Tissue Repair: Regeneration and Fibrosis

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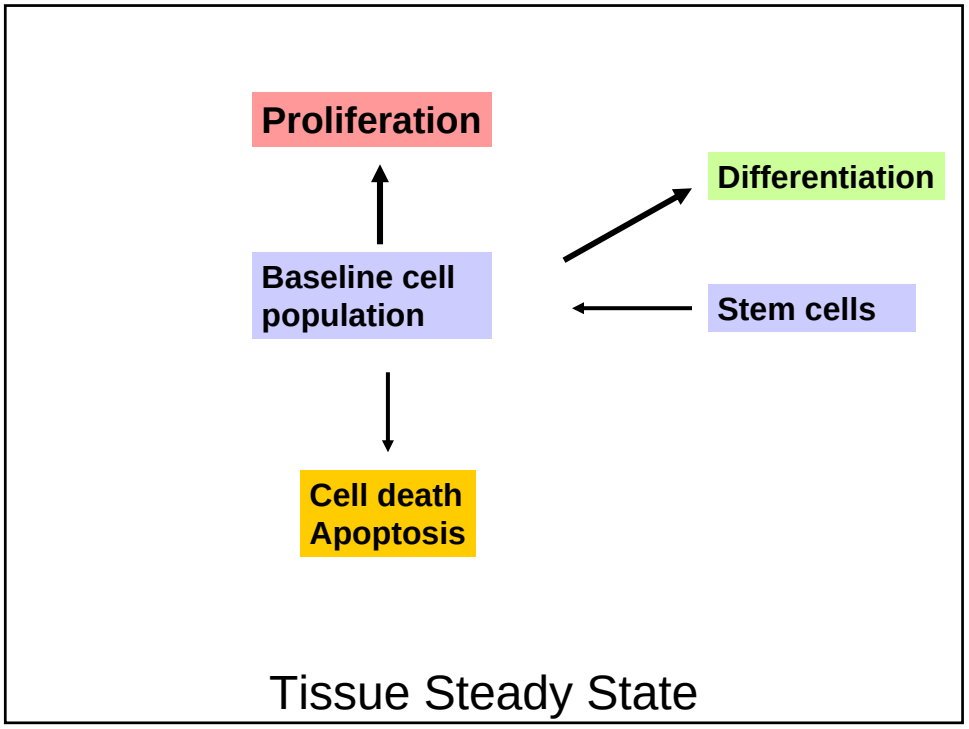
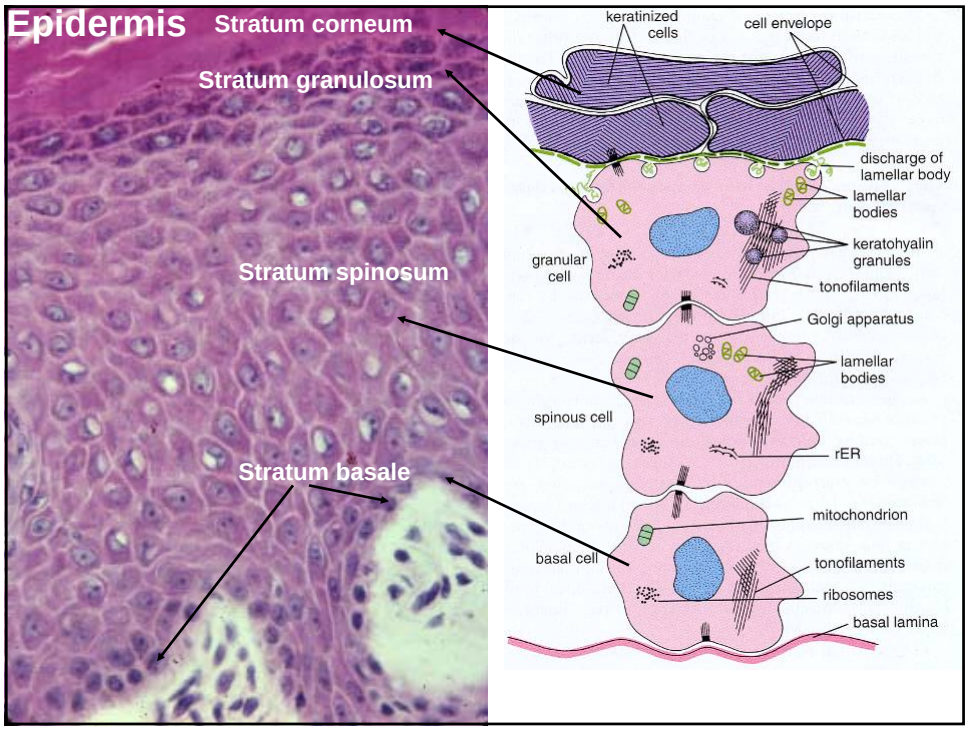
Lecture Outline

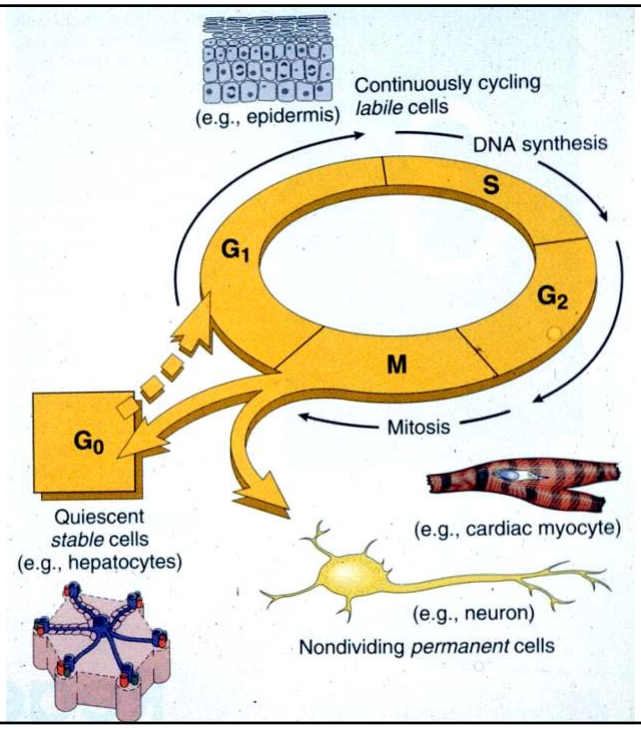
- Control of Cell Proliferation – cell cycle
- Growth Factors
- Extracellular matrix
- Cell and Tissue Regeneration
- Repair (scar)
- Cutaneous wound healing
- Pathologic repair


Proliferation
Differentiation
Baseline cell population
Stem cells
Cell death Apoptosis
Tissue Steady State


Epidermis
Stratum corneum
Stratum granulosum
Stratum spinosum
Stratum basale
keratinized cells
cell envelope
discharge of lamellar body
lamellar bodies
keratohyalin granules
granular cell
tonofilaments
Golgi apparatus
lamellar bodies
spinous cell
rER
mitochondrion
basal cell
tonofilaments
ribosomes
basal lamina

Tissue Types

- Continuously Dividing (labile)
  - Hematopoietic and surface epithelia
- Stable
  - Liver, kidney, pancreas, smooth muscle, endothelial cells, fibroblasts
- Permanent
  - Neurons and cardiac muscle



Signaling of Growth Factor Receptors

- Autocrine – lymphocytes, liver
- Paracrine – macrophages in wound healing
- Endocrine - hormones

Growth Factors in Tissue Repair

- Vascular Endothelial growth factor (**VEGF**) – increased vascular permeability
- Transforming Growth Factor-Beta (**TGF**-**B**)
- Platelet Derived Growth Factor (**PDGF**)
- Epidermal Growth Factor (**EGF**)
- Fibroblast Growth Factor (**FGF**)

VEGF

- Produced by mesenchymal cells
- Increases vascular permeability
- Mitogenic for endothelial cells

TGF- beta

- Produced by:
  - Platelets and macrophages

  MOST IMPORTANT FACTOR IN WOUND HEALING
- Actions:
  - Monocyte chemotaxis
  - Fibroblast migration and proliferation
  - Angiogenesis and fibronectin synthesis
  - Collagen and ECM:
    - Increased synthesis
    - Decreased degradation by MMP's, increased TIMP's

PDGF

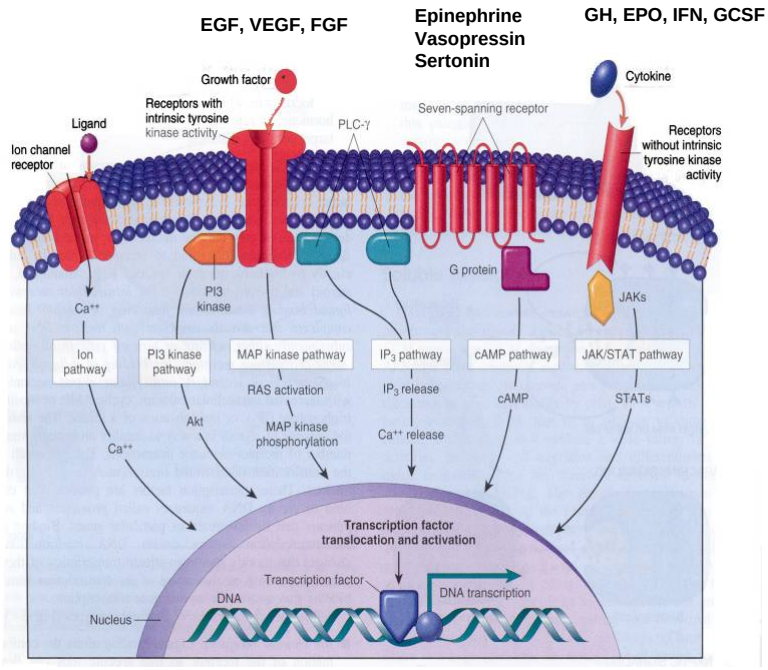
- Produced by platelets, macrophages, endothelial cells
- Chemotactic for neutrophils, macrophages, fibroblasts, smooth muscle cells
- Stimulates production of MMP's, fibronectin and hyaluronic acid
- Stimulates angiogenesis

EGF

- Produced by activated macrophages
- Mitogenic for keratinocytes and fibroblasts
- Stimulates granulation tissue formation

FGF

- Produced by macrophages, T cells
- Chemotactic for fibroblasts
- Mitogenic for fibroblasts and keratinocytes
- Stimulates keratinocyte migration, angiogensis, wound contration and matrix production



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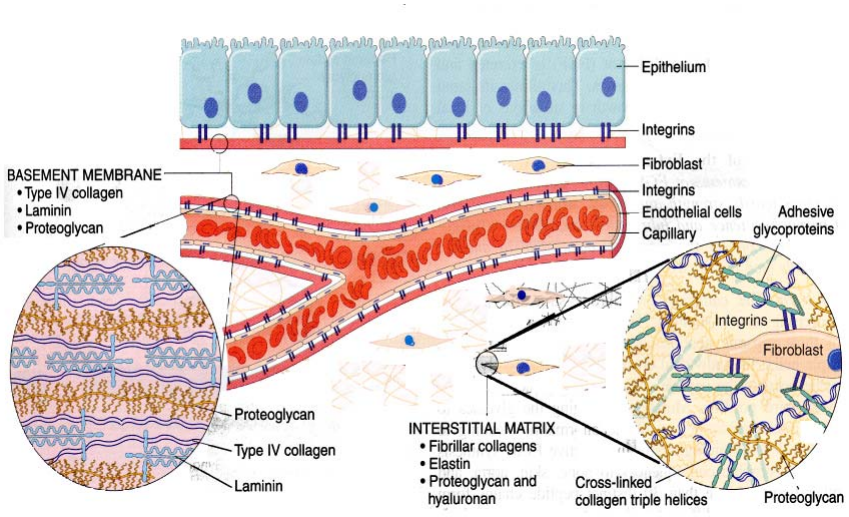
Role of ECM

- Mechanical Support – anchorage, cell migration, cell polarity
- Cell growth control
- Maintenance of cell differentiation -



