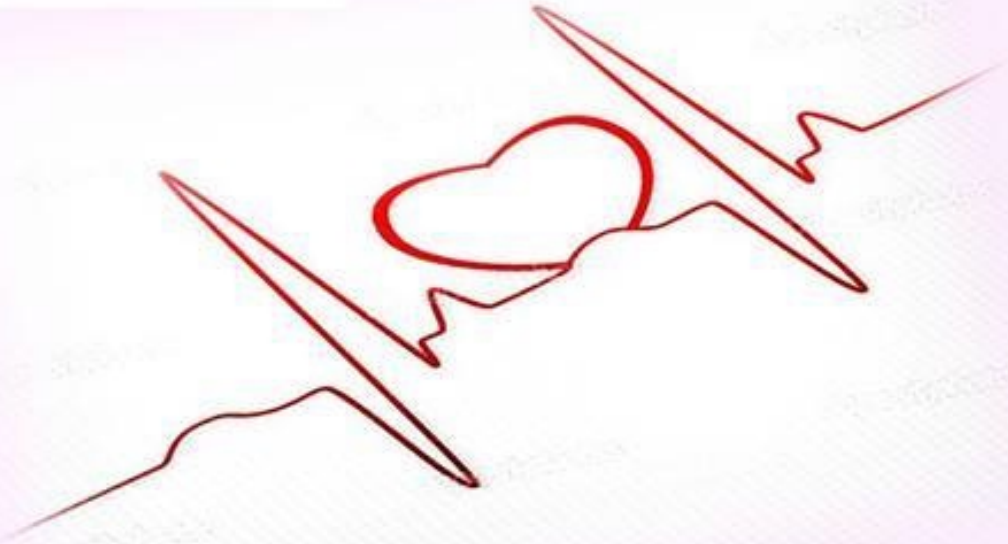
PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SlIDE



Lecture Number: 14

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Scar Formation

Today, we will talk about scar formation and end off our lecture with examples.

We will focus on the last two steps of figure 2-29, "formation of granulation tissue" and "scar formation".

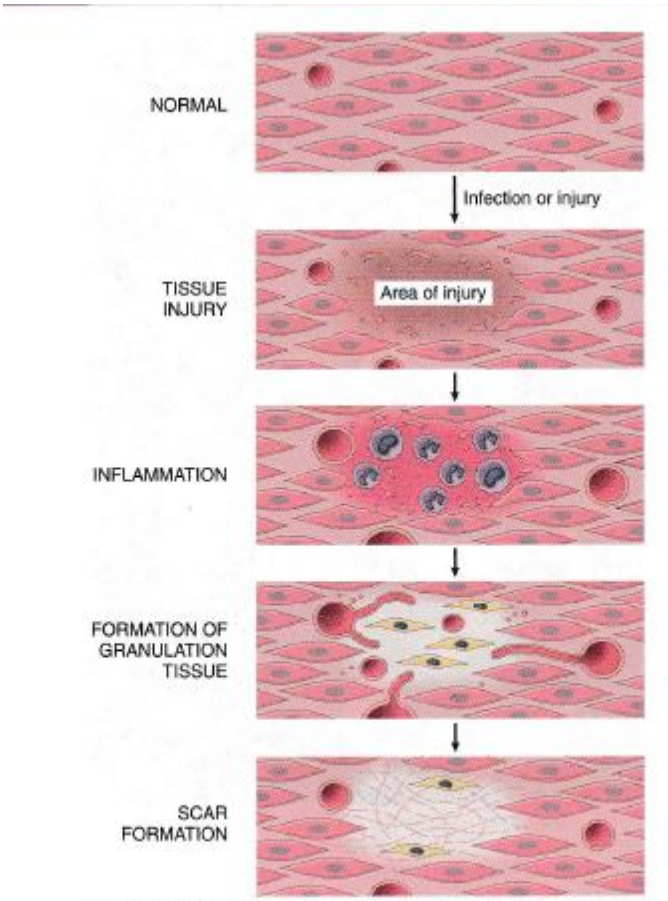

Figure 2–29 Steps in repair by scar formation. Injury to a tissue that has limited regenerative capacity first induces inflammation, which clears dead cells and microbes, if any. This is followed by formation of vascularized granulation tissue and then deposition of ECM to form the scar. ECM, extracellular matrix.

Steps of Scarring:

1) Angiogenesis: the formation of new vessels from preexisting ones.

