Pathology

Subject :

Lec no : lecture-7

Done By : Hala AL Beshtawe

وَقُل رَّبِّ زِدْنِي عِلْمًا

Functions of ECM

(1) **Mechanical support for Cell anchorage (fixation) + migration + Maintenance of cell polarity.**

(2) **Control of cell growth.**

ECM components can regulate cell proliferation.

(3) **Maintenance of cell differentiation.**

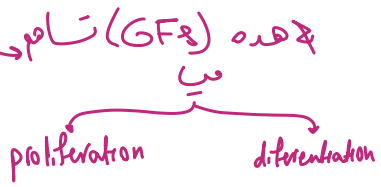
Type of ECM proteins affects the degree of differentiation of the cells in the tissue, acting via cellular receptor of integrin family.

(4) **Scaffolding for tissue renewal.**

The maintenance of normal tissue structure requires BM for stromal scaffold.

(5) **BM acts as a boundary between epithelium & underlying connective tissue.**

(6) **Storage & presentation of GFs like FGF & HGF, both are excreted & stored in the ECM in some tissues.**



Collagen :

This is the most abundant of the matrix protein, it is synthesized by the fibroblasts & osteoblasts .

Collagens are fibrous structural proteins, that confer tensile strength.

The collagen s are composed of three separate polypeptide chains braided into rope-like triple helix . More than 30 types have been identified, some of which are unique to specific cells & tissues .

Can be fibrillar collagen like type I,II, III,&V .

Collagen types I & III form a major proportion of the connective tissue in healing wounds & particularly in scars.

The tensile strength of the fibrillar collagen derives from their cross-linking, which is the result of covalent bonds catalyzed by the enzyme lysyl-oxidase , his process requires vitamin C .

That is why individuals with vitamin C deficiency have skeletal deformities .

Other are non-fibrillar & may form :

(a) BM (type IV)

(b) or be component of other structures like intervertebral discs (type IX), or dermal-epidermal junctions (type VII).

Genetic defects in collagen causes diseases like osteogenesis Imperfecta & Ehlers-Danlos syndrome .

ELASTIN

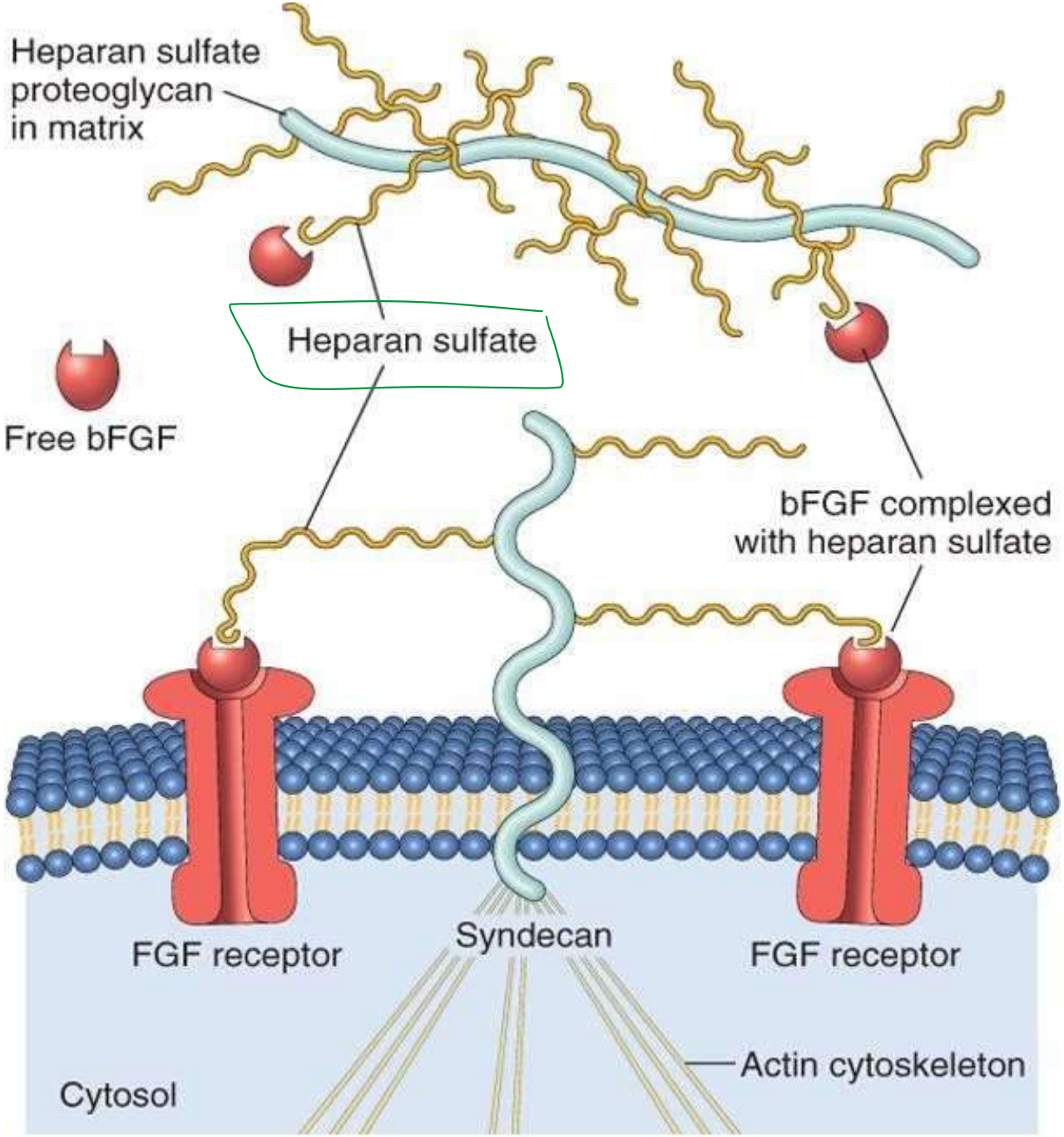
After physical stress, the ability of tissue to recoil & return to a baseline structure is conferred by elastic tissue, especially in the walls of large blood vessel (e g aorta, which must accommodate recurrent pulsatile blood flow), uterus, skin, & ligaments. Morphologically elastic fibers consist central core of elastin surrounded by meshwork of fibrillin glycoprotein . Defects in fibrillin synthesis leads to weakening of arterial walls & skeletal deformities like Marfan's syndrome .

PROTEOGLYCANS & HYALURONAN

These are highly hydrated compressible gel conferring resilience and lubrication such as cartilage in joints .

They consist of long polysaccharides, called glycosaminoglycans , or mucopolysaccharides , (examples are dermatan sulfate & heparan sulfate)

Also serve as reservoirs for Growth Factors s secreted into the ECM (e. g Fibroblast Growth Factor).



F 63 : Proteoglycans in the ECM & on cells act as reservoirs for GF.

Adhesive Glycoprotein , & Adhesion Receptors

Both are involved in:

(1) cell-cell adhesion

(2) the linkage between cells & ECM, &

(3) binding between ECM components.

④ Control of proliferation, differentiation & movement

- The adhesive glycoproteins include:

  (a) fibronectin (major component of the interstitial ECM) , synthesized by fibroblasts monocytes & endothelial cells . It binds the extracellular matrix components together also attach to integrins & to fibrin in blood clot, necessary in healing .

  (b) laminin (major constituent of BM).

  The adhesion receptors, also known as cell adhesion molecules (CAMs), can modulate cell proliferation, differentiation & motility . .

INTEGRINS → adhesion receptors

Are a family of transmembrane glycoproteins that are the main cellular receptor for ECM components, like fibronectins & laminins. Integrins are present in the plasma membrane of most animal cells, with the exception of RBCs.

Function

→ They bind to many ECM components initiating signaling cascades that can affect cell locomotion, proliferation, & differentiation.

(1) (2) (3) (4)

لا يوجد على سطحها integrin لكي لا تلتصق ببعضها

اشارات تؤثر على حركة الخلايا

* مواد تتحكم في انقسام الخلايا & تمايزها & يوجد عدة أنواع (GFs)

IV- The Nature & Mechanisms of Actions of Growth Factors

Cell proliferation can be triggered by many chemical mediators, such as

(1) hormones .