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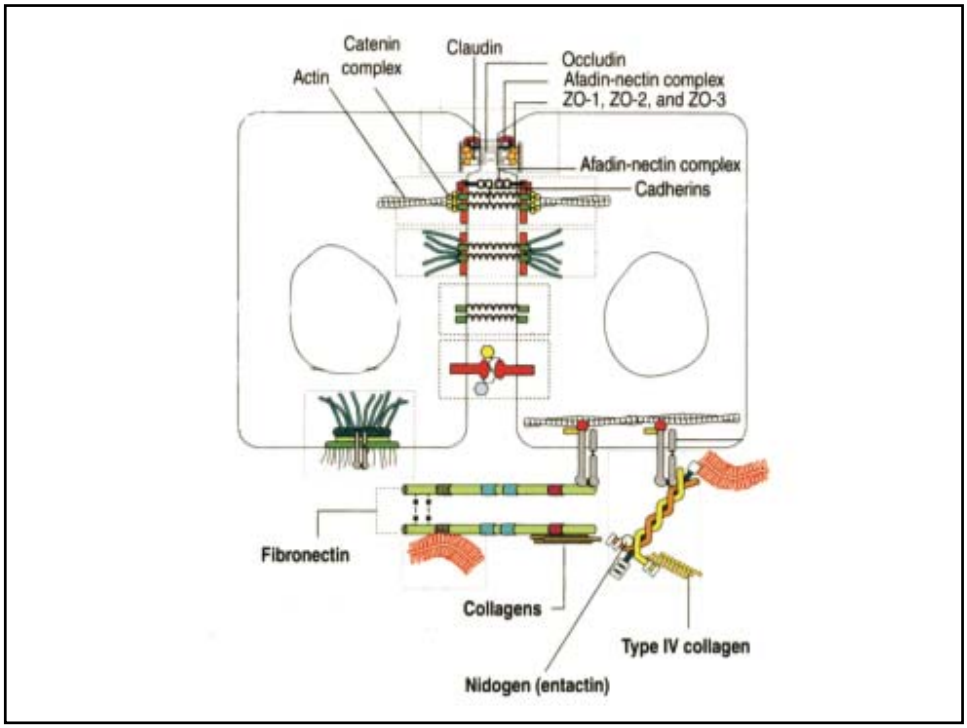
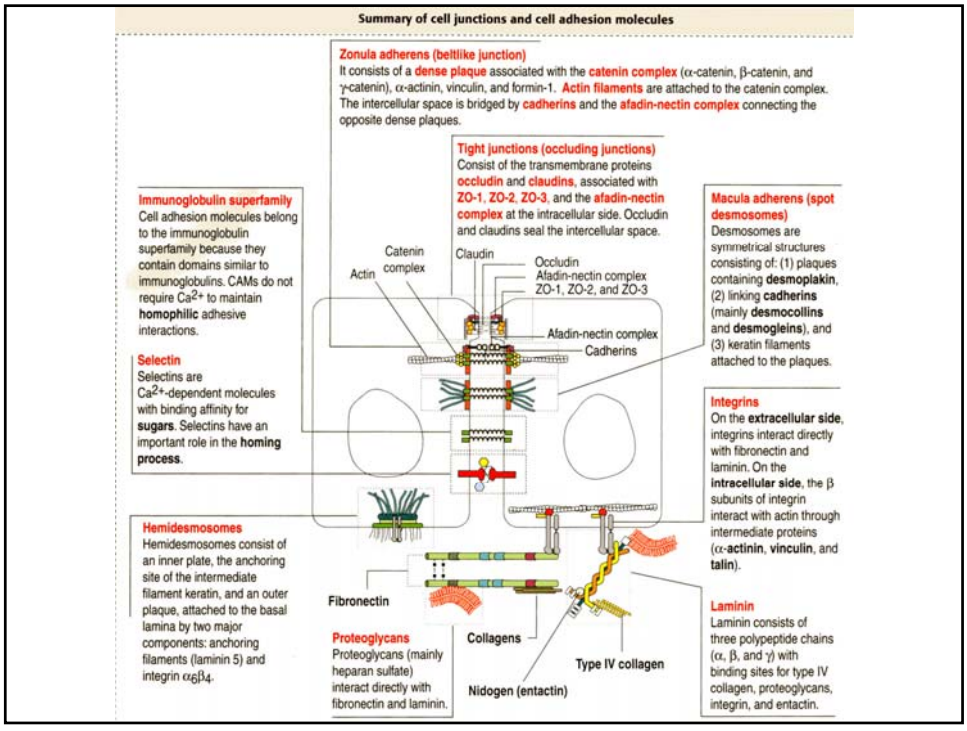
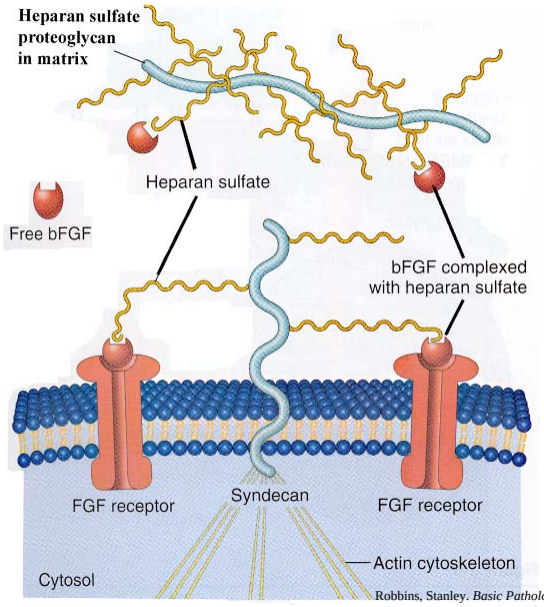

Catenin complex
Actin
Claudin
Occludin
Afadin-nectin complex
ZO-1, ZO-2, and ZO-3
Afadin-nectin complex
Cadherins
Fibronectin
Collagens
Type IV collagen
Nidogen (entactin)


Summary of cell junctions and cell adhesion molecules
Zonula adherens (beltlike junction)
It consists of a dense plaque associated with the catenin complex (α-catenin, β-catenin, and γ-catenin), α-actinin, vinculin, and formin-1. Actin filaments are attached to the catenin complex. The intercellular space is bridged by cadherins and the afadin-nectin complex connecting the opposite dense plaques.
Tight junctions (occluding junctions)
Consist of the transmembrane proteins occludin and claudins, associated with ZO-1, ZO-2, ZO-3, and the afadin-nectin complex at the intracellular side. Occludin and claudins seal the intercellular space.
Immunoglobulin superfamily
Cell adhesion molecules belong to the immunoglobulin superfamily because they contain domains similar to immunoglobulins. CAMs do not require Ca^{2+} to maintain homophilic adhesive interactions.
Selectin
Selectins are Ca^{2+}-dependent molecules with binding affinity for sugars. Selectins have an important role in the homing process.
Macula adherens (spot desmosomes)
Desmosomes are symmetrical structures consisting of: (1) plaques containing desmoplakin, (2) linking cadherins (mainly desmocollins and desmogleins), and (3) keratin filaments attached to the plaques.
Integrins
On the extracellular side, integrins interact directly with fibronectin and laminin. On the intracellular side, the β subunits of integrin interact with actin through intermediate proteins (α-actinin, vinculin, and talin).
Hemidesmosomes
Hemidesmosomes consist of an inner plate, the anchoring site of the intermediate filament keratin, and an outer plaque, attached to the basal lamina by two major components: anchoring filaments (laminin 5) and integrin $\alpha_6\beta_4$.
Laminin
Laminin consists of three polypeptide chains (α, β, and γ) with binding sites for type IV collagen, proteoglycans, integrin, and entactin.
Proteoglycans
Proteoglycans (mainly heparan sulfate) interact directly with fibronectin and laminin.
Catenin complex
Actin
Claudin
Occludin
Afadin-nectin complex
ZO-1, ZO-2, and ZO-3
Afadin-nectin complex
Cadherins
Fibronectin
Collagens
Type IV collagen
Nidogen (entactin)

FGF stimulates keratinocyte migration, wound contracture and matrix depostion



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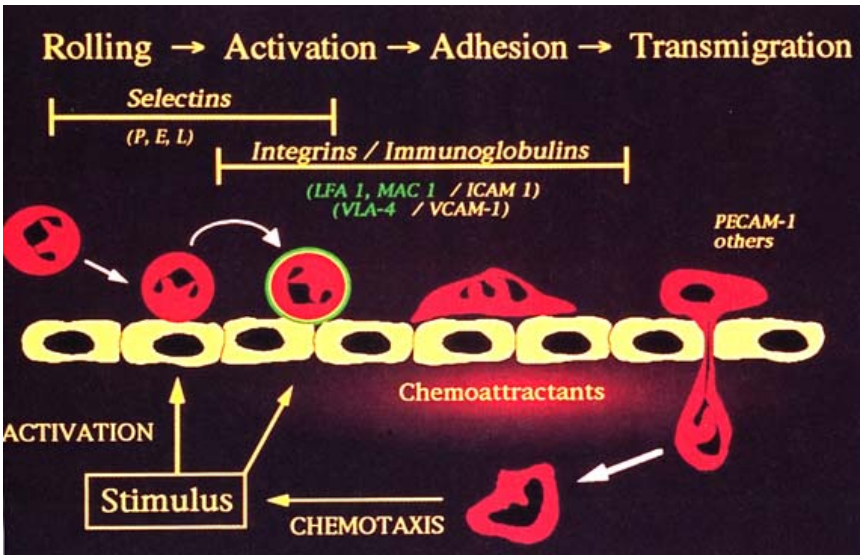
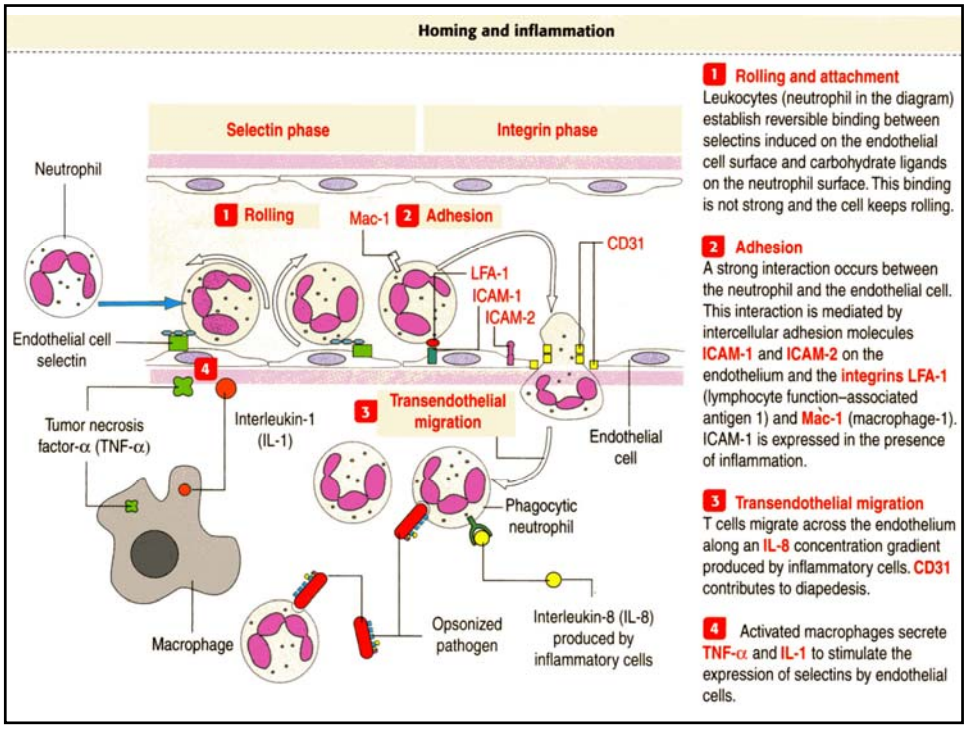
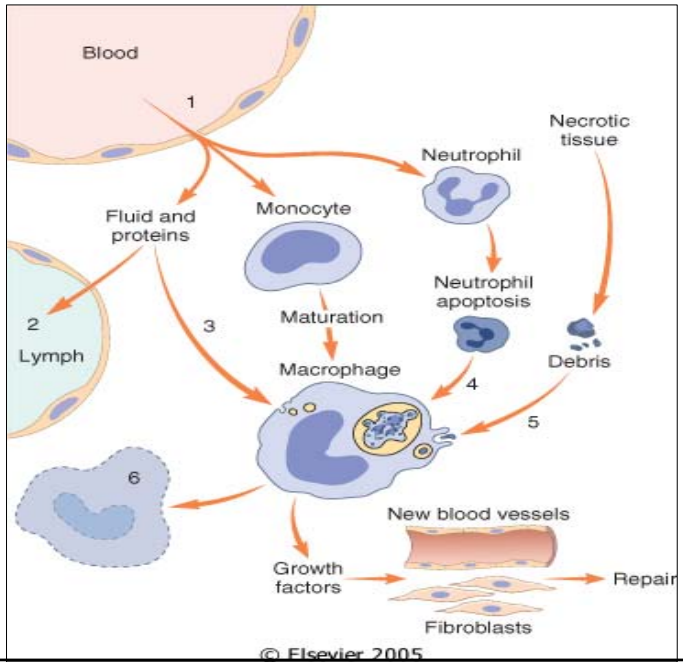
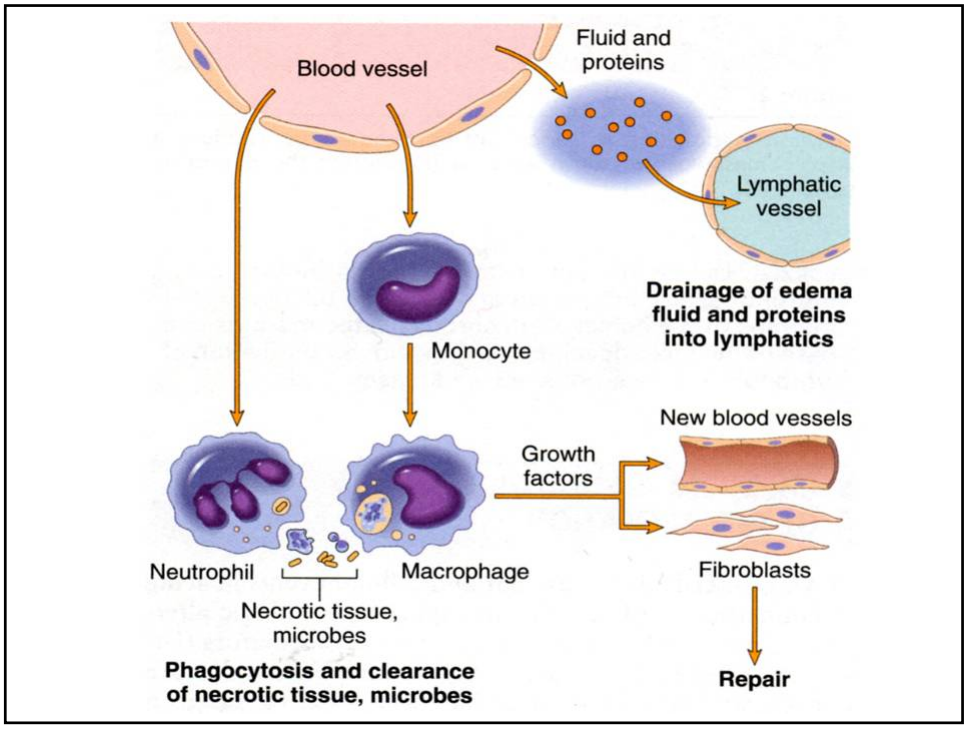
Model of Leukocyte Transmigration

