General Pathology

Tissue Repair
Healing by Regeneration and Fibrosis

Tissue Repair

Restoration of tissue architecture & function after an injury.

Repair → proliferation of various cells

↓

close interactions between cells & (ECM).

Types of repair

Repair occurs by two type of reactions :-

1- **Regeneration: R**eplacement of the damaged components by the same original tissue so returning to a normal state.

2- **Fibrosis**: Extensive deposition of collagen fibers. (scar formation)

Factors determined type of repair :

1. The tissue capacity for proliferation.

2. The severity of damage to the supporting structures of the tissue.

THE CONTROL OF CELL PROLIFERATION

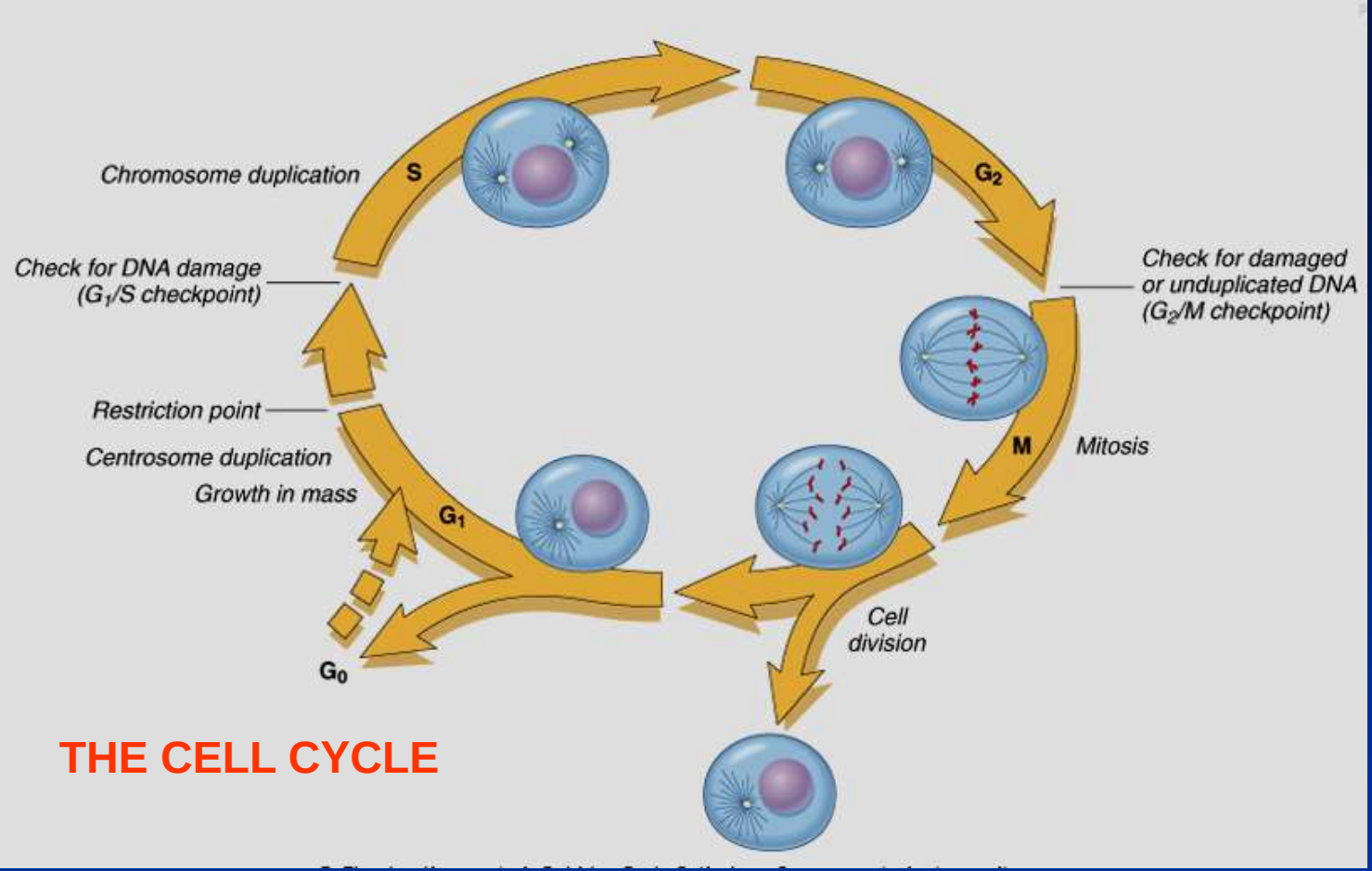
Several cell types proliferate during tissue repair. These include

1. **The remnants of the injured tissue (which attempt to restore normal structure)**
2. **Vascular endothelial cells (to create new vessels that provide the nutrients for the repair process)**
3. **Fibroblasts (the source of the fibrous tissue that fills defects).**

- **The proliferation of the above cell types is driven by growth factors**

THE CELL CYCLE

- The *cell cycle represents* ***the sequence of events that control DNA replication & mitosis in the proliferation of cells*****.**
- **It consists of a series of steps at which the cell checks for the accuracy of the process and instructs itself to proceed to the next step.**
- **The cycle consists of:**
  - **the presynthetic growth phase 1 *(G_1)***
  - **the DNA synthesis phase *(S)***
  - **the premitotic growth phase 2 *(G_2)***
  - **and the mitotic phase *(M)*.**
- **Non-dividing cells are either in cell cycle arrest in G_1 or they exit the cycle to enter a phase called G_0.**



THE CELL CYCLE

G0 G1 S

G2: growth premitotic, M: mitotic

The G1 and S stages generally constitute the majority of the time of the cell cycle; the mitotic (M) phase is typically brief.

Note the G1 restriction point, and the G1/S and G2/M checkpoints.

- ✓ **Any stimulus that initiates cell proliferation, such as exposure to growth factors, needs to promote the G_0/G_1 *transition* and the entry of cells into the G_1.**
- ✓ **Further progression is determined by the ability of the cell to pass through an intrinsic quality control mechanism for cell integrity, known as *checkpoint control*.**
- ✓ ***Checkpoint controls prevent DNA replication or mitosis of damaged cells and either transiently stop the cell cycle to allow for DNA repair or eliminate irreversibly damaged cells by apoptosis.***

- ✓ **Progression through the cell cycle from G_1 is regulated by proteins called *cyclins*, which form complexes with enzymes called *cyclin-dependent kinases (CDKs).***

- ✓ **These complexes regulate the phosphorylation of proteins involved in cell cycle progression leading to DNA replication and mitosis, and thus are required for cell cycle progression.**

Proliferative Capacities of Tissues:

1. Labile tissues: (Continuously Dividing Tissues)

2. Stable Tissues: (Quiescent cells)

3. Permanent Tissues: (Non dividing cells)

1. Labile tissues: (Continuously Dividing Tissues):

Continuously being lost and replaced by maturation from stem cells and proliferation of mature cells.

(Repair by regeneration)

Stem cells

The original embryonic cells that have the inherent property of proliferation.