(2) cytokines,

(3) growth factors (GF); * مواد (GFs) ترتبط بمستقبلات (receptor) على سطح الخلية تحفز انقسام الخلية ← تسرع دخولها في cell cycle

The first two have many other functions & are discussed separately.

In this section, we focus on **polypeptide GF** whose major role is to **promote cell survival** & **proliferation** & which are **important in regeneration & healing.**

Signaling Mechanisms of GF Receptors -R- (GFR)

The major intracellular signaling pathways, induced by GFR are similar to those of many other cellular receptors that recognize extracellular ligands .

The binding of a ligand to its receptor triggers a series of events, by which extracellular signals are transduced into the cell, leading to the stimulation or repression.

Signaling may occur

(1) directly, in the same cell, (autocrine)

(2) between adjacent cells, (paracrine)

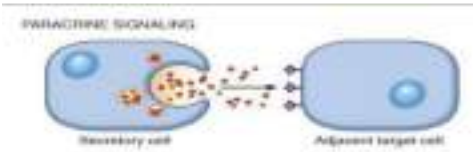
(3) over greater distances (endocrine)

Autocrine signaling:

In which a soluble mediator acts predominantly on the cell that secretes it.

وعالبا تحدث حين ← من الامثله عليها تنشيط الـ T cells يرتبط عليها الـ2

This pathway is important in the immune response (eg lymphocyte proliferation induced by some cytokines), & in compensatory epithelial hyperplasia (e g liver regeneration).



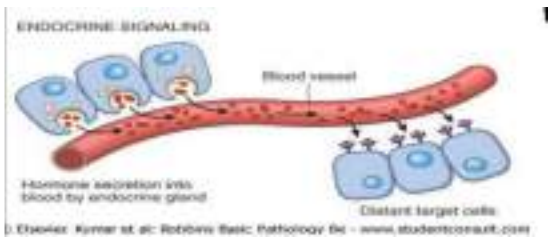
Paracrine signaling: → present in inflamatory response

In which, a substance affect cells in the immediate vicinity of the cell that released the agent.

This pathway is important for recruiting inflammatory cells to the site of infection, and in wound healing.

Endocrine signaling :

In which a regulatory substance, such as a hormone , is released into the blood stream & acts on target cells at a distance.



Growth factors of repair process

Growth factor	Source	Function
1. Epidermal growth factor (EGF)	Activated macrophages (M2), keratinocytes	It is mitogenic for keratinocytes & fibroblasts , stimulates keratinocytes migration and stimulates granulation tissue formation
2. Transforming growth factor alfa: (TGF-α)	activated macrophages (M2) , T lymphocytes and keratinocytes	It stimulates replication of hepatocytes & epithelial cells
3. Hepatocyte growth factor (HGF) (scatter factor)	fibroblasts, stromal cells in the liver and endothelial cells	Enhances proliferation of hepatocytes & other epithelial cells , and enhance cell mobility.
4. Vascular endothelial cell factor (VEGF)	mesenchymal cells (macrophages and fibroblasts)	It stimulates proliferation of endothelial cells & increases vascular permeability (angiogenesis).
5. Fibroblast growth factor (FGF) :	macrophages, mast cells , T lymphocytes , endothelial cells	It is chemotactic & mitogenic for fibroblasts ,keratinocytes. It stimulates keratinocytes migration , angiogenesis , wound contraction matrix deposition.
6. Transforming growth factor beta (TGF-β)	platelets , T lymphocytes , macrophages (M2) , endothelial cells , fibroblasts & smooth muscle cells.	It is chemotactic for neutrophils ,macrophages, lymphocytes, fibroblasts, smooth muscle cells and stimulates ECM synthesis and suppresses acute inflammation (anti-inflammatory)
7. Keratinocyte growth factor (KGF)	fibroblasts.	It stimulates keratinocyte migration , proliferation and differentiation.
8. Platelets –derived growth factor (PDGF)	platelets , macrophages , endothelial cells , keratinocytes and smooth muscle cells.	It is chemotactic to neutrophils , macrophages ,fibroblasts & smooth muscle cells. Stimulates the production of extra cellular matrix protein.

* هذا السلايد من تلاجيص الدكتور حمزه طراد

Growth factors & cytokines involved in regeneration & wound healing are :

Epidermal growth factor : (EGF) :

Released from activated macrophages , keratinocytes & other cells. It is mitogenic for keratinocytes & fibroblasts , stimulates keratinocytes migration and stimulates granulation tissue formation.

Transforming growth factor alfa : (TGF-α)

Released from activated macrophages, T lymphocytes & keratinocytes & other cells. It stimulates replication of hepatocytes & epithelial cells.

Hepatocyte growth factor (HGF) (scatter factor)

Released from fibroblasts, stromal cells in the liver & endothelial cells . Enhances proliferation of hepatocytes & other epithelial cells , and enhance cell mobility.

Vascular endothelial cell factor (VEGF) (isoform A,B,C,D)

Released from mesenchymal cells . It stimulates proliferation of endothelial cells & increases vascular permeability .

Platelets –derived growth factor (PDGF)

Released from platelets , macrophages , endothelial cells , keratinocytes & smooth muscle cells. It is chemotactic to neutrophils , macrophages , fibroblasts & smooth muscle cells. Stimulates the production of extra cellular matrix protein.

Fibroblast growth factor (FGF)1&2:

Released from macrophages, mast cells , T lymphocytes , endothelial cells & other cells.

It is chemotactic & mitogenic for fibroblasts , & keratinocytes. It stimulates keratinocytes migration , angiogenesis , wound contraction & matrix deposition.

Transforming growth factor beta (TGF-β)

Released from platelets , T lymphocytes , macrophages , endothelial cells , fibroblasts & smooth muscle cells.

It is chemotactic for neutrophils ,macrophages, lymphocytes fibroblasts & smooth muscle cells & stimulates ECM synthesis & suppresses acute inflammation .

Keratinocyte growth factor (KGF)

Released from fibroblasts.

It stimulates keratinocyte migration , proliferation & differentiation.

*Factors affecting type of healing

Fibrosis

Regeneration & repair

The relative roles of *regeneration & repair* vary between the type of tissues affected and also depends on the nature , the severity & the duration of the injury.

I - Type of tissue :

حسب قدرتها على التجديد
- regeneration → التجدد
- repair → ليس بالضرورة التجديد

The "Proliferative Potential of Different tissues" is based on the proliferative capacity & thereby, the ability of tissues to repair themselves.

The ability of the surviving cells to divide is the key factor in this response .

Permenant cells → Fibrosis
labile cells → Mostly (regeneration)
Stable cells → it depend ↔ regeneration / repair (Fibrosis)

II- Severity & duration of injury:

- Severe → Fibrosis (هذا يعني انها تضررت الخلايا architecture/scaffold)
- mild → regeneration (تضررت الخلايا فقط) (عندما يكون التضرر يصل بعد إلى هيئة النسيج)

Mild injury may be followed by complete restoration of normal cellular architecture , especially in tissues having labile or stable cells like skin & liver.

The liver cells have a remarkable capacity to regenerate . (شرط ١: (ECM) لم تتضرر ② وجود (GFs))

In experimental animals up to 90% of liver can be removed surgically and the remaining parenchyma will regenerate to the original mass having normal cellular structure & function.