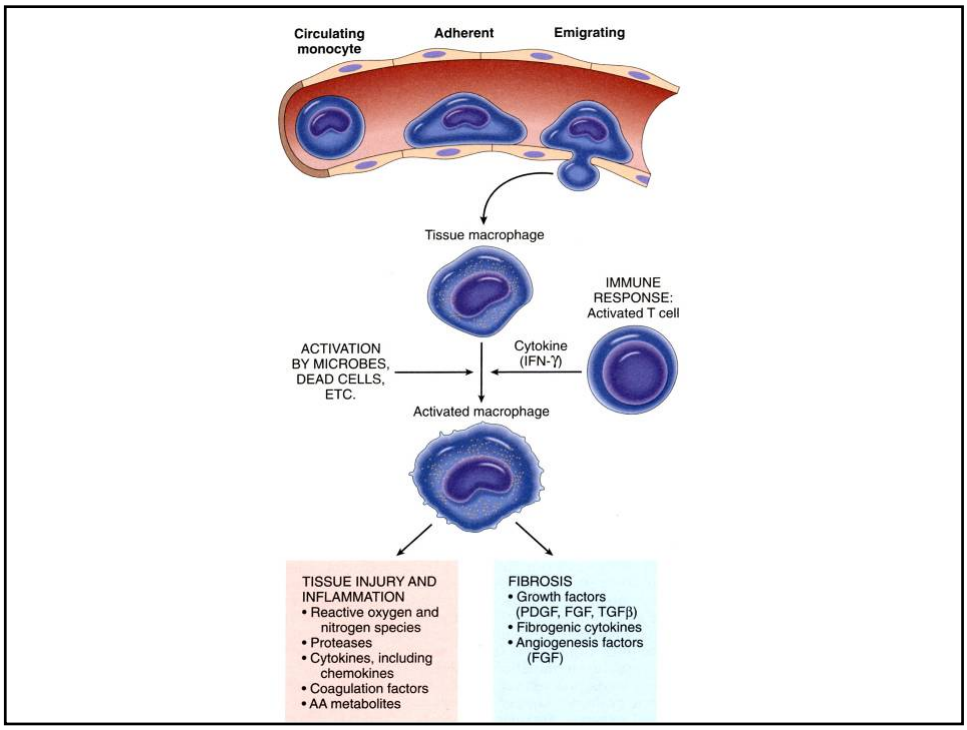




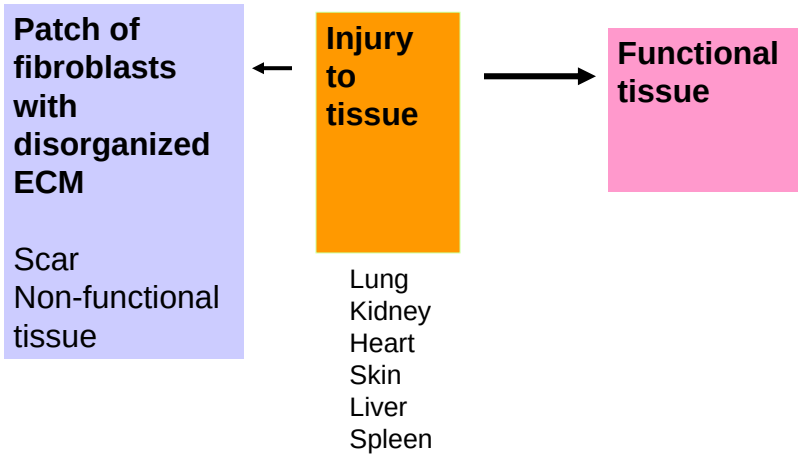
Macrophages in inflammation and repair




Blood vessel
Fluid and proteins
Lymphatic vessel
Drainage of edema fluid and proteins into lymphatics
Monocyte
New blood vessels
Growth factors
Neutrophil
Macrophage
Fibroblasts
Necrotic tissue, microbes
Phagocytosis and clearance of necrotic tissue, microbes
Repair


Circulating monocyte
Adherent
Emigrating
Tissue macrophage
IMMUNE RESPONSE: Activated T cell
ACTIVATION BY MICROBES, DEAD CELLS, ETC.
Cytokine (IFN-γ)
Activated macrophage
TISSUE INJURY AND INFLAMMATION
• Reactive oxygen and nitrogen species
• Proteases
• Cytokines, including chemokines
• Coagulation factors
• AA metabolites
FIBROSIS
• Growth factors (PDGF, FGF, TGFβ)
• Fibrogenic cytokines
• Angiogenesis factors (FGF)

Wound Repair and Regeneration



Key Points

- How does each tissue restore itself to prevent scar?
- Humans lose the ability to prevent scar after fetal life
- Scar prevents tissue regeneration
- What is the purpose of the scar?

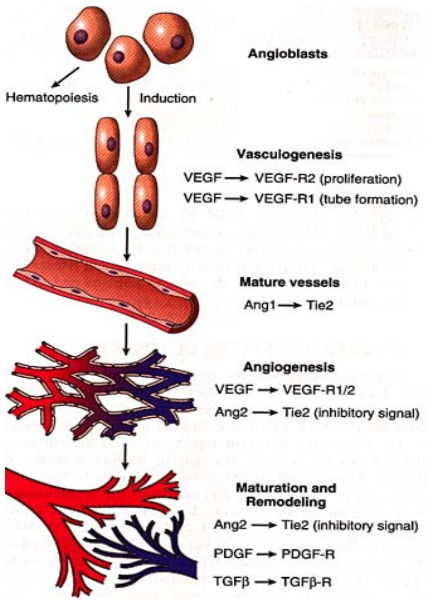
Repair By Connective Tissue

- Formation of new blood vessels (angiogenesis)
- Migration and proliferation of fibroblasts
- Deposition of ECM (scar)
- Maturation and reorganization of fibrous tissue (remodeling)

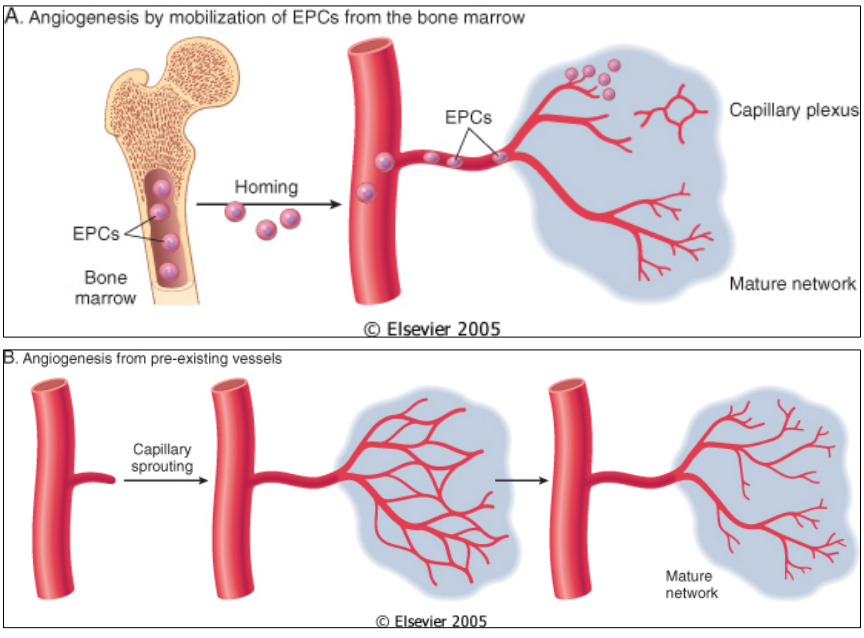
Angiogenesis

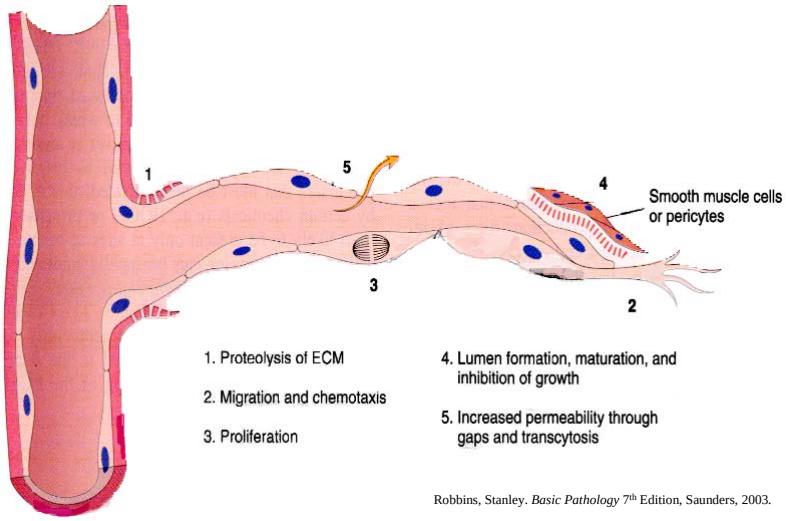
- Proteolysis of vessel basement membrane
- Endothelial cell migration and proliferation
- Pericyte recruitment

Growth factor receptors in angiogenesis



Two types of angiogenesis





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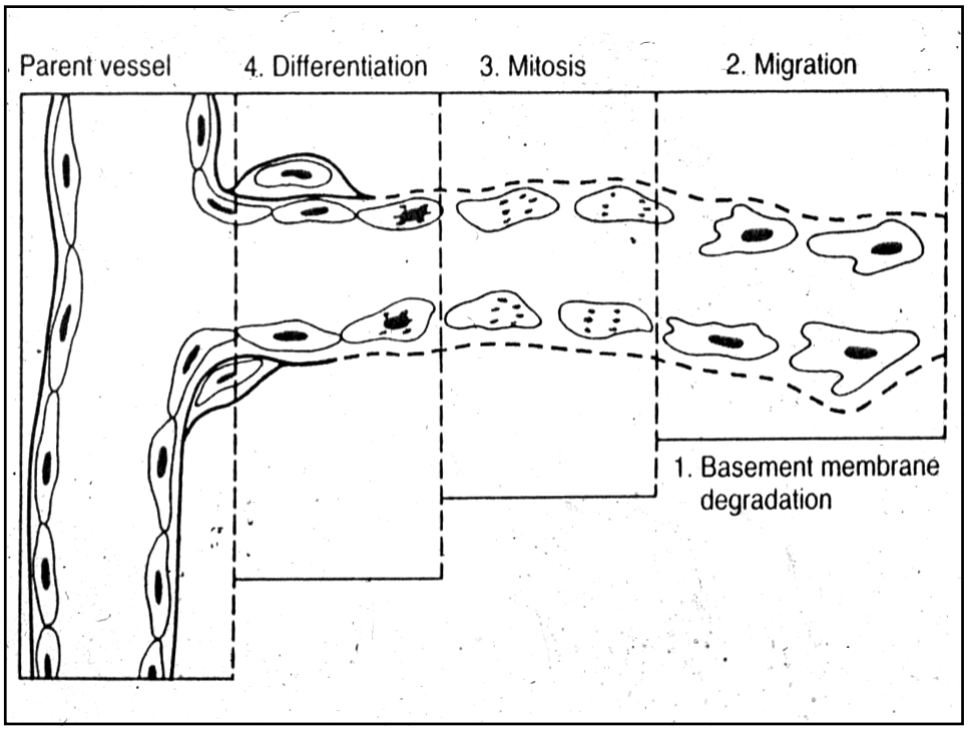
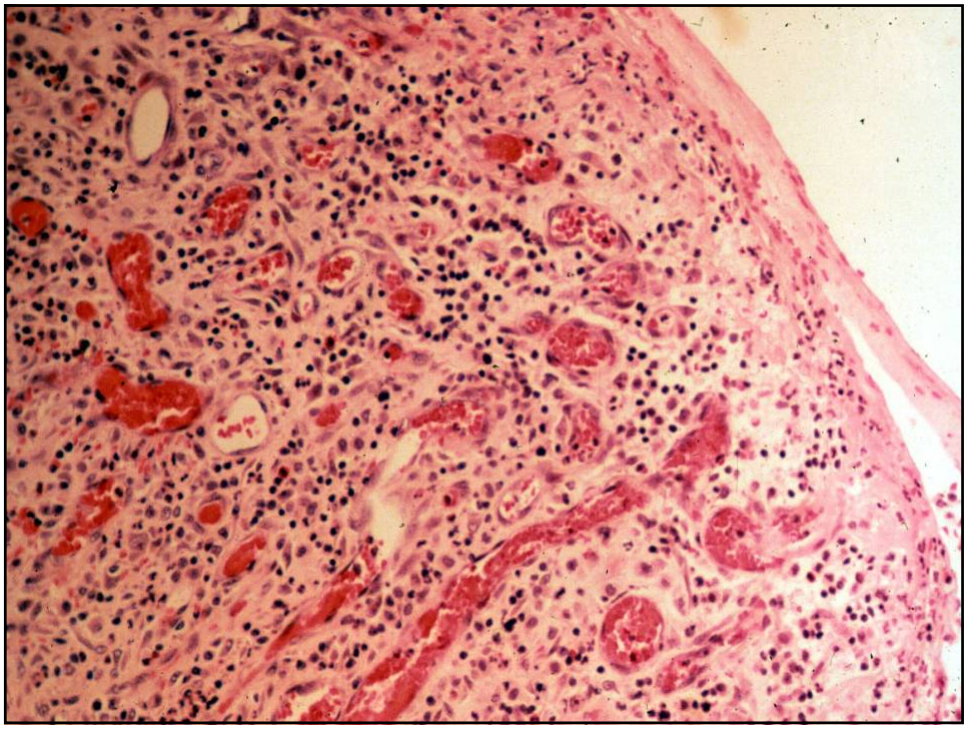
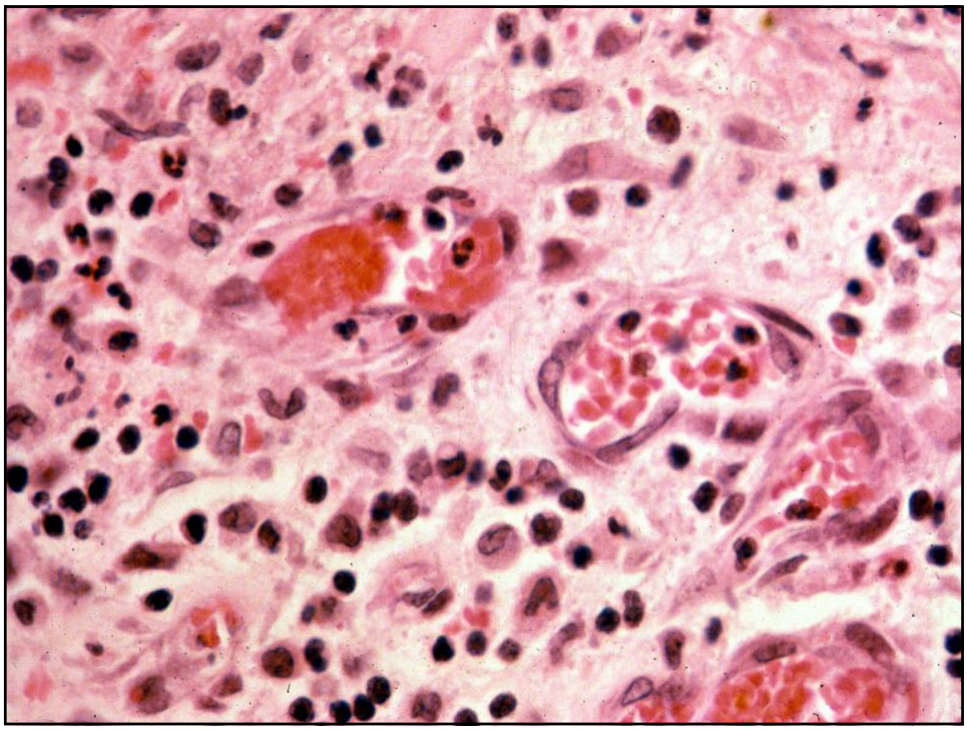