New blood vessel formation also occurs in the embryo, and is called vasculogenesis.

Bone marrow contains cells that are precursors of hemangioblasts. -
Hemangioblasts, in turn, are precursors for the endothelium involved in new vessel formation in vasculogenesis.

Written by Zahraa Al-Tamimi

Whether hemangioblast precursors are involved in angiogenesis or not is not known. But we know that they are there.

2) Migration and proliferation of fibroblasts and deposition of connective tissue:

This typically occurs within the first 24 hours. In the previous lecture, we mentioned that the extracellular matrix (ECM) is formed by epithelial cells and fibroblasts.

So, how do we have new ECM deposition? During wound repair, new epithelial cells are forming. New fibroblasts that move into the area deposit the ECM as they move along.

3) Maturation and reorganization (remodeling) of fibrous tissue:

New ECM needs to mature. Maturation, in this case, means that the newly deposited collagen needs to be reorganized. When collagen is first deposited, with elastin and all other components of the ECM, it is pointing along different directions / has different orientations. Thus, its tensile strength is low. In order to gain tensile strength, collagen needs to be reorganized/remodeled to point in the correct direction. Remember, in the previous lecture we mentioned that tensile strength is mainly due to collagen.

During wound repair, granulation tissue is formed. Granulation tissue is named so because of how it looks like under the microscope. It's granular (rough) and pinkish. It's pinkish because of the new blood vessels that are forming. The newly formed blood vessels are relatively immature and leaky, which leads also to edema. Some of the growth factors required for repair (like VEGF), make these vessels leaky.

VEGF = leaky vessels = edema.

Let us now look at these steps one by one, and in more detail.

1) Angiogenesis:

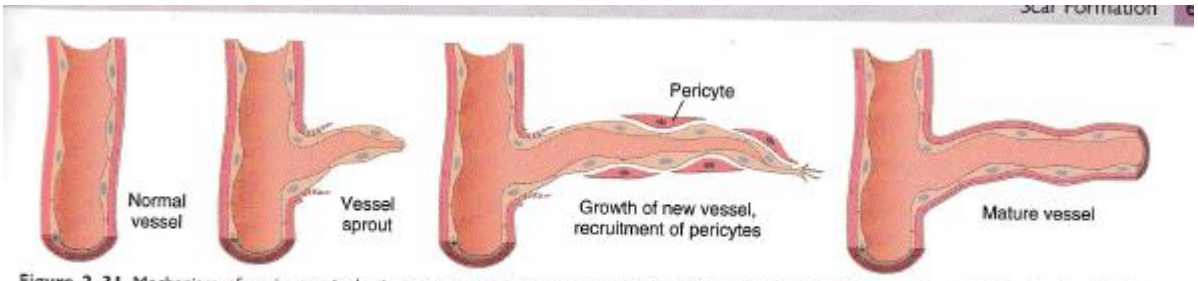

Figure 2–31 Mechanism of angiogenesis. In tissue repair, angiogenesis occurs mainly by growth factor–driven outgrowth of residual endothelium, sprouting of new vessels, and recruitment of pericytes to form new vessels.

Dr. Mazen has simplified in our slides (in graphical form, as he mentions) what the book goes on about for many pages.

a) Vasodilation and increased permeability of existing vessels must occur.

Vasodilation = NO (nitric oxide)

Increased permeability = VEGF (vascular endothelial growth factor)

b) Periendothelial cells (cells that surround and shield blood vessels) separate from the abluminal surface. Abluminal simply means that it's not the luminal surface, but rather the surface on the opposite /other side. These periendothelial cells are different depending on the size of the vessels they surround.

Large vessels = smooth muscle cells

Capillaries = pericytes

c) Endothelial cells start to migrate in a new direction, namely towards the area of tissue injury. Once migration occurs, the cells right behind the migratory front start to proliferate. That way, cells are not taken away from the original blood vessel. Migrating cells are always replaced thanks to this proliferation. Migrating and proliferating cells form new capillary tubes.

d) Recruitment of periendothelial cells to form a mature blood vessel.

Once all this has been accomplished, proliferation and migration need to be stopped. Uncontrolled proliferation is bad, as will be discussed in more detail later.

e) Deposition of the ECM.

The ECM is important throughout this process. In order for the endothelial cells to migrate they have to interact with the matrix. The ECM also contains growth factors that endothelial cells need to proliferate. Angiogenesis is an active process of cell-cell interactions, cell-matrix interactions and deposition of the matrix.