* في تجربة على الحيوانات ازالو 90٪ من حجم الكبد بعد فترة عاد الكبد إلى حجمه الطبيعي وشكلها ظهرت وكرة التبرع بالكبد من اشخاص على قيد الحياة

living-donor transplantation (عملية التبرع بالكبد) **in which portion of the liver is resected from a normal individual & is transplanted into a recipient with end-stage liver disease .**

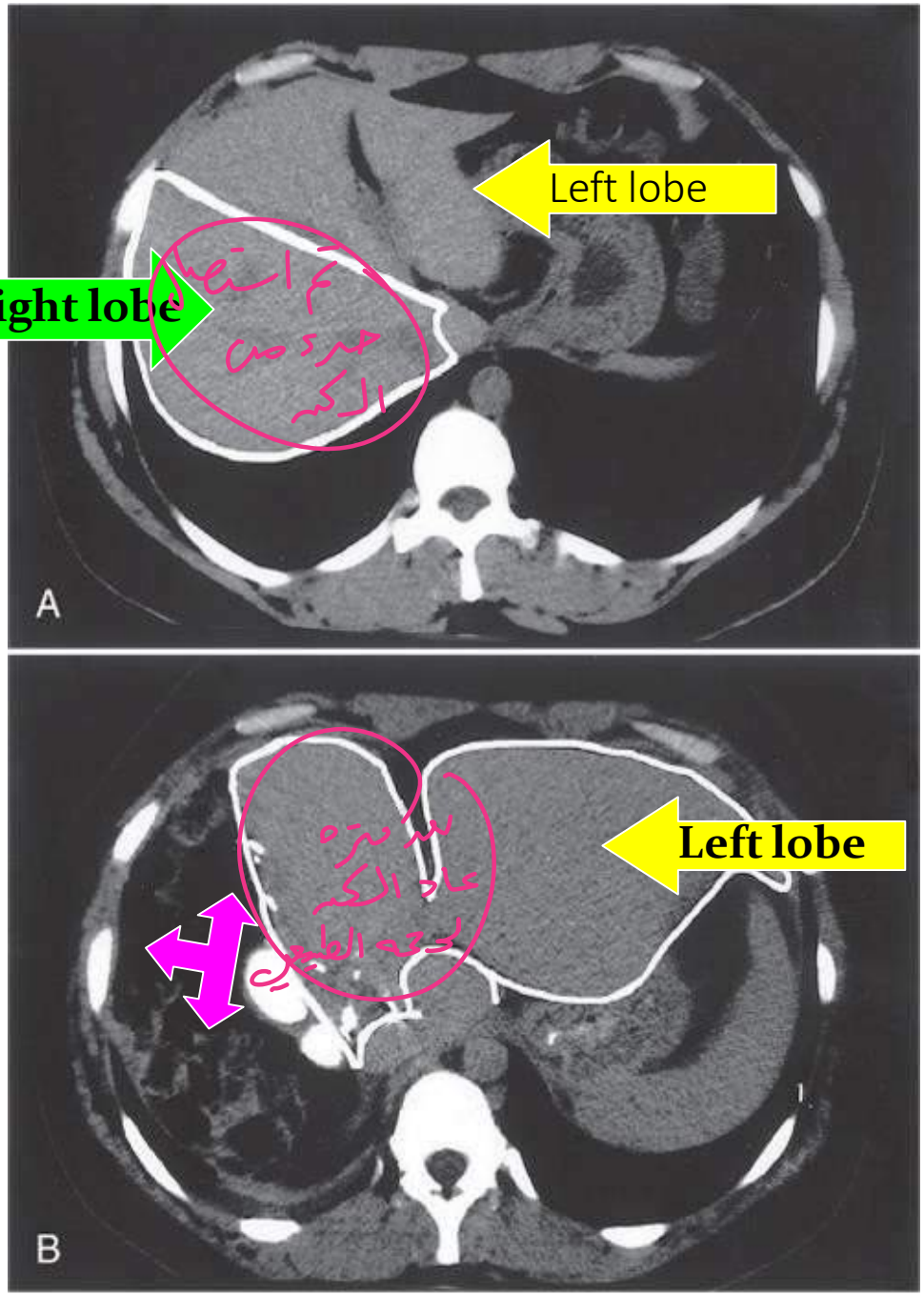
خاصة اذا كان (Benign tumor) ورم حميد يزيل الجراح جزء من الكبد المصاب * يعود الكبد بعدها للوضع الطبيعي

استئصال جزء من الكبد

Patients with liver tumor, treated by partial hepatectomy, in both conditions the tissue resection triggers a dramatic, proliferative response of the remaining hepatocytes (which are normally quiescent) & the subsequent replication of the surgical removal of 40% to 60% of the liver, in hepatic cells.

In humans when there is massive central necrosis of hepatocytes , with minimal collapse of the matrix as in viral hepatitis , however, in most cases the hepatocytes regenerative responses ensure restoration of the liver architecture and function when infection subsides.

* في حالات (viral hepatitis) خصوصاً النوع (A) لا يحدث تدمير ل ECM بل فقط في الخلايا مما يجعل الكبد يدخل عليه (regeneration) * ينتج خلايا تؤدي وظائفها المعتادة



F 66: Regeneration of human liver. CTS of the donor liver in living-donor liver transplantation.

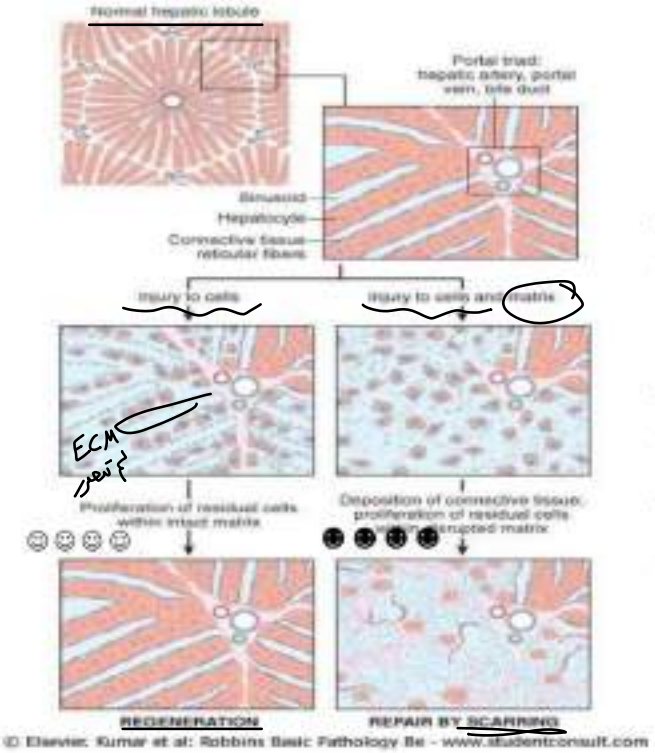
A, The liver of the donor before the operation. **Note the right lobe *(outline),* which will be resected & used as a transplant.**

B, Scan of the same liver 1 week after resection of the right lobe; note the **enlargement of the left lobe *(outline)*** without regrowth of the right lobe.

While in chronic liver cell injury, when the amount of fibrosis is quite substantial, the regeneration develops in form of regenerating nodules surrounded by fibrous tissue as in liver cirrhosis.

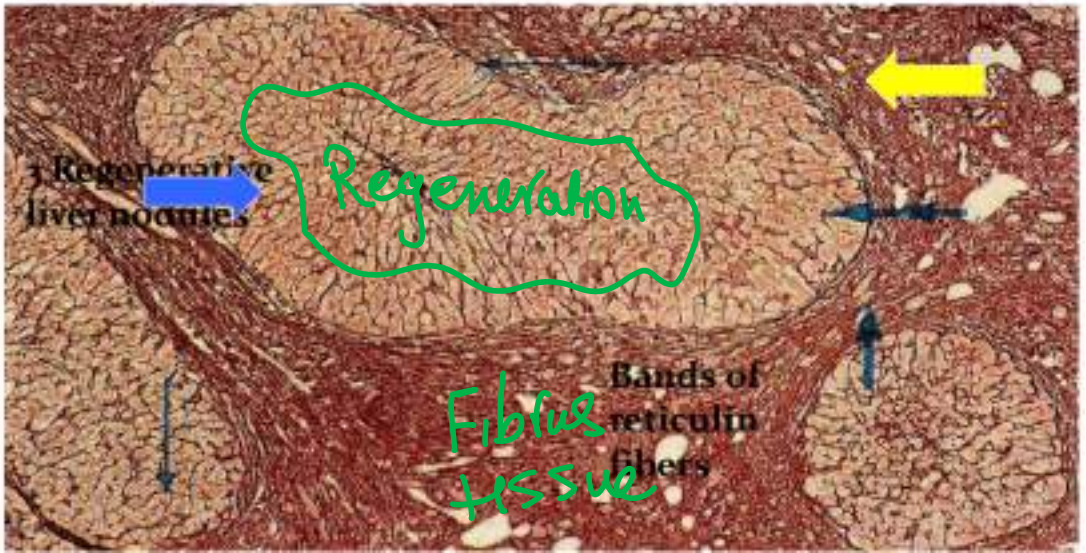
It seems that the tendency of chronic inflammatory process to produce excessive fibrosis is related to continuing production of macrophages & lymphocytic- derived cytokines like interleukin-6 (IL-6) & tumor necrosis factor (TNF) & growth factors like HGF & EGF & TGF- ά which act as a mediator of the healing process.