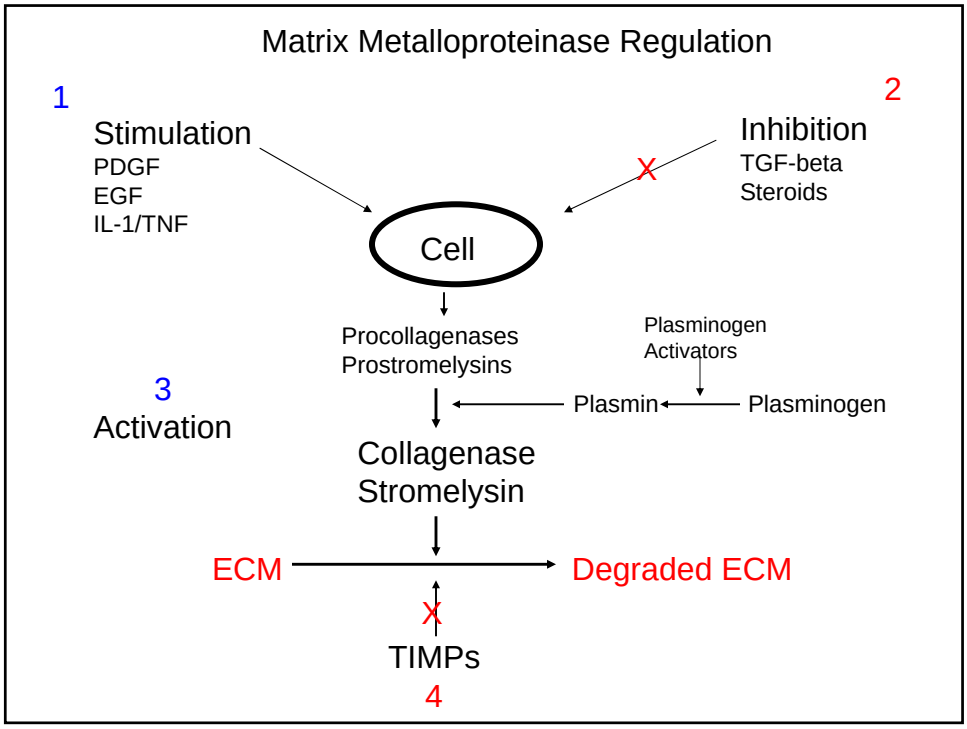


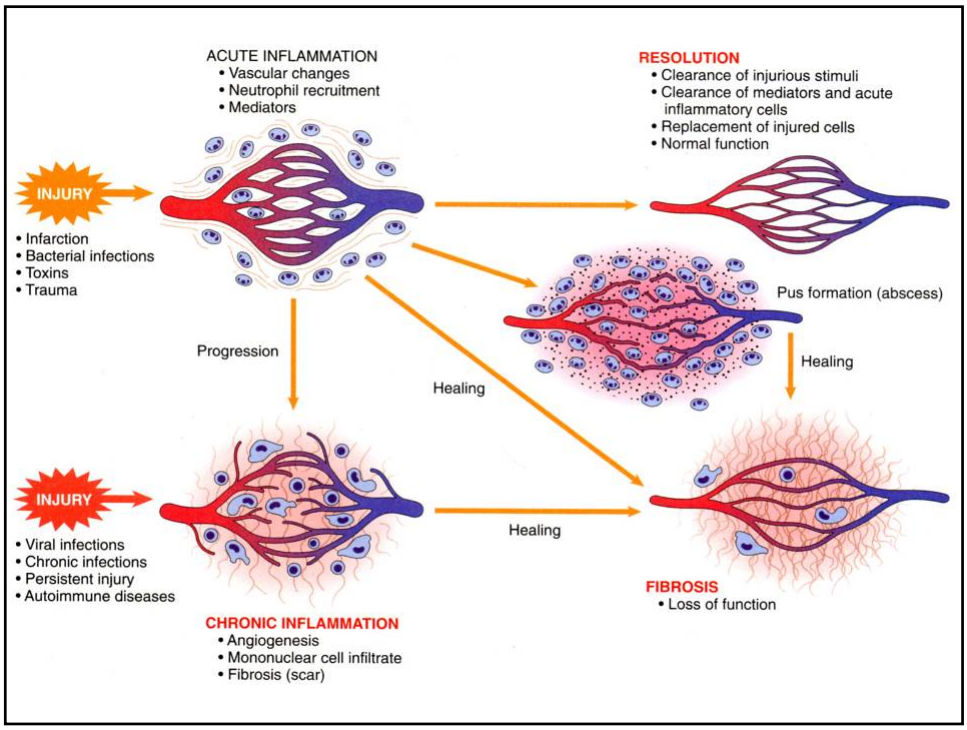
Scar Formation

- Fibroblast proliferation and migration
  - PDGF, FGF, TGF-beta mainly from macrophages
- ECM deposition
  - TGF-beta – potent agent of fibrosis

ECM and Tissue Remodeling

- Outcome of repair: **balance** between synthesis and degradation of matrix
- **MMP's** are synthesized by fibroblasts, macrophages, neutrophils, epithelial cells, etc destroy matrix (inactive form) activated by proteases and plasmin and inhibited by **TIMP's**-synthesized by mesenchymal cells

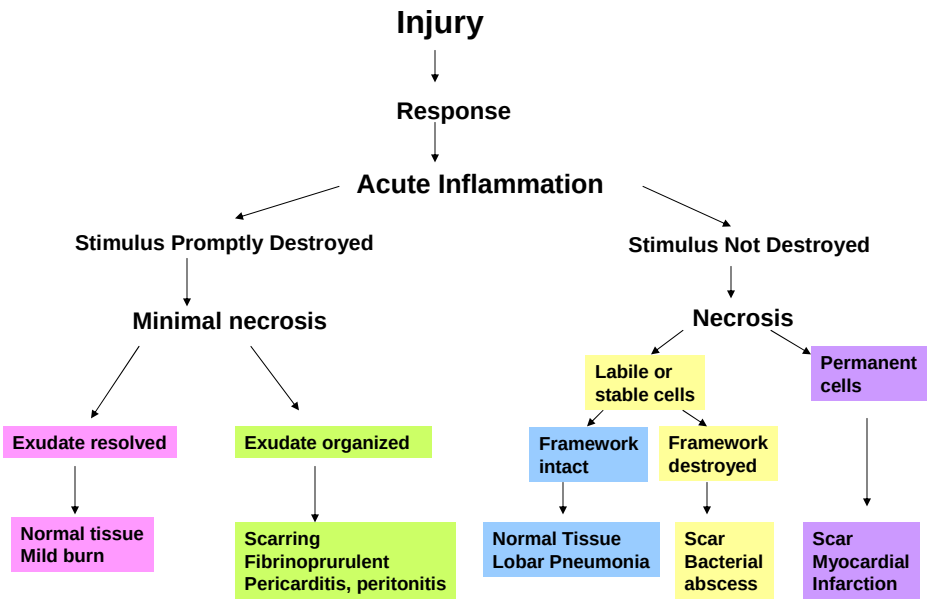

Matrix Metalloproteinase Regulation
1
Stimulation
PDGF
EGF
IL-1/TNF
2
Inhibition
TGF-beta
Steroids
X
Cell
Procollagenases
Prostromelysins
Plasminogen
Activators
3
Activation
Plasmin
Plasminogen
Collagenase
Stromelysin
ECM
Degraded ECM
X
TIMPs
4


ACUTE INFLAMMATION
• Vascular changes
• Neutrophil recruitment
• Mediators
RESOLUTION
• Clearance of injurious stimuli
• Clearance of mediators and acute inflammatory cells
• Replacement of injured cells
• Normal function
INJURY
• Infarction
• Bacterial infections
• Toxins
• Trauma
Pus formation (abscess)
Progression
Healing
Healing
INJURY
• Viral infections
• Chronic infections
• Persistent injury
• Autoimmune diseases
Healing
FIBROSIS
• Loss of function
CHRONIC INFLAMMATION
• Angiogenesis
• Mononuclear cell infiltrate
• Fibrosis (scar)

Classic Stages of Wound Repair

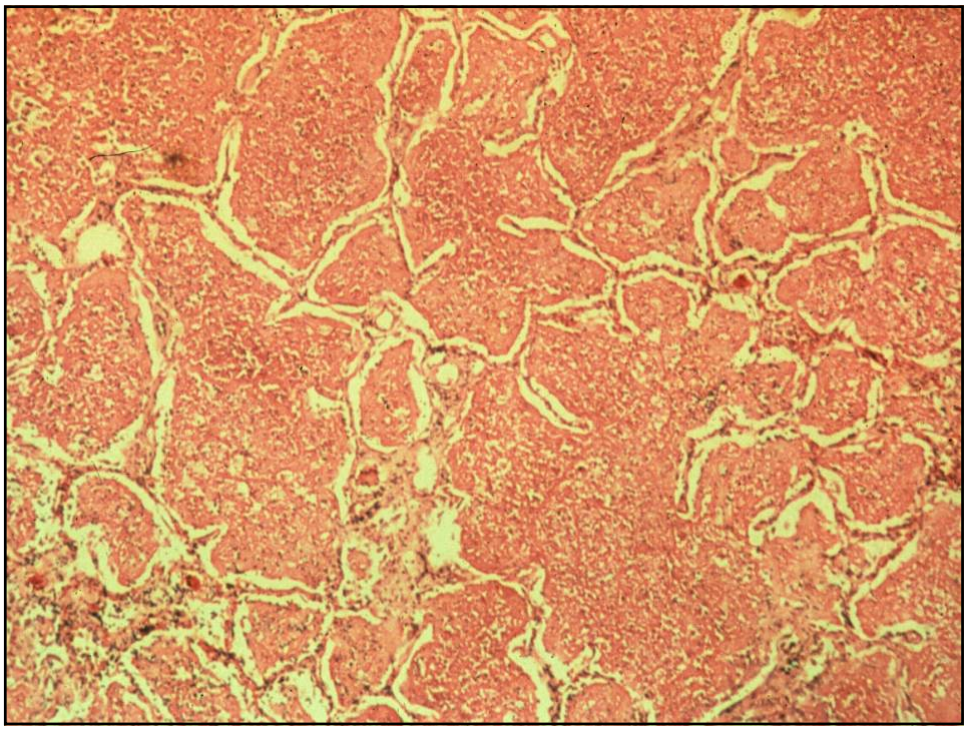
- Inflammation – until 48 hrs. after injury
- New tissue formation – 2-10 days after injury
- Remodeling – 1-12 months after repair

Possible Outcomes after Injury



Regeneration

- If the connective tissue framework is intact
- If the cells are not post-mitotic
- THEN:
- Complete restoration of the structure and function of the tissue is possible



