Wound Healing

Tissue Repair: Regeneration and Fibrosis

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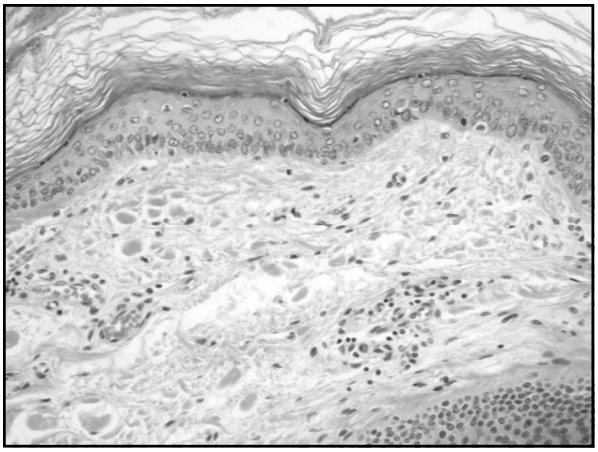
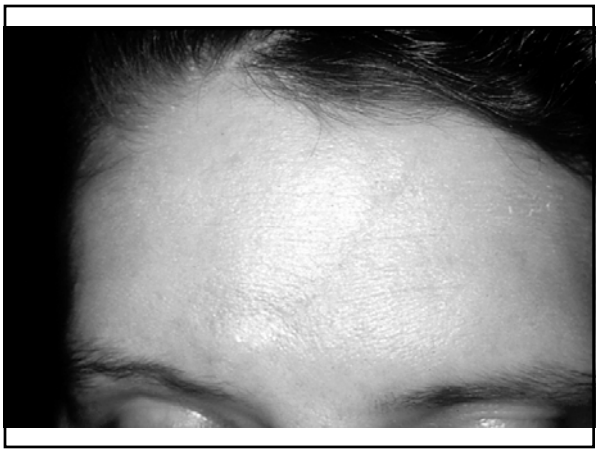
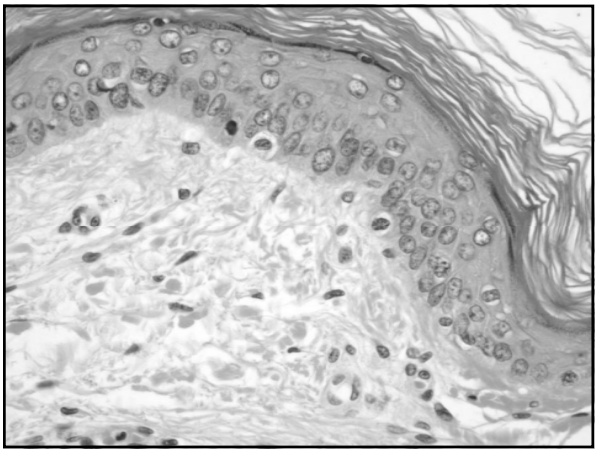
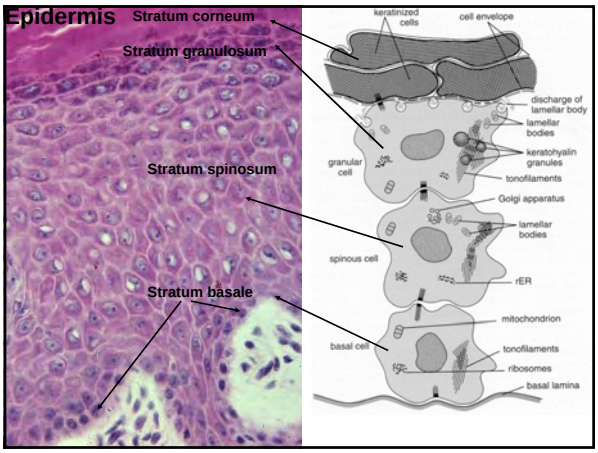
Lecture Outline

- Cutaneous wound healing
- Control of Cell Proliferation – cell cycle
- Growth Factors
- Extracellular matrix
- Regeneration or Repair (scar) **Outcomes**
- Primary and Secondary Intention
- Pathologic repair









Events 2

- **Monocytes** recruited next and become macrophages
  - produce cytokines, growth factors and angiogenic factors
- **New blood vessels** develop and organize **granulation tissue (four days after injury)**
- **Reepithelialization** starts when keratinocytes of stratum basale migrate in from edges using F-actin containing lamellopodia
  - Leading edge keratinocytes disrupt hemidesmosomes and dissolve fibrin clot barrier by upregulating **plasminogen activator** (plaminogen to plasmin)
  - **MMP's** produced by dermal fibroblasts help to free migrating keratinocytes
  - **Epidermal growth factor family** : EGF, TGFα, heparin binding EGF and **keratinocyte growth factor drive reepithelialization**

Cutaneous Wound Healing

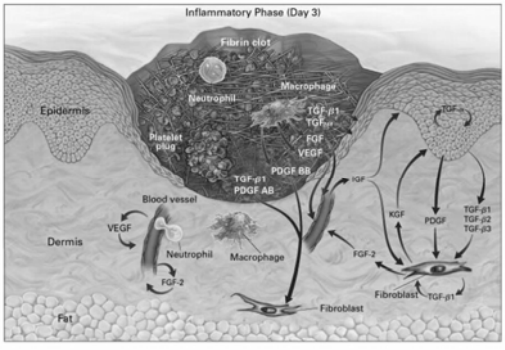
- Formation of fibrin-platelet clot
- Leukocyte recruitment
- Neovascularization and cell proliferation
- Tissue remodeling

Events 3

- Within 3-4 days after injury connective tissue of dermis contacts bringing wound margins closer
- Local PDGF and TGFβ drive local fibroblasts to proliferate, infiltrate clot and deposit ECM and type III collagen
- After 1 week, some wound fibroblasts become myofibroblasts (resemble smooth muscle) and wound contracture results

Events in Wound Healing

- **Blood clot** temporarily closes wound
- **Platelets** in a fibrin mesh of cross-linked fibrin formed when thrombin cleaves fibrinogen
  - **PDGF** stored in alpha granules of platelets released on platelet degranulation
- **Leukocytes** arrive at wound site
  - Keratinocytes and endothelial cells express cytokine CXC and CXC receptor which recruits neutrophils, monocytes, and lymphocytes to wound site (CXC receptor gene deletion results in delayed wound healing)
- **Neutrophils** arrive within minutes of injury
  - release proinflammatory cytokines to activate local fibroblasts in dermis keratinocytes in epidermis



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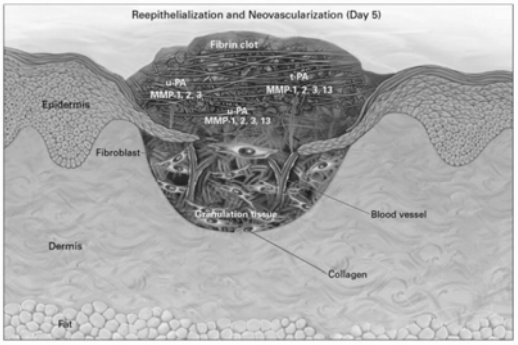

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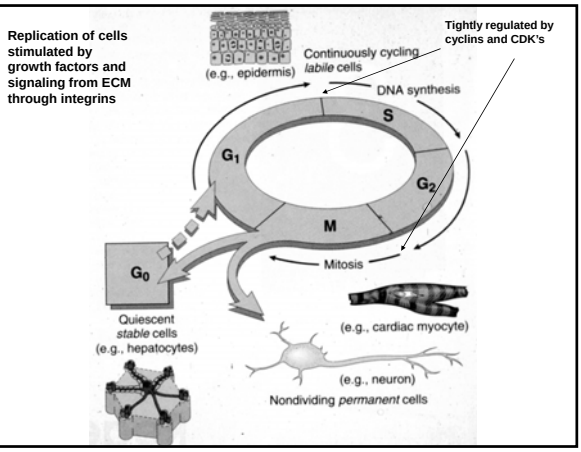
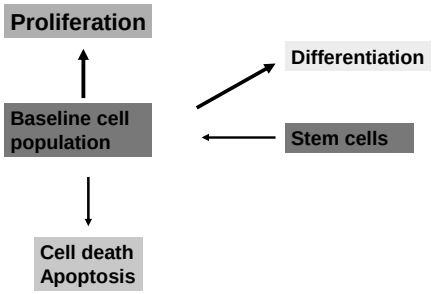




Tissue Steady State

Signaling of Growth Factor Receptors

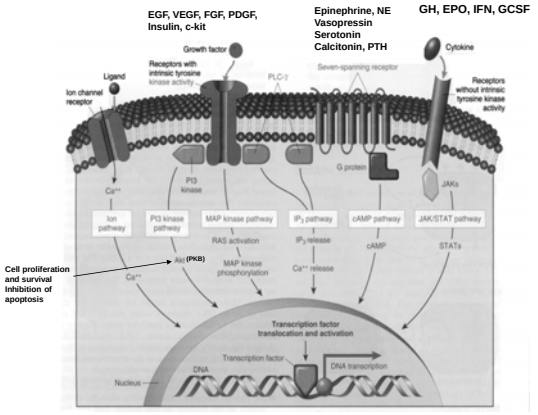
- Autocrine – lymphocytes, liver
- Paracrine – macrophages in wound healing (fibroblasts)
- Endocrine – endocrine organs - hormones

Tissue Types

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

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**)



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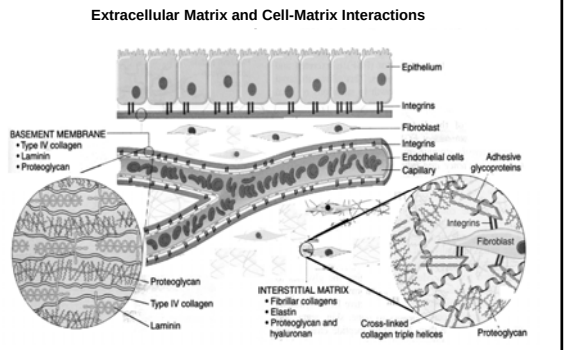

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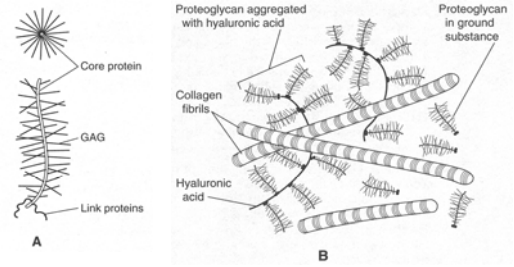
Extracellular Matrix

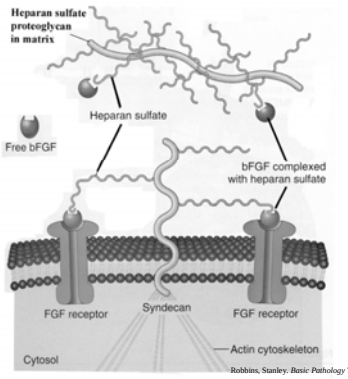
- Interstitial matrix – fibers, cells and ground substance
- Basement membrane – nonfibrillar collagen and laminin underlying epithelium and surrounding blood vessels

Role of ECM

- Mechanical Support – collagen and elastin
- Provides anchorage, cell migration, cell polarity
- Substrate for cell growth with tissue microenvironments
- Controls cell proliferation and differentiation –
  - PG's bind growth factors and sequester them in high concentration
  - Fibronectin and laminin stimulate cells via integrin receptors



FGF stimulates keratinocyte migration, wound contracture and matrix depostion



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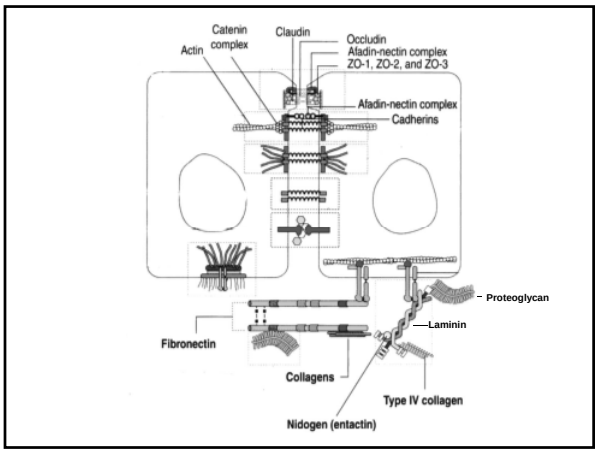
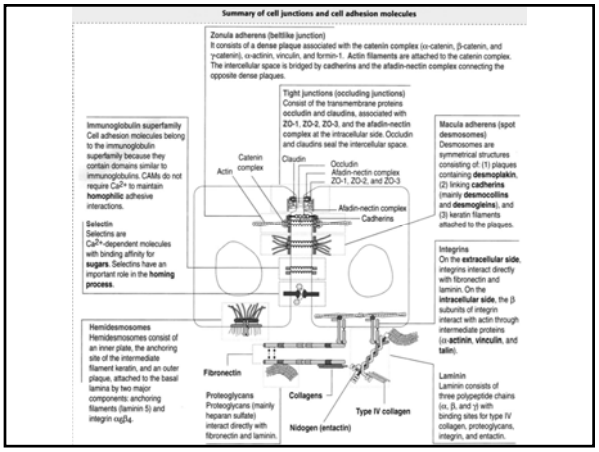
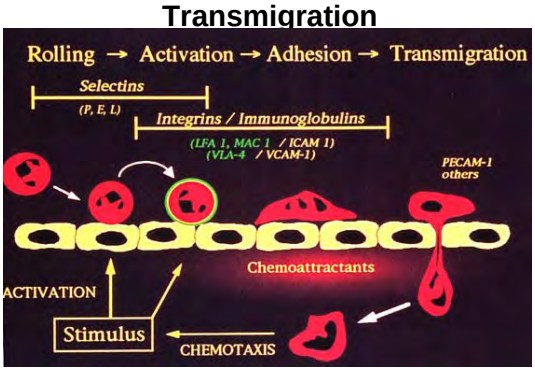


Classic Stages of Wound Repair

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



Model of Leukocyte Transmigration



Wound Repair and Regeneration

Patch of fibroblasts with disorganized ECM

Scar
Non-functional tissue

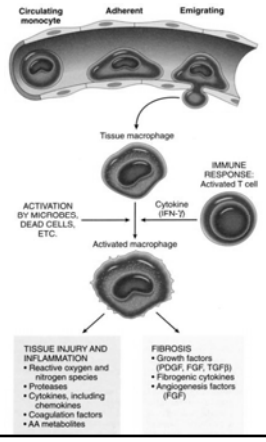
←

Injury to tissue

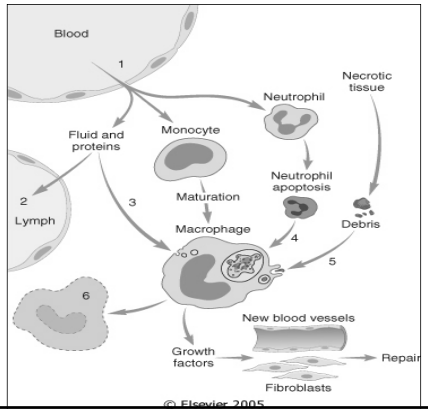
Lung
Kidney
Heart
Skin
Liver
Spleen

→

Functional tissue

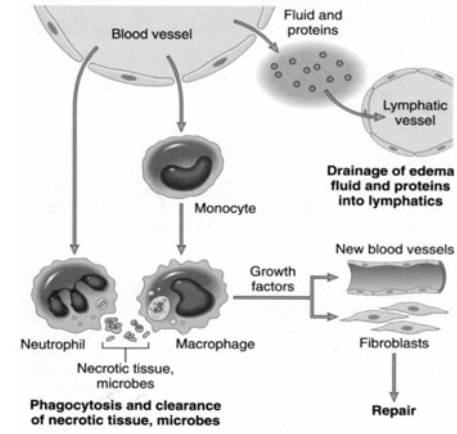


Macrophages in inflammation and repair



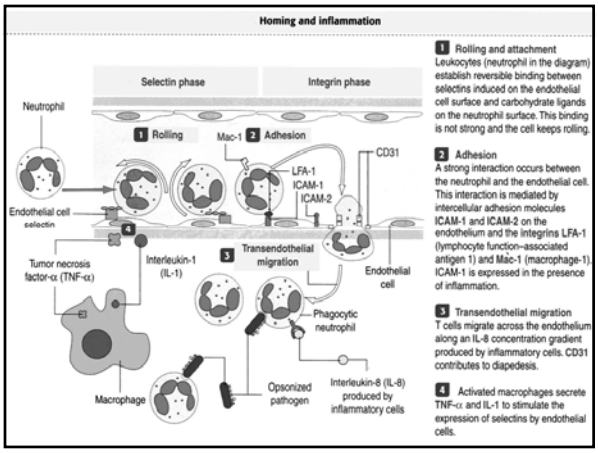
Repair By Connective Tissue

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

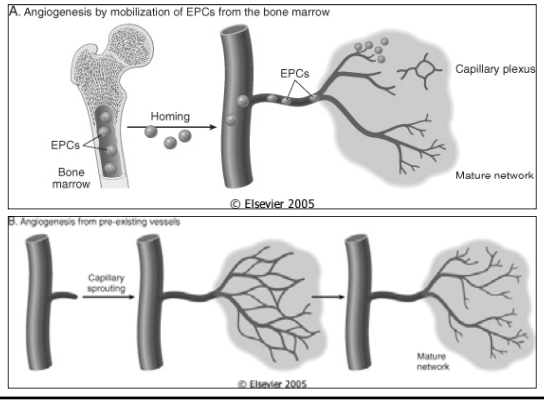


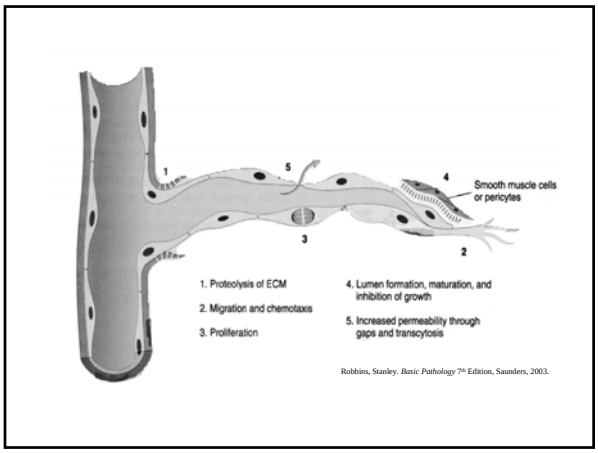
Angiogenesis

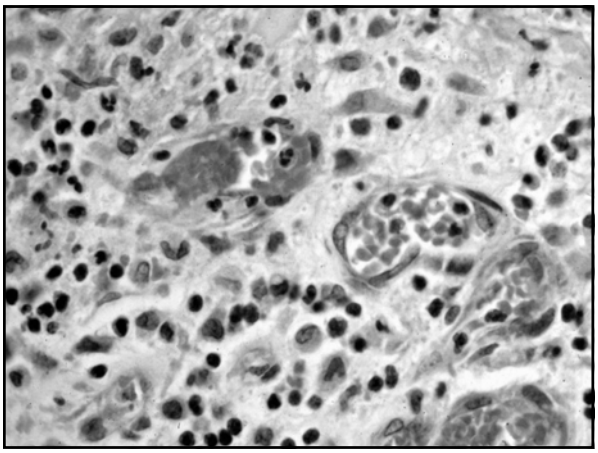
- Proteolysis of vessel basement membrane
- Endothelial cell migration and proliferation
- Pericyte recruitment
- New blood vessels sustain granulation tissue

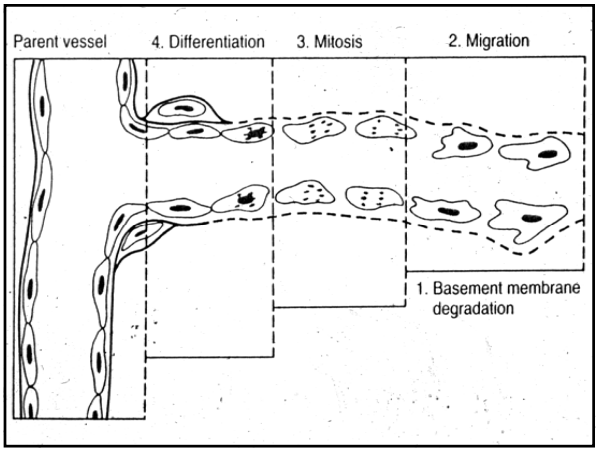


Two types of angiogenesis



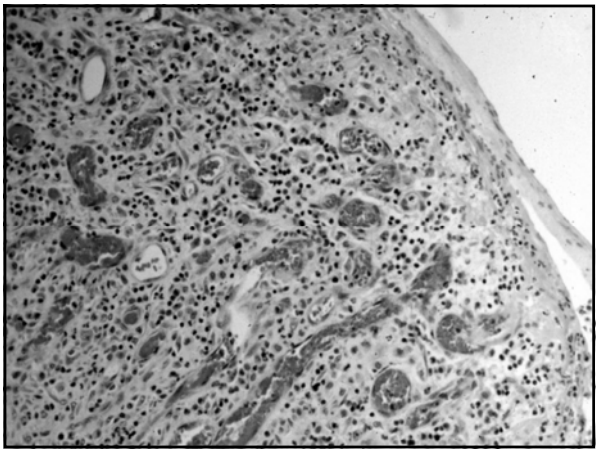






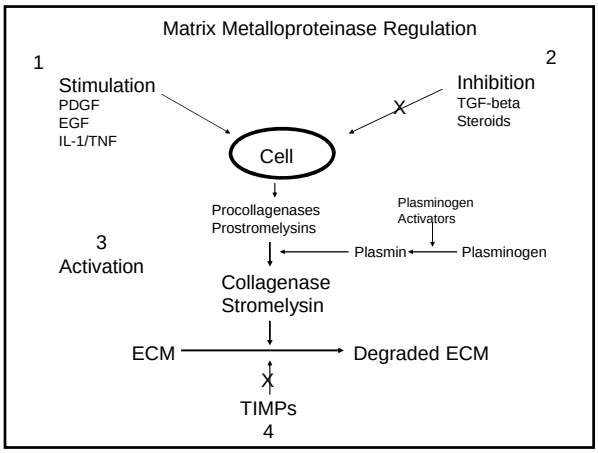
Scar Formation

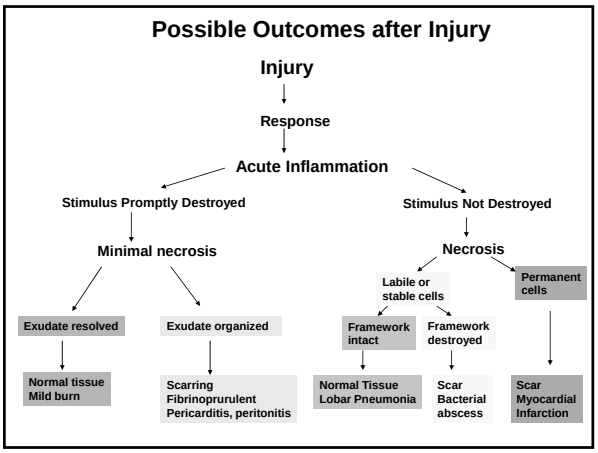
- Fibroblast proliferation and migration
  - PDGF, FGF, TGF-beta mainly from macrophages
- ECM deposition
  - TGF-beta – **potent agent of fibrosis**

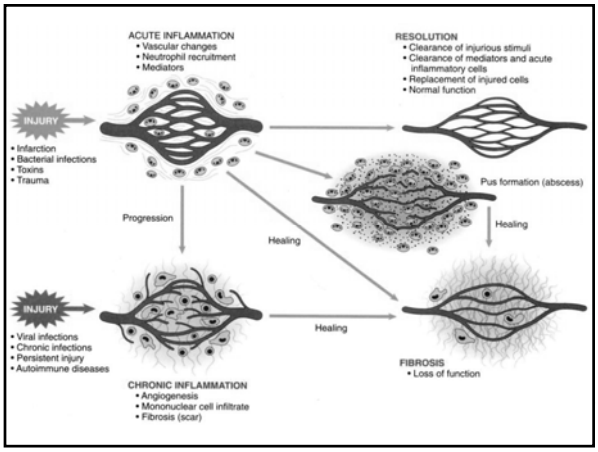


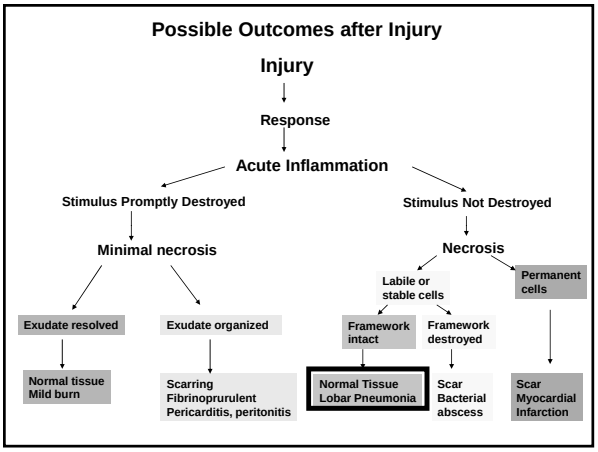
ECM and Tissue Remodeling

- Outcome of repair: **balance** between **synthesis** and **degradation** of matrix
  - **MMP's:** matrix metalloproteinases: made by fibroblasts, macrophages, neutrophils, epithelial cells destroy matrix
    activated by proteases and plasmin and inhibited by
  - **TIMP's**: tissue inhibitors of matrix metalloproteinases: synthesized by mesenchymal cells to control degradation









Classic Stages of Wound Repair

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- New tissue formation – 2-10 days after injury
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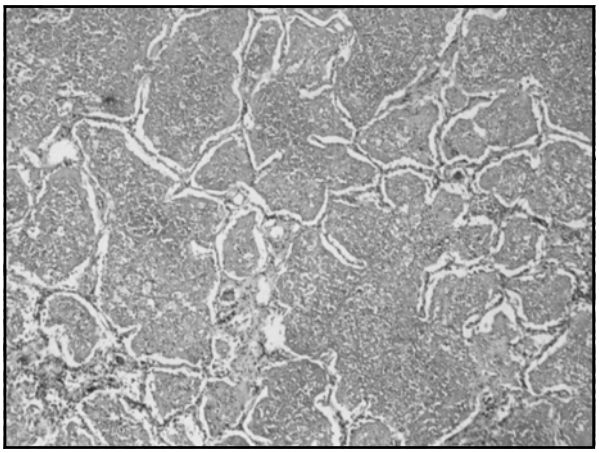
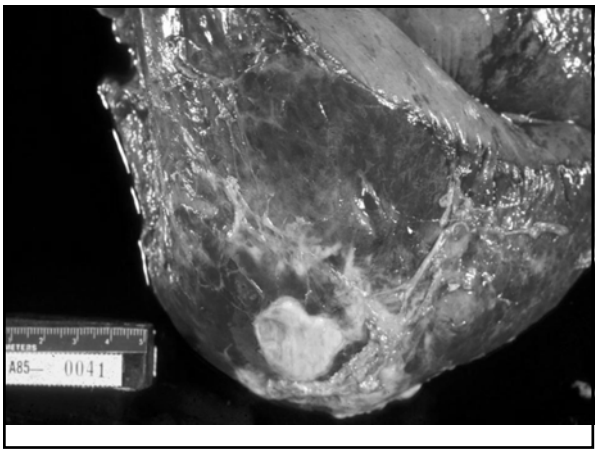
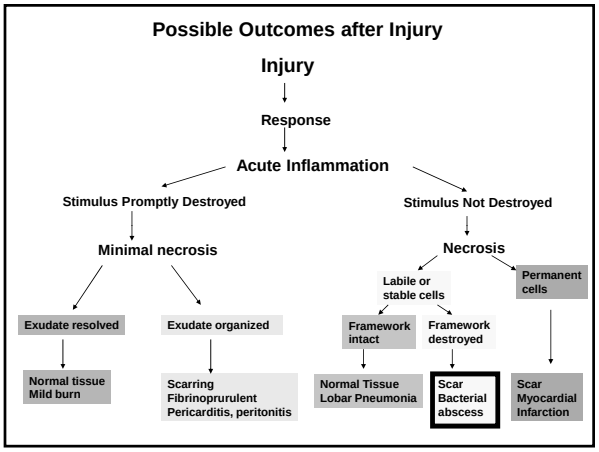
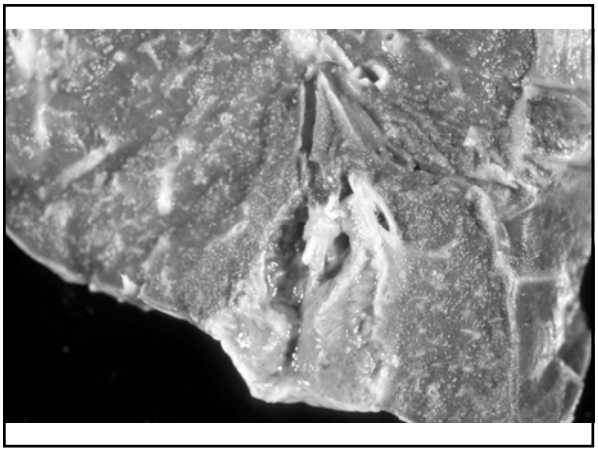
Regeneration

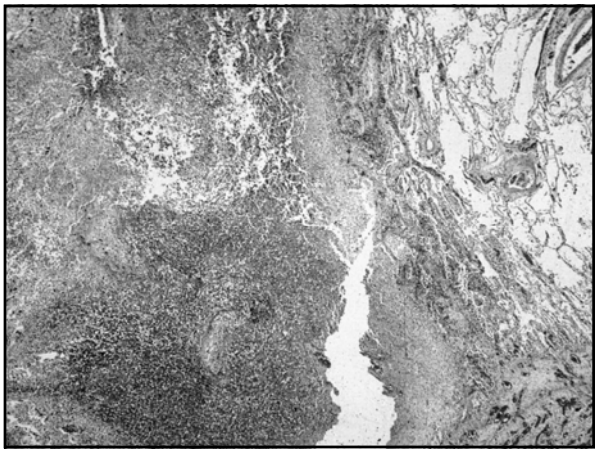
- If the connective tissue framework is intact
- If the cells are not post-mitotic
- THEN:
- Complete restoration of the structure and function of the tissue is possible

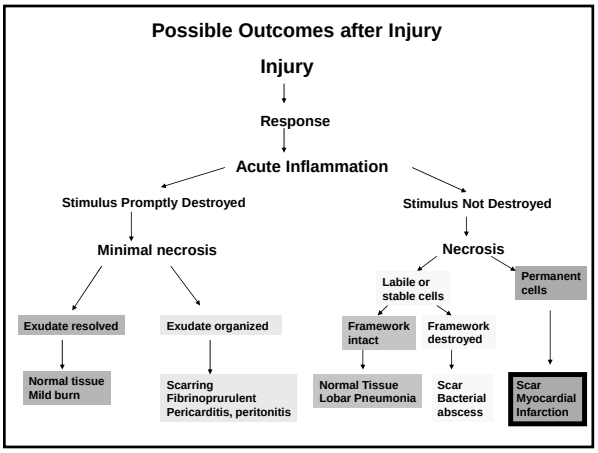





Possible Outcomes after Injury
Injury
Response
Acute Inflammation
Stimulus Promptly Destroyed
Stimulus Not Destroyed
Minimal necrosis
Necrosis
Labile or stable cells
Permanent cells
Exudate resolved
Exudate organized
Framework intact
Framework destroyed
Normal tissue Mild burn
Scarring Fibrinoprurulent Pericarditis, peritonitis
Normal Tissue Lobar Pneumonia
Scar Bacterial abscess
Scar Myocardial Infarction



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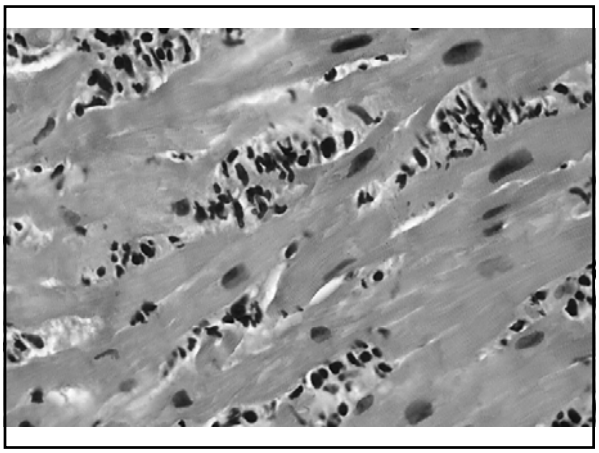

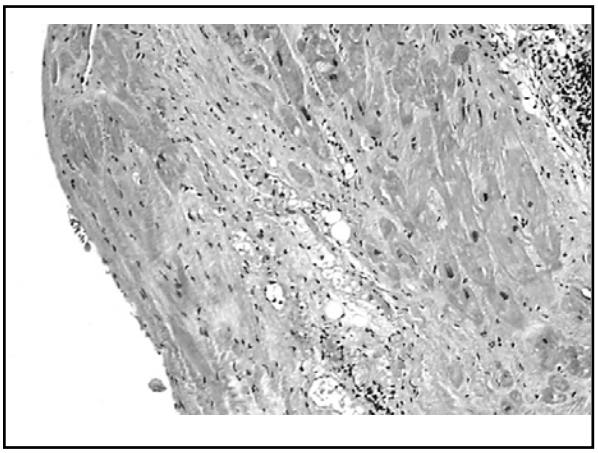

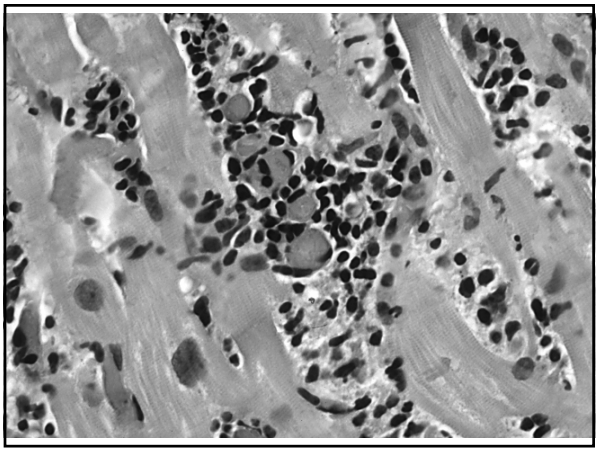

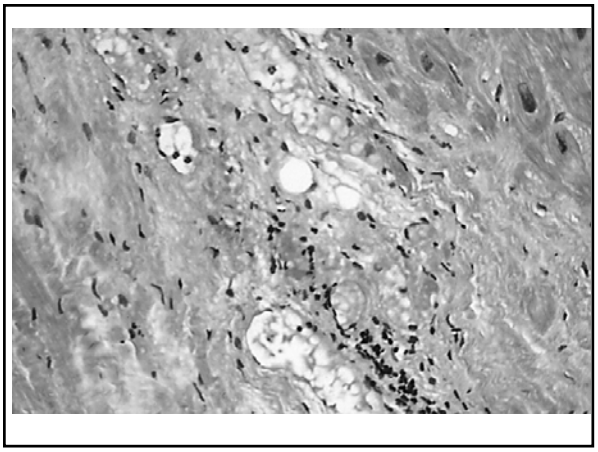

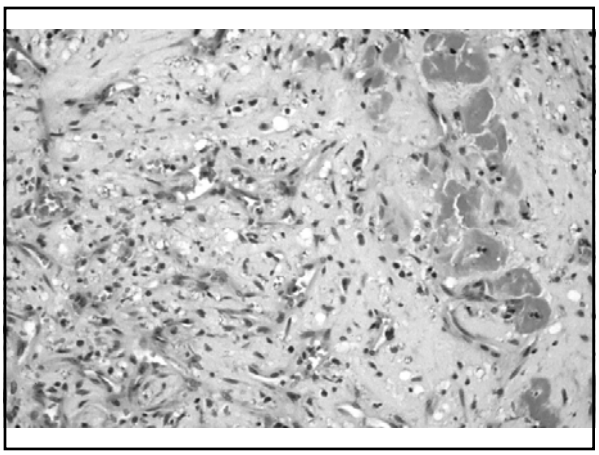

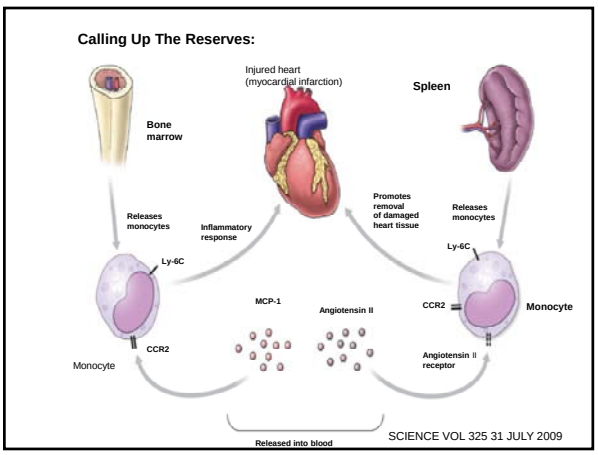
Calling Up The Reserves:
Injured heart
(myocardial infarction)
Spleen
Bone
marrow
Releases
monocytes
Inflammatory
response
Promotes
removal
of damaged
heart tissue
Releases
monocytes
Ly-6C
Ly-6C
MCP-1
Angiotensin II
CCR2
Monocyte
Monocyte
CCR2
Angiotensin II
receptor
Released into blood
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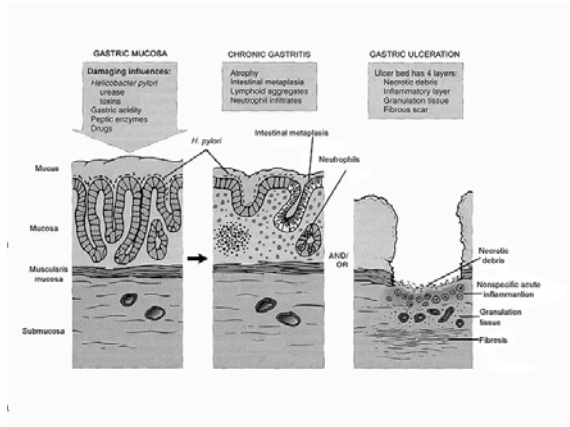
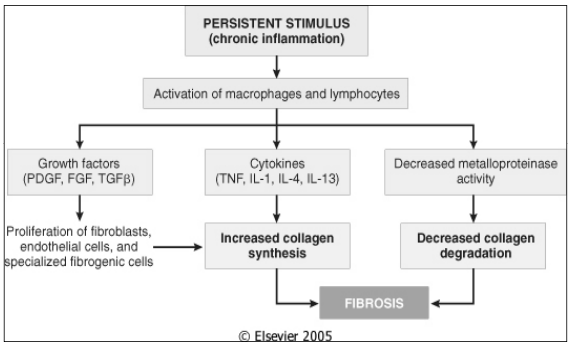
Repair by Fibrosis

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



Macrophages in healing and fibrosis



Fibrotic response to toxin-mediated injury

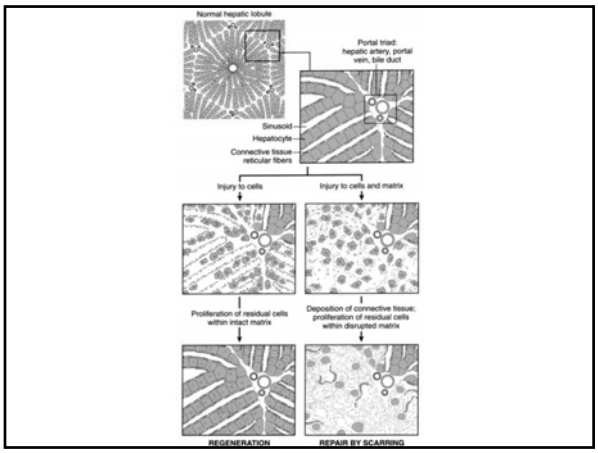
- Poorly understood:
  -Liver Hepatitis B,C
  -Pulmonary fibrosis

Chronic Peptic Ulcer

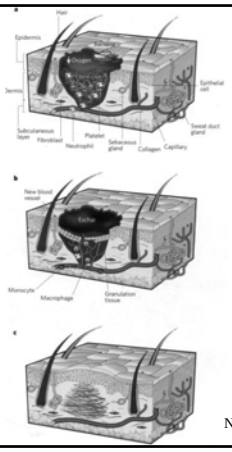
Fibrosis below the ulcer bed

Scarring in the Liver

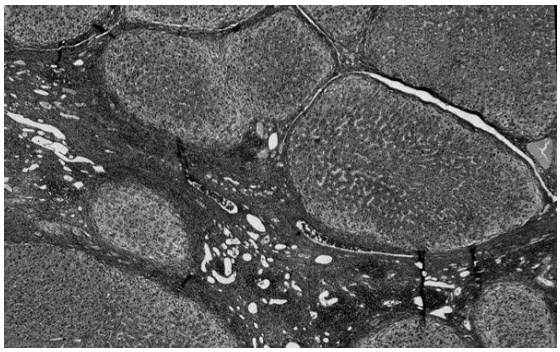
- Healing by fibrosis after inflammation
- **TGF beta** implicated in excessive collagen formation



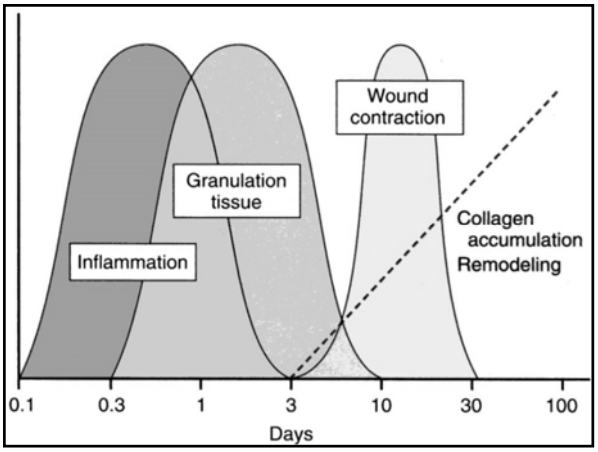
Cutaneous Wound Healing



Nature Vol. 453;15 May 2008



Cirrhosis

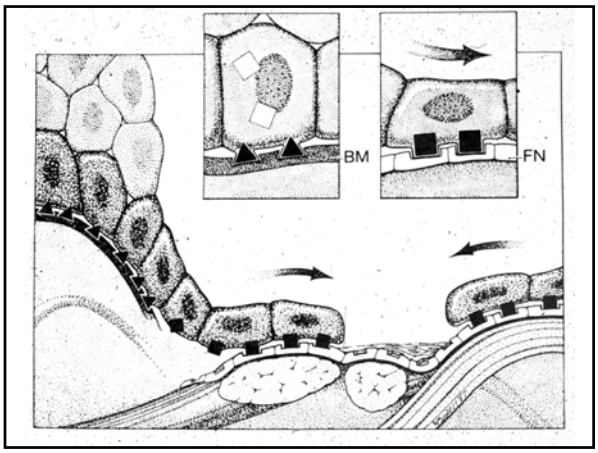


Overview of Cutaneous Wound Healing

- A defect in the skin occurs
- Fibrin clot 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 up to 24 hrs
- **Macrophages** move in (by 48-96 hrs) 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)

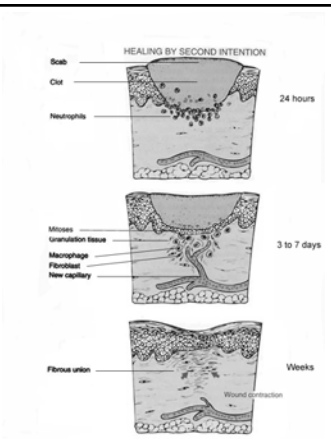


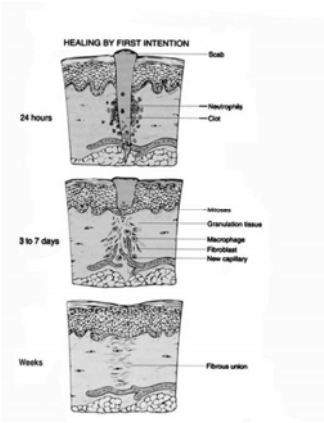
Healing by Second Intention

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

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





Inhibition of Repair

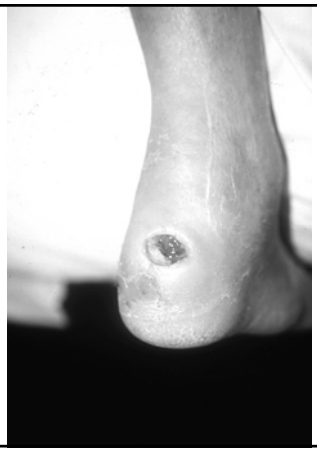
- Infection with inadequate nutrition (Vitamin C is essential for collagen)
- Glucocorticoids inhibit inflammation with decreased wound strength and less fibrosis.
- Poor perfusion due to diabetes or atherosclerosis.
- Foreign bodies left in the wound.
- Chronic inflammation leads to excess, disabling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

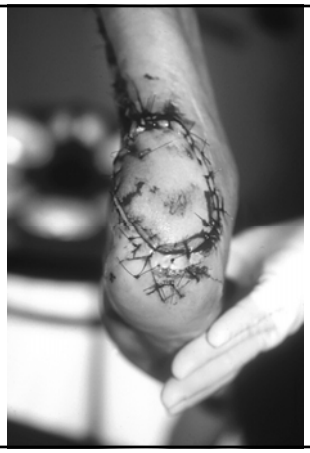
Possible New Therapy

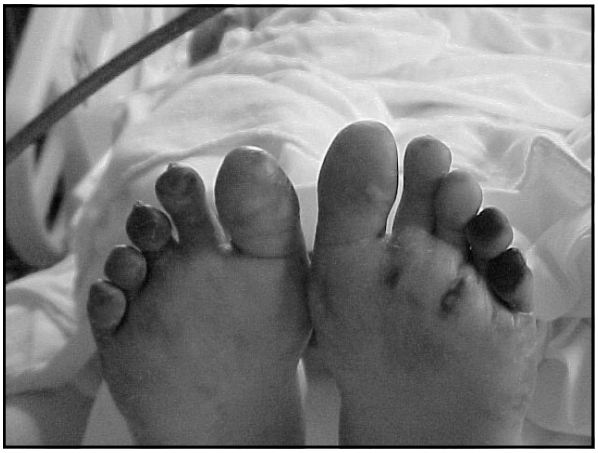
- Application of VEGF alone to wounds in an animal model of diabetes (wound repair is dysregulated in DM) can normalize healing



Diabetic Foot Ulcer Case #2

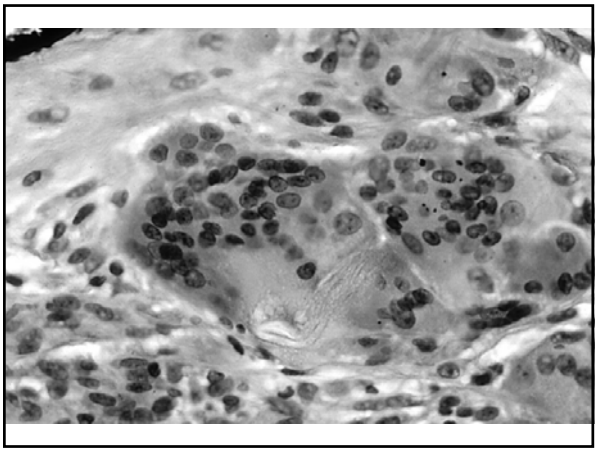
- A 63 year old male has had Type 2 diabetes mellitus for the past 10 years.
- He requires insulin.
- He presents to you with the complaint of a painless sore on the sole of his foot directly beneath a metatarsal head.
- He asks why his foot has difficulty healing.