(Growth Factors involved in hepatic Fibrosis/liver fibrosis)



F 67 : Mechanisms of tissue repair. In this example, injury to the liver is repaired by regeneration if only the hepatocytes are damaged, or by **laying down of fibrous tissue (scarring) if the matrix is also injured.**

Figure 68 : Liver cirrhosis : Liver section stained by reticulin stain . There are three regenerative liver nodules (double arrow), separated by broad bands of reticulin fibers (thick arrow) . An example of healing by combine regeneration & fibrosis which follows injury to the liver cells & stroma .



Angiogenesis :

Is a process of new blood vessel development from existing vessels , primarily venules .

It is critical in healing at site of injury , in development of collateral circulation at sites of ischemia ,& in allowing tumors to increase in size beyond the constraints of their original blood supply .

Steps of angiogenesis include :

1 **Vasodilation occuring in response to NO & increased permeability induced by vascula r endothelial growth factor (VEGF) .**

2 **Migration of proliferating endothelial cells from pre-existing blood vessels towards the area of tissue injury forming solid tube .**

3 **Remodelling of proliferating endothelial cells into capillary tubes attached to the lumen of the pre-existing vessel .**

4 **Recruitment of periendothelial cells (pericytes) & smooth muscle cells around the new capillaries .**

5 **Suppression of endothelial cells proliferation & deposition of basement membrane .**

The major growth factors involved in angiogenesis : the most important are vasacular endothelial growth factor (VEGF) & basic fibroblast growth factors (basic FGF) .

* Major growth factor involved in angiogenesis are

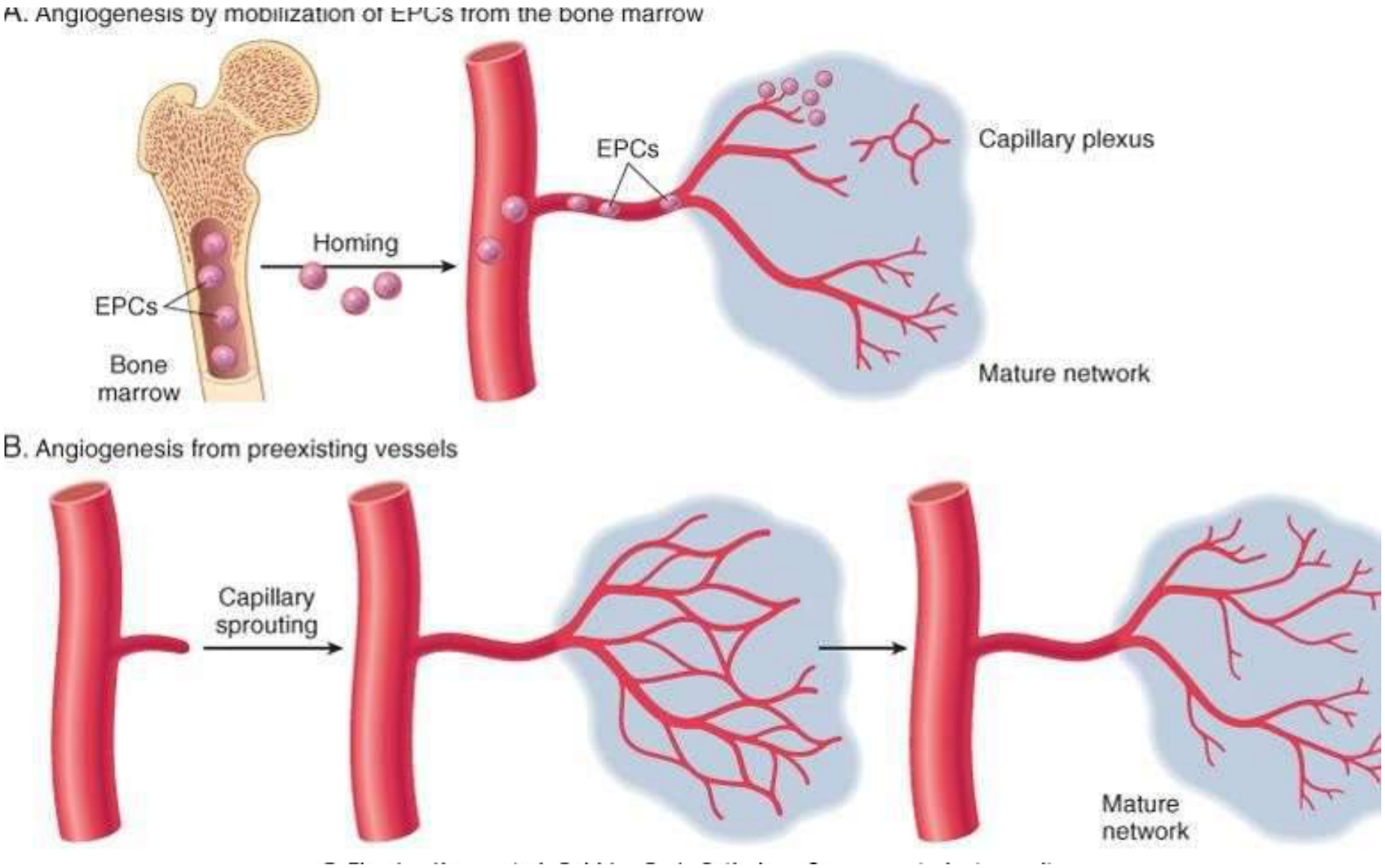


Figure 69 : Diagrammatic demonstration of steps of angiogenesis .

HEALING OF SKIN WOUND

Here, we specifically describe the healing of skin wounds.

As it involves both epithelial regeneration & the formation of connective tissue scar, it is thus illustrative of the general principles that apply to wound healing in all tissues.

Healing of skin wounds : Either

I. Healing by Primary intention. (Primary union) Or

II. Healing by secondary intention (Secondary union)

Healing by Primary Intention

شروط حدوثه

Occurs in an uninfected clean sterile wound without tissue loss as in surgical incision approximated by surgical sutures .

مثال عليها مرضى العمليات الجراحية

The incision causes only focal disruption (loss of continuity) of epithelial BM & death of relatively few epithelial & connective tissue cells.

minimal tissue loss or minimal damage

As a result, epithelial regeneration predominates over fibrosis.

يحدث تجدد يكون اكثر من النسيج الليفي fibrosis

A small scar is formed , but there is minimal wound contraction.

When an incision is made in the skin & subcutaneous tissue , **blood escapes from the cut vessels**, it clots on the wound surface & fills the gap between the wound edges , which is narrow in sutured wound .

Within **24 hours: neutrophils** are seen at the incision margin, migrating toward the fibrin clot.

This is called **traumatic inflammatory response** .

Mean while the **Basal cells** at the cut edge of the epidermis begin to exhibit **mitotic activity**.

Within **24 to 48 hours** , epithelial cells from both edges have begun to migrate & **proliferate** along the dermis, depositing **basement membrane** components as they progress .

The cells meet in the midline beneath the surface scab, yielding a thin but continuous epithelial layer. The basal cell proliferation stops by contact inhibition.

By day 3 neutrophils have been largely replaced by macrophages, followed by angiogenesis & granulation tissue, which consists of proliferating capillaries & fibroblasts progressively invades the incision space.

Collagen fibers being layed down by the fibroblasts are now evident at the incision margins, but these are vertically oriented & do not bridge the incision.

Epithelial cell proliferation continues, yielding a thickened epidermal covering layer.