Possible Outcomes after Injury

Injury

Response

Acute Inflammation

Stimulus Promptly Destroyed

Minimal necrosis

Exudate resolved

Normal tissue
Mild burn

Exudate organized

Scarring
Fibrinoprurulent
Pericarditis, peritonitis

Stimulus Not Destroyed

Necrosis

Labile or stable cells

Framework intact

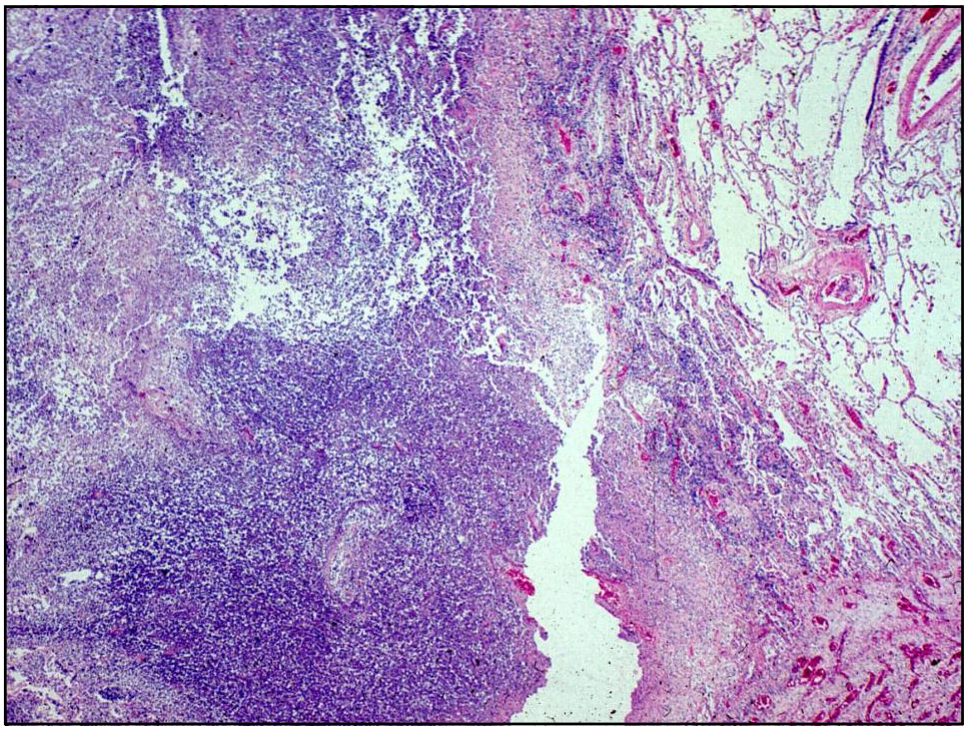
Normal Tissue
Lobar Pneumonia

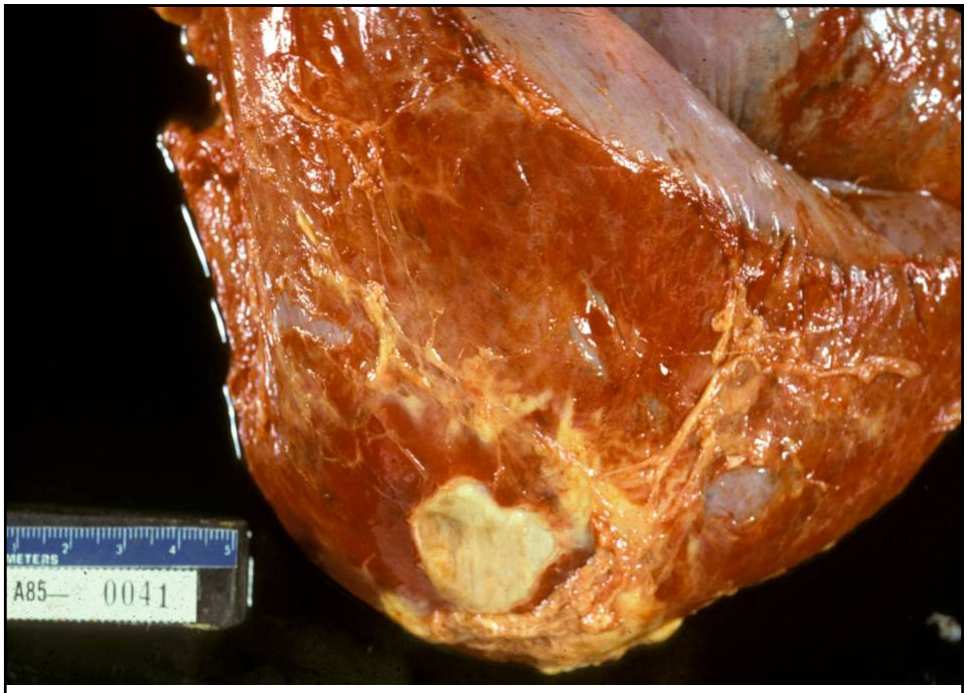
Framework destroyed

Scar
Bacterial abscess

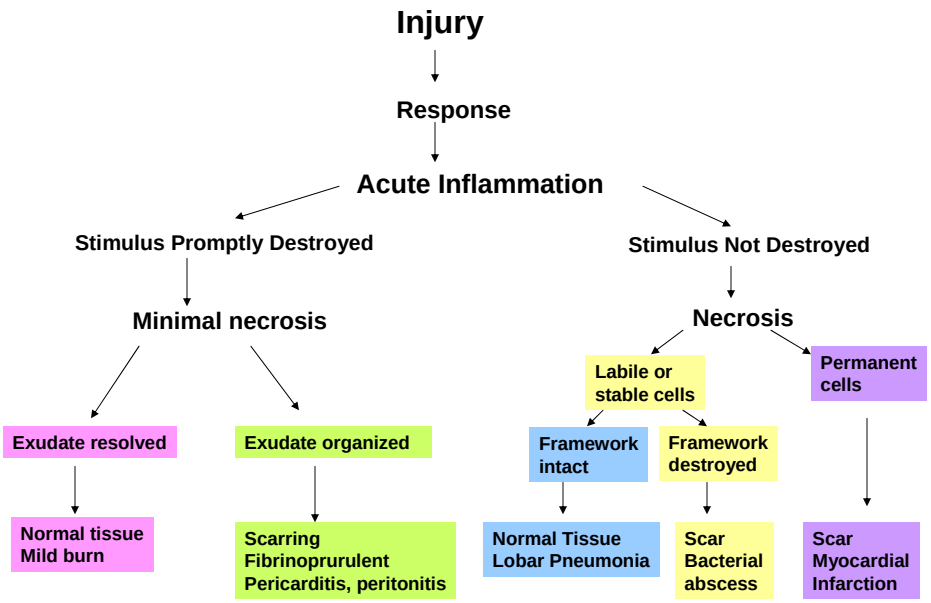
Permanent cells

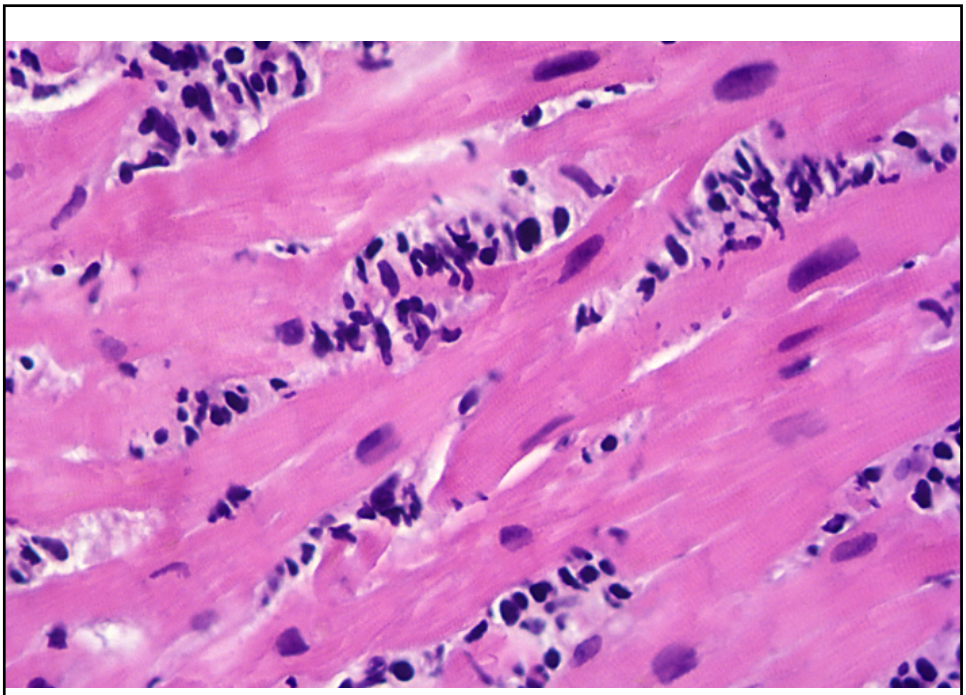
Scar
Myocardial Infarction

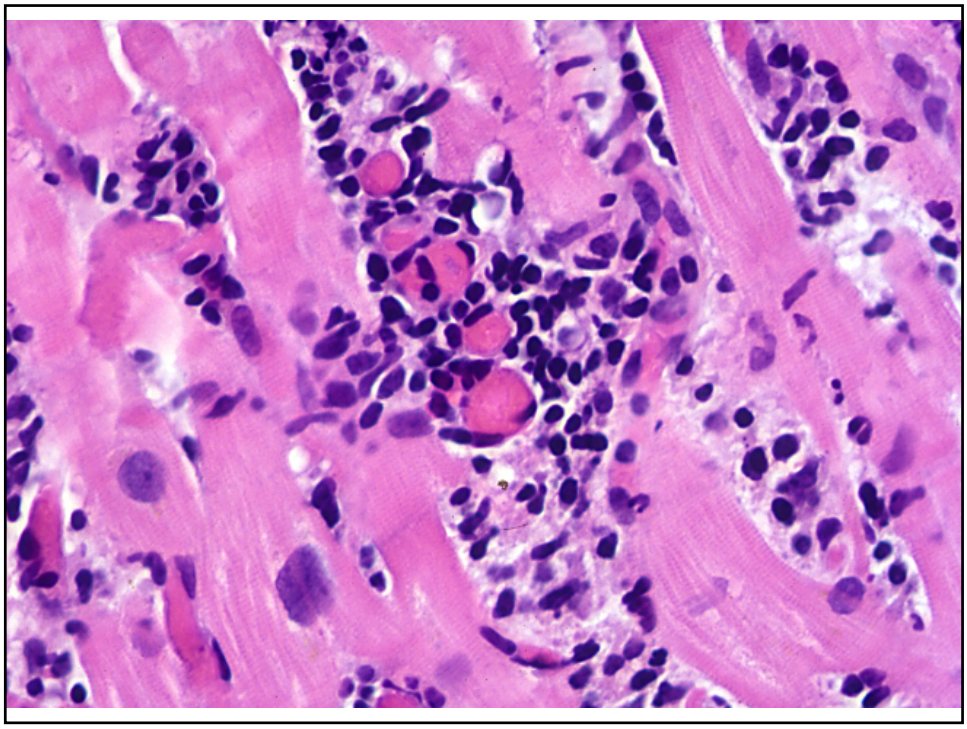


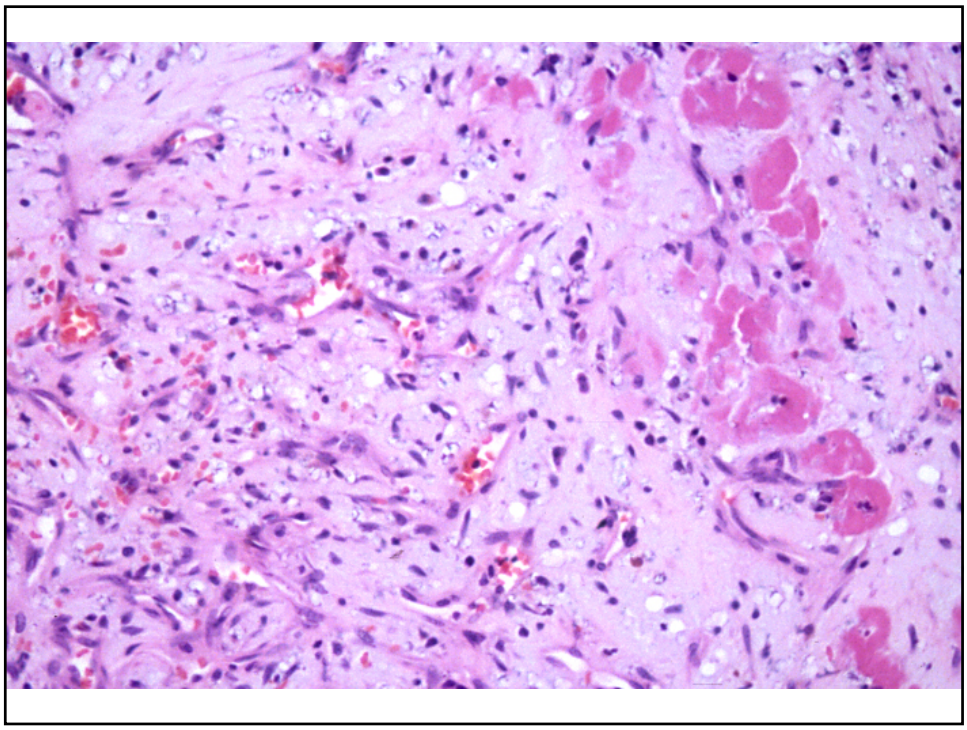

METERS
A85— 0041

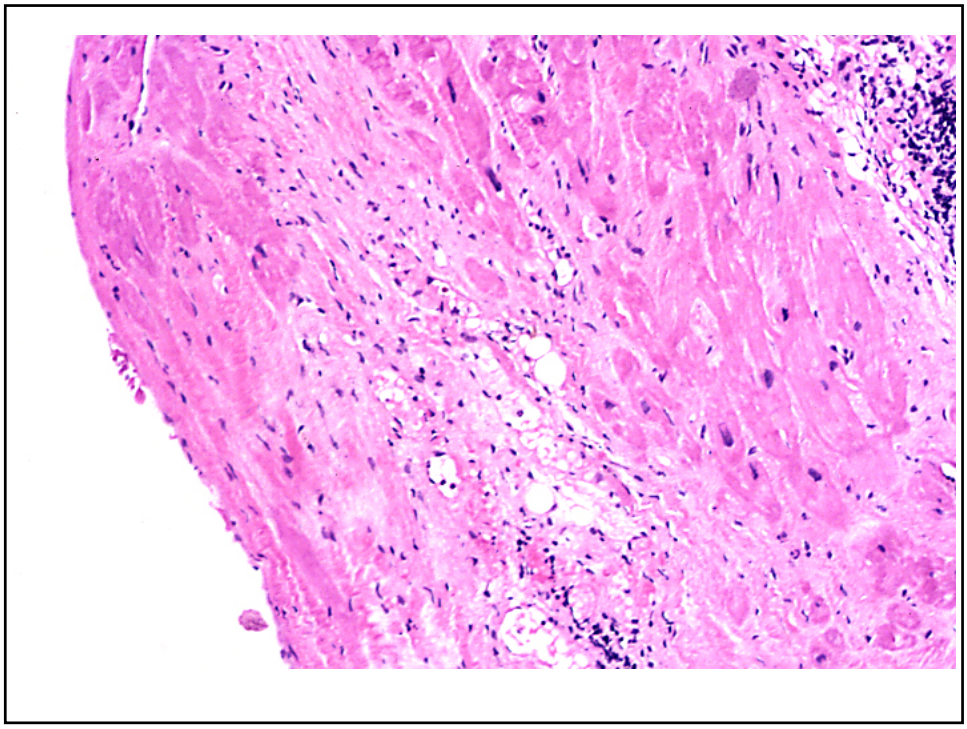
Possible Outcomes after Injury

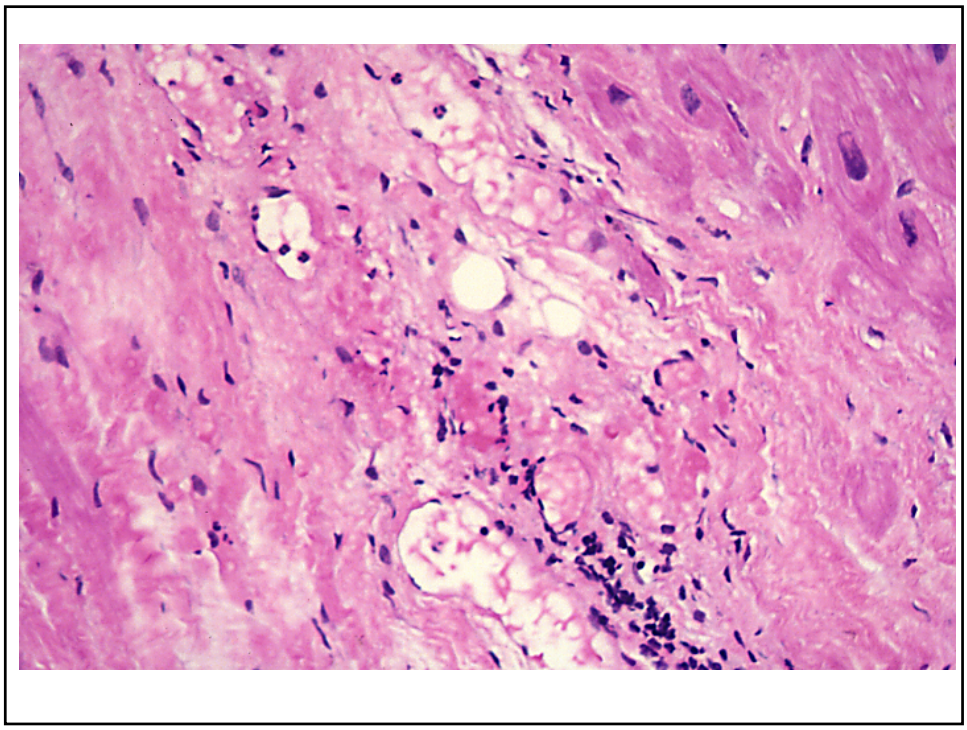








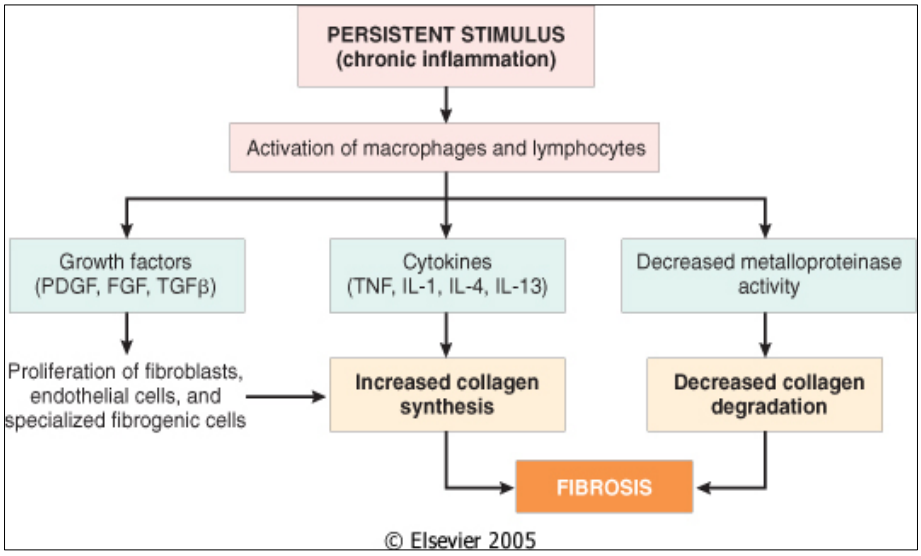




Repair by Fibrosis

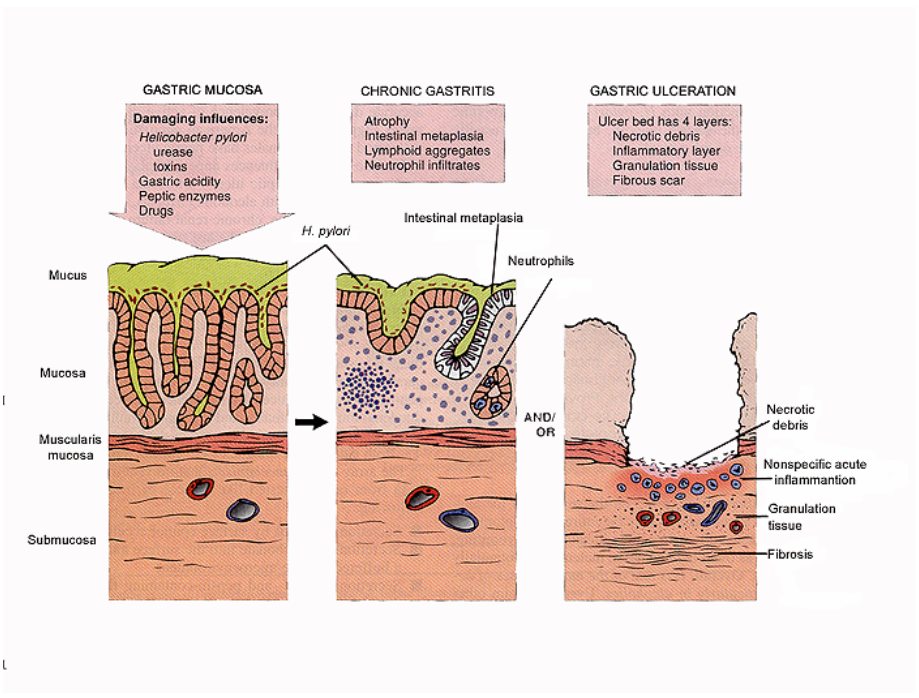
- Angiogenesis
- Granulation tissue
- Migration and proliferation of fibroblasts
- Deposition of extracellular matrix
- Organization of collagen “remodeling”
- Fibrosis – scar formation

Macrophages in healing and fibrosis



Chronic Peptic Ulcer

Fibrosis below the ulcer bed

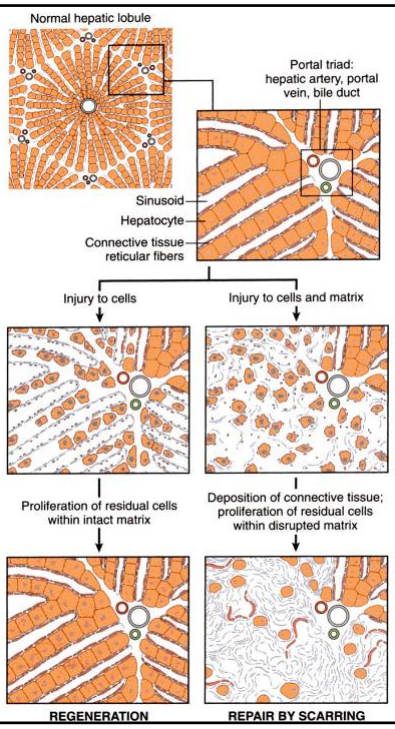


