Tissue repair: Cell Regeneration, Fibrosis, and Wound Healing

Dr. Marwan Qubaja
Al-Quds University
Faculty of Medicine
Pathology Department

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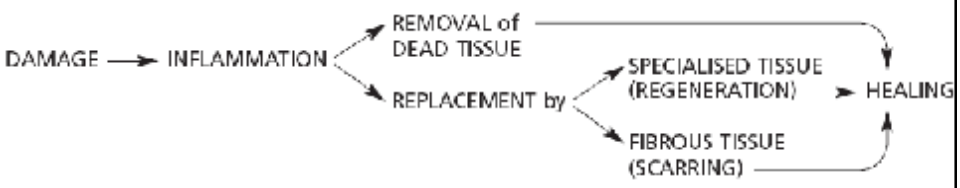
Tissue repair (HEALING)

- **Healing is the final stage of the response of tissue to injury.**



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Tissue repair

- **Repair involves the combination of two processes:**
  - **Regeneration: healing** of the injured tissue **by regrowth** of the parenchymal cells of the same type.
  - **Fibrosis (scarring): healing** of the injured tissue **by connective tissue** resulting in scar formation.

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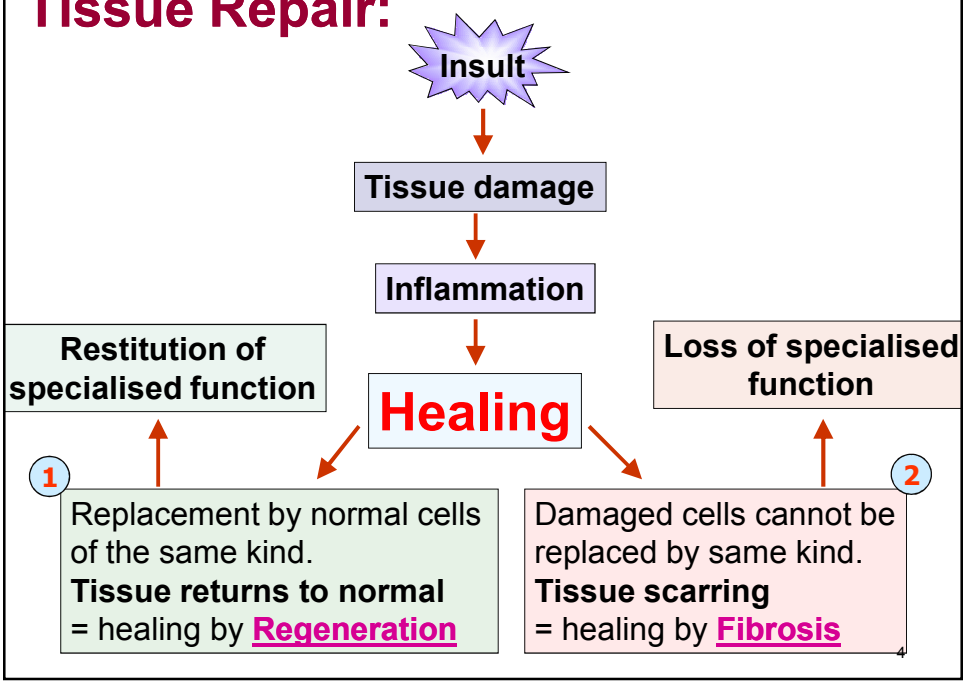
Tissue Repair:



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(1) Repair by Cell Regeneration

- **Definition:**

  Parenchymal cell proliferation & differentiation, which involves interaction of the proliferating cells with soluble **chemical mediators** and insoluble extracellular matrix.

- **Regeneration involves two processes:**

1. **proliferation** of surviving cells to replace lost tissue.
2. **migration** of surviving cells into the vacant space.