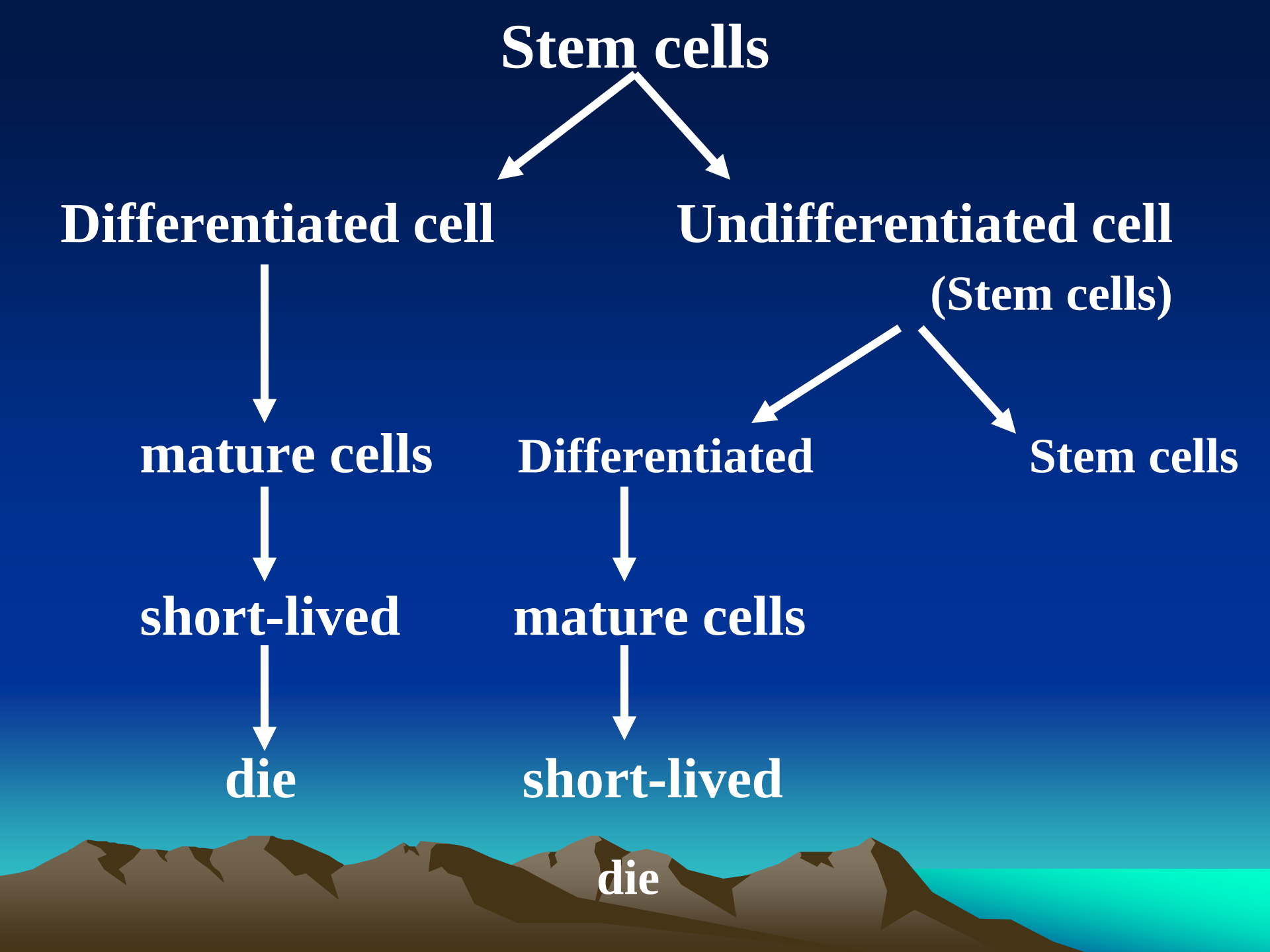
- **Characteristic features:**

1- Prolonged self-renewal capacity.

2- After each cell division, one cell will be differentiated while other cell remain undifferentiated, retaining their self-renewal capacity.

3- They have very broad differentiation capabilities, being able to generate any cell.

(fat, cartilage, bone, endothelium, and muscle)


Stem cells
Differentiated cell
Undifferentiated cell
(Stem cells)
mature cells
Differentiated
Stem cells
short-lived
mature cells
die
short-lived
die

Example of labile tissues

1- Hematopoietic cells in the bone marrow.

2- The majority of surface epithelium.

(skin, oral cavity, vagina, and cervix).

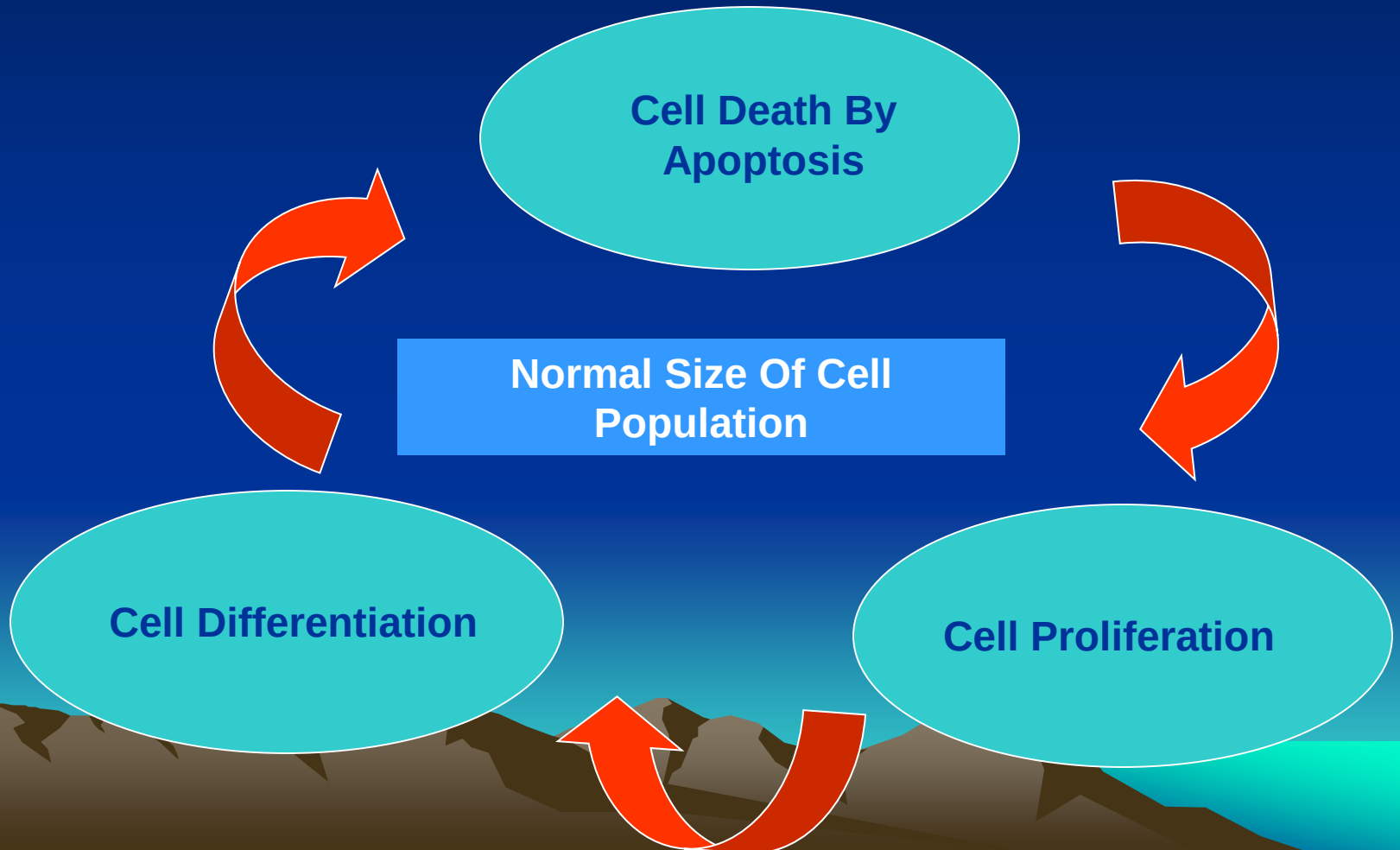
3- The cuboidal epithelia of the ducts draining exocrine organs.

(salivary glands, pancreas, biliary tract)

- **4- The columnar epithelium of the GIT, uterus, fallopian tubes & the transitional epithelium of the urinary tract.**

Homeostasis

Keeping a constant number of cells and tissue size to get normal shape and function.



2. Stable Tissues (Quiescent cells)

- Cells are quite
- Minimal proliferative activity in normal state.
- Capable of proliferating in response to injury.

Examples:

1. The parenchyma of most solid tissues.
   (liver, kidney, and pancreas).
2. The endothelial cells.
3. The fibroblasts.
4. The smooth muscle cells.

3. Permanent Tissues: (Non dividing cells)

Terminally differentiated and non-proliferative in postnatal life

(neurons and cardiac muscle cells)

-
-

↓

injury

↓

irreversible

↓

scar formation

Cells involved in repair

1- The remnants of the injured tissue.

2- Vascular endothelial cells.

3. Fibroblasts.

Control of cell proliferation:

Cell proliferation can be triggered by:

1. Chemical mediators:

Growth factors, Hormones, Cytokines

stimulation or inhibition of cell growth.

2. Signals from the extracellular matrix (ECM).

Growth factors & Repair

Growth factor are produced by:-

1- Leukocytes.

2- Connective tissue cells

1. Chemical mediators:- Growth factor

Growth factor: (Protein)

- **Stimulate cell division.**
- **Promoting cell survival.**
- **Stimulate migration.**
- **Stimulate differentiation.**
- **Enhance the synthesis of collagen in fibroblasts.**

2. Extracellular matrix (ECM) and cell-matrix interactions:

ECM:

Dynamic, constantly remodeling macromolecular complex synthesized locally as a network surrounding the cells.

ECM occurs in two basic forms:-

1. **Interstitial matrix.**
2. **Basement membrane.**

ECM send signals controlling cell proliferation.

ECM occurs in two basic forms:

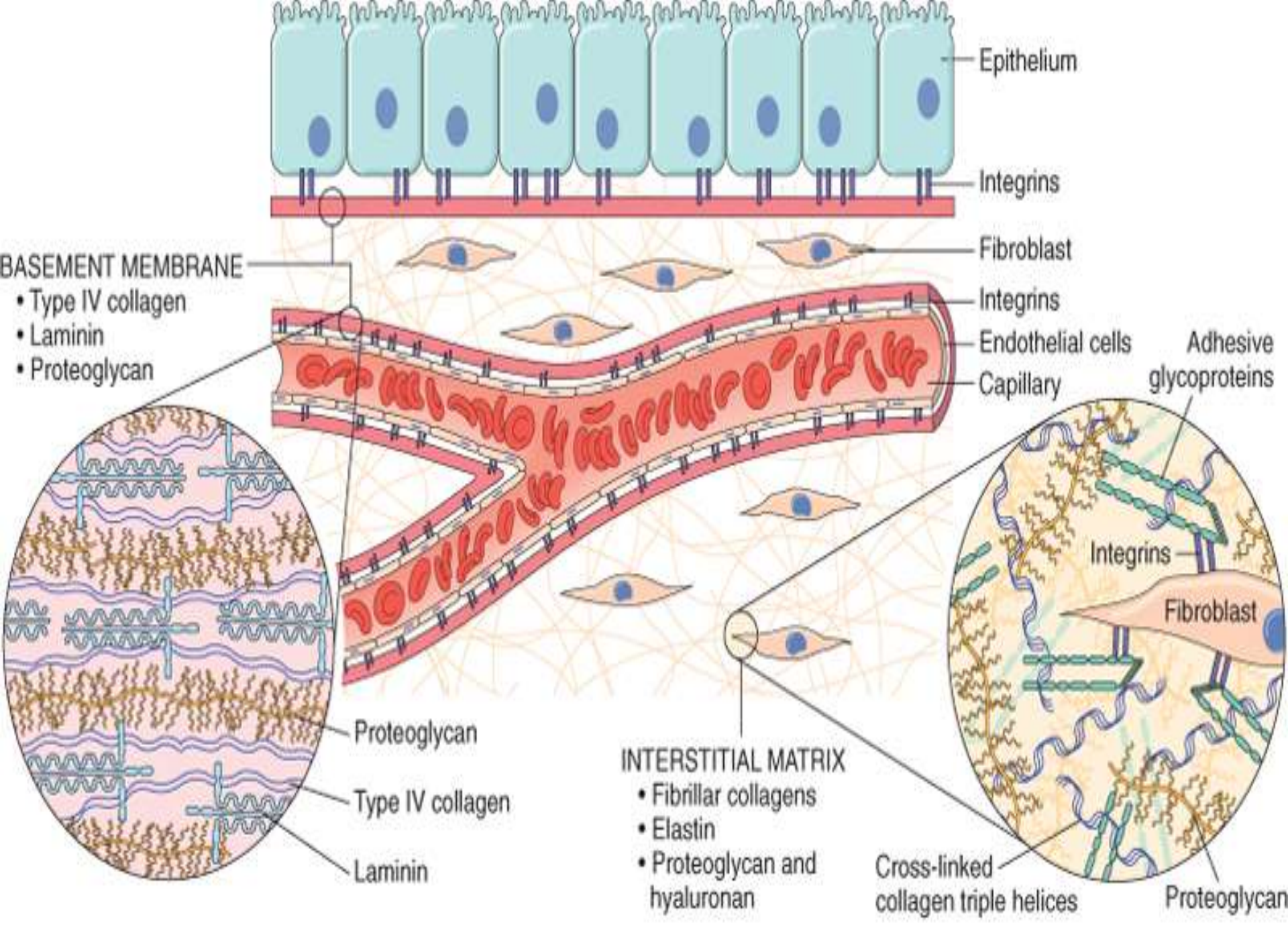
1. ***Interstitial matrix:***

- **Present in the spaces between mesenchymal (connective tissue) cells, and between epithelium and supportive vascular & smooth muscle structures.**
- **It is synthesized by the mesenchymal cells (e.g., fibroblasts).**
- **Its major constituents are:**

Collagens (fibrillar and nonfibrillar), Fibronectin, Elastin, Proteoglycans, Hyaluronate & Other elements.

2. Basement membrane:

- ***W****hich lies beneath the epithelium and is synthesized by overlying epithelium and underlying mesenchymal cells*
- **Its major constituents are amorphous nonfibrillar type IV collagen and laminin.**



Functions of the ECM

1. ***Mechanical support*** **for cell anchorage and migration, and maintenance of cell polarity**

2. ***Control of cell growth*** **by signaling through cellular receptors**

3. ***Maintenance of cell differentiation*** **through the type of ECM proteins.**

4. ***Scaffolding for tissue renewal*** **: the maintenance of normal tissue structure requires a basement membrane or stromal scaffold.**

5. ***Establishment of tissue microenvironments:*** basement membrane acts as a boundary between epithelium and underlying connective tissue and also forms part of the filtration apparatus in the kidney.

6. ***Storage and presentation of regulatory molecules.*** For example, growth factors like FGF is excreted and stored in the ECM in some tissues. This allows the rapid release of growth factors after local injury, or during regeneration.

Components of the Extracellular Matrix

1. ***Fibrous structural proteins*** **(collagens and elastins).**

2. ***Water-hydrated gels*** **(proteoglycans and hyaluronan).**

3. ***Adhesive glycoproteins*** **(that include *fibronectin* and *adhesive receptors* (selectins, integrins and cadherins).**

Wound healing

Healing process in general involve:

(1) Inflammation

(2) Formation of granulation tissue

(3) ECM deposition and remodeling

Types of Wound healing

1. Healing by **First Intention** (primary union).

2. Healing by **Second Intention** (secondary union).

1. Healing by First Intention (primary union):

1- Healing of a clean, uninfected small wound.

2- Surgical incision approximated by surgical sutures.

In Primary union

Focal disruption of epithelial basement membrane continuity.

Death of few epithelial & connective tissue cells

epithelial regeneration > fibrosis.

(Very small scar is formed + minimal wound contraction)

Steps of primary union

<u>In the first step:</u>

The incision space

is filled with fibrin clotted blood

Then

It is rapidly invaded by granulation tissue

Then

Covered by new epithelium

Steps of primary union

Within 24 hours

Neutrophils:

migrate toward the fibrin clot.

Basal cells:

increased mitotic activity at the cut edge of the epidermis.

Steps of primary union

After 24 up to 48 hours

Epithelial cell migrate from both edges & proliferate along the dermis

+ depositing basement membrane components.

Cells meet in the midline beneath the surface cut Forming a thin but continuous epithelial layer.

Steps of primary union

By the 3rd day

1. **Neutrophils replaced by macrophages.**
2. **The granulation tissue progressively invades the incision space.**
3. **Collagen fibers are now evident at the incision margins.**
4. **Epithelial cell proliferation continues, forming a thick epidermal covering layer.**

Steps of primary union

By 5th day,

1. **Neovascularization reaches its peak**
2. **Collagen fibrils more abundant.**
   forming a bridge in the area of incision.
3. **The epidermis recovers its normal thickness**
   with surface keratinization.

Steps of primary union

in the Second week
Collagen continue to accumulation
Fibroblast continue to proliferation.

leukocyte infiltrate, edema, vascularity

collagen deposition

Regression of vascular channels

Blanching

Steps of primary union

By the end of the 1st month

Features of the scar:

1. **Formed of cellular connective tissue**
2. **Devoid of inflammatory cells**
3. **Covered by normal epidermis.**

2. Healing by Second Intention (secondary union):

Secondary union occurs in :

1. Extensive tissue loss (large wounds)
2. Chronic inflammation
3. Abscess formation
4. Ulceration

Healing by Second Intention

In Secondary union there is :

1. **Intense inflammatory reaction.**
2. **Abundant development of granulation tissue.**
3. **Large scar formation.**
4. **Wound contracts by action of myofibroblasts.**

2. Healing by Second Intention (secondary union):

Begins within 24 hours of injury

Start by Emigration of fibroblasts

Induction of fibroblast and endothelial cell proliferation.

2. Healing by Second Intention

3rd – 5th days:

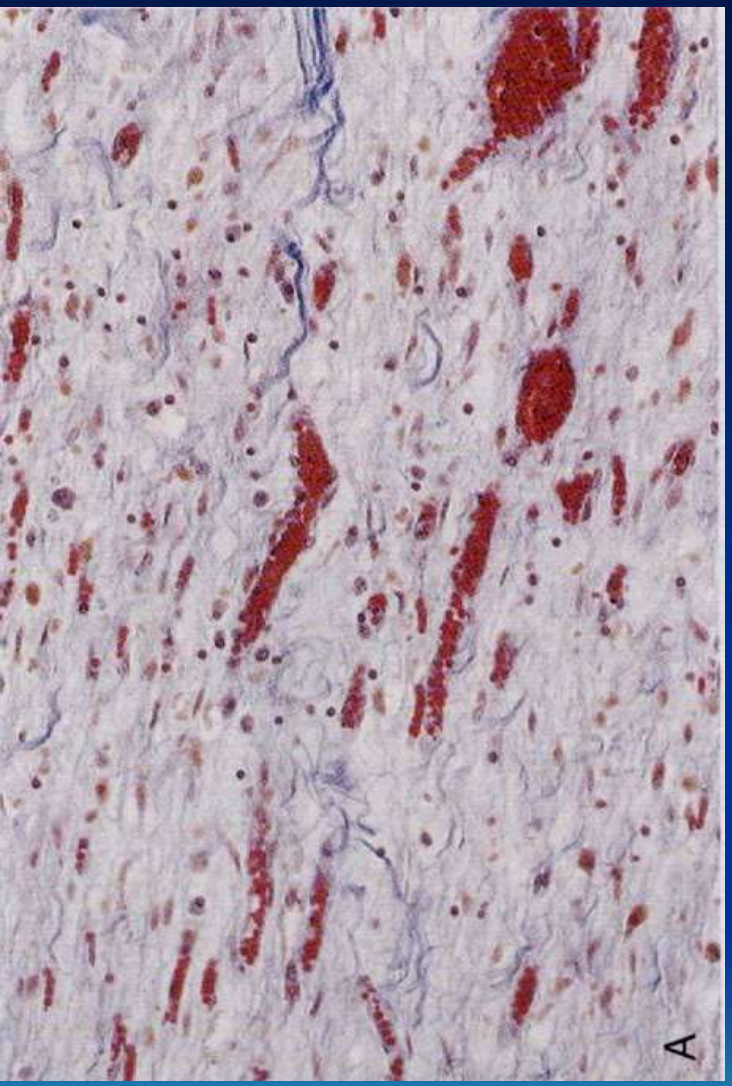
Granulation tissue formation:

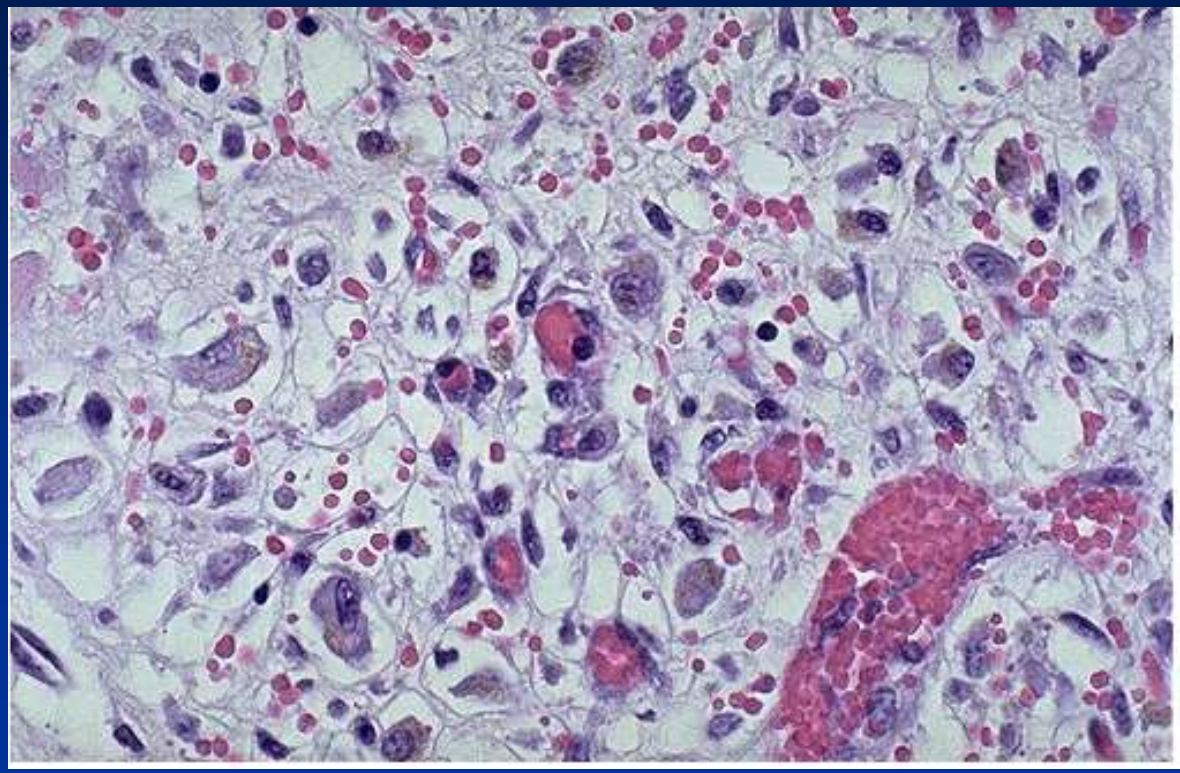
Specialized type of tissue that is characteristic of healing. The term granulation tissue derives from the pink, soft, granular gross appearance.

Histologic appearance:

1. Proliferation of fibroblasts
2. Formation of new thin-walled, delicate capillaries (angiogenesis)
3. Loose ECM

Granulation tissue formation in wound healing





Rt. There are numerous blood vessels, edema, and a loose extracellular matrix containing occasional inflammatory cells.

Lt. at high magnification, granulation tissue has capillaries, fibroblasts, and a variable amount of inflammatory cells.

Steps of scar formation

Repair by connective tissue deposition consists of four sequential processes:

1- Formation of new blood vessels **(angiogenesis)**

2- Migration & proliferation of **fibroblasts** with deposition of ECM.

3- Maturation and reorganization of the fibrous tissue **(remodeling)**

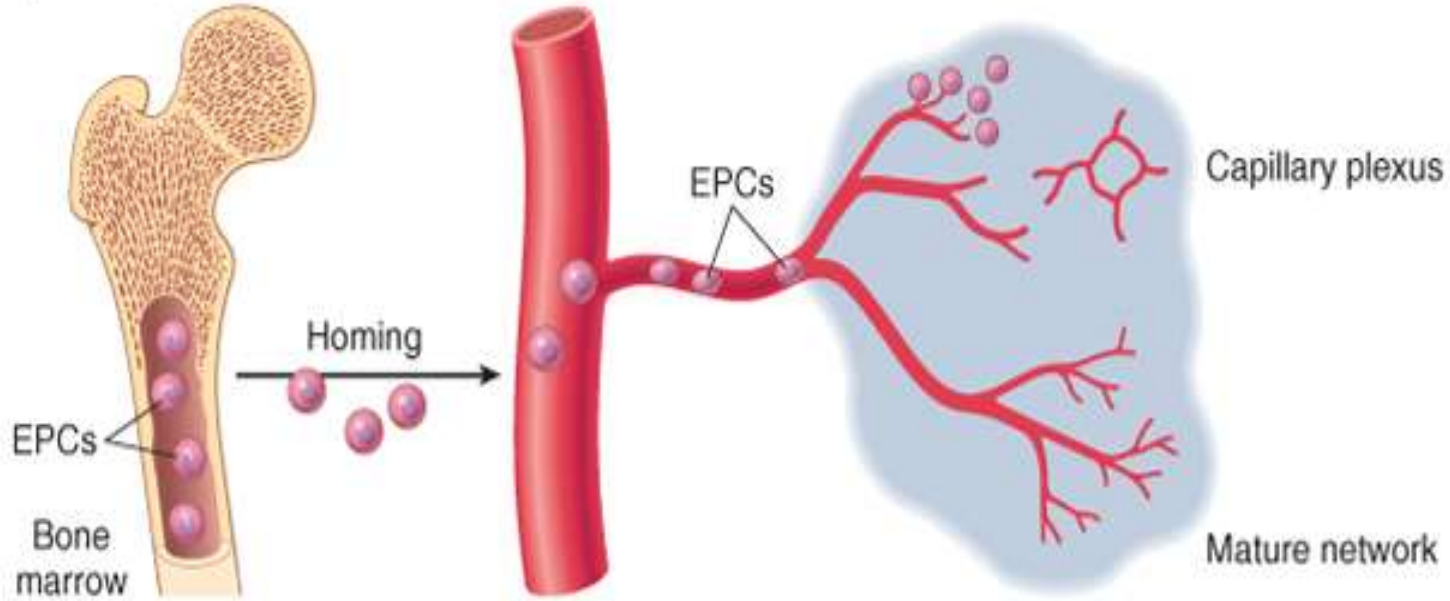
4- Wound contraction.

1- Formation of new blood vessels:

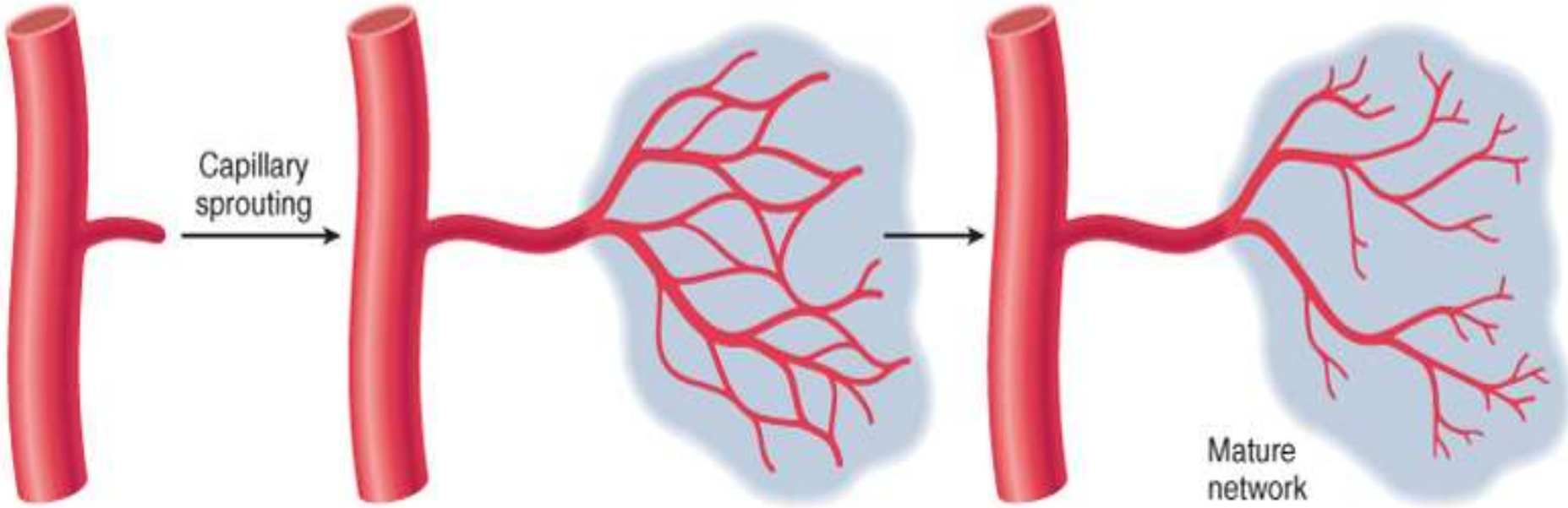
1. From preexisting vessels that send out capillary sprouts to produce new vessels.

2. B.M. may send Endothelial precursor cells which migrate to the areas of injury & participate in angiogenesis.

A. Angiogenesis by mobilization of EPCs from the bone marrow



B. Angiogenesis from preexisting vessels



1. Angiogenesis (neovascularization)

- **New vessels formed during angiogenesis are leaky. This leakiness explains why granulation tissue is often edematous.**

- **Several factors induce angiogenesis, but the most important are *VEGF* and *basic fibroblast growth factor (FGF-2).***

2. Migration of Fibroblasts & deposition of ECM

(1) Migration and proliferation of fibroblasts:

A- formation of a framework of new vessels and loose ECM

B- Recruitment & stimulation of fibroblasts.