By day 5, angiogenesis reaches its peak as granulation tissue fills the incisional space & collagen fibrils become more abundant & begin to bridge the incision.

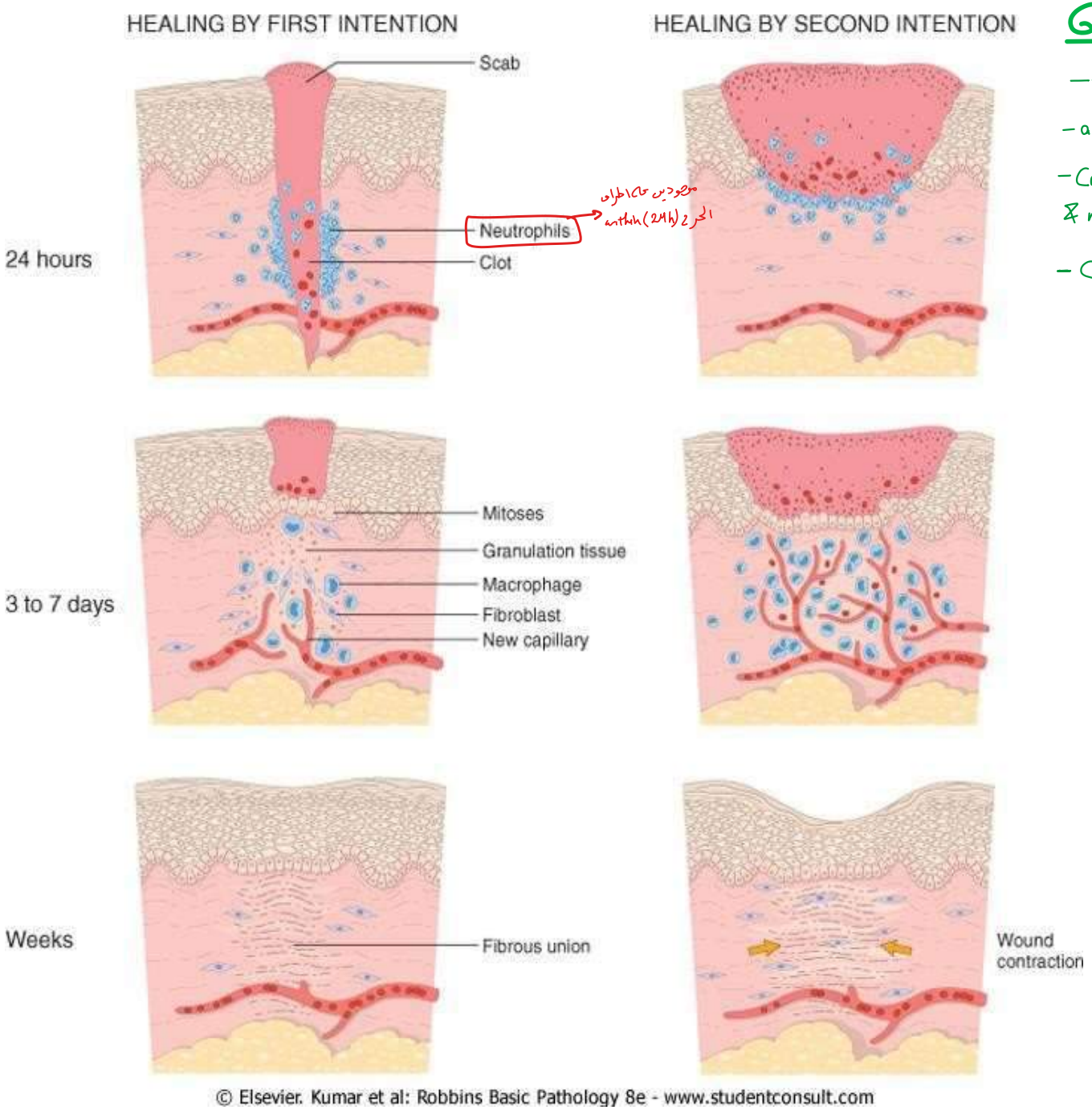
The epidermis recovers its normal thickness as differentiation of surface cells yields a mature epidermal architecture with surface keratinization.

During the second week:

There is continued collagen accumulation & fibroblasts proliferation. The WBC infiltrate, edema, & the vascularity are substantially diminished. The long process of "blanching" (pallor) begins, accomplished by: collagen deposition within the incisional scar & the regression of vascular channels.

By the end of the first month the scar comprises acellular connective tissue, devoid of inflammatory cells & covered by an essentially normal epidermis.

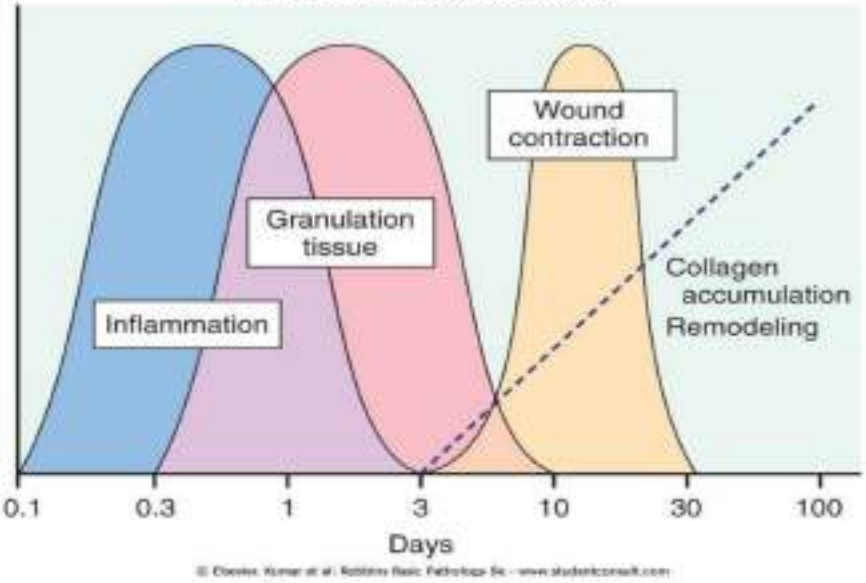
Hair follicles & sebaceous glands which are destroyed in the line of incision are permanently lost.



Granulation	scar tissue
– fibra blast ↑	– fibroblast ↓
– angeogenesis ↑	– Colagen ↑ + type 1
– Colagen type (3) & not mature	tri chrome stain
– Capilaries ↑	– Capilaries ↓

F 70 : Steps of wound healing by first intention *(left)* & second intention *(right).*
In the latter, note the large amount of granulation tissue & wound contraction.

F 71 : Phases of wound healing



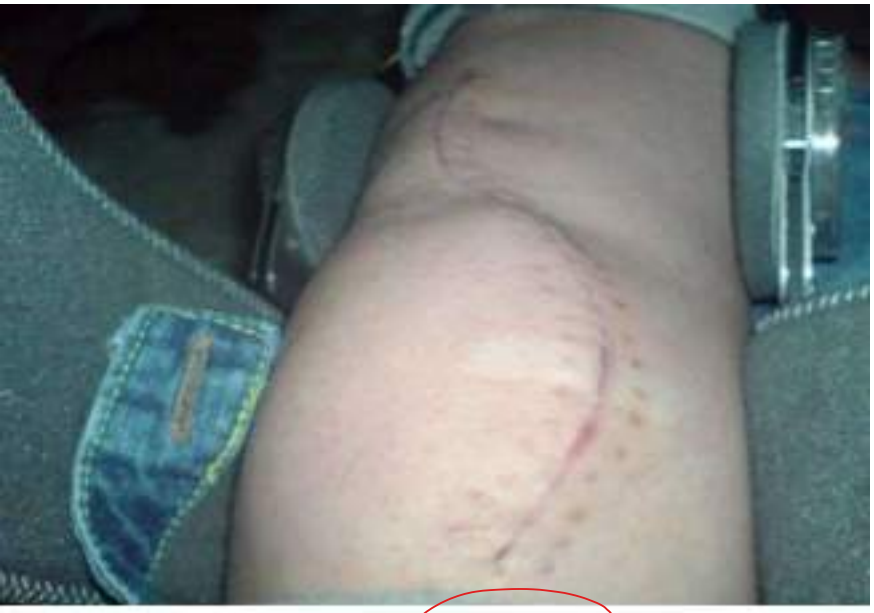


Figure 72 :Healing of surgical wound by primary intention or union .

scar → ندبة

Figure 73 : Healed wound: Cornea . The healed wound is visible as a 'gap' in the stroma, filled with a connective tissue & many fibrocytes (double A), the epithelium covering the gap in it (thin A) is much thinner than the normal epithelium on each side of the wound.