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(2) Repair by Connective Tissue (Fibrosis/Scarring)

- **Definition:**
  - Repair by replacement of the non-regenerated parenchymal cells with connective tissue.
  - Occurs if **the parenchymal cells can't regenerate**, or if the stromal framework is damaged.
- **Fibrosis involves four main processes:**
  1) **Angiogenesis**
  2) **Proliferation and Migration of fibroblasts**
  3) **Deposition of ECM**
  4) **Remodeling of ECM**

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Tissue repair

- **Both processes involve essentially similar mechanisms including:**
  - Cell **proliferation, differentiation** and **migration**
  - Synthesis of and interaction with the extracellular matrix (ECM).

However, the cell types involved are different.

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Tissue repair

- **In any tissue healing, both processes are involved but in different proportions depending on:**
  - **Type of tissue** injured or the capacity of a tissue for regeneration
  - **The severity of injury**
  - **The type of injury**

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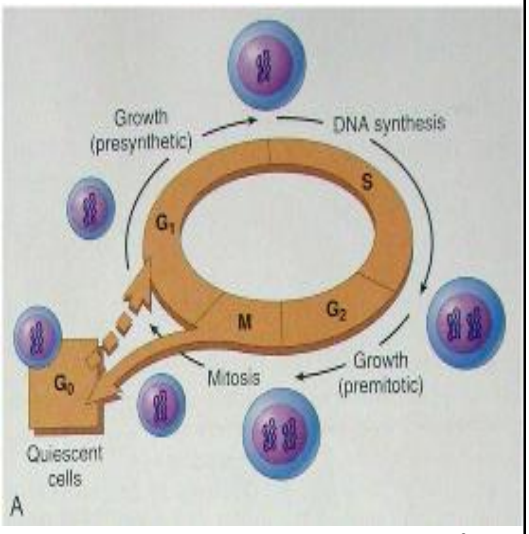
Phases of the cell cycle

- G0 phase: pool of **resting** cells "**quiescent cells**" are present. Most cells in the body are quiescent.
- G1 phase: **presynthetic** growth phase.
- S phase: **DNA-synthetic** phase. Both of those two phases constitute the majority of the cell cycle.
- G2 phase: **premitotic** growth phase.
- M phase: **mitotic phase**. This is the shortest phase.



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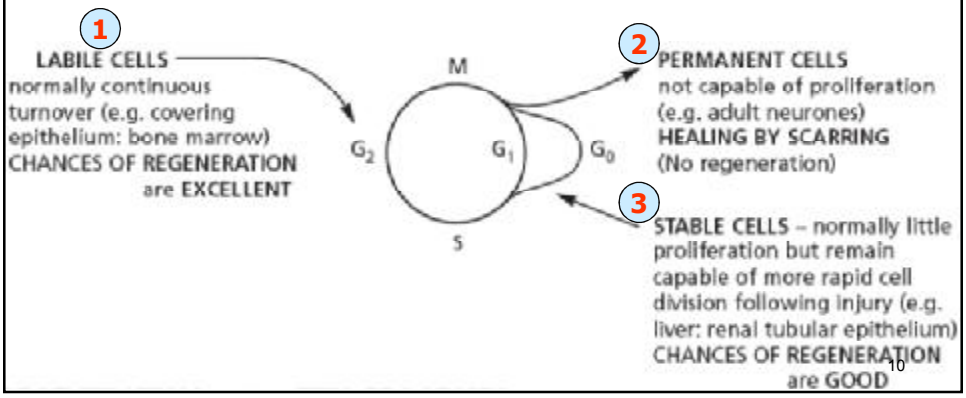
Types of cells in relation to the cell cycle

According to the proliferative capacity and relation to the cell cycle, the different cells in the body are divided into three types:



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Proliferative potential of different cell types:

1) Labile cells:

- Continuously dividing and dying cells.
- Stem cells are the source of this ability. The stem cell divides to produce one daughter cell retaining the ability to divide, and one cell that differentiates to carry out the normal function.
- E.g. **Hematopoietic cells, surface epithelial cells, mucosal surfaces**.

