inserted into the membrane as integral cell membrane proteins , and because of this, they can transmit signals from the outside of the cell to the inside, or vise versa. That's why proteoglycans are important factors in signaling proliferation, migration, and differentiation.



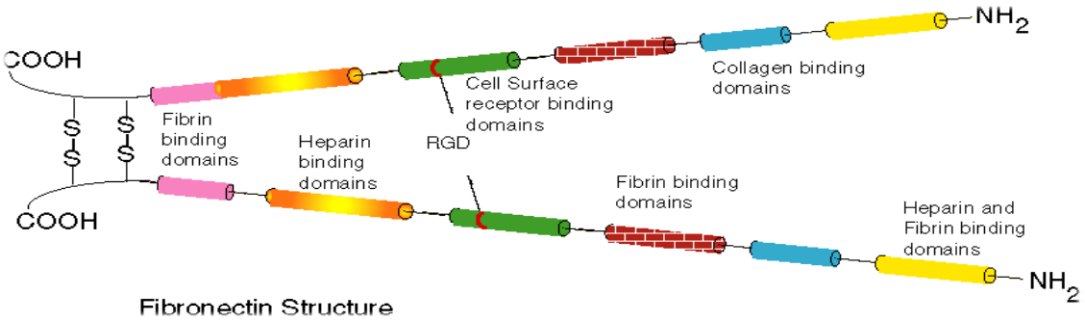
4. Hyaluronic Acid:

It is basically a single mucopolysaccharide (like the ones bound to proteoglycans), however it is very large in comparison to the proteoglycan ones, and it retains water and hence assists in forming a gel-like matrix. So it also functions in lubrication(such as the one in the cartilage of the bone , growth factor sequestration (binding growth factors), and signaling effects mentioned previously in proteoglycans.

5. Adhesive Glycoproteins and Adhesive Receptors:

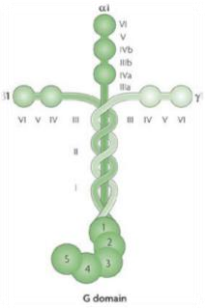
These are what stick the other ECM components together (collagen, elastin, proteoglycans and hyaluronan). Adhesive glycoproteins are part of the ECM, while adhesive receptors are part of the cells. We are going to discuss two main adhesive glycoproteins; one present in the basement membranes, and one in the interstitial matrix.

- **Fibronectin**: binds to fibrin. So when we have a wound, and the blood begins clotting, accumulating fibrin, fibronectin binds to fibrin and provides a source of ECM so we can repair the defect by the prevailing growth factors found inside the ECM. As you can see in the image below, fibronectin is comprised of two proteins bound together by a single disulfide bridge. However these two proteins are not identical to one another, and so fibronectin is commonly called a 'hetero-dimer'; hetero meaning different. It also has collagen binding domains, fibrin binding domains, heparin binding domains and so it binds to the components of the ECM. It also has a cell-surface-receptor binding domain, this has what is called an RGD motif, which is the single letter designations for amino acids (Arginine, Glycine, and Aspartic Acid). And this sequence is what binds to the cell surface receptor. So through fibronectin we have a way of binding the ECM components and the cells together.



Fibronectin Structure

- **Laminin**: it is a hetero-trimer. It is a cross-like protein made of 3 components. It has a collagen binding domain, a proteoglycan binding domain, and an integrin binding domain. They can also bind to each other. This is the main mechanism through which the basement membrane underlying the cells is bound to the cells themselves. Similarly, it can also transmit signals across the cells and through the ECM.



Note: fibronectin is in the interstitial matrix, while laminin is in the basement membrane.