C- The dominant leukocyte is Macrophage which:

1- Clearing extracellular debris & fibrin.

2- Elaborate mediators that induce fibroblast proliferation and ECM production.

D- Other leukocytes; mast cells, and lymphocytes contribute to fibroblast proliferation and activation.

2. Migration of Fibroblasts

- **The recruitment and stimulation of fibroblasts is driven by many growth factors,**

including PDGF.

(2) Deposition of ECM by fibroblast:

With progress of healing

↓ number of proliferating fibroblasts & new B.V.

Fibroblasts → synthetic phenotype

↑ deposition of ECM.

Structure of Scar

Firstly scar is formed of:

- Inactive, spindle-shaped fibroblasts.
- Dense collagen.
- Fragments of elastic tissue.
- Other ECM components.

Mature scar is characterized by:

- Vascular regression convert the scar into a pale avascular tissue.

3- Maturation and reorganization of the fibrous tissue (remodeling)

Degradation of ECM components need:

Metalloproteinases (MMPs) enzyme.

Which is produced by:

(fibroblasts, macrophages, neutrophils, synovial cells, and some epithelial cells)

4. Wound contraction:

1. Occurs within 6 weeks
2. Reduced large skin defect into 5% to 10% of their original size.

Myo-fibroblast:

Modified fibroblasts have many of the ultrastructural and functional features of contractile smooth muscle cells.

Differences between primary & Secondary healing

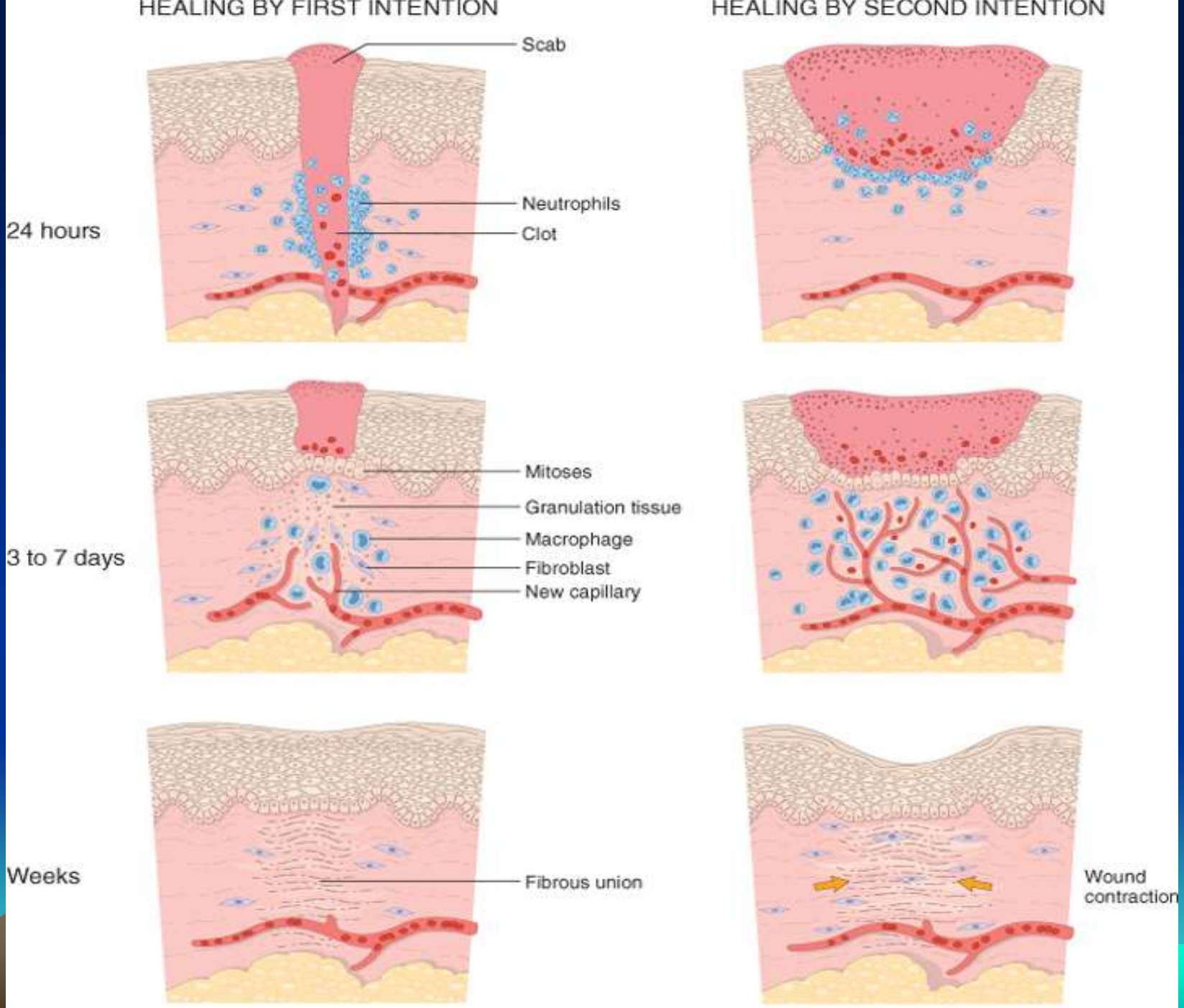
1- A larger clot rich in fibrin & fibronectin forms at wound surface.

2- More inflammation because of the large tissue defects with a greater volume of necrotic debris, exudate, and fibrin.

3- More chance for secondary infection due to the large defects.

4- Larger amounts of granulation tissue are formed to fill gaps & provide the underlying framework for the re-growth of epithelium.

5- A greater volume of granulation tissue generally results in a greater mass of scar tissue.



Types of repair according to the type of the tissue

Labile cells: (B. M., GIT & skin epithelium)

Repair by regeneration

Proliferation and differentiation of stem cells

Types of repair according to the type of the tissue

Stable cell (Parenchymal organs):

Regeneration is a limited process occur only if the residual tissue is structurally & functionally intact.

Extensive tissue damage end with scarring.

Types of repair according to the type of the tissue

Repair by connective tissue: (scar formation)

1- Severe or chronic tissue injury.

There is damage to:

- Parenchymal cells
- Epithelium
- Stromal framework.

2- Injury to the perminant cells (nondividing cells)

CUTANEOUS WOUND HEALING

- **This is a process that involves both epithelial regeneration and the formation of connective tissue scar and to general principles of wound healing in all tissues.**

Cutaneous wound healing has three main phases:

- ***inflammation***
- ***formation of granulation tissue***
- ***ECM deposition and remodeling***

- ***Based on the nature of the wound, the healing of cutaneous wounds can occur by first or second intention.***

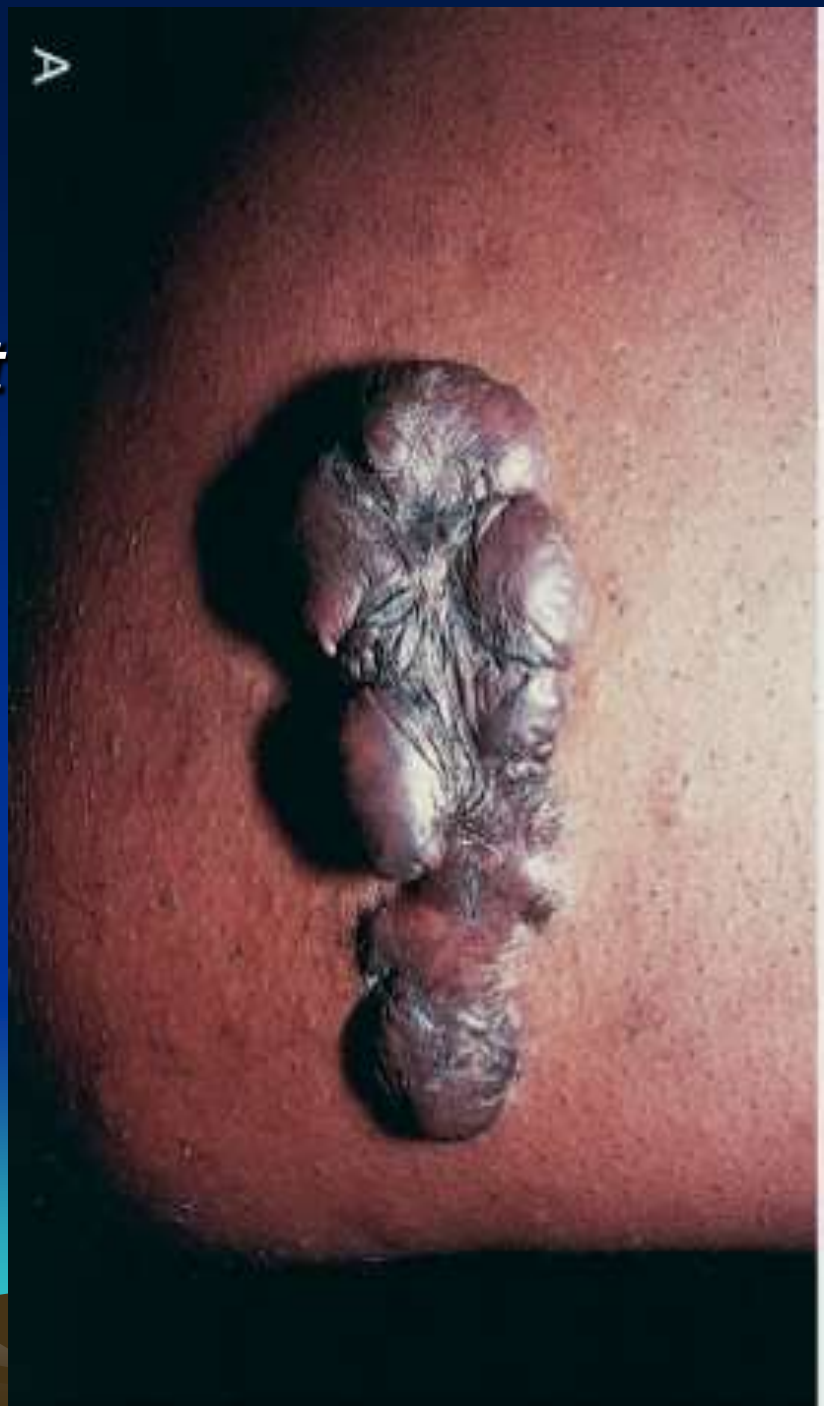
Aberrations of cell growth & ECM production

This may occur even in what begins as normal wound healing.