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Proliferative potential of different cell types:

2) Stable (quiescent) cells:

- They are normally non dividing but are capable of undergoing rapid division in response to injury.
- E.g. Parenchymal cells of most solid organs are of this kind as well as the endothelial cells, the fibroblasts and smooth muscle cells, (e.g. liver, renal tubules)

3) Permanent cells:

- Terminally differentiated non-proliferating cells in the post natal life.
- E.g. **Neurons** and **cardiac** muscles

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Proliferative potential of different cell types:

- **Cell growth (proliferation) and differentiation involves at least two signals:**
  1) soluble chemical mediators like **growth factors and inhibitors**
  2) insoluble elements of **the ECM**.

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Extracellular matrix (ECM):

Definition:

- A macromolecular complex that is synthesized locally and constitute a large proportion of any tissue.

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Components of the ECM

1. Basement membrane:

- A highly organized and specialized matrix, present around epithelial, endothelial and smooth muscle cells.
- Synthesized by epithelial and mesenchymal cells
- **Type IV collagen** and adhesive glycoproteins are the major constituents.

2. Interstitial matrix:

- A three dimensional amorphous gel, present in the spaces between cells in connective tissue, and between epithelium and supportive vascular and smooth muscle structures
- Synthesized by mesenchymal cells
- **Collagens** (fibrillary and nonfibrillar), **proteoglycan** and **glycoproteins** are the major constituents.

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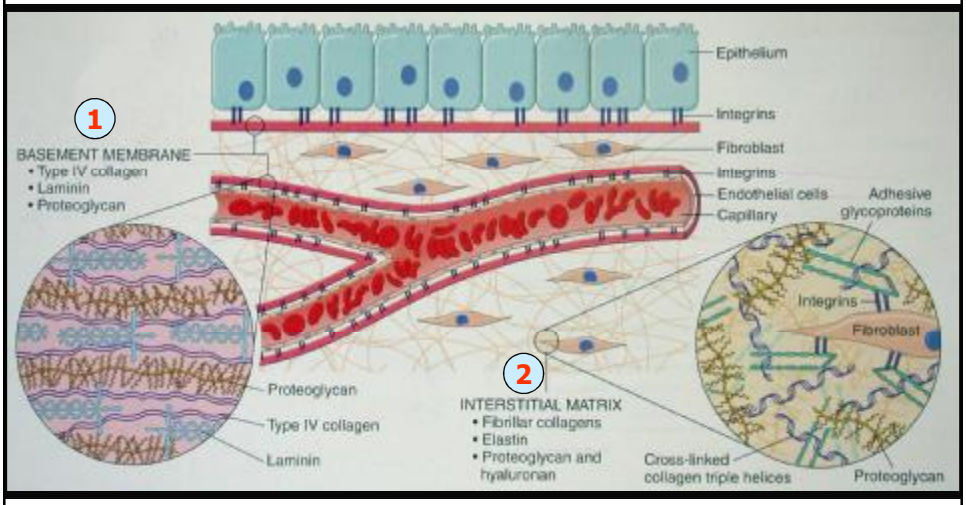
Components of the ECM:



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Components of the ECM:

- **Fibrous structural proteins:** that confer tensile **strength** (**collagen**) & recoil (**elastin**)
- **Water-hydrated gels:** that permit the **elasticity and lubrication** (**proteoglycan & hyaluronan**).
- **Adhesive glycoproteins & integrins:** that **connect the matrix elements** to one another and to cells (**fibronectin, Laminin**).

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Biological Roles of the ECM

- **Mechanical support**
- **Determination of cell polarity (cell orientation)**
- **Control of cell growth**
- **Control/maintenance of cell differentiation**
- **Establishment of tissue microenvironment**
- **Storage and presentation of regulatory proteins**

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Growth Factors

- Mostly soluble growth factor proteins derived from the serum or cells.
- Secreted in extremely low concentrations.
- They bind to a specific high-affinity receptors on the target cells
- **Pleiotropic effect:** not only **stimulate cell growth** but **migration**, **differentiation** and **remodeling**.
- They induce cell **proliferation** by affecting the expression of genes involved in normal growth, protooncogenes.
- Some have growth inhibition effects, e.g. TGF-beta.