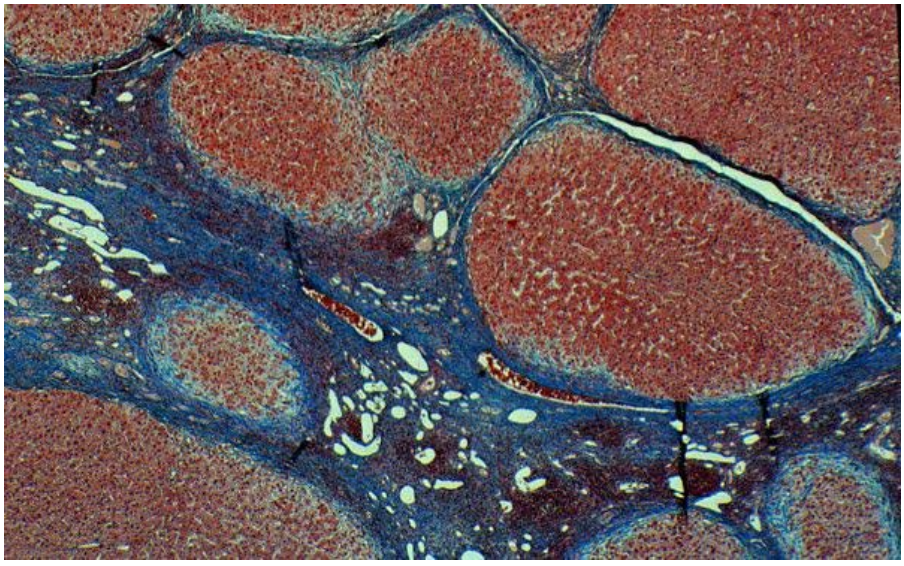
Fibrotic response to toxin-mediated injury

- Poorly understood:
  -Liver Hepatitis B,C
  -Pulmonary fibrosis

Scarring in the Liver

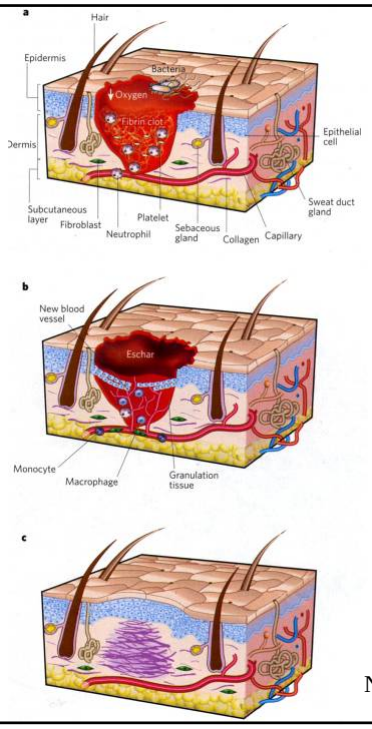
- Healing by fibrosis after inflammation
- TGF beta implicated in excessive collagen formation


Normal hepatic lobule
Portal triad:
hepatic artery, portal
vein, bile duct
Sinusoid
Hepatocyte
Connective tissue
reticular fibers
Injury to cells
Injury to cells and matrix
Proliferation of residual cells
within intact matrix
Deposition of connective tissue;
proliferation of residual cells
within disrupted matrix
REGENERATION
REPAIR BY SCARRING



Cirrhosis

Cutaneous Wound Healing



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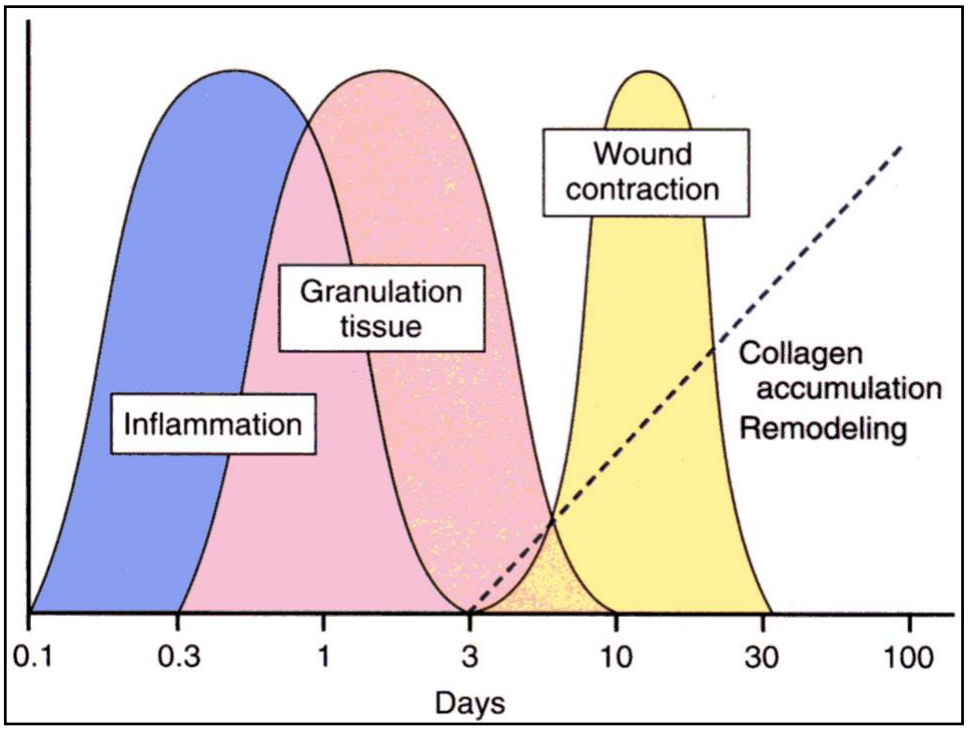
Classic Stages of Wound Repair

- Inflammation – until 48 hrs. after injury
- New tissue formation – 2-10 days after injury
- Remodeling – 1-12 months after repair

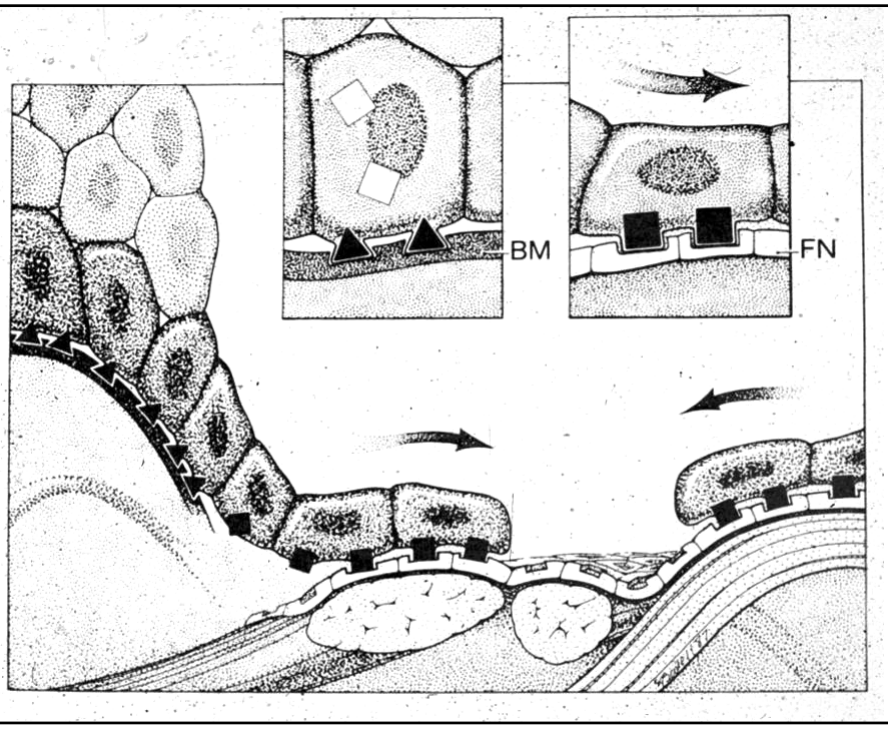
Overview of Cutaneous Wound Healing

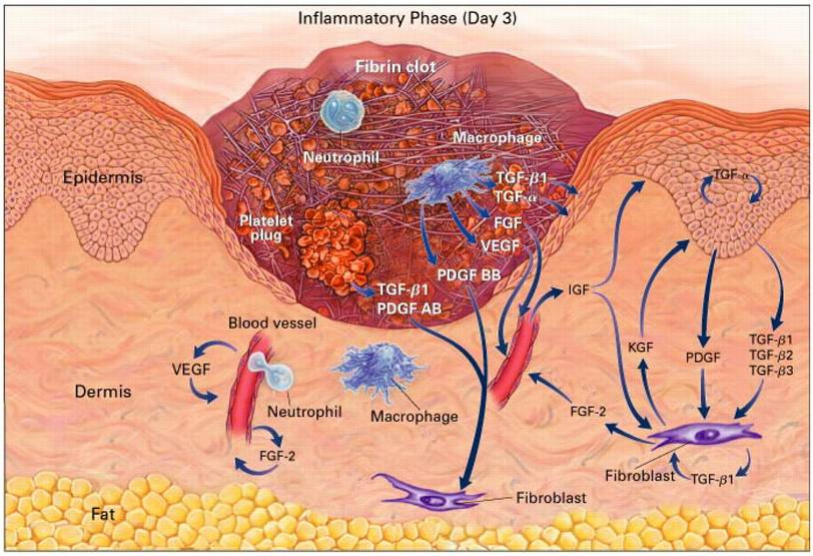
- A defect in the skin occurs
- Fibrin fills in defect – scab forms
- Epithelial regeneration beneath scab
- Granulation tissue – angiogenesis
- Wound contraction
- Collagen remodeling