1. ***Keloid*** **: is the accumulation of exuberant amounts of collagen that give rise to prominent, raised scars.**

Heritable predisposition exists.

It is commoner in blacks.

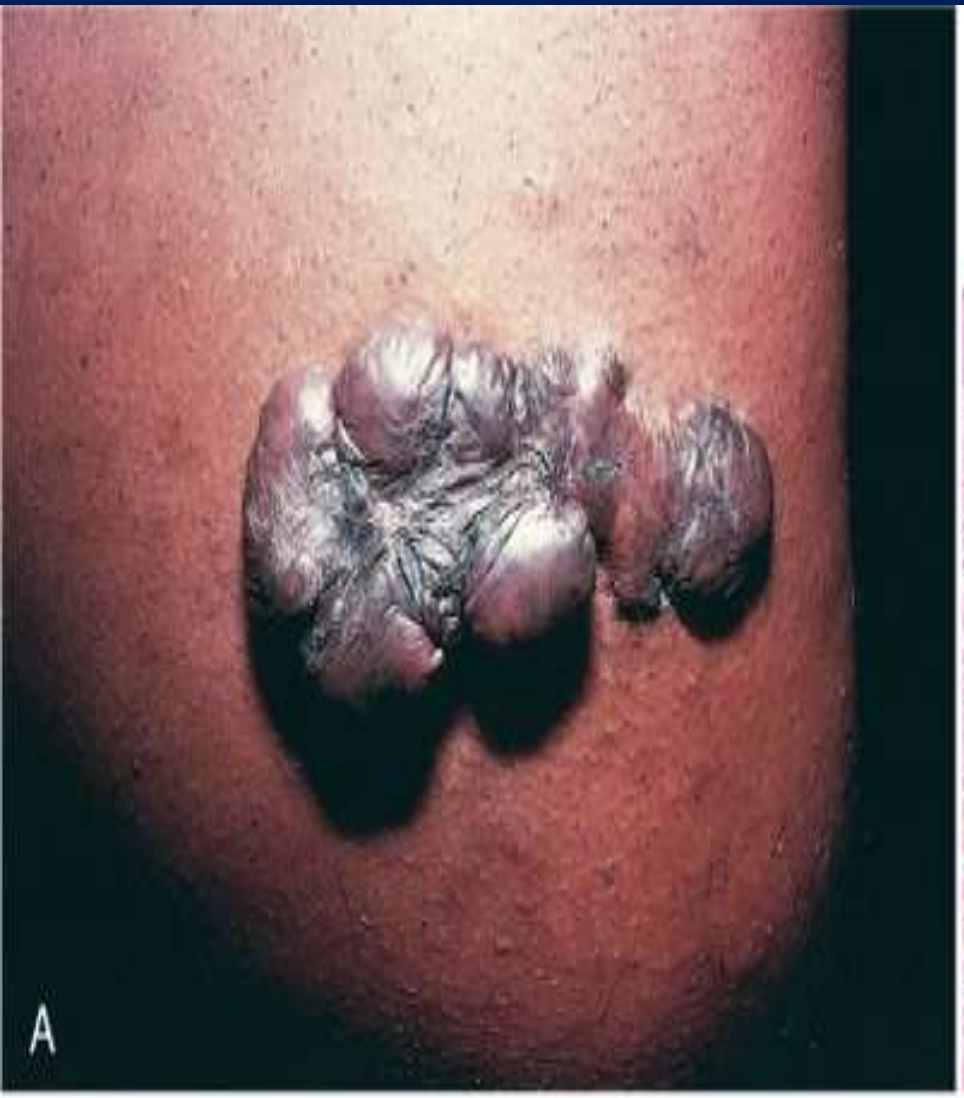


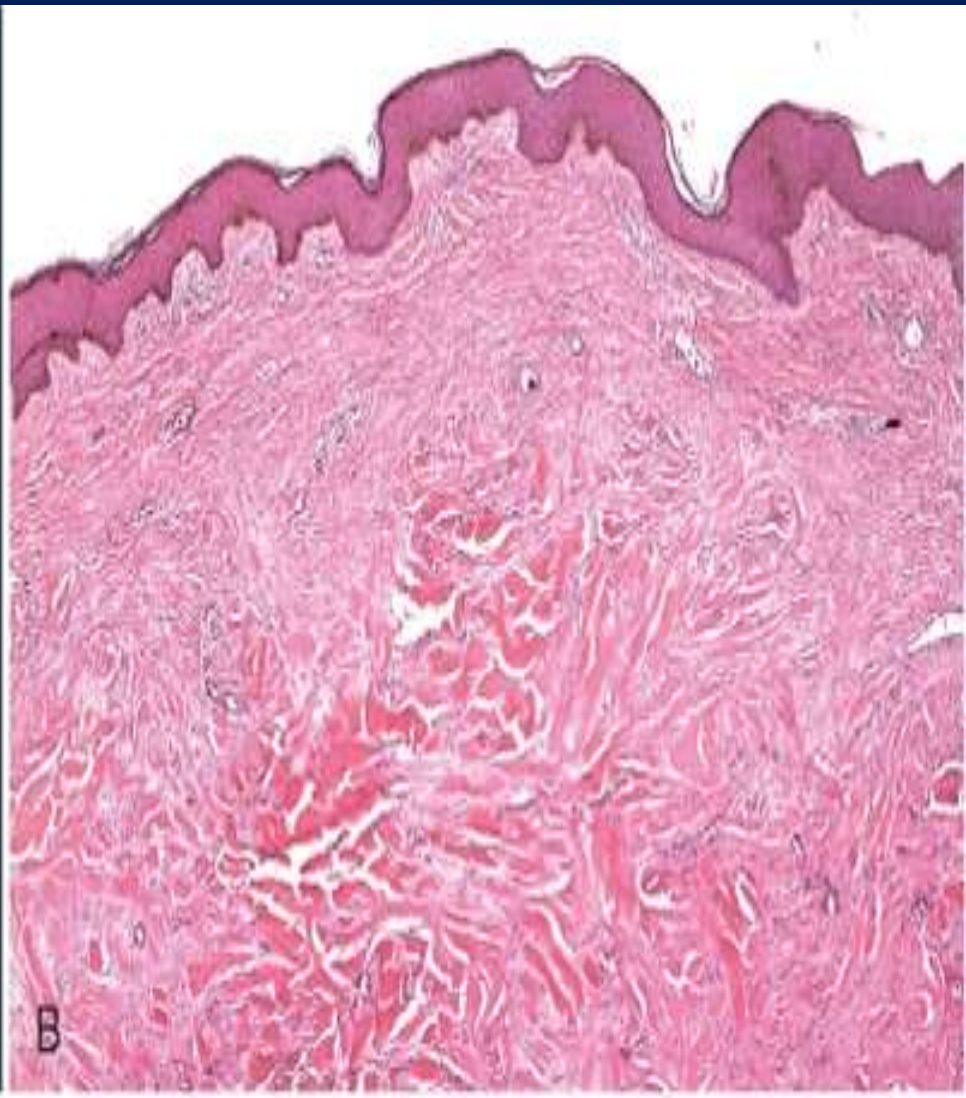
Keloids:

The accumulation of large amounts of collagen that give rise to prominent, raised scars occurred due to aberrations of cell growth and ECM production.

More common in blacks.