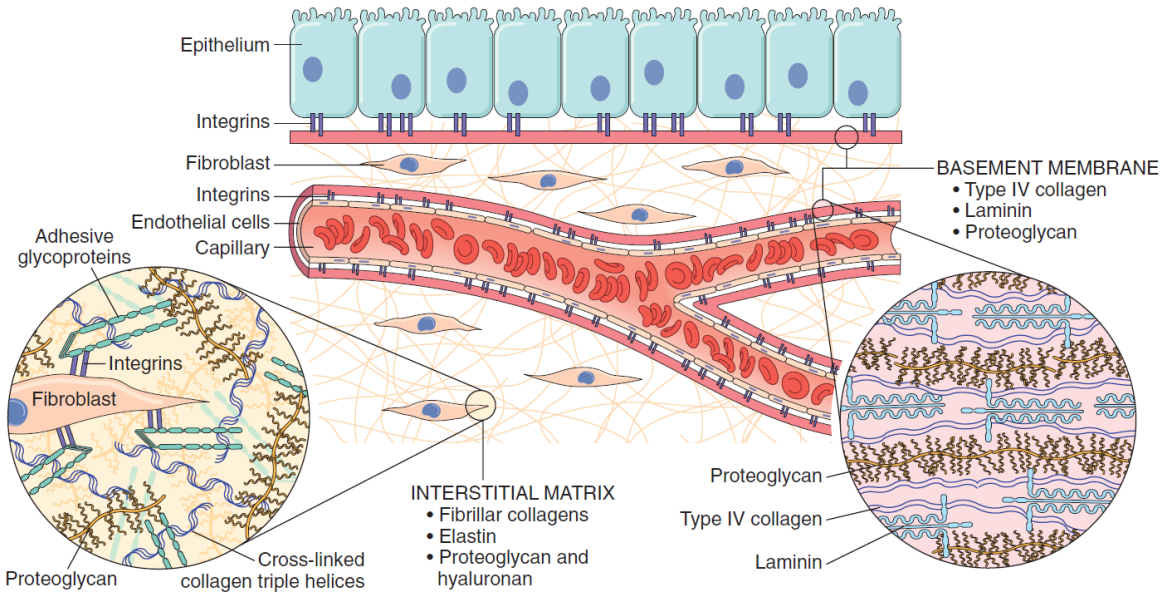
So we mentioned that integrins are one of the cell adhesion molecules, the other three adhesion molecules are:

1. Selectins
2. Immunoglobulins
3. Cadherins

These are what provide very strong adhesion between cells. However what we really care about is the integrins, which attach the cells to the extracellular matrix.

Integrins are the main cellular receptors for extracellular matrix components like fibronectins and laminins .

After looking into each component of the ECM, look at the image below, this should help summarize pretty much everything we have been discussing.



So just by looking at this image you should be able to recall that laminin is in the basement membrane, it has a cross-like structure, it binds to collagen type IV, and you should remember that fibronectin is a hetero-dimer that binds to integrins in the interstitial matrix. Also by looking at the basement membrane you can see that it is attached to the endothelium of the blood vessels by integrins and provides polarity. The ECM provides mechanical support and scaffolding for the suspended fibroblasts and other cells in order to swim in the matrix. One last thing you can notice in this image is

the fact that the basement membrane forms a sort of 'micro environment' between the cells and the ECM. However the basement membrane is NOT only a structural barrier, it can also be a functional one, for example in the glomerular filtration of the kidney in which the basement membrane plays an active role.

Keep in mind that the ECM is responsible for proliferation of cells, motility, and of course repair. So if we have some sort of defect in which motility in the ECM is impaired, then what disease do you think will arise other than inflammation? The answer is Neoplasia. Without migration, cancer cells cannot metastasize (spread to other regions of the body). So in the coming chapter you will see that the ECM plays an active role in carcinogenesis and neoplasia.

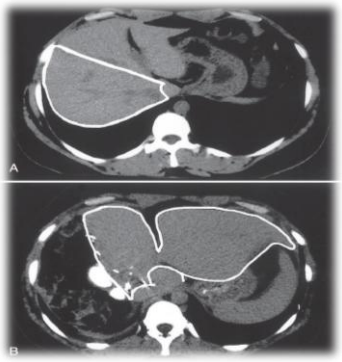
Part 2: Regeneration of Cells

Labile tissues: tissues which have the ability to continuously divide, like the epithelium, due to constant wear-and-tear. An intact basement membrane is essential, however, for this continuous division to proceed, because without the basement membrane, there will be no scaffolding. In other words the epithelium won't know which way to divide towards (remember polarity). That's why in cases of severe injury, regeneration of epithelium doesn't happen and instead the ECM takes control and forms what we call a 'scar'.

Stable tissues: a good example of this type of tissue is the kidney. Stable tissue is tissue that doesn't normally undergo constant division, in contrast to the labile tissue, because there is no constant wear-and-tear in these tissues. There's an example in Robbins that mentions that under certain circumstances, one of your kidneys may undergo hyperplasia if the other kidney has been damaged or removed (although it is not fully understood how this occurs). The one exception to the stable tissues is the Liver, which has unlimited capacity to regenerate. The reason why the liver is a stable tissue and not a labile one is due to the fact that there isn't constant wear-and-tear in the liver, so the parenchyma and hepatocytes are normally quiescent, however when induced, they can actively divide.