What are the growth factors responsible for angiogenesis?

1) VEGF: it's induced by hypoxia. We got a wound, it needs oxygenation.

Hypoxia > more VEGF

VEGF is widely expressed but it's expressed highest near epithelial cells adjacent to fenestrated (a German word. Literally translates to "windowed") capillaries. Hence, epithelium next to fenestrated capillaries produces VEGF.

The VEGF family of growth factors includes:

- VEGF-A: injury and tumor angiogenesis. *
- VEGF-B: vasculogenesis (in the embryo).
- VEGF-C &VEGF-D: angiogenesis of lymph (lymphogenesis). **
- VEGF-E: a **VEGF-like** substance produced by certain **viruses**.
- VEGF-F: a **VEGF-like** substance expressed in **snake venom**.
- PIGF: placental.

*VEGF-A is the one we usually refer to as only VEGF.

**Lymph vessels are also destroyed in injury, not just blood vessels

How do VEGFs exert their effects?

They bind to receptor tyrosine kinases called Vascular Endothelial Growth Factor Receptors (VEGFR). There are three such receptors: VEGFR-1, -2, -3. We care about VEGFR-2, in this context. It's the one expressed on endothelium.

Once VEGF binds VEGFR-2, two things happen.

1) Migration and proliferation of epithelium.

2) Production of NO.

If you want to stimulate angiogenesis, like in wound repair, VEGF mimics can be used.

Antibodies directed against VEGF may be therapeutic, as in the case of cancer or Age Related Macular Degeneration (AMD or ARMD).

ARMD occurs because of neovascularization on the macula (a spot near the center of the retina). In this case, it's called "wet" macular degeneration, because the new vessels that are formed are leaky, and thus induce edema. Inhibiting VEGF through antibodies may potentially prevent macular degeneration. Anti-VEGF antibodies are also being studied in macular degeneration of the premature, and diabetic macular degeneration. We don't know whether it's effective in those yet.

2) PDGF: Platelet Derived Growth Factor.

As the name suggests, it's derived from activated platelets. Activated platelets are deposited in a wound to coagulate the blood, and thus, prevent bleeding. They also produce PDGF, which leads to the production of VEGF.

3) FGF: Fibroblast Growth Factor is an angiogenic growth factor (a growth factor involved in angiogenesis).

It stimulates the growth (migration, proliferation and recruitment) of fibroblasts. It also stimulates the proliferation of endothelial cells.

The book mentions two FGFs, acidic and basic (FGF-1 and FGF-2, respectively). We care about FGF-2. FGF-2 recruits macrophages to the injured area. Therefore, switch from neutrophils to macrophages is related to FGF.

-**Angiopoietin 1** and **TGF-β** are also angiogenic growth factor (not just FGF and VEGF).

-Angiopoietin 1 and PDGF recruit periendothelial cells. They also (especially PDGF) are responsible for recruitment of fibroblasts.

TGF- β is responsible for stopping angiogenesis. It can also turn on VEGF. So, the same growth factor does different things in a time or concentration responsive manner.

TGF- β and PDGF also recruit fibroblasts.

Some growth factors come from inflammatory cells, especially macrophages. So, you can see that there exists an abundance of positive and negative feedback loops. One turns on another and turns it back off again.

2&3) Migration and proliferation of fibroblasts and deposition of connective tissue +Remodeling of connective tissue:

The ECM, as we know from our last lecture, is not passive in the process of wound healing. It's involved in the following ways:

1) The interactions of integrins: cells are anchored to the ECM, and use it during migration (through the interaction of integrins).

2) Retention and presentation of growth factors: creating microenvironments. Proteoglycan and heparin sulfate can bind certain growth factors, such as FGF.

3) Scaffold for new vessel formation. Endothelial cells need ECM to find their way. They can't migrate in empty space.

4) Remodeling the ECM into a new structure allows the wound to gain strength. But in order to remodel the ECM, we need to destroy. One way to destroy is through Metalloproteinases.

Metalloproteinases are proteases that need metal ions (especially zinc) to function. They allow cells to lose polarity, proliferate and create a new structure. By degrading the ECM, the growth factors are released and can function in migration of endothelial cells, proliferation, and recruitment of fibroblasts and macrophages. Metalloproteinases are how we release the high concentrations of growth factors that are found in the ECM.

The difference between a mature scar and granulation tissue is obvious. A mature scar has more collagen, less blood vessels, and less inflammatory cells.
The text below will make sense when looking at figure 2-30.