Keloids:



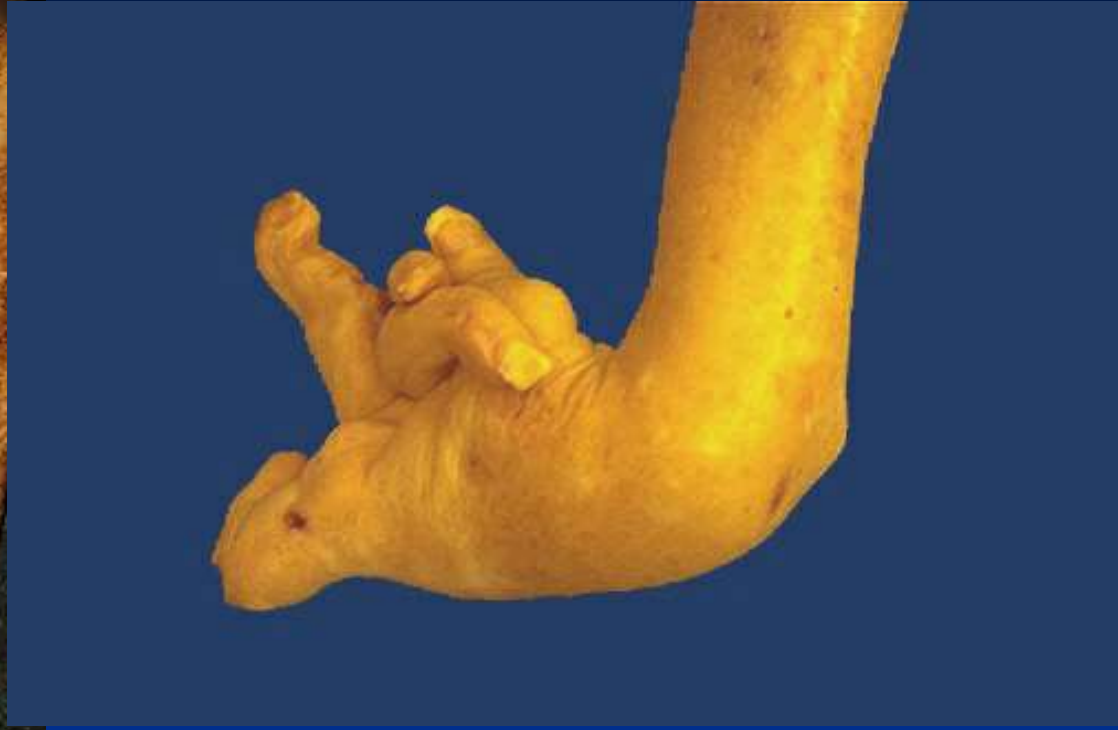


2. Exuberant granulation:

healing wounds may also generate excessive granulation tissue that protrudes above the level of the surrounding skin and hinders re-epithelialization.

The restoration of epithelial continuity requires cautery or surgical resection of the granulation tissue.

3. ***Disabling fibrosis*** **is associated with chronic inflammatory diseases such as rheumatoid arthritis, pulmonary fibrosis and liver cirrhosis have many similarities to those involved in normal wound healing. In these diseases, however, persistent stimulation of fibrogenesis results from chronic immune reactions that sustain the synthesis and secretion of growth factors, fibrogenic cytokines, and proteases.**



rheumatoid arthritis

autoimmune disease leads to synovial proliferation and joint destruction, typically in a symmetrical pattern involving small joints of hands and feet, followed by wrists, ankles, elbows, and knees. Rheumatoid factor can be identified serologically in most, but not all, RA patients.

Factors causes delay healing

1- **Infection:** **Prolongs the inflammation + increases the local tissue injury**.

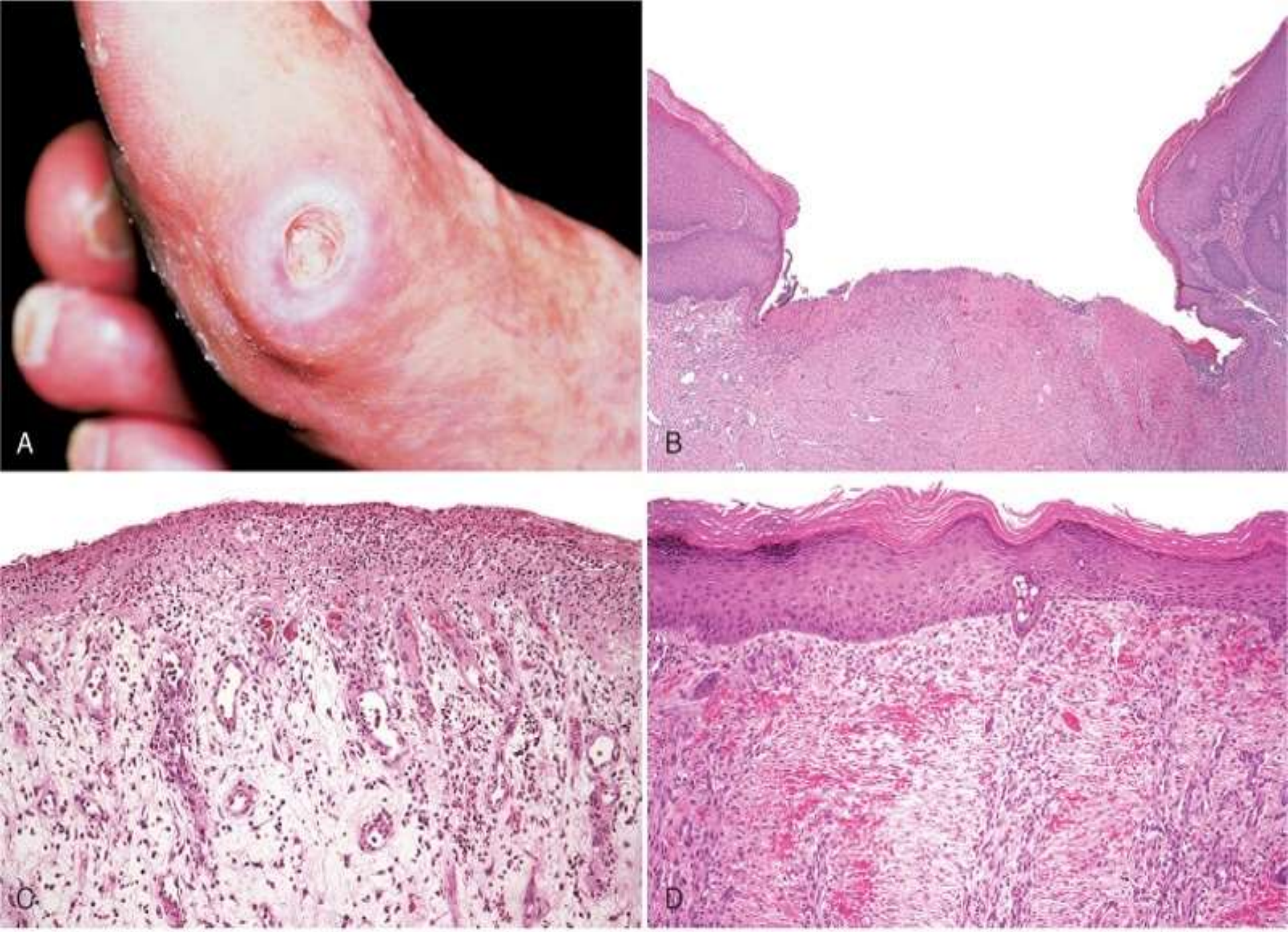
2- **Nutrition deficiency**: **Vitamin C deficiency, inhibits collagen synthesis**.

3- **Glucocorticoids: S**teroids = anti-inflammatory

4- **Mechanical injuries:** Increased local pressure, torsion.

5- **Diminished blood supply:** Arteriosclerosis, diabetes or to obstructed venous drainage

6- **Foreign bodies**: Fragments of steel, glass, or fractured bone.



HEALING OF BONE FRACTURES

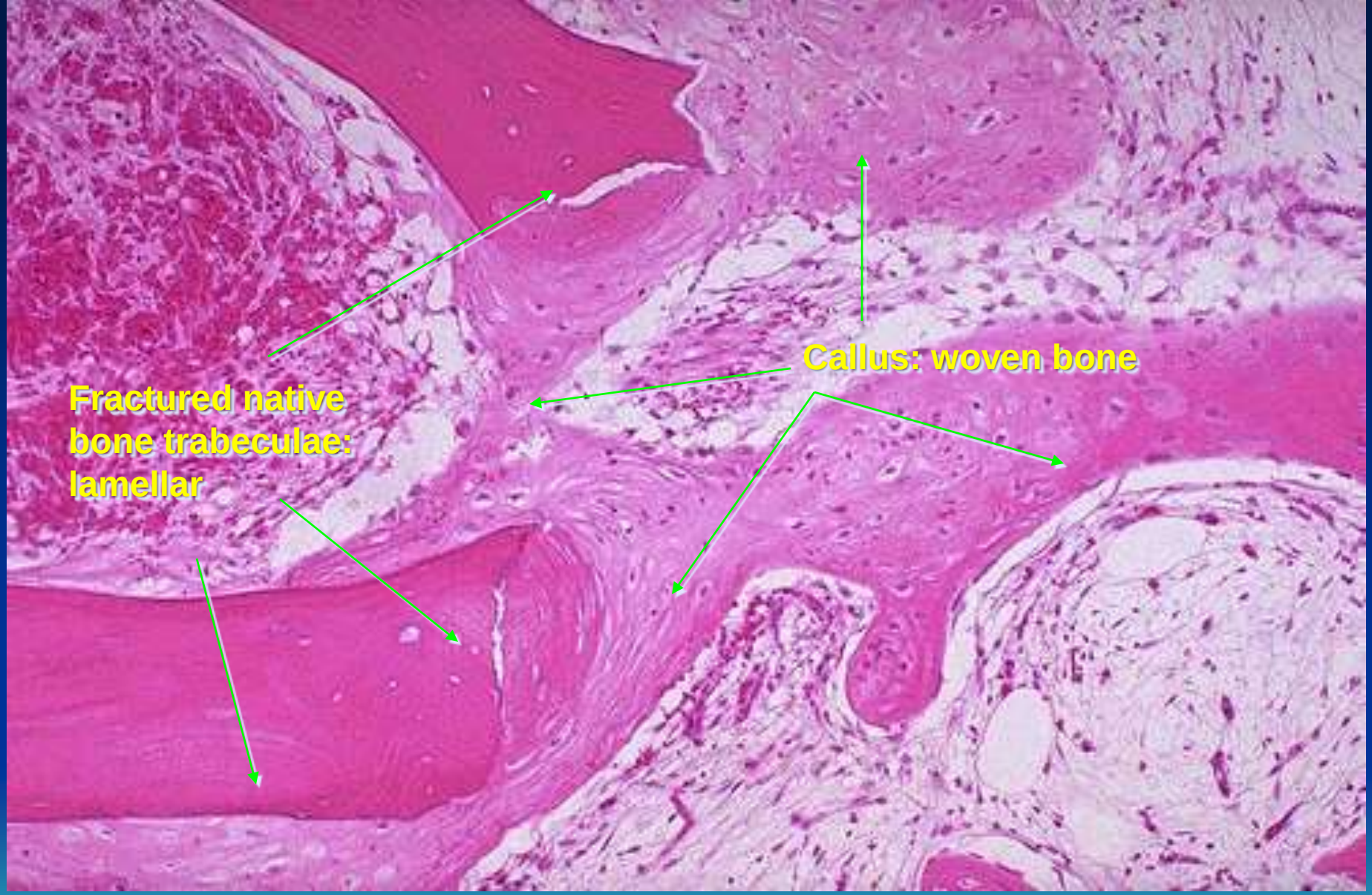
Bone fracture is caused by physical trauma, leading to discontinuity of the bone. The separation of fractured ends may be **complete** or **incomplete**. The latter is common in young children and called **greenstick fracture**. The fracture may be a **closed** one i.e. with an intact overlying skin or **open** i.e. the overlying skin is also injured so that the fractured bone is exposed through a gaping wound. A **comminuted** fracture is the one in which the bone is divided into multiple fragments.