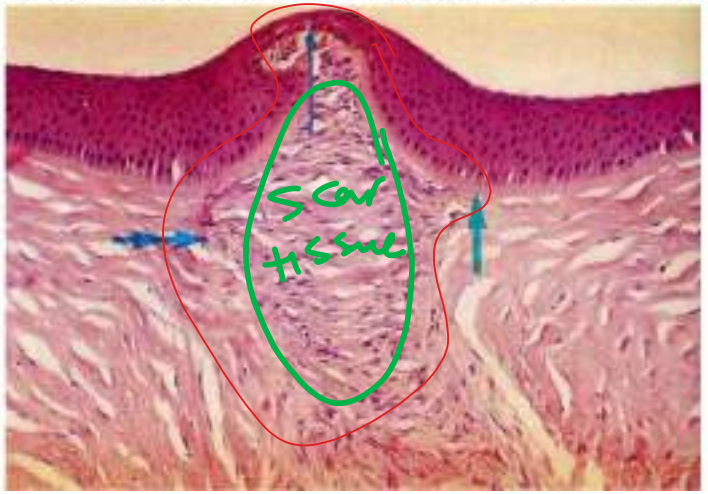
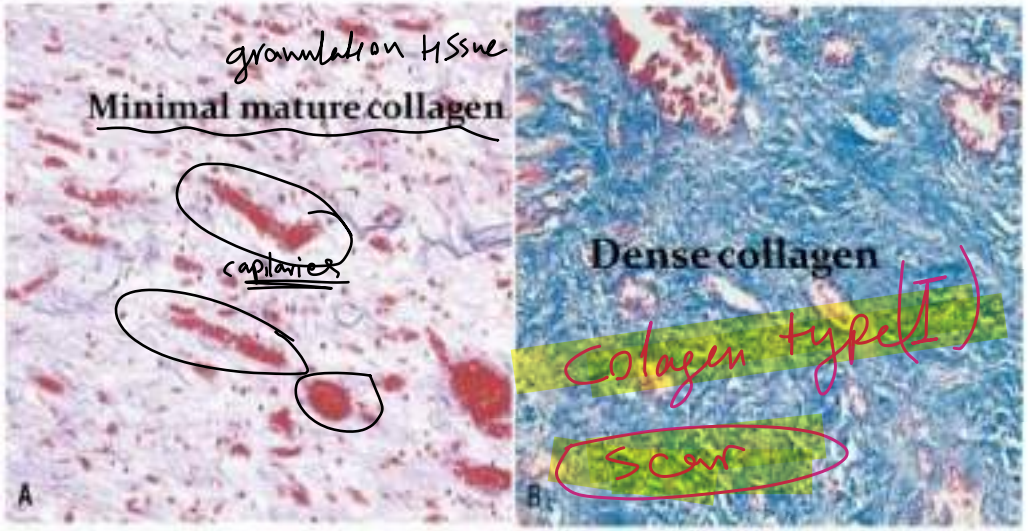


Figure 74 : A, Granulation tissue showing numerous blood vessels, **edema, & a loose ECM ; minimal mature collagen .**
B, Trichrome stain of mature scar, showing dense collagen(blue) with only scattered vascular channels.



Healing by Secondary Intention

When cell or tissue loss is more extensive, as in Infarction, Abscess, Ulcer or Large wound, the reparative process is more complex.

The regeneration of parenchymal cells alone cannot restore the original architecture, therefore, there is an extensive ingrowth of granulation tissue from the wound margins, followed by ECM accumulation & scarring.

This is called secondary union or healing by second intention

Very Imp

Secondary union differs from the primary in several aspects:

(I) A larger clot or scab rich in fibrin and fibronectin forms at the surface of the wound .

(2) Inflammation is more intense because large tissue defects have a greater volume of necrotic debris , exudate , and fibrin that must be removed .

Consequently large defects have a greater potential for secondary inflammation – mediated injury .

(3) Larger defects require greater volume of granulation tissue to fill in the gaps & provide the underlying framework for the re growth of tissues epithelium . A greater volume of granulation tissue generally results in a greater mass of scar tissue .

(4) Secondary healing involves wound contraction

Within 6 weeks large skin defects may be reduced to 5%-10% of their original size largely by contraction .

This process is due to the presence of myofibroblasts, a modified fibroblasts exhibiting many of the ultrastructural & functional features of contractile smooth muscle cells.

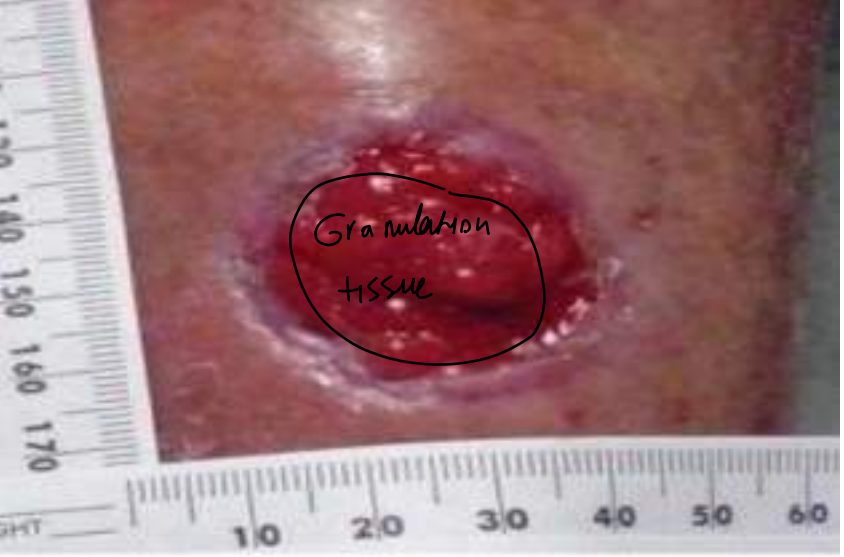


Figure 75 : Healing by secondary intention of a large wound with excessive tissue necrosis .