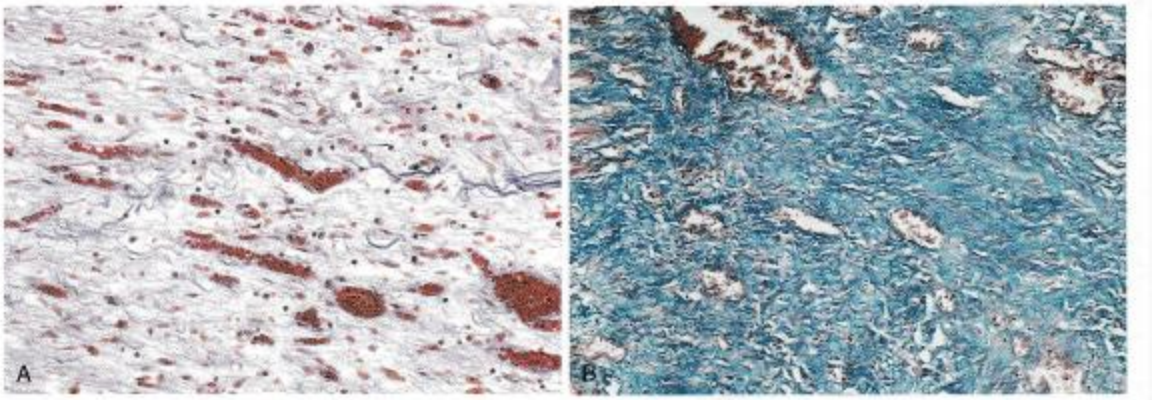

Figure 2–30 A, Granulation tissue showing numerous blood vessels, edema, and a loose ECM containing occasional inflammatory cells. Collagen is stained blue by the trichrome stain; minimal mature collagen can be seen at this point. **B,** Trichrome stain of mature scar, showing dense collagen with only scattered vascular channels. ECM, extracellular matrix.

Compared to the picture on the left (A), the picture on the right (B) has less proliferating fibroblasts. Fibroblasts synthesize a lot of ECM at the beginning and then become inactive. Once they are inactive, we remodel collagen to become dense and start creating the cross-links we talked about last lecture. The vasculature that was required at the beginning (to provide cells and nutrients to the injured area) is no longer required and therefore regresses. Reddishness goes away because there is less vasculature in the scar. That's why granulation tissue is pink and edematous, whereas the scar looks like the original colour of the skin (or even a bit fainter).

There are growth factors involved in ECM deposition and remodeling. We already mentioned several of them.

PDGF and FGF – recruiting fibroblasts.

TGF-β – stops angiogenesis but also recruits fibroblasts, increases production of components of ECM (collagen, fibronectin, proteoglycans)

How does TGF-β do this?

It's part of a family of growth factors that do different things depending on the context. In the context of wound repair, they increase production of ECM

components. Usually, there is a balance between collagen production and degradation.

TGF-β can decrease the degradation of collagen by:

- Reducing proteolytic activity
- Increasing Tissue Inhibitors of Metalloproteinases (TIMPs).

TGF-β is also an anti-inflammatory. It stops inflammation once the injury stimulus is gone. It does this by reducing proliferation of lymphocytes and reducing activity of leukocytes.

What's TGF-β's mechanism of action?

It binds receptor serine-threonine kinase (similar to receptor tyrosine kinase, but instead of adding the phosphate to a tyrosine, it adds it to a serine or threonine). It signals through proteins called SMADS (SMA –in flies, a mutation gene causing small flies. MAD is found in worms).

SMADs act as transcription factors. They turn on or off genes.

Now that we've deposited the ECM, we want to mature it. The way we do that is by degrading components to rearrange them in a new way.

We mentioned metalloproteinases (MMPs), enzymes that depend on metal ions. First they are produced as zymogens (inactive form). They need a protease like plasmin to activate them. They are produced by macrophages, fibroblasts and inflammatory cells. They are under the control of growth factors and cytokines like IL1, IL13. Once activated, they can degrade basically all ECM components.

Matrix Metalloproteinase types:

- Interstitial Collagenases (MMPs 1, 2, 3): can degrade collagen.
- Stromeolysins (MMP 3, 10, 11): can degrade proteoglycans, laminin, fibronectin, and amorphous collagen.
- Gelatenases (MMP 2, 9): can degrade amorphous collagen and fibronectin.