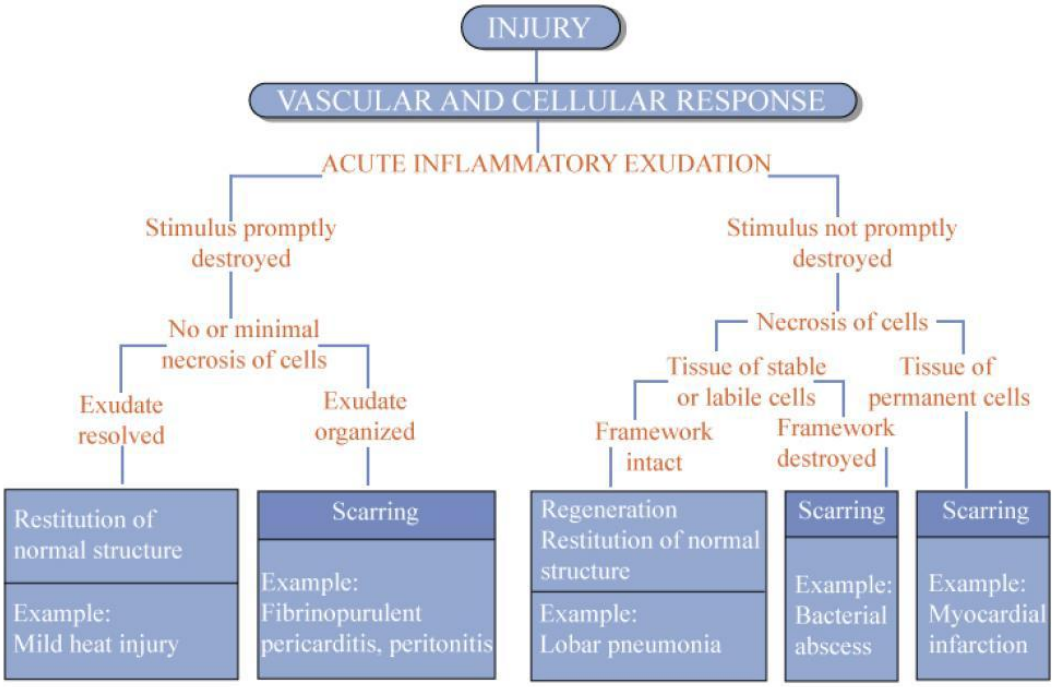
The chemokines and factors used to initiate regeneration are the same as those used during inflammation; e.g. TNF (tumour necrotic factor), IL-6 (interleukin 6), and even some EGF growth factors. These factors induce cell proliferation in the liver and allow its

cells to pass through the cell cycle. However you need to remember that none of this can happen without an intact underlying basement membrane!



As you can see in the image to the right, this part of the liver was resected, the part of the liver that underwent hyperplasia was the opposite lobe. This is because when you resect part of the liver, you remove both the cells AND the ECM, and as we said, without the ECM you can't regenerate cells, so the opposite lobe will in turn proliferate (as it has an intact ECM) and compensates for the loss of tissue.

The following diagram is a very useful one as it differentiates between whether fibrosis or regeneration is going to occur.



If you have an injury, and you end up with acute inflammation, one of two things is going to happen;

(1) You get rid of the injurious insult quickly, the stimulus is destroyed promptly and there is minimal necrosis, in other words there isn't a lot of residual damage and not many damage byproducts are present. If the exudate is resolved, normal tissue structure is restored.
(2) On the other hand, if the stimulus is not actively removed, necrosis will prevail, and 2 things can occur: if we're talking about labile tissues and the framework is

intact, regeneration occurs. But if the framework is destroyed, cell fibrosis will occur (scarring), and this is true for both labile and stable tissue.

(3) If we're talking about permanent cells, then we don't have any other option but for scarring to happen, unless we medically intervene as previously mentioned in the left ventricular assistance device (LVAD), some cells have stem cells which when elicited may proliferate and replace any damaged cells, however this cannot happen in the body on its own.

And that's it for today's lecture :D

Best of luck ☺