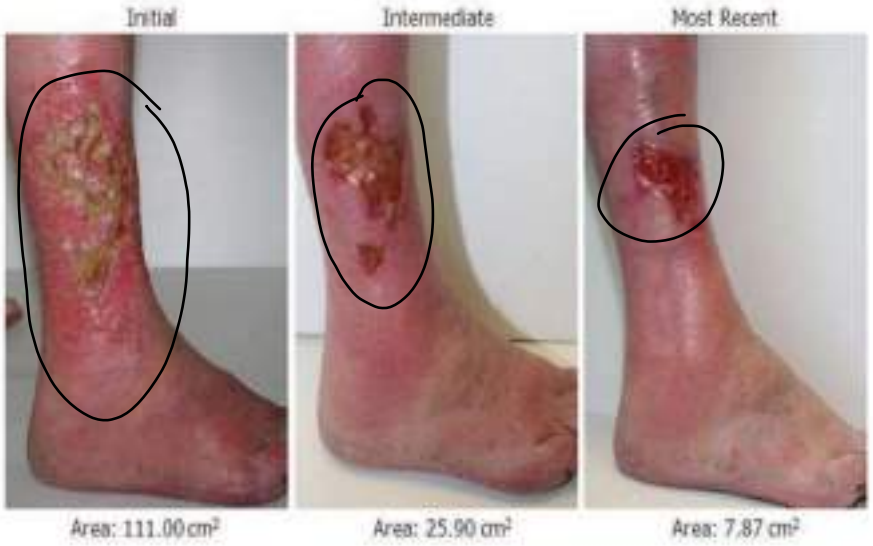


Figure 76 :Healing of skin wound by secondary intention

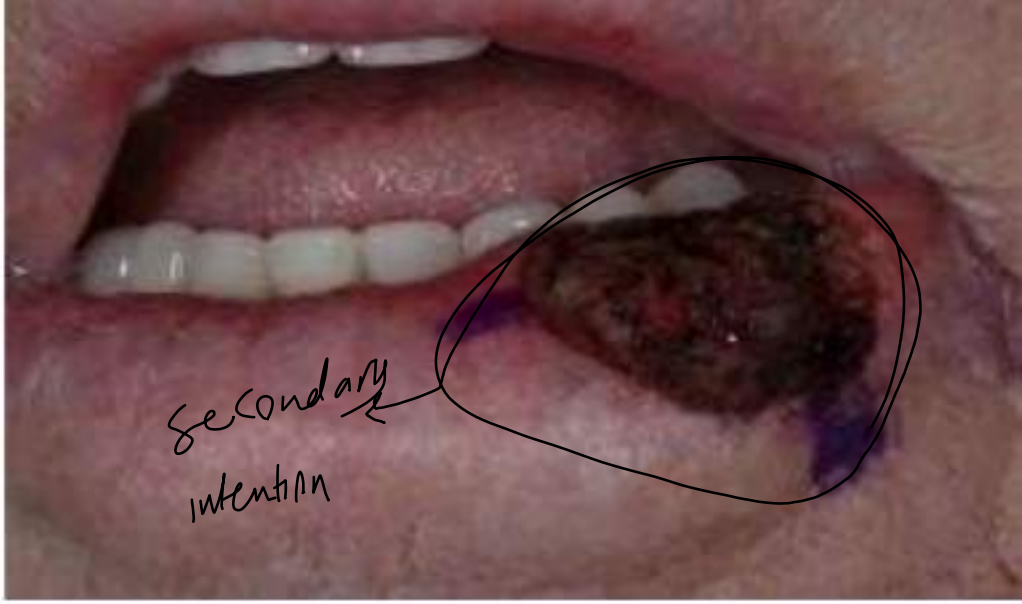


Figure 77 : Healing by secondary intention : showing a large irregular permanent scar .

Wound Strength → قوه الارتباط بين حواف الجرح ومقارنة بالجلد الطبيعي

Carefully sutured wounds have approximately 70% of the strength of unwounded skin, largely because of the placement of the sutures.

When sutures removed after one week, wound strength is approximately 10% of that of unwounded skin, but this increases rapidly during the next 4 weeks .

* قوه ارتباط الجلد الطبيعي 100%
* * = = الجلد عند وجود (suture) من 70% يقل الى 10% بعد ازالة ال suture
استعادة قوة ال tissue تحدث من خلال

The recovery of tensile strength results from:

(1) Collagen synthesis exceeding degradation during the first 2 months, & from ← زيادة الكمية

(2) structural modifications of collagen (e . g cross-linking & increased fiber size) when synthesis declines at later times . type 3 → type 1 (اقوى)

Wound strength reaches 70% to 80% of normal by 3 months , but usually does not improve beyond that point.

لا تعود الى اكثر من هذا الحد

عوامل تؤدي الى تأخير healing عليه

Factors that cause delay of healing process :

In wound healing, normal cell growth & fibrosis may be altered by a variety of factors, frequently reducing the quality or adequacy of the reparative process:

1. **Infection, is the single most important cause of delay in healing, by prolonging the inflammation phase of the process, & potentially increases the local tissue injury.**

2. **Nutrition has profound effects on wound healing, for example protein deficiency & especially, vitamin C deficiency, inhibit collagen synthesis & retard healing.**

الاشخاص الذين يأخذون الكورتيزون

3. **Glucocorticoids (steroids): have well-documented anti-inflammatory effects, & their administration may result in poor wound strength owing to diminished fibrosis.**

4. **Mechanical factors** such as increased local pressure or torsion may cause wounds to pull apart (separate), or dehisce (e. g abdominal wound dehiscence after laporatomy).

5. **Poor blood perfusion**, due either to **atherosclerosis** (which reduce arterial blood supply), or to obstructed venous drainage, e . g **varicose veins**, both impairs healing.

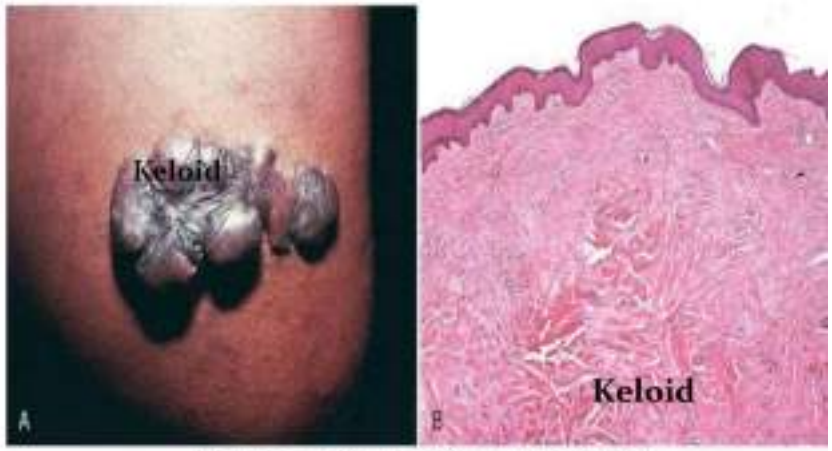
6. **Foreign bodies** such as fragments of steel (e . g gun-shot , glass, wood, or even bone, impede (delay) healing process .

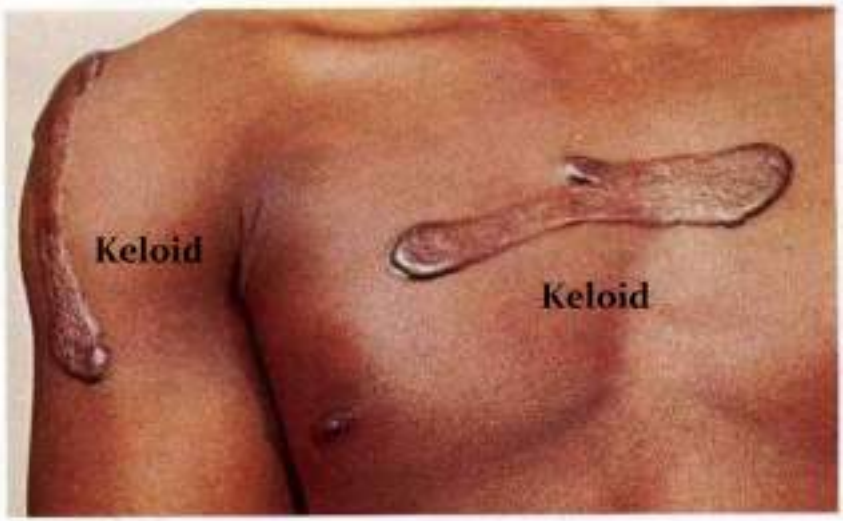
Healing wounds may also generate excessive granulation tissue that protrudes above the level of the surrounding skin & in fact, prevent re-epithelialization. This is called exuberant granulation, or proud flesh.

Sometimes, the accumulation of excessive amounts of collagen can give rise to prominent raised scars known as Keloids, more commonly seen in blacks .

ذوي البشرة السوداء

F 78 : Keloid.
A, Excess collagen deposition in the skin forming a raised scar known as a keloid. B, Thick collagen deposition in the dermis (pink color).