Fracture healing

- Due to tearing of blood vessels in the medullary cavity, cortex and periosteum, a hematoma forms at the site of fracture. The periosteum is stripped off form the bone surfaces. The bone with haemopoietic marrow around the fracture site undergoes ischemic necrosis. Bone death is recognized histologically by loss of osteocytes from lacunae (empty lacunae).

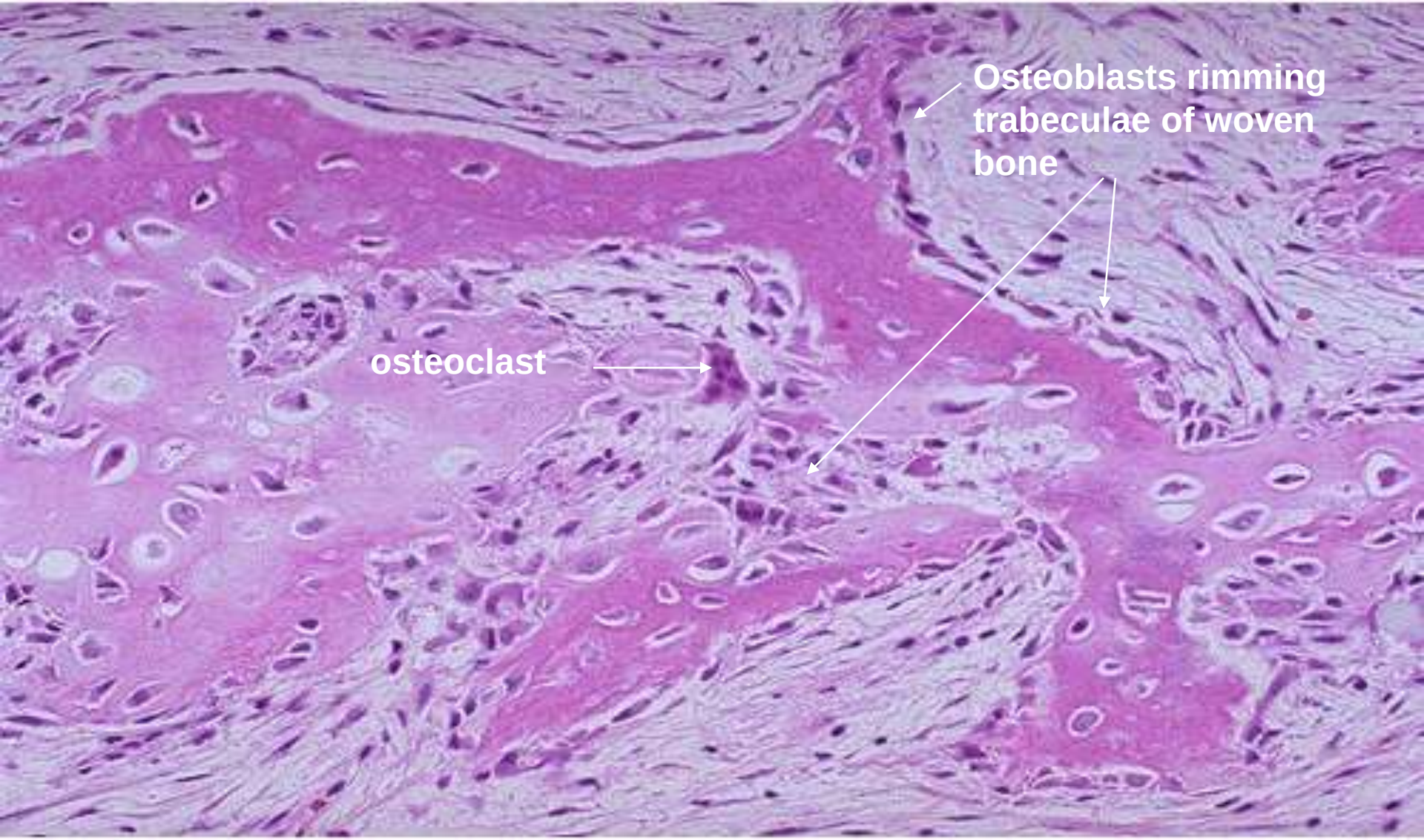
- **Organization of the hematoma is associated with a local inflammatory response. Neutrophils & macrophages phagocytose the hematoma & necrotic debris.**
- **This is followed by in-growth of capillaries & fibroblasts, producing fibrovascular granulation tissue**
- **At the end of the 1st week** Osteoblasts derived from osteoprogenitor cells of the inner layer of the periosteum will migrate into the granulation tissue and deposit large quantities of osteoid in a haphazard way, producing a **woven bone** pattern.
- **External callus** is thus formed by the periosteum and tends to immobilize the bone fracture site. The two enlarging cuffs of callus advance towards each other until finally unite to bridge the fracture gap outside externally.

- If there is a significant gap between the bone ends, it may induce cartilage formation. The
- **The internal callus is derived from endosteal osteoprogenitor cells** bridges the fracture from within the medullary cavity, and rarely contains cartilage due to better vascularization.
- The cartilaginous component of calus is converted to bone by endochondral ossification
- Callus is usually formed by the 3rd week after the incident of fracture, but the **initial bony union is by woven bone, which is mechanically weak.**
- The amount of periosteal callus formed (external callus) depends on the site of fracture & the degree of immobilization. It tends to be abundant in poorly immobilized fracture e.g. clavicle & ribs.



In this region of a **recent fracture**, **callus is seen forming at the broken ends** of bony trabeculae that extend to the center from the left

Here is a region of fracture with remaining disrupted trabeculae at the left and bottom. The new bone is forming at



This is irregular new bone, or woven bone, which is forming in the region of a fracture. Osteoblasts are seen lining the irregular trabeculae, and there is an osteoclast near the center.

Remodeling of callus occurs once the defect between the two bone ends is bridged by bony callus, so the newly laid down bone is reconstructed to restore full mechanical strength.

- The newly formed woven bone is resorbed and gradually replaced by **lamellar bone** (compact).
- The cortex is re-formed across the fracture gap & gradually the medullary callus is removed with restoration of the marrow cavity.
- **Remodeling is done by the osteoblasts & osteoclastes.**
- The whole reparative process may take about a year, although the time varies from site to site. It is also more rapid and more complete

Factors Affecting Fracture Healing

- These are basically similar to those of affecting healing in general. However, **mobility of fracture ends** and **mal-alignment** play a detrimental role in interfering fracture healing.

  Vitamin D deficiency leads to abundant callus, which fails to calcify & remains soft.

Complications of fractures

- **Delayed union,** after fibrous union, bony conversion is slow.
- **Non-union,** in which the fractured bone ends do not join by bone. This occurs if the fibrous tissue becomes very dense.
- **Fat embolism**, which may follow damage to the bone marrow. In such cases globules of fat embolize to such sites as the lungs, brain, and kidneys with the ultimate result of ischemic necrosis (infarction).
- **Osteo-necrosis**; this refers to local bone necrosis after fracture. It may occur depending on local peculiarities of the blood supply, e.g. fracture of femoral neck is often followed by osteonecrosis of the femoral head.
- **Osteoarthritis;** this degenerative joint disease may occur when the fracture line has involved the articular surface that result in the production of an incontinuity of the articular cartilage.

Pathological Fracture

- Fracture of normal bone require considerable trauma.
- However, trivial trauma may cause fracture when the underlying bone is diseased e.g. presence of **osteoporosis** (reduced bone mass) that occurs in the elderly may be associated with pathological fractures particularly in the femur & vertebral column. **Osteomalacia** (vitamin D deficiency lead to inadequate bone mineralization→ soft weak bone). **Primary or metastatic tumors** (from carcinoma of breast, bronchus, thyroid & kidney) may be associated with pathological fractures

THANK YOU