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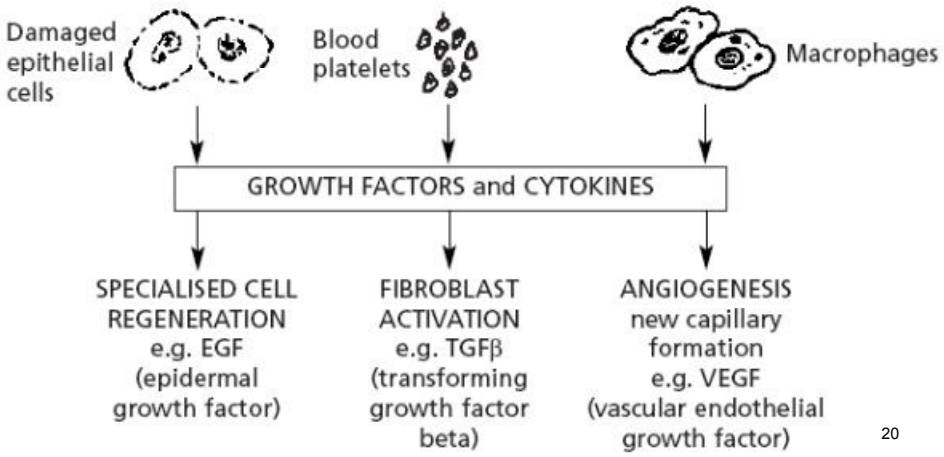
Growth Factors

- There are multiple sources for the growth factors, activated **macrophages** are the most important.



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Repair by Connective Tissue (Fibrosis/Scarring)

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  - This occurs if the parenchymal cells can't regenerate, or if the stromal framework is damaged.
- **Fibrosis involves four main processes:**
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Repair by connective tissue (Fibrosis)

- Repair begins **within 24 hours** by **emigration of the fibroblasts** and induction of fibroblast and endothelial cells proliferation.
- By **3-5 days**: **granulation tissue is formed**; specialized type of tissue that is characteristic of healing. Derived from the granular, pink, soft gross appearance.
- When it matures; it results in the formation of fibrosis (scar)

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Components of Fibrosis:

1. **Angiogenesis:** formation of new blood vessels
2. **Migration of fibroblasts**
3. **Deposition of ECM**

} **scar formation**

4. **Remodeling of ECM:** maturation and reorganization of the fibrous tissue

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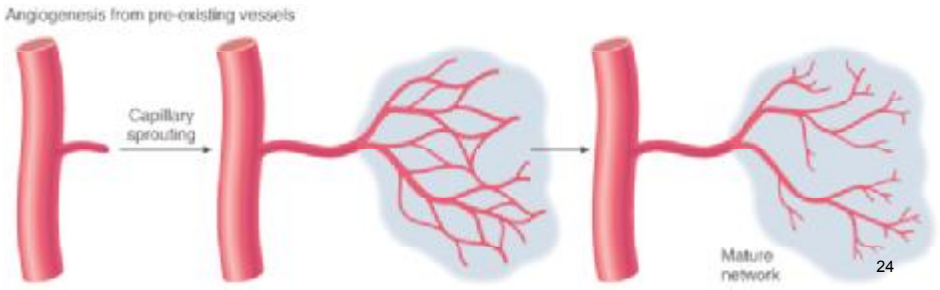
1. Angiogenesis "Neovascularization"

One important feature of the newly formed blood vessels is that they are **leaky** due to the immature junctions between the endothelial cells and the increased transcytosis.