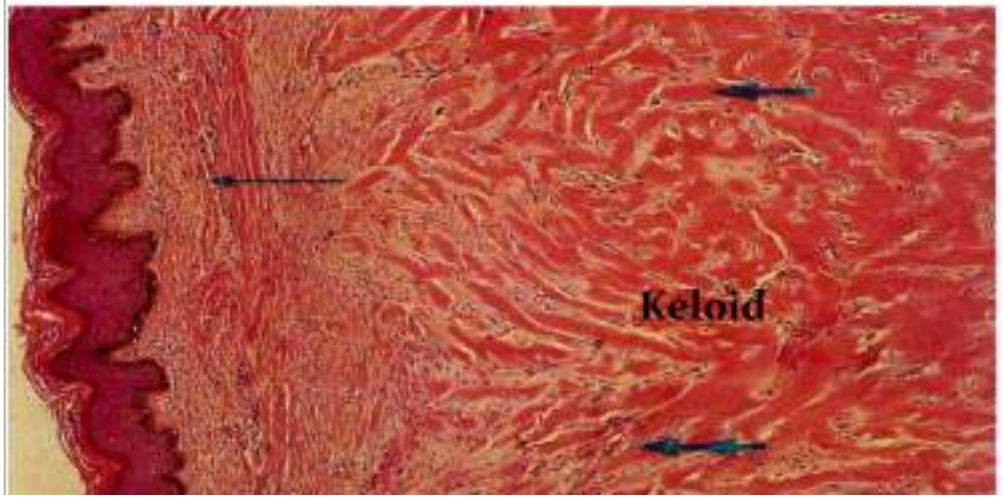


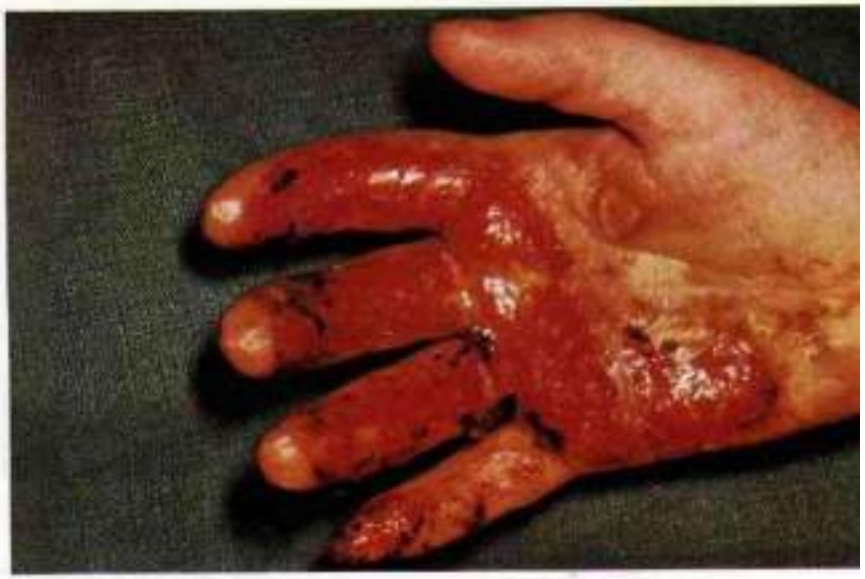
14.5 Keloid

Figure 79 : Keloid .

Figure 80 : Keloid in a healed wound in the Skin.

The epidermis &dermis (thin arrow) appear normal, but the deeper dermis & subcutaneous tissues are replaced by very broad bands of hyaline eosinophilic collagen (thick arrow) .





1.12 Granulating burn

Figure 81 : Exuberant granulation tissue .

Figure 82 : Foreign-body granuloma : healed wound of skin , showing granulation tissue, consisting of (1) large & greatly dilated capillaries, (2) lymphocytes & plasma cells, (3) fibroblasts (thin arrow), (4) very large giant cells enclosing nylon suture material, (thick arrow) from the original surgical incision

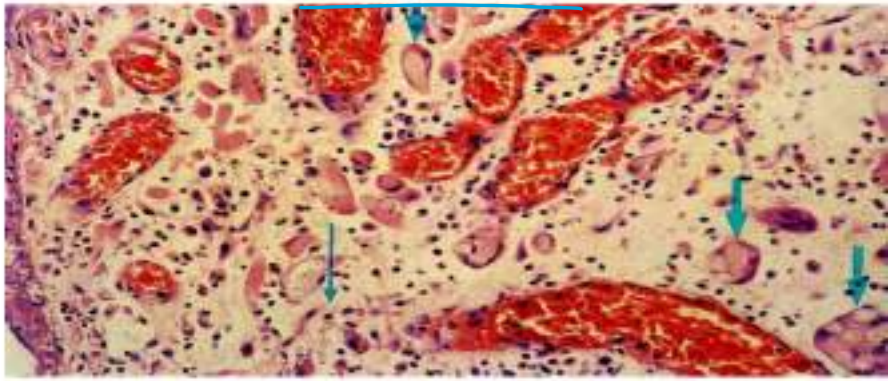
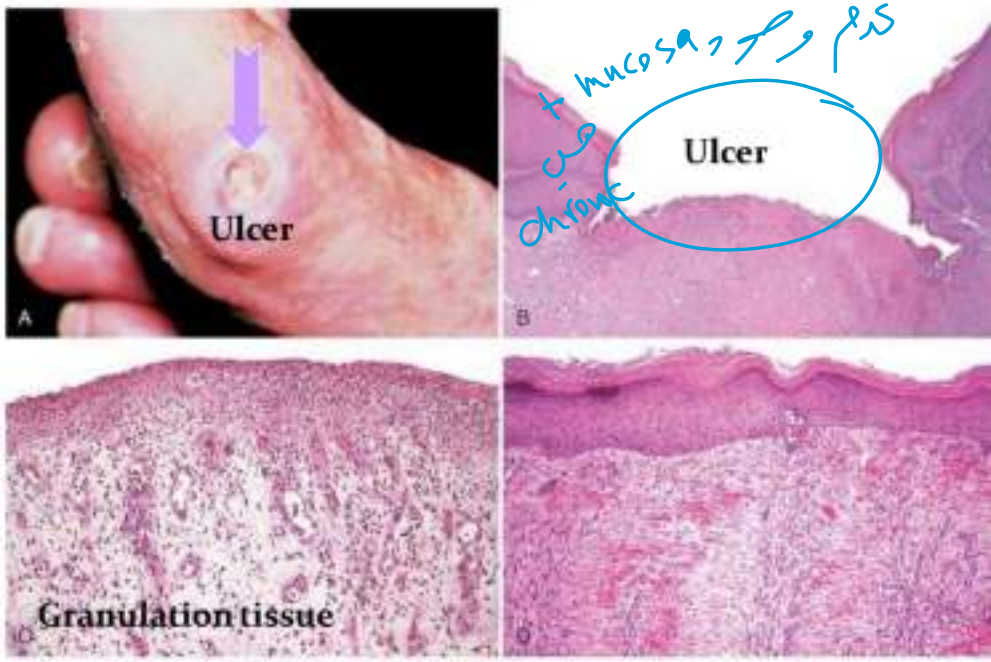
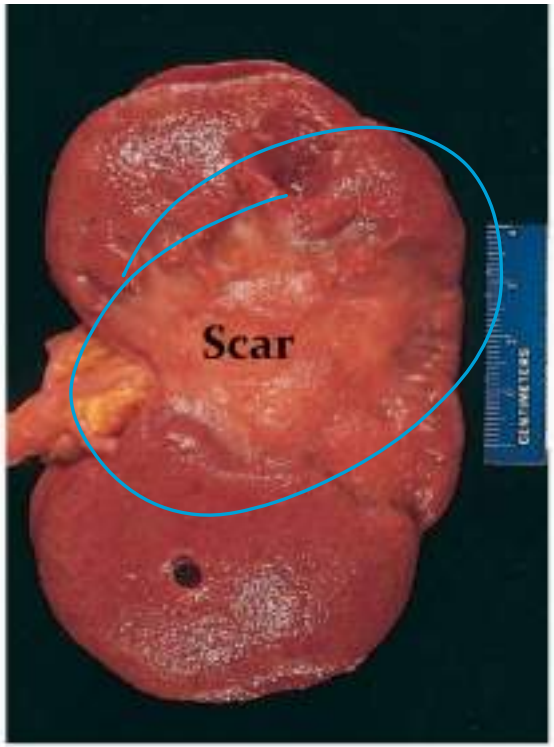


Figure 84 : Healing of diabetic skin **ulcer.**





F 83 : Large old kidney infarct, now replaced by a large fibrotic scar.