These MMPs don't work unopposed. Most of our mesenchymal cells also produce things called Tissue Inhibitors of Metalloproteinases (TIMPs). They oppose MMPs when we no longer want to degrade the ECM.

This is a note Dr. Mazen made regarding some confusion about a previous lecture:

Ectoderm, mesoderm and endoderm are your germ cell layers. They will create your body. Once you're an adult, your cells will be classified as either epithelial cells or mesenchymal cells. Your epithelial cells arise from all 3 of the germ layers. Mesenchymal cells only arise from the mesoderm. When we talk about neoplasia, you will know that cancers are divided into epithelial cancers and mesenchymal cancers.

What other factors do you think influence tissue repair?

- Type of tissue: stable, labile, or permanent.
- Nutrition: vitamin C for proper collagen cross-linking.
- Infection: inflammatory byproducts will continue injury.
- ECM: is it intact enough to heal wound?
- Mitochondrial damage: no energy for repair, like in radiation damage.
- Mechanical: if you keep moving and reopening wound, it will slow down repair.
- Glucocorticoids: inhibit immune system. It also inhibits TGF-β.
- Old age: less proliferative capacity.
- Body temperature: if you live in a cold region or your wound is at a cold temperature, that will affect blood supply and thus, healing.

Note: Glucocorticoids can be good or bad depending on the situation; if you use them in a case where the injurious stimulus has been removed, then they can facilitate inhibition of inflammation, however in cases where the injurious agent is persistent, then this will only exacerbate the situation.

The difference between location and tissue type is that location means if you get a cut on your shin (anterior part of your lower leg), does it heal quicker than other places? No. Why? It's more prone to movement and has a colder temperature.

In people of African descent, scarring can go abnormal (keloid scar). The scar grows out abnormally instead of just bridging the gap the wound creates. Before it becomes a scar, it's also called "proud flesh", granulation tissue that has gone above the epithelial layers. The only way to allow it to scar normally is to cut off everything above the epithelial level, however this doesn't mean that it can't grow back (sometimes numerous repetitive procedures are required if they keep growing back).

Clinical examples of Repair:

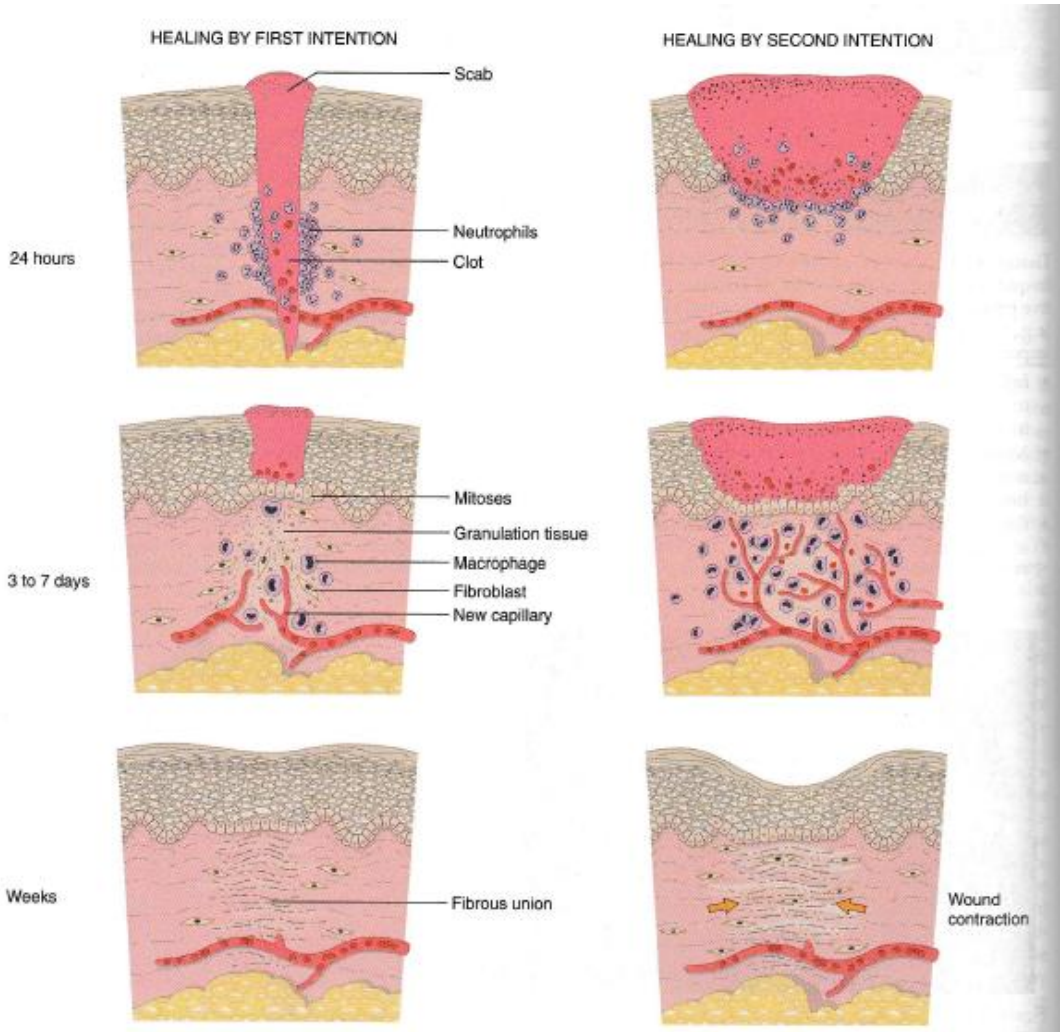

Figure 2–33 Steps in wound healing by first intention (*left*) and second intention (*right*). In the latter case, note the large amount of granulation tissue and wound contraction.

Healing by first intention (primary union) occurs when you have a very nice, clean cut that is sutured. You only have a focal destruction of the epithelial basement membrane and death of only a few epithelial connective tissue cells. Thus, the principle mechanism of repair is regeneration.

Blood vessels are cut and a clot forms. This clot has fibrin, so you can redeposit the ECM.

- Within the first 24 hours you get neutrophils.
- Within the first few days, if the injurious stimulus is not persistent, you will have macrophages replacing the neutrophils. Fibroblasts will be recruited and will proliferate. That gap in epithelium will be bridged by migrating epithelial cells. Those epithelial cells will have lots of mitosis in the basal layer and will replicate all the above layers quick. By day 3, collagen is deposited on the margins of the clot. It's in the vertical orientation, so not very useful. By day 5, once angiogenesis is finished, most of the collagen will be horizontal because we degraded and remodeled the ECM, and tensile strength is regained in the direction that it should be.
- By the second week, blanching occurs with increased collagen deposition and the regression of vascular channels. We no longer need the cells to proliferate, so they don't need as much blood. What we do need is for the fibroblasts that are already there to deposit ECM to recreate the layers.

Secondary union occurs with much bigger scars. There is a bigger clot, lots more fibrin and fibronectin, and more ECM deposition. Because it's such a big gap, you start getting differentiation into myofibroblasts instead of fibroblasts. Myofibroblasts are fibroblasts that have contractile elements that help with wound contraction. When you're injured, after a small while you notice that it the injury becomes smaller. This doesn't mean that the epithelium has grown very quickly and filled the gap. Rather, it's because the wound is big and contraction is one of the defense mechanisms of the body and an attempt to prevent further ingress of pathogens. It's an attempt to close the wound as much as possible. That's when u start to see the irregularities we tend to see in scarring.

Even with the best sutures, the wound will typically only be 70-80% of the original strength, at best. By the time sutures are removed, wound strength is only 10% of

original strength. If the wound reopens, it will most likely heal by secondary intention and leave a scar. Within the first two months, there is a lot more collagen synthesis than degeneration. Once we reach the critical mass of the ECM, and we don't need more, we switch to collagen structural modification. Within the first one month there is a rapid increase of wound strength. However, by the third month there won't be much gain of wound strength.

Glucocorticoids may be beneficial in certain cases. For example, in your cornea, you don't want to have fibrosis because it would prevent u from seeing efficiently. So, you can use glucocorticoids with antibiotics to maintain vision. However, if you have a patient on anti-rejection medication, you need to know that this patient won't heal as quickly or resolve an infection as efficiently as a normal patient.

For extra info: surgeons (especially plastic surgeons) pay a huge amount of attention to these details after performing a surgical procedure. They can manipulate the methods of healing in a way that allows them to change the ratio of regeneration to scarring, so as to minimize the visible scarring. However you should note that even if a surgeon altered the integrity of the scarring to an extent that it is no longer visible, this doesn't necessarily mean that the features of scarring are not present underneath the skin, and the scars will remain palpable upon touching.