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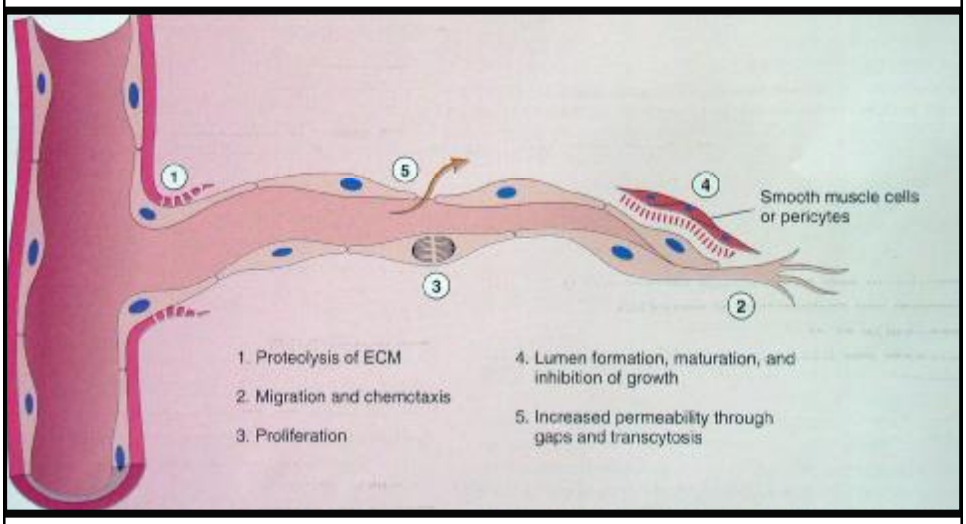
Steps in Angiogenesis



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Steps in Angiogenesis

- Proteolytic **degradation of the parent vessel BM**, allowing the formation of a capillary sprout.
- **Migration of the endothelial cells** from the original capillary toward an angiogenic stimulus
- **Proliferation of the endothelial cells** behind the leading edge of migrating cells
- **Maturation of the endothelial cells** with inhibition of growth and organization into capillary tubes. This includes recruitment of pericytes for capillaries and smooth muscles for larger vessels.

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Factors that induce angiogenesis:

- **basic Fibroblast Growth Factor (bFGF)**
- **Vascular Endothelial Growth Factor (VEGF)**

- These factors are produced by a variety of cells. They bind to proteoglycans in the BM, to be released once needed.
- The **receptors** are restricted to the **endothelial** cells

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Why is angiogenesis important ?

- **Healing** at the wound sites.
- Development of **collateral circulation** at the sites of ischemia.
- **Tumor growth** beyond the constrains of the their original vascular supply.

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2 & 3. Fibrosis; "scar formation"

Definition:

- Change of the granulation tissue into an inactive fibroblasts, dense collagen and fragments of elastic fibers.
- **Composed of two steps:**
  - **A. emigration and proliferation of fibroblast** at the site of injury
  - **B. deposition of ECM** by these cells

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A. Emigration and proliferation of fibroblast at the site of injury

- **Factors that are important in fibrosis:**
  - PDGF
  - bFGF
  - TGF-beta
- **Sources of the mediators:**
  - Activated endothelium
  - Inflammatory cells, macrophages

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B. Deposition of ECM

- The proliferating fibroblasts change into synthesizing fibroblasts.
- Increased **synthesis and deposition of ECM, mainly collagen**. This process starts by **the 3rd - 5th day** of wound healing.
- Collagen deposition and maturation is critical in the development of the strength of the wound.

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Factors that mediate collagen synthesis:

- **Growth factors:** PDGF, bFGF and TGF-beta
- **Cytokines:** IL-1 and TNF

Net collagen accumulation depends not only on increased synthesis but also on diminished collagen degradation

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4. Scar Remodeling:

- **Degradation of the collagens and other ECM** components is accomplished by a family of enzymes “metalloproteinases”, as well as non serine proteinases.
- Derived from a variety of cells including fibroblasts, macrophages, neutrophils, synovial cells and some epithelial cells.

