The end of the inflammatory process: Tissue regeneration- Healing

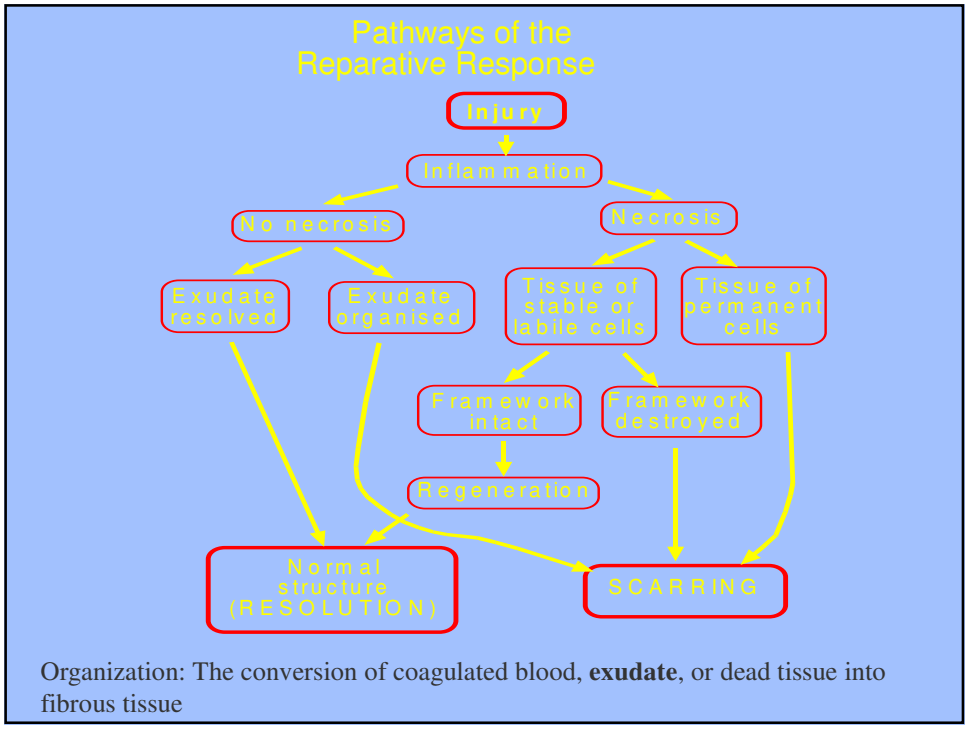
1

Wound Healing Process

The wound healing process leads to a restoration of the integrity and function of the tissue:

- injury elicits acute inflammation and demolition
- migration and proliferation of parenchymal and connective tissue cells
- synthesis of ECM proteins by fibroblasts and other cells
- regeneration of parenchymal cells into functional tissue
- remodeling of connective tissue and parenchymal cell components
- collagenization and development of wound tensile strength

2



Organization: The conversion of coagulated blood, **exudate**, or dead tissue into fibrous tissue

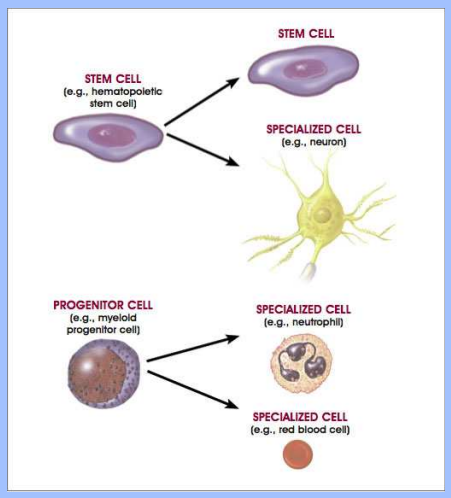
3

The ability of a tissue to rigenerate itself is dependent on the presence of :

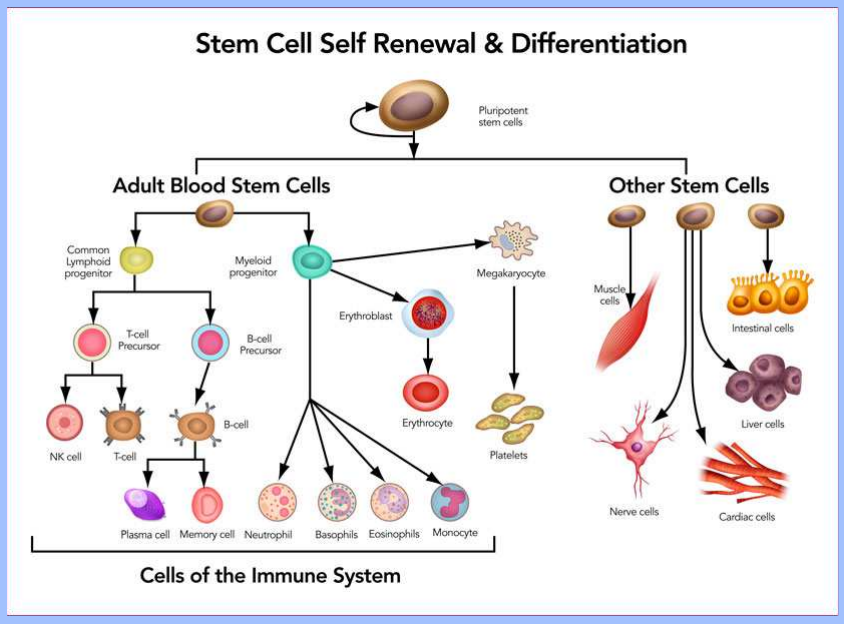
Stem cells (in particular Adult Stem cells), which are cells:

- Able to renew theirselves (symmetric division)
- Able to generate a progeny of cells that may differentiate into different tissue (asymmetric division)

4


STEM CELL
STEM CELL
(e.g., hematopoietic stem cell)
SPECIALIZED CELL
(e.g., neuron)
PROGENITOR CELL
(e.g., myeloid progenitor cell)
SPECIALIZED CELL
(e.g., neutrophil)
SPECIALIZED CELL
(e.g., red blood cell)

5

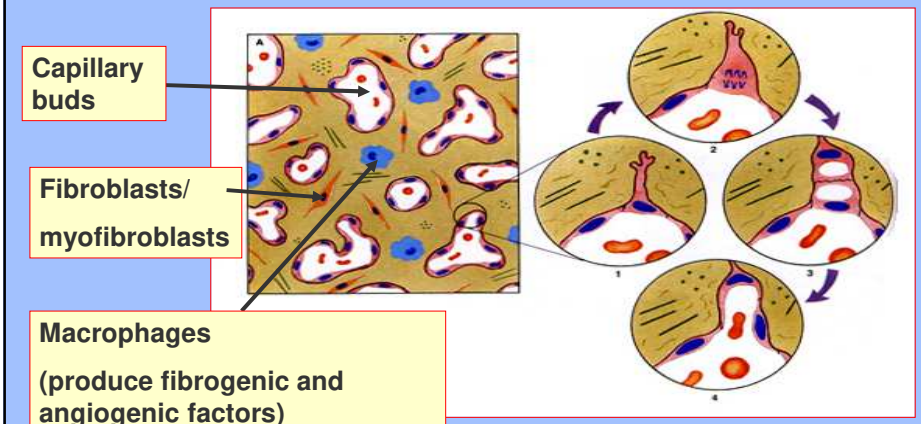

Stem Cell Self Renewal & Differentiation
Pluripotent stem cells
Adult Blood Stem Cells
Other Stem Cells
Common Lymphoid progenitor
Myeloid progenitor
Megakaryocyte
Erythroblast
T-cell Precursor
B-cell Precursor
Erythrocyte
B-cell
NK cell
T-cell
Platelets
Plasma cell
Memory cell
Neutrophil
Basophils
Eosinophils
Monocyte
Cells of the Immune System
Muscle cells
Intestinal cells
Liver cells
Nerve cells
Cardiac cells

6

Organization and repair

a) Debris removal by macrophages

b) Vascular granulation tissue



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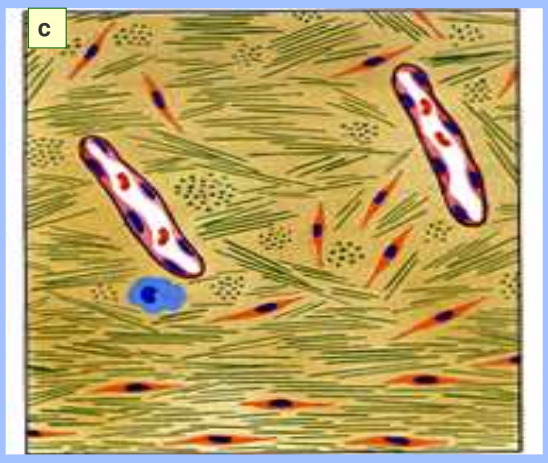
c) Vascular granulation tissue

(Fibroblasts proliferate and deposit collagen fibers)



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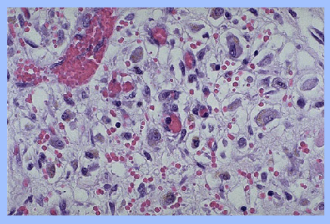
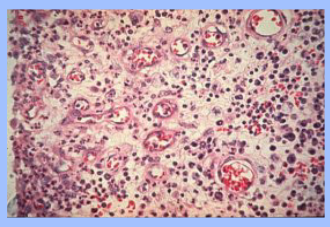
Granulation Tissue





The initial repair response to a wound, consisting of:

- richly vascular connective tissue (capillary sprouts (leaky new blood vessels)
- collagen
- reactive **fibroblasts** and myofibroblasts,
- variable numbers of inflammatory cells (especially macrophage)
- enzymes and mediators.

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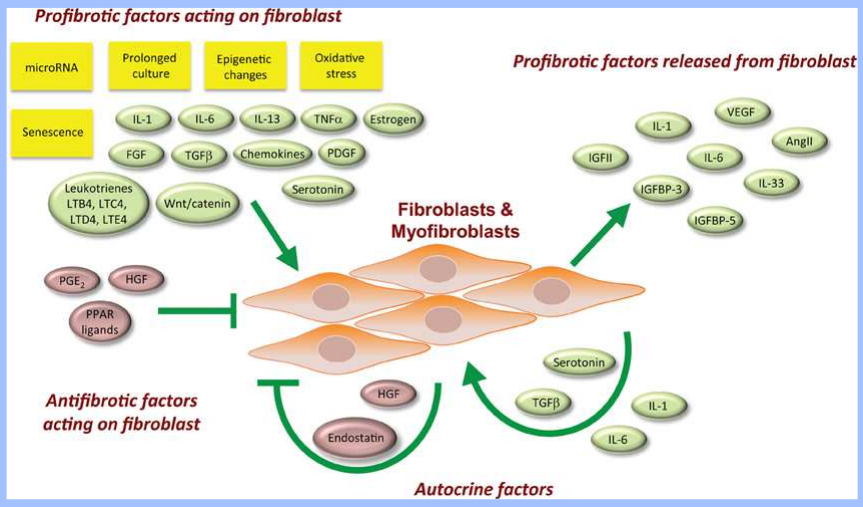
Fibroblasts in fibrosis



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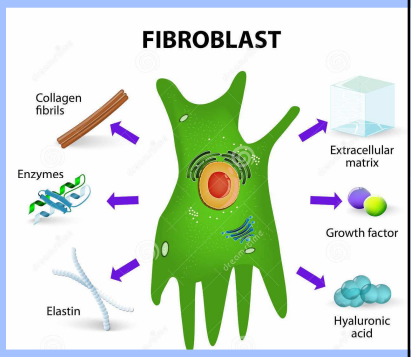
Fibroblasts

- Migration and proliferation of fibroblasts are stimulated by growth factors:
  - PDGF (platelet derived growth factor)
  - EGF (epidermal growth factor)
  - FGF (fibroblast growth factor)
  - TGF-B (transforming g. f. beta):
    - Monocyte chemotaxis
    - Fibroblast migration and proliferation
    - Increases synthesis of collagen, ECM, and fibronectin
    - decreases degradation of collagen and ECM
    - Growth inhibitor for most epithelial cell types



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REPAIR :

Extracellular Matrix

structural fibrous proteins : **collagen** **elastin**

Adhesion glicoproteins : **fibronectin** **laminin**

proteoglicans

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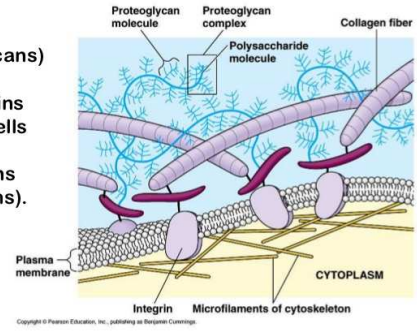
The **ExtraCellular** Matrix

The ECM contains 3 classes of molecules:
- structural proteins (collagens and elastins)
- protein-polysaccharide complexes to embed the structural proteins (proteoglycans)
- adhesive glycoproteins to attach cells to matrix (fibronectins and laminins).



- Stable complex of macromolecules underlying epithelial cells and surrounding connective tissue cells.
- Provides a structure into which replacement cells can come for wound resolution, creating order and tissue strength.
- Migrating cells attach to it.
- A special form of ECM forms basement membranes.

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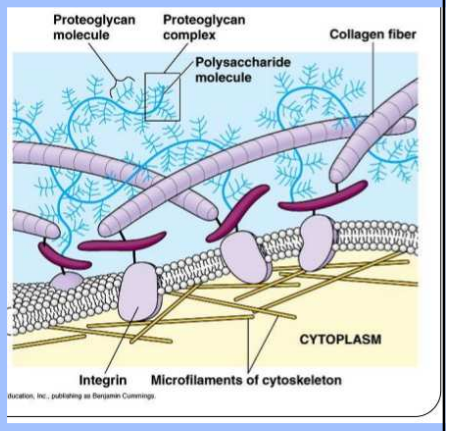
Extracellular Matrix (ECM) Composition

- **collagens** (10 types, vary in location):
  Type I - Bone, tendon, scars.
  Type III - 'tissue scaffold'.
  Type IV - non-fibrous, basement membranes ...

- **Matrix metalloproteases** (zinc dependent) degrade and remodel collagen
- **elastin** provides recoil after stretching for skin, etc.
- Glycoproteins (Fibronectin, Osteonectin, Tenascin..)
- Proteoglycans (e.g. Heparan sulphate proteoglycan..)
- laminin



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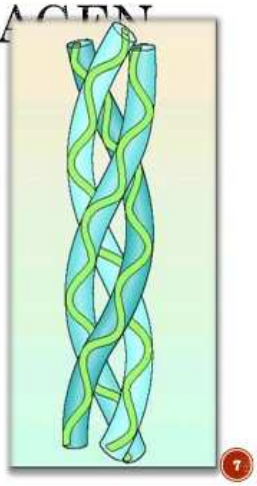
Collagen

STRUCTURE OF COLLAGEN

- All collagens are composed of 3 polypeptide alpha chains coiled around each other to form the tripe helix configuration.
- The α chains are left handed helices that wrap around each other into a right handed rope like triple helical rod.
- Each such helix is around 1.4 nanometers in diameter and 300 nanometers in length
- The triple helix may be of a continuous stretch or it may be interrupted by non collagenous elements.



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Collagen

1. intracellular procollagen is hydroxylated (Vit C dependent), glycosolated, secreted by fibroblasts
2. enzymatic modifications:
3. - terminal peptide chains are removed by procollagen peptidases
4. complete fibrils/fibers/bundles form in the extracellular space
5. cross linkages between alpha chains and adjacent molecules form by oxidation rxn over weeks
6. Wounds are filled with Type 3, later replaced by Type 1
7. Remodeling requires MMP (matrix metallo proteases)

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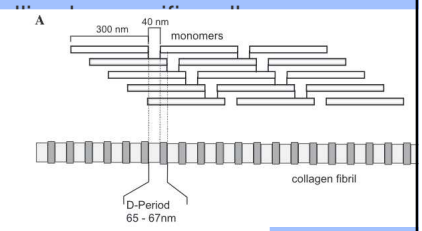
Collagens :

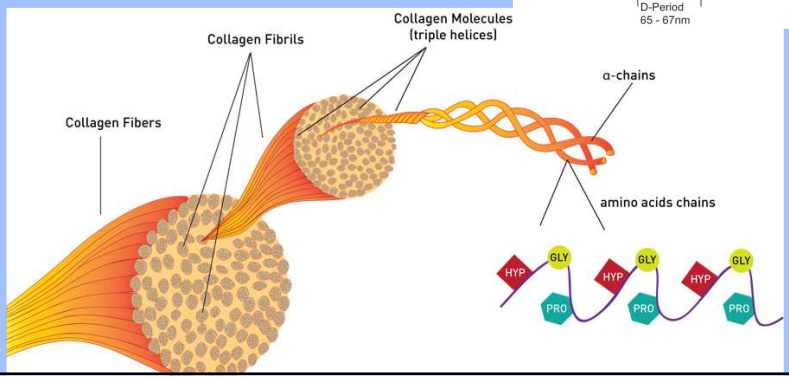
- Triple helical fibrils
- Fibrils arrange in 'quarter stagger' mode to form insoluble fibres
- Relatively resistant to general proteases; slow remod

Collagen Biosintesis:

Collagene molecules

Fibrils → fibers





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Fibronectins

- Derived from fibroblasts, monocytes, and endothelial cells
- Chemotactic for fibroblasts and macrophages
- opsonin
- Organizes endothelial cells into channels
- Cross links with fibrin, fibrinogen and collagen to increase tensile strength of collagen
- Binds to ECM components via integrin receptors
- Directly involved in attachment, spreading, and migration of cells

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Wound Healing

- **Proliferative phase** occurs after the neutrophils have removed cellular debris and release further cytokines acting as attracting agents for macrophages.
  - Fibroblasts now migrate into the wound, and secrete collagen type III.
  - Angiogenesis occurs by 48 hours.
  - The secretion of collagen, macrophage remodeling and secretion, and angiogenesis continues for up to 3 weeks.
  - The greatest increase in wound strength occurs during this phase.

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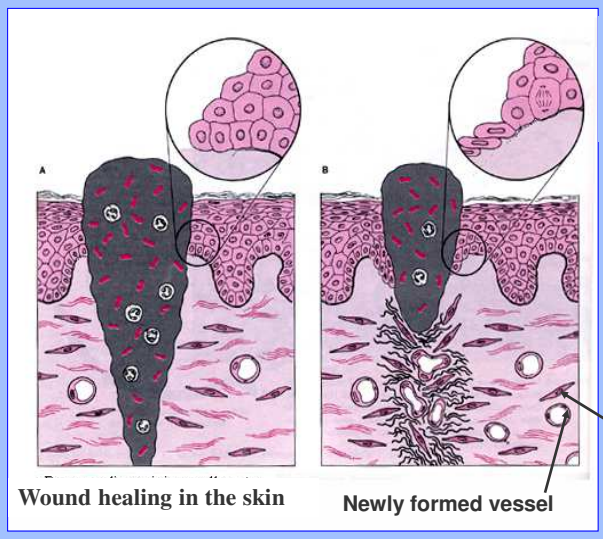

Degradation products of damaged cells, fibronectin, lysosomal enzymes, growth factors

chemiotaxis

Macrophages (removal of clot)

Fibroblasts/ miofibroblasts (collagen deposition)

fibrin → **Wound stabilization**

Plasmatic Fibronectin + **Extracellular Matrix Fibrin** → **Bridge between clot and tissue**

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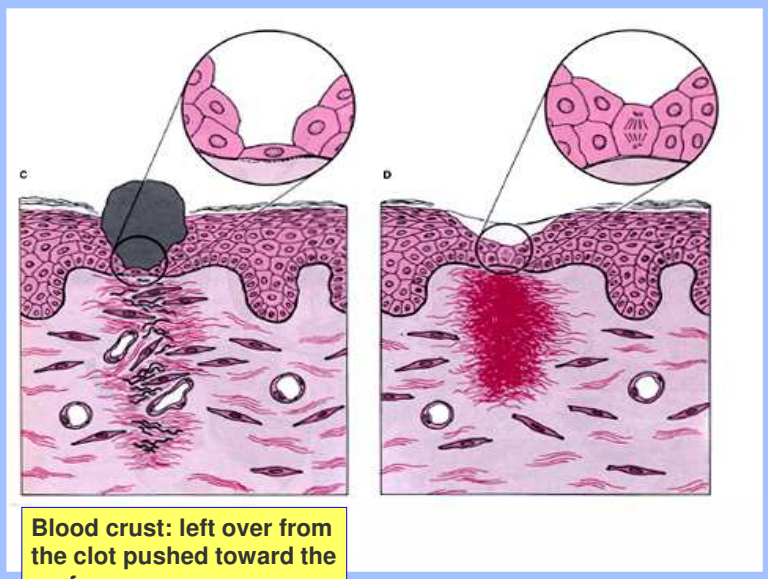

Blood crust: left over from the clot pushed toward the surface.

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Angiogenesis: New blood vessels

2-3 days after wounding, vascular proliferation in granulation tissue begins, lasting several days

induction by

- Fibroblast Growth Factor (FGF)
- Vascular permeability factor (VPF)
- Vascular Endothelial growth factor (VEGF)

phases in the process

- Proteolytic degradation of the basement membrane allowing for formation of a capillary sprout
- Migration of endothelial cells toward an angiogenic stimulus
- Proliferation of endothelial cells
- Maturation of endothelial cells and organization into capillary tubes

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Types of Cells and Repair potential

- Labile cells
  - replaceable from stem cells
  - e.g., squamous epithelium of skin, mucous membranes, bone marrow
- Stable cells
  - replacement requires forcing G0 cells to cycle
  - e.g. kidney tubule cells, hepatocytes, smooth muscle cells
- Permanent cells
  - nondividing, replaced by a scar or a cavity
  - e.g. brain

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SKIN WOUNDS

Classification of Wounds Closure

- **Healing by Primary Intention:**
  - All Layers are closed. The incision that heals by first intention does so in a minimum amount of time, with no separation of the wound edges, and with minimal scar formation.
- **Healing by Secondary Intention:**
  - Deep layers are closed but superficial layers are left to heal from the inside out. Healing by second is appropriate in cases of infection, excessive trauma, tissue loss, or imprecise approximation of tissue.
- **Healing by Tertiary Intention:**
  - Also referred to as delayed primary closure.

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SKIN WOUNDS: Healing by "Primary Intention"

- Epidermis regenerates
- Dermis undergoes fibrous repair.
- Sutures out at 5-10 days: approx. 10% normal strength.
- Maturation of scar continues up to 2 years.
- Minimal scarring, good strength
- Risk of trapping infection under skin - produces abscess.

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Healing by "primary union", or "first intention"

Early events during Days 1-3:

- Wound occurs: hemorrhage, clotting, cell injury, minor acute inflammation
- Neutrophils appear at margins.
- Mitotic epithelial cells at margins migrate across the wound
- Macrophages infiltrate the defect and break down fibrin. Thin surface epithelial layer forms by cell migration and fusion.
- Surface layer is thickening. Macrophage predominate.
- Vertical collagen fibrils appear along the margins.
- Granulation tissue progressively enters the tissue space

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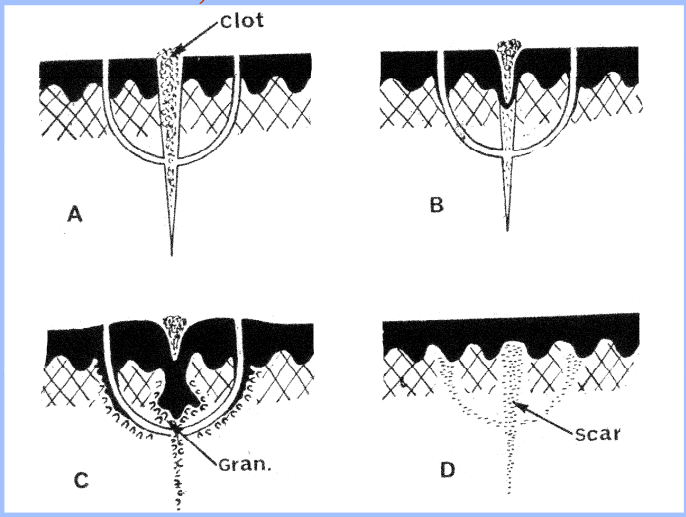
Healing by 'primary intention': A clean, sutured wound.



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Wound Healing:Week 1 and 2

Day 5: Wound space is filled with granulation tissue, collagen fibrils begin to bridge the site, surface epithelium is normal thickness and architecture, acute inflammation is regressing.

Day 7: Sutures are removed. Wound has 10% of tensile strength of normal skin.

Day 7-15: More fibroblast proliferation and collagen deposition follows the lines of tissue stress with an increase in wound strength; Leukocytic infiltrate, edema, increased vascularity decreases.

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Wound Healing: Final Stages

Day 30: Wound now is devoid of infiltrate, largely covered by intact normal epidermis; 50% strength, Type III collagen is slowly being replaced by Type I via action of collagenases and MMPs

3 Months: Wound is 80% of normal. Complete blanching of a scar takes longer

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Wound Healing

- **Maturation phase** is the final phase and starts from the 3rd week and continues for up to 9-12 months.
- This is where collagen III is converted to collagen I, and the tensile strength continues to increase up to 80% of normal tissue.

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SKIN WOUNDS: Healing by "Secondary Intention"

- Quantitative differences.
  - Initial contraction (in furry animals!)
  - Clot dries to form a 'scab' or ESCHAR
  - Epidermis regenerates beneath.
  - Repair process produces GRANULATION TISSUE
- Comparison with primary intention:
  - Takes longer
  - Produces a larger scar; not necessarily weaker
  - Produces more late contraction

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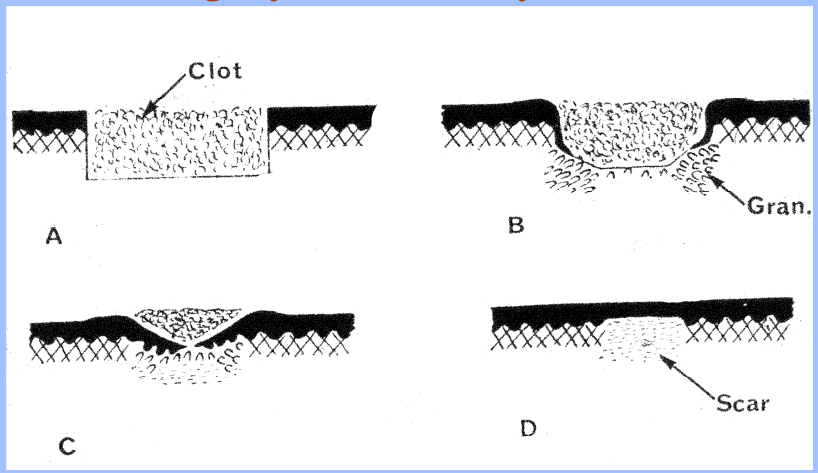
An open wound: Healing by 'secondary intention'



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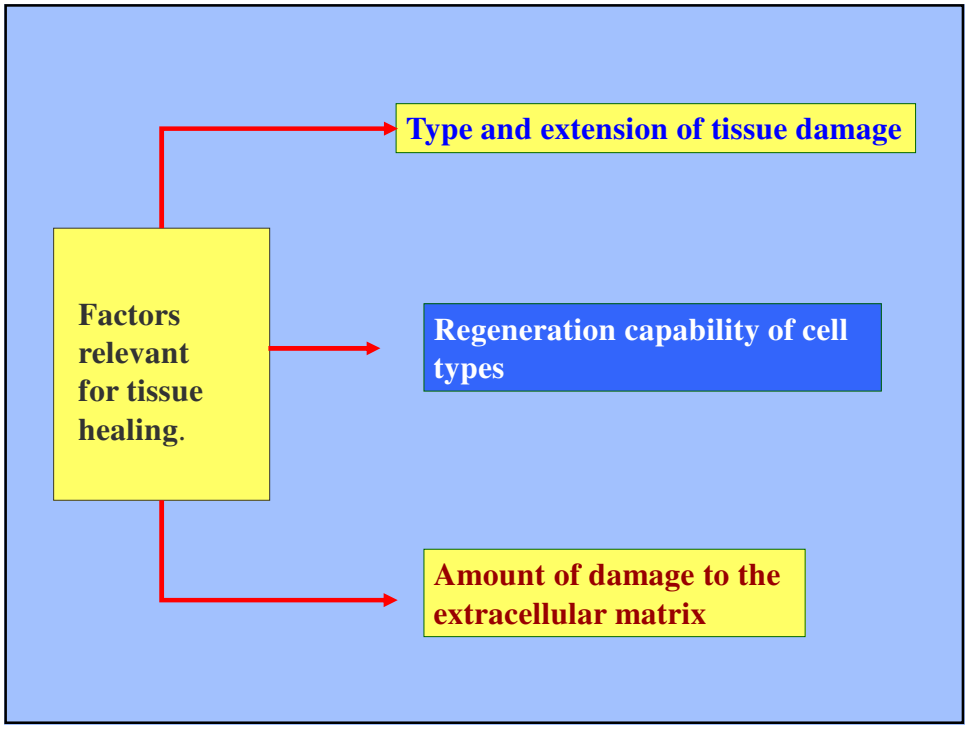

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Issues in Successful Resolution

- extent of tissue necrosis
  - Support stroma must be intact
- type of parenchymal cells involved
  - cells must be able to regenerate
- adequacy of demolition
  - debris and fibrin impair healing, induce scarring
- arterial perfusion and venous drainage
  - e.g., scaphoid bone (hand) has poor circulation
- approximation- rough or tight
  - Hint: evert wound edges

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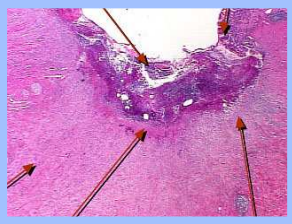
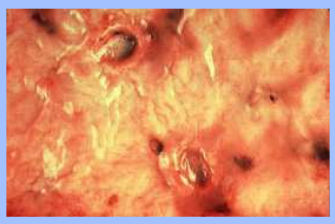
An Ulcer





- This slide shows in lining of the stomach with two punched out areas
- An erosion which extends down past the muscularis mucosa is an ulcer

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Occurrence of Larger Deficits

More extensive tissue loss means a loss of ECM upon which to pattern tissue architecture

Objective of the response:

- seal the surface, and when possible, fill the hole to preserve overall structure

Examples:

- e.g. ragged wound edges, ulcer or an infarct
- permanent parenchymal cells which cannot divide

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How is ‘secondary union’ different than primary?

- large gaps have more fibrin and more necrotic debris, thus more intense inflammatory reactions
- more granulation tissue occurs and thus more scarring
- patent wound contraction is possible, esp. in skin (to 90%) (myofibroblasts)
- result is healing by "secondary union, secondary intention”
- progression into chronic inflammation is possible

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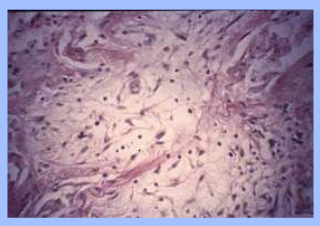
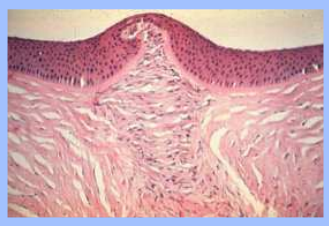
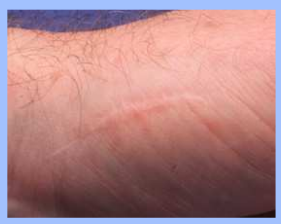
Scars



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Physiological impairments to normal healing

Physiological impairments weakening the immune system and healing:

- malnutrition or specific deficiency of Vitamin C, Copper, or zinc
- tissue hypoxia
- leukopenia (monocytes or neutrophils)
- Fibrinogen deficiency
- Diabetes
- severe anemia
- hormonal imbalances and various syndromes

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Other Interferences to Normal Healing

- **Infection** (most common), or **foreign bodies** can lead to "chronic inflammation"
- **Excessive contractures**, e.g. burns
- **Genetic and idiopathic predispositions** lead to improper healing
  - **Keloid**: genetically predisposed production of hypertrophic scar tissue (type III collagen).
  - **exuberant granulation** tissue formation (Proud flesh) protrudes above the skin and blocks re-epithelialization
  - **Desmoids** (aggressive fibromatosis) reoccurring proliferation of fibroblasts follows incision of scars or traumatic injuries

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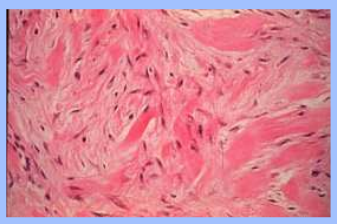
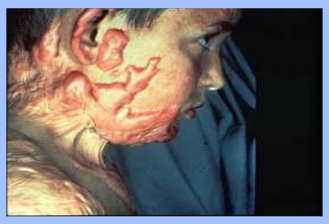
Keloid





- hypertrophic scarring
- an over abundant deposition of Type III collagen
- Influenced by genetic predisposition (is more common in blacks)

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Healing in various tissues :

Liver: **We can have complete parenchymal regeneration and/or fibrosis/scar**

Biopsy:	**cicatrization**
focal Necrosis:	**complete regeneration.**
massive Necrosis:	**lethal in few days, if survives the acute phase, residual epatocytes form irregular regeneration regions (nodules) separated by large scars (cirrhosis)**

Kidney : **limited regeneration capability:**
high regeneration in cortical tubules,
minimal in medullar tubules ,
absent in glomerula

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Lung: :

The lining epithelium has ample capacity for regeneration unless it has been damaged the underlying extracellular matrix.
If the damage involves Basal Membrane, repair occurs by scarring and fibrosis.

Heart :

Myocardial cells have not regenerative capacity; Tissue damages heal by deposition of granulation tissue and scarring.

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CNS :

Damage to the brain or spine leads to the proliferation of astrocytes and microglia.
Gliosis is equivalent to scarring in other tissues.

PNS :

Axonal regeneration is up to 2 weeks from the injury if there is alignment of the extremities.

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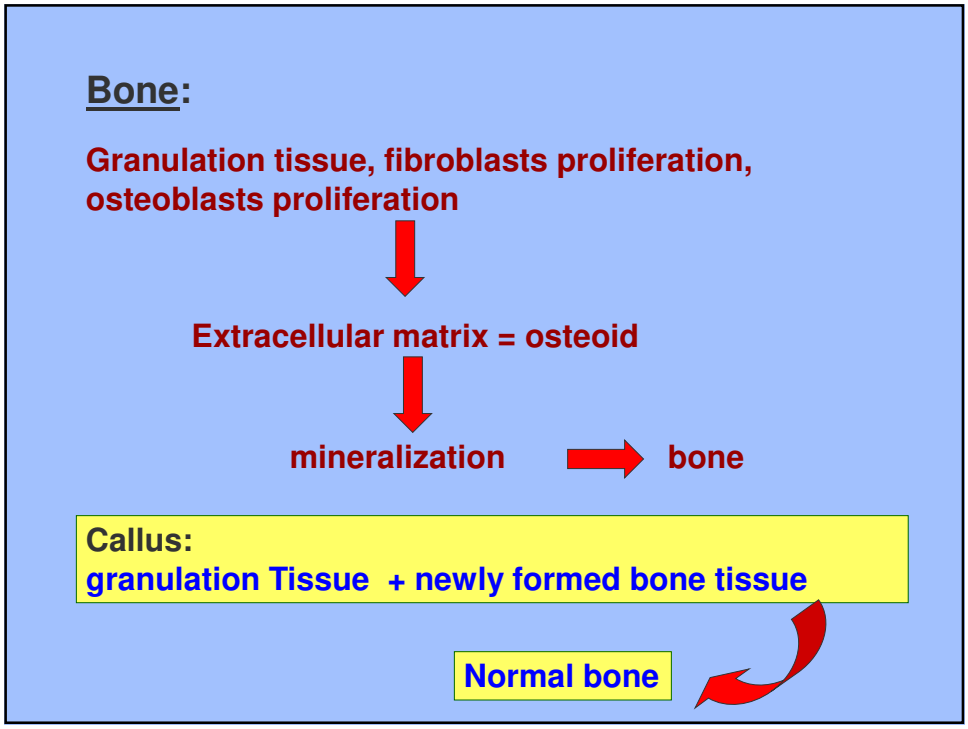
Bone:
Granulation tissue, fibroblasts proliferation, osteoblasts proliferation
Extracellular matrix = osteoid
mineralization
bone
Callus:
granulation Tissue + newly formed bone tissue
Normal bone

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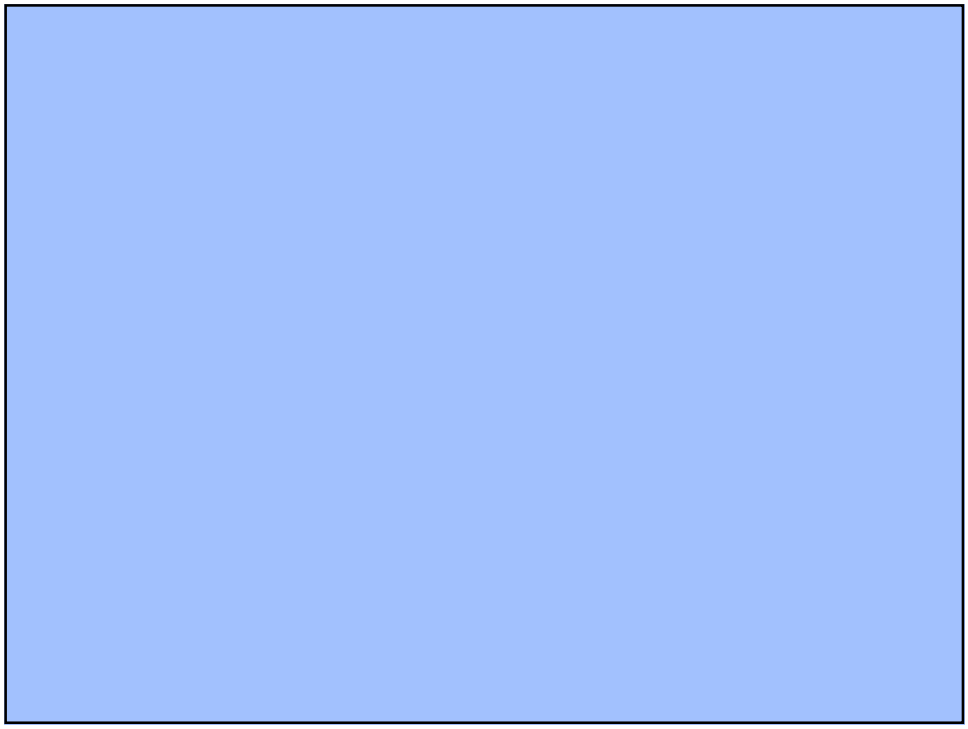

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Sum-up: Regeneration and Fibrous Repair/Replacement

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Tissue Repair: REGENERATION AND REPLACEMENT

- **Regeneration:**
  replacement of functional, differentiated cells: new growth completely restores portions of damaged tissue to their normal state

- **Replacement:**
  production of a fibrous scar: severely damaged or non-regenerable tissues are repaired by the laying down of connective tissue, a process commonly referred to as "scarring"

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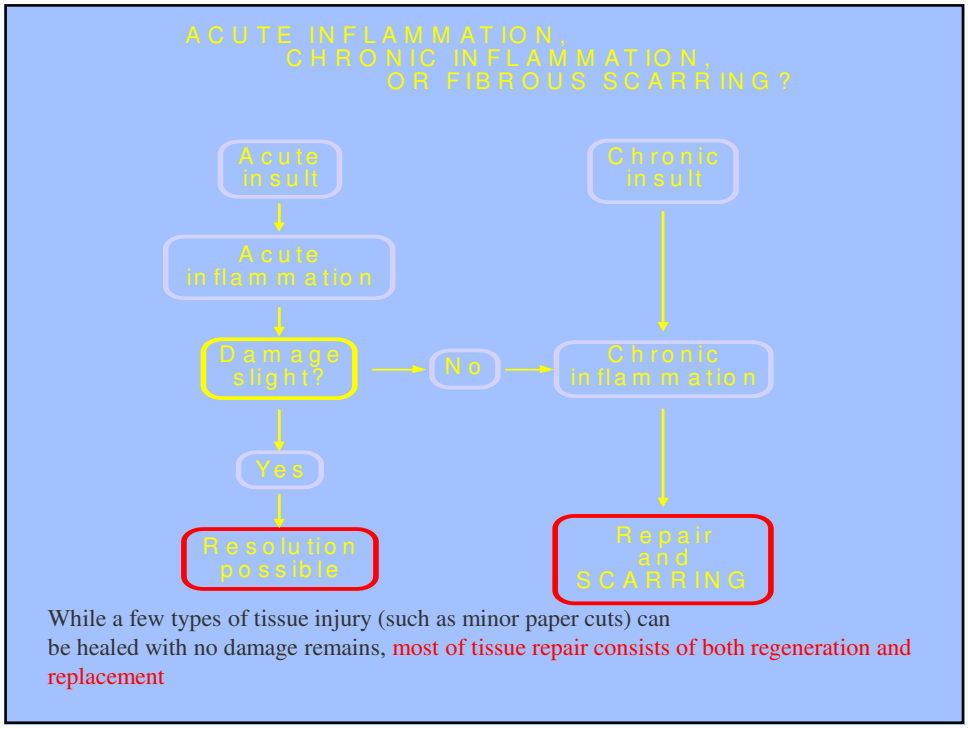

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FIBROUS REPAIR:

The development of a fibrous scar.

- Rabbit ear chamber example.

  - Blood clot forms.
  - Acute inflammation around the edges.
  - Chronic inflammation: Macrophages infiltrate the clot.
  - Capillaries and lymphatics sprout and infiltrate.
  - Myofibroblasts infiltrate and differentiate.
  - Glycoproteins and Collageb are produced
  - Cell population falls, vessels differentiate and are reduced in number.
  - Collagen matures and contracts.

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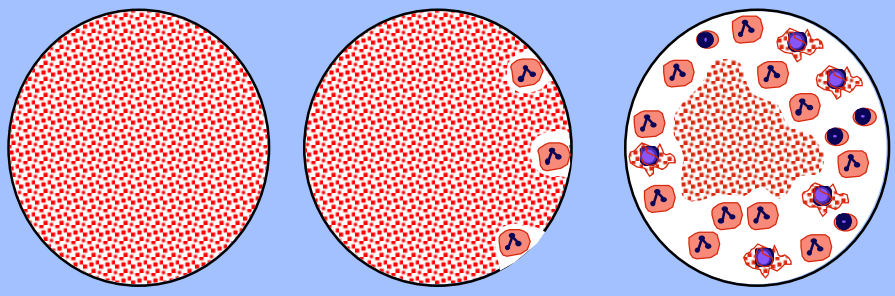
Rabbit Ear Chamber: Direct observation of fibrous repair.



1) Exudate clots. 2) Neutrophils infiltrate and digest clot 3) Macrophages and lymphocytes are recruited

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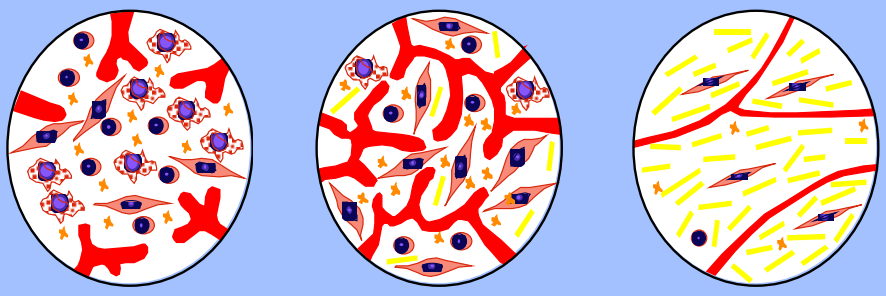
Rabbit Ear Chamber: Direct observation of fibrous repair.



4) Vessels sprout, myofibroblasts make glycoproteins 5) Vascular network; collagen synthesised; macrophages reduced 6) Maturity. Cells much reduced; collagen matures, contracts, remodels

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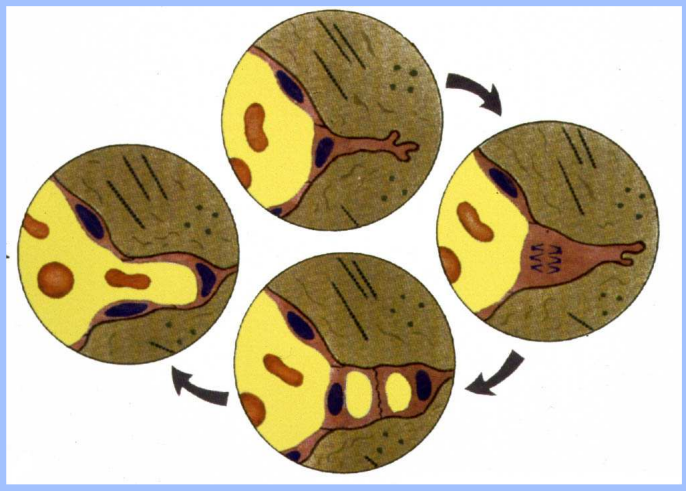
Angiogenesis



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REGENERATION

- **Labile cells:**
  - normal state is active cell division
  - usually rapid regeneration
  - These tissues contain pools of stem cells
- **Stable cells:**
  - not normally dividing at a significant rate
  - may enter the cell cycle in response to certain stimuli, such as cell injury
  - speed of regeneration variable
- **Permanent cells:**
  - Have left the cycle permanently
  - unable to divide
  - No regeneration: replacement

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CONTROL OF REPAIR PROCESSES

- Angiogenesis
  - Macrophages - Various angiogenic cytokines, e.g. VEGF, bFGF ...
- Fibrosis
  - Macrophages- various pro-fibrotic cytokines, e.g. TGF beta, PDGF, ...
- Limitation of fibrosis and remodelling
  - Macrophages - through the production of TGF-β, PDGF, tumor necrosis factor (TNF), osteopontin (OPN), IL-1, collagenase and matrix metalloproteinases (MMPs

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Major steps in wound healing and their associated growth factors

	EGF	FGF	KGF	PDGF	TGF-α	TGF-β	TNF	VEGF
Fibroblast migration		X		X		X		
Fibroblast proliferation	X	X		X	X		X	
Monocyte migration		X		X		X	X	
Macrophage activation							X	
Epithelial migration	X	X	X		X			
Epithelial proliferation	X	X	X		X			
Angiogenesis		X		X	X		X	X
Collagen synthesis				X		X		
Collagenase synthesis	X	X		X			X	X
Wound contraction		X		X				

EGF, epidermal growth factor; FGF, fibroblast growth factor; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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FACTORS INFLUENCING WOUND HEALING

- General Factors:
  - Age
  - General state of health
    - chronic diseases e.g. diabetes, rheumatoid arthritis etc.
  - Drugs (e.g. steroids) and hormones
  - General cardiovascular status
  - General dietary deficiencies e.g. protein
  - Specific dietary deficiencies
    - Vitamin C
    - sulphur-containing amino acids

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FACTORS INFLUENCING WOUND HEALING

- Local factors:
  - Type, size, location of wound
  - Apposition, lack of movement
  - Infection: Suppuration, Gangrene, Tetanus
    - (Secondary hæmorrhage)
  - Blood supply: Arterial, Venous
  - Foreign material: dirt, glass, sutures, necrotic tissue
  - Radiation damage

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Complications of Repair

- Insufficient fibrosis:
  - Wound dehiscence; hernia; ulceration
- Excessive fibrosis:
  - Cosmetic scarring; hypertrophic scars; keloid
- Excessive contraction:
  - Limitation of joint movement (Contractures); obstruction of tubes & channels (Strictures)

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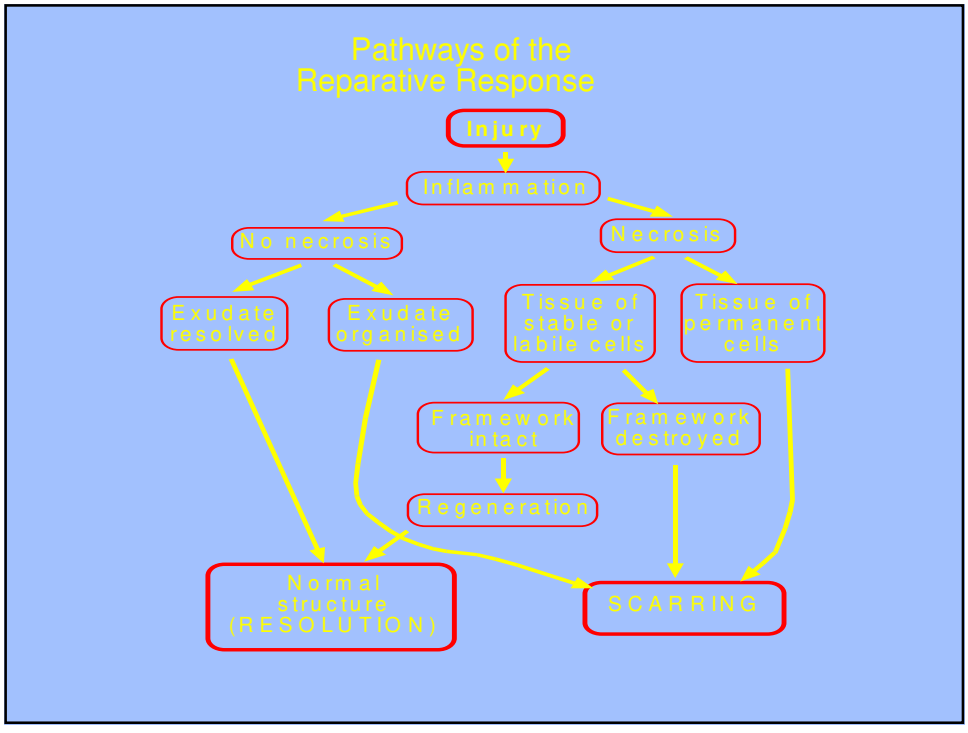

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