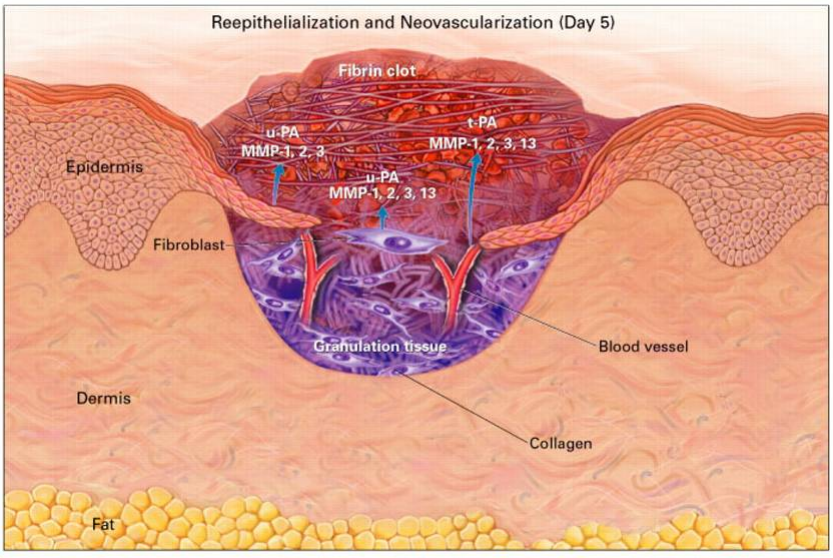
Cell Migrations in Wound Healing

- **Platelets** form a blood clot and secrete fibronectin (FN), PDGF and TGF-beta
- **Neutrophils** arrive within minutes
- **Macrophages** move in as part of granulation tissue and secrete fibronectin
- **Keratinocytes** or other epithelial cells detach from the basement membrane at wound edge and migrate on fibronectin rich matrix across wound to fill in defect (cells switch receptors from those for BM to FN receptors)





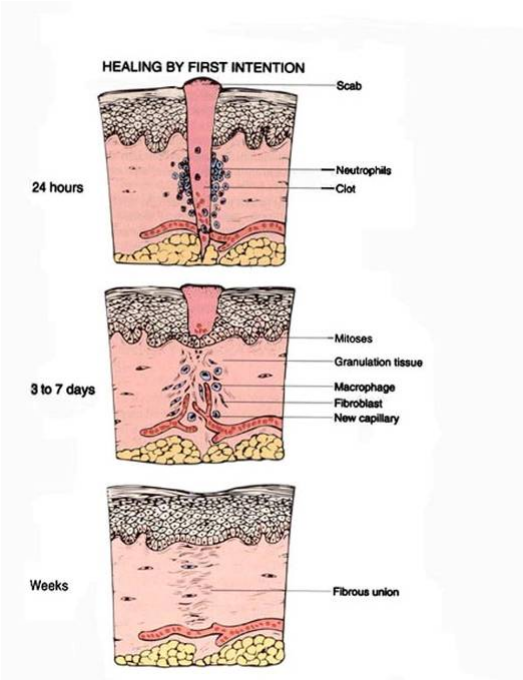
New England Journal of Med 341:738-746, 1999



New England Journal of Med 341:738-746, 1999

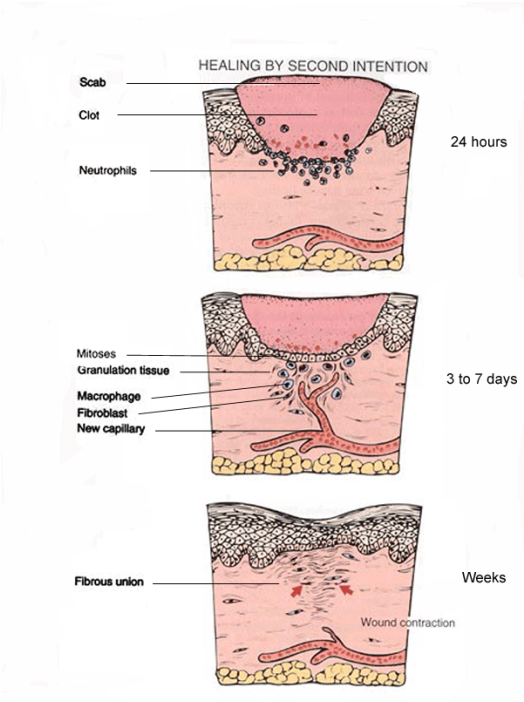
Healing by Primary Intention

- Surgical incision
- Edges easily joined together
- Small amount of granulation tissue
- Little fibrosis
- Wound strength 70-80% of normal by 3 months



Healing by Second Intention

- Large wound, may be infected
- Edges **not** brought close together
- Large amount of granulation tissue
- Scar formation and contracture

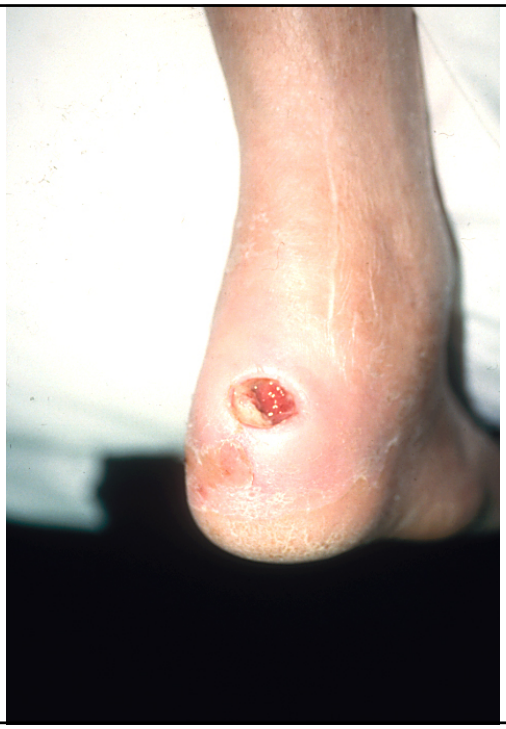


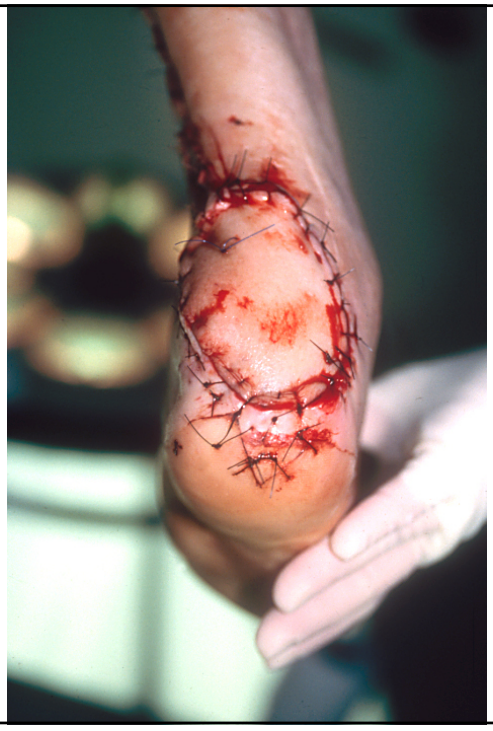
Inhibition of Repair

- Infection with inadequate nutrition (Vitamin C is essential for collagen)
- Glucocorticoids inhibit inflammation with decreased wound strength and less fibrosis.
- Poor perfusion due to diabetes or atherosclerosis.
- Foreign bodies left in the wound.
- Chronic inflammation leads to excess, disabling fibrosis as in rheumatoid arthritis, pulmonary fibrosis and cirrhosis.

Diabetic Foot Ulcer Case #1

- A 52 year old woman has had fairly well controlled type 2 diabetes mellitus for the past 20 years.
- In the last three months, she has noticed a non-healing ulcer on her heel.
- She asks you what can be done to make it heal better.



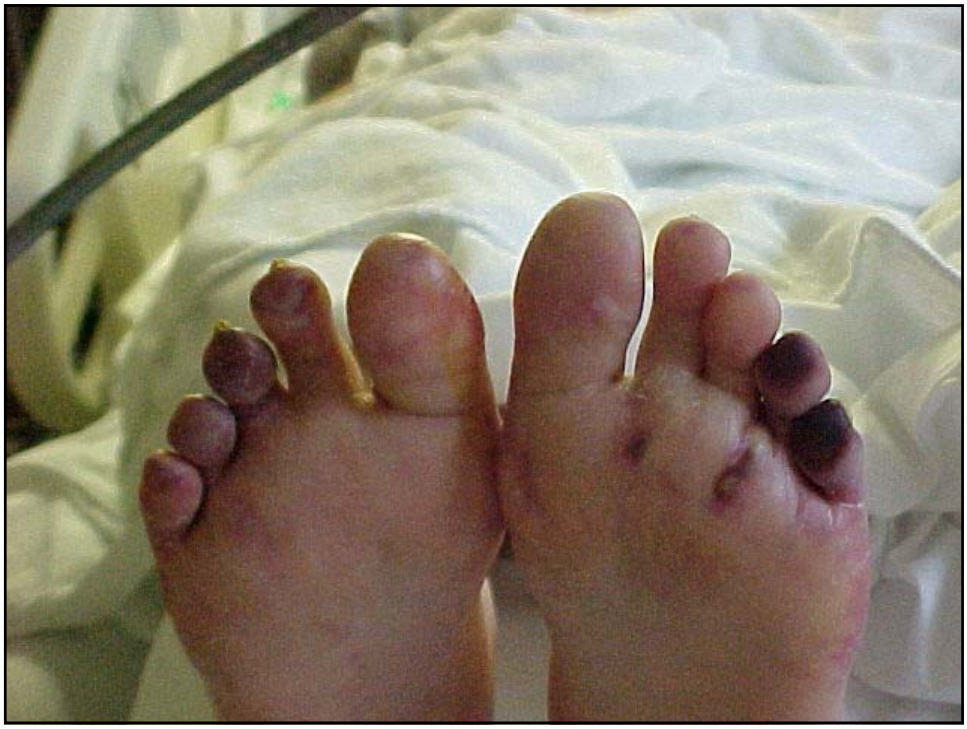


Possible New Therapy

- Application of VEGF alone to wounds in an animal model of diabetes (wound repair is dysregulated in DM) can normalize healing

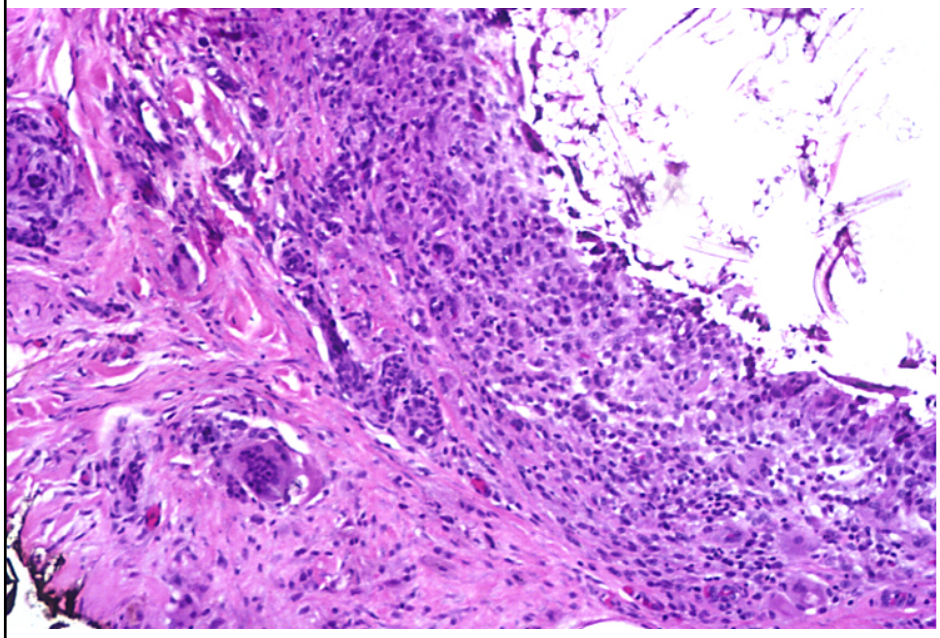
Diabetic Foot Ulcer Case #2

- A 63 year old male has had Type 2 diabetes mellitus for the past 10 years.
- He requires insulin.
- He presents to you with the complaint of a painless sore on the sole of his foot directly beneath a metatarsal head.
- He asks why his foot has difficulty healing.

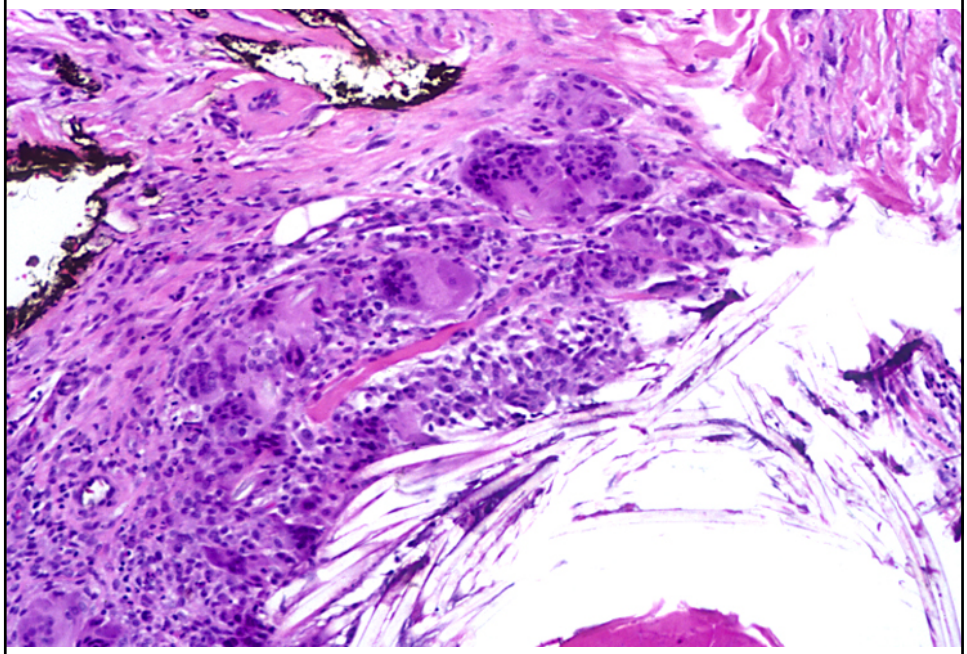


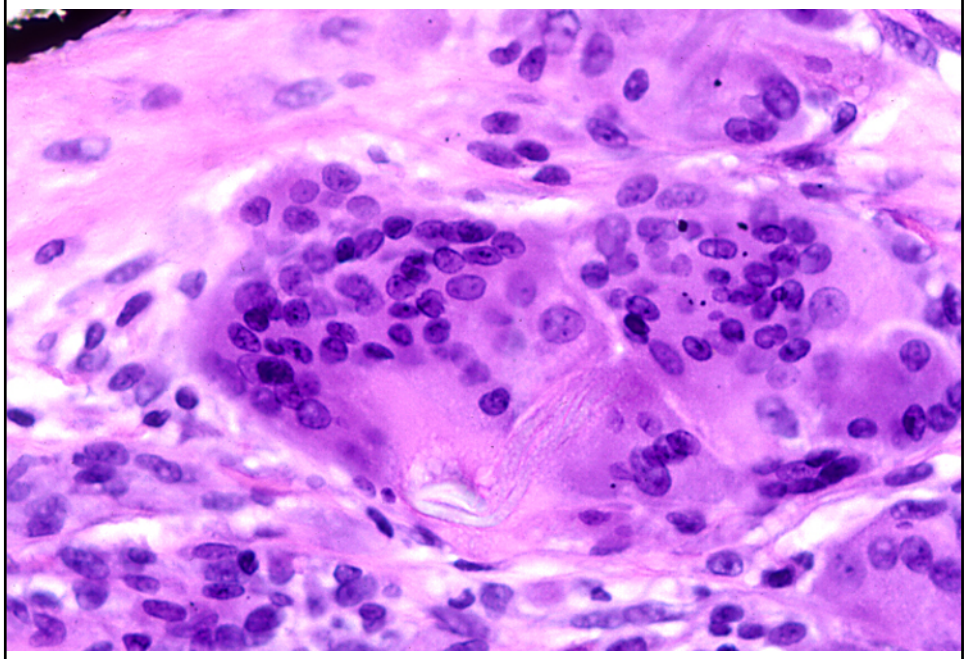
Inhibition of Repair

- Foreign body in wound



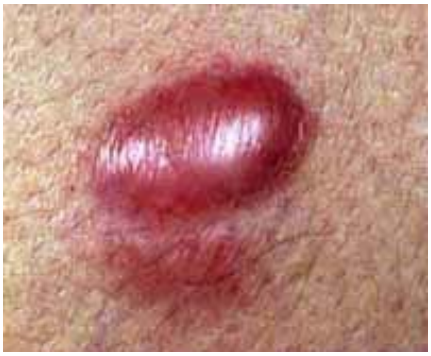
Foreign body - suture

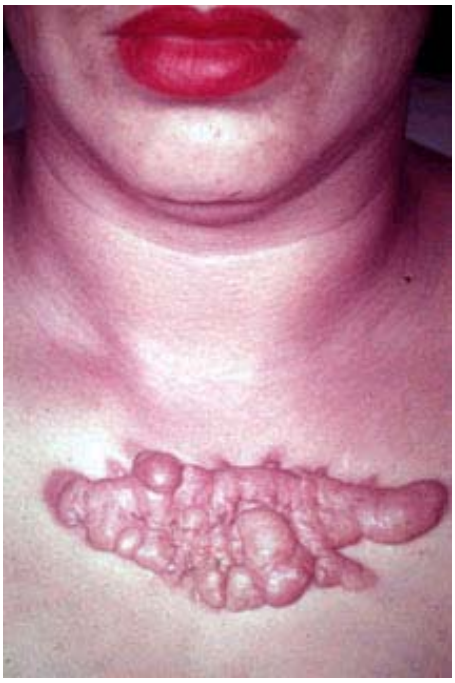


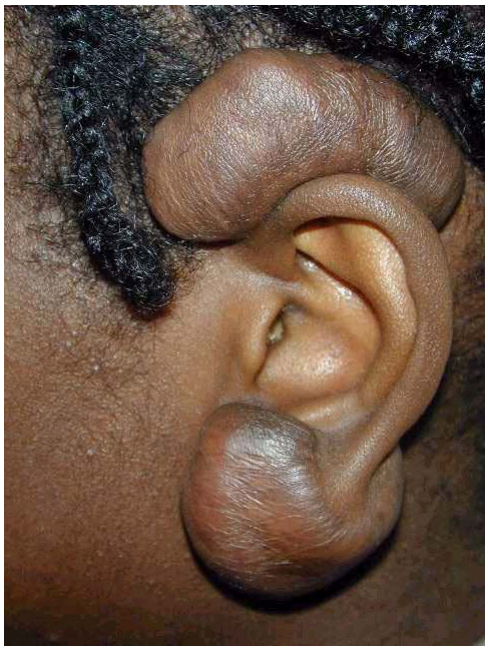


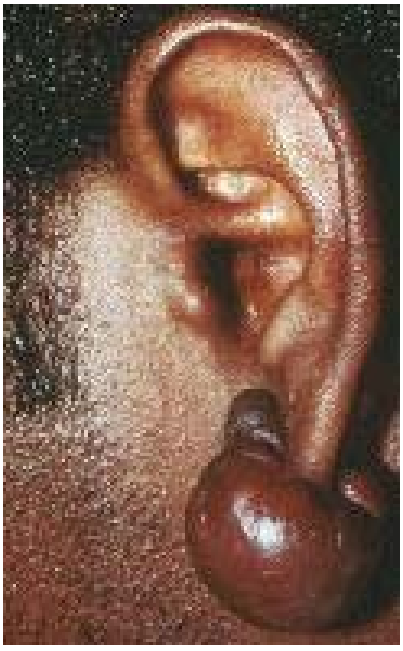
Abnormal Repair Processes

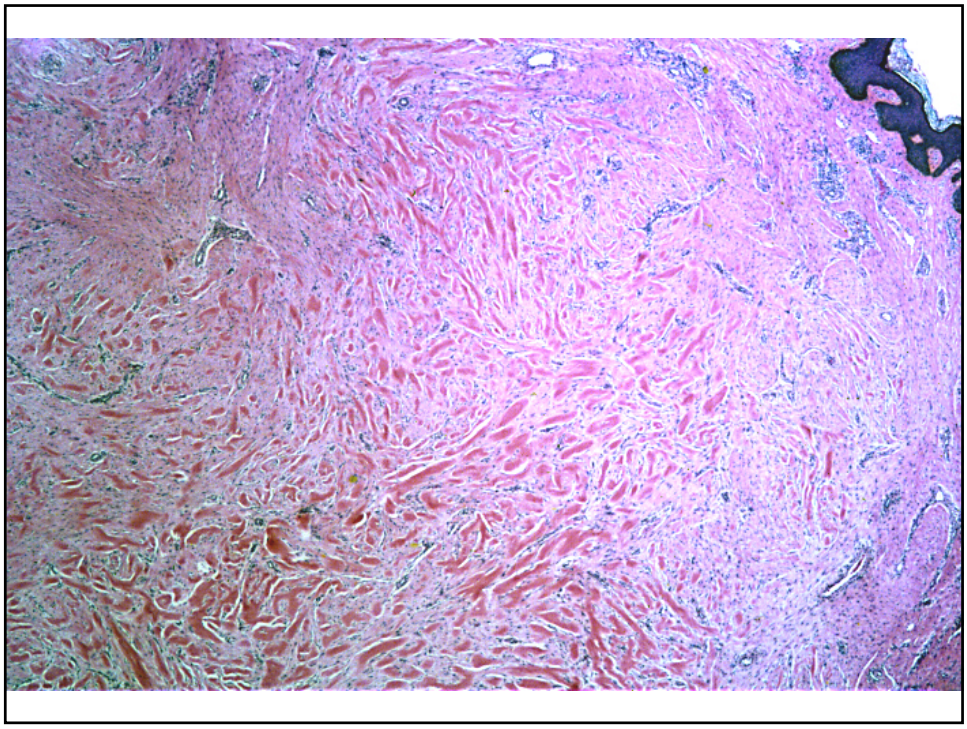
- Inadequate scar formation - dehiscence, ulceration
- Excessive scar formation – keloids
- Contracture – exaggeration of normal process (soles, palms, thorax) especially with serious burns

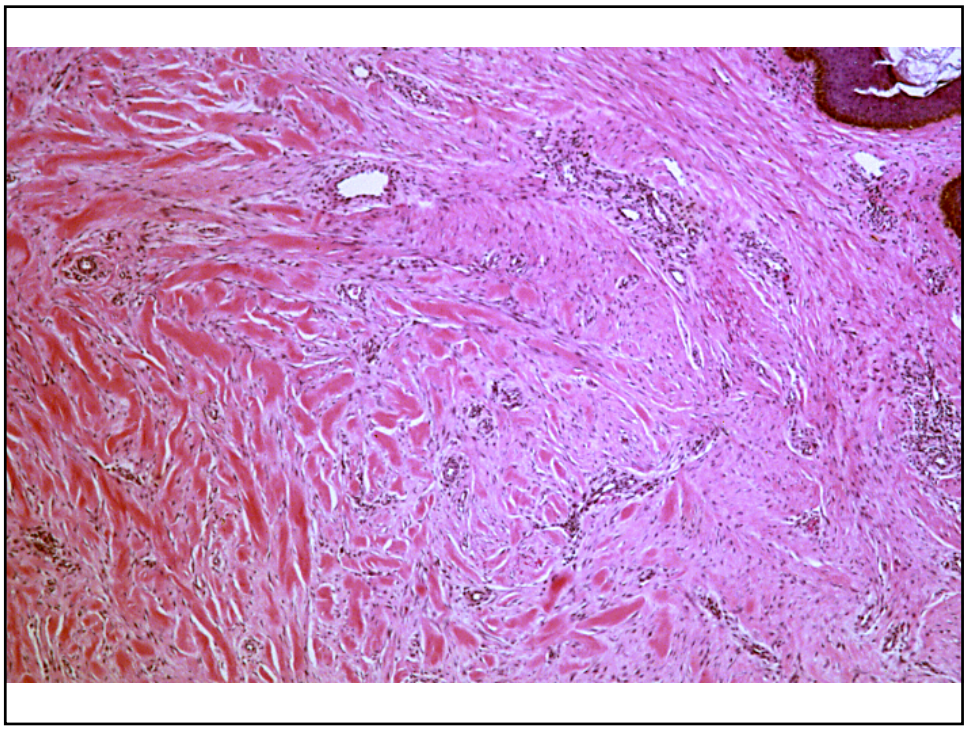


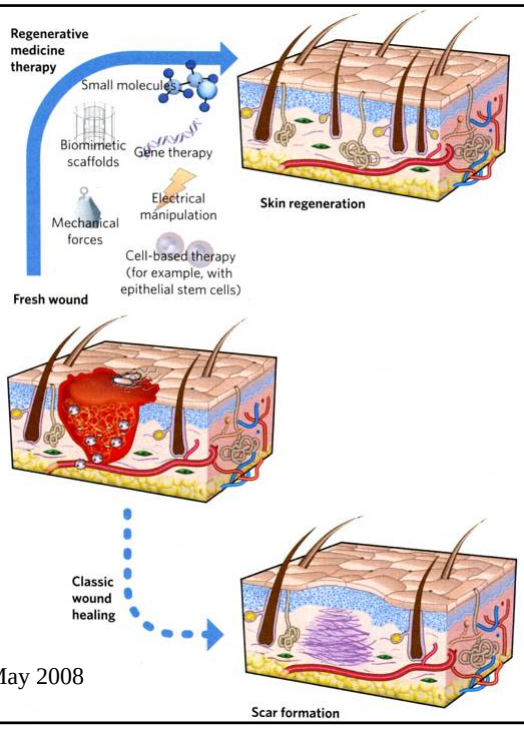












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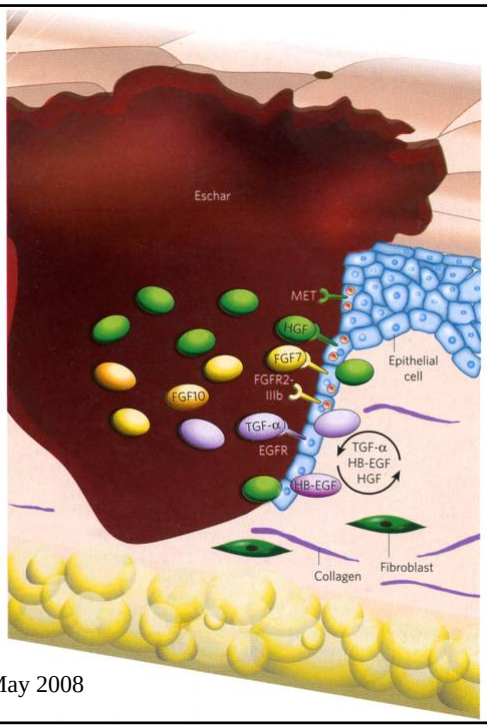

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