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Wound healing

Can be viewed as a sequence of processes:

- Induction of acute inflammatory response by the stimulus
- Parenchymal cell regeneration, when possible
- Migration and proliferation of both parenchymal cells and connective tissue cells
- Synthesis of ECM
- Remodeling of the parenchymal elements to restore tissue function
- Remodeling of the connective tissue to achieve wound strength

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Wound Healing

Healing of skin wound is taken as an example:

- **Healing by first (primary) intention:**
  - Clean, uninfected surgical wound
  - Edges apposed
  - E.g. planned surgical incisions, with sutures
- **Healing by second (secondary) intention:**
  - Extensive loss of tissue
  - Wound edges not apposed
  - E.g. large haematoma, infection

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Wound healing

Healing by first intention "primary union":

- **Definition:**

  healing of a **clean**, uninfected surgical incision approximated by surgical sutures.

- **Epithelial regeneration predominates over fibrosis**, as the amount of tissue loss is minimal

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Healing by first intention

- The narrow incision space rapidly fills with fibrin-clotted blood. "scab: dehydrated fibrin-clot"
- **Within 24 hours:**
  - **neutrophils migrate** at the incision margin
  - **proliferation of the basal cells** at the edges of the incision
- **Within 24-48 hours:**
  - **epithelial cells migrate** towards the centre, forming a thin continuous layer

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Healing by first intention

- **by 3rd day:**
  - thick epidermis
  - **macrophages** are the predominant cells
  - **granulation tissue** is seen at the incision
  - **collagen fibers** seen at the edges of incision
- **by 5th day:**
  - full thickness epidermis
  - **peak granulation tissue and neovascularization**
  - **collagen fibrils bridge the incision**

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Healing by first intention

- **during 2nd week:**
  - fibroblast proliferation and collagen deposition
  - **inflammation and edema subside**
  - **remodeling begins**
- **by the end of the first month:**
  - **normal epidermis**
  - **scar in the dermis "fibrous tissue without inflammatory cells"**

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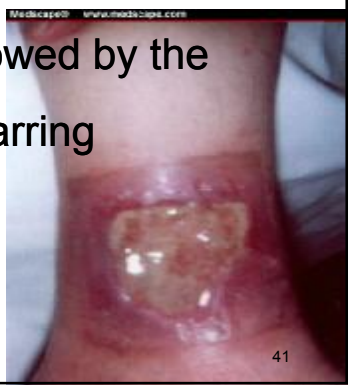
Healing by second intention: "secondary union"

Definition:

- **extensive** ingrowths of granulation tissue from the wound margins, followed by the accumulation of ECM and scarring



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Healing by second intention: "secondary union"

Secondary healing differs from primary healing in several aspects:

- **inflammatory reaction is more intense**
- **larger amounts of granulation tissue**
- **wound contraction:**
  - reduction of the size of the wound by 5-10% of the original size
  - achieved by myofibroblasts

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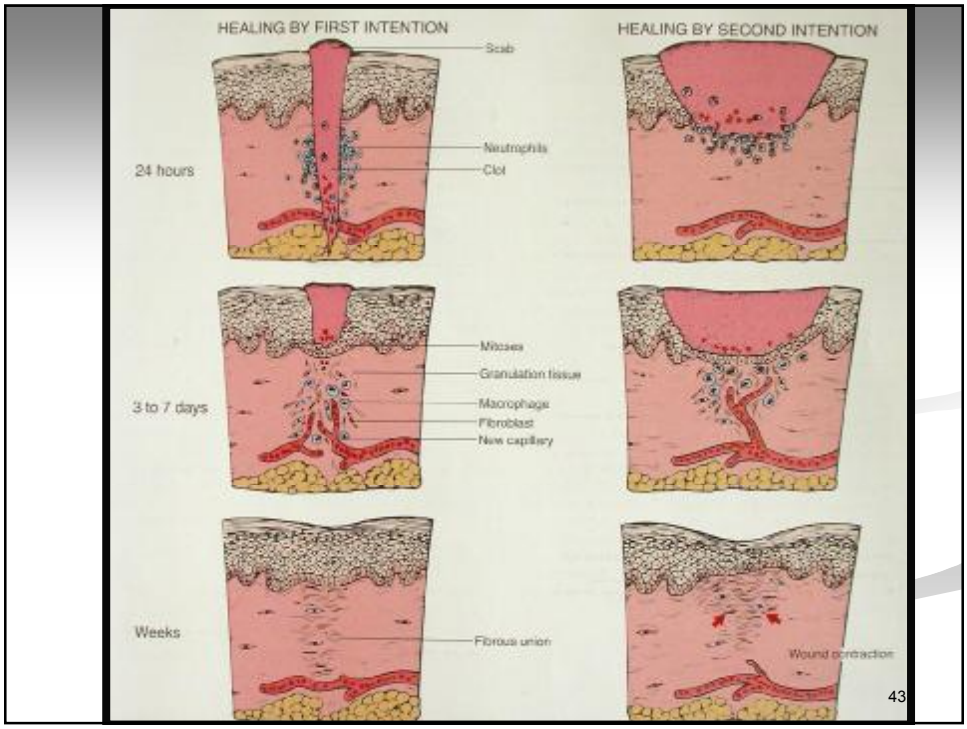

Wound strength

- **With sutures:** 70% of the strength of unwounded skin
- **When sutures are removed**
  - **At 1 week:** 10% of the strength
  - **By 3 months:** 70-80% of the strength
- Wound strength results from collagen synthesis exceeding degradation in the first 2 months, and from remodeling of the collagen by cross linking and increase fiber size later on.

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Factors that interfere with the process of wound healing?

- **Infection**: the single most important cause of delay in wound healing
- **Nutrition**: deficiency of proteins, vit C or metals like zinc
- **Medications**: glucocorticoids
- **Mechanical factors**: increased local pressure or torsion, leading to dehiscence
- **Poor perfusion**: ischemia or venous blockage
- **Foreign bodies**

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Factors inhibiting healing

Local	Systemic
Infection	Age
Hematoma	Drugs
Blood supply	Anemia
Foreign bodies	Diabetes
Mechanical stress	Malnutrition
	Vitamin C deficiency
	Trace metal deficiency

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Pathological aspects of wound healing

- **Keloids:** accumulation of **excess amount of collagen**, leading to prominent, raised scars. More common in blacks, **unknown** cause
- **Exuberant granulation** "**proud flesh**": generation of **excessive amount of granulation tissue** that protrudes above the level of the surrounding skin and prevent proper healing

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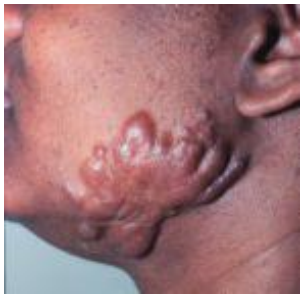
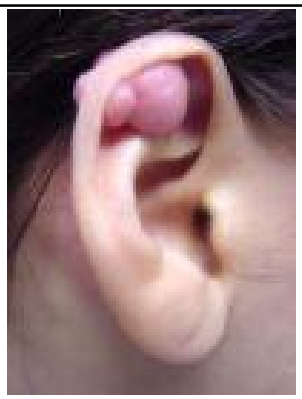
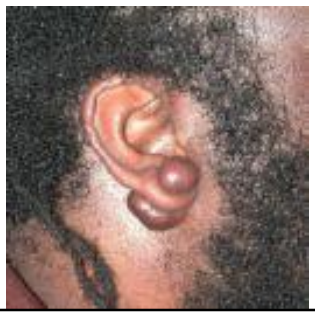
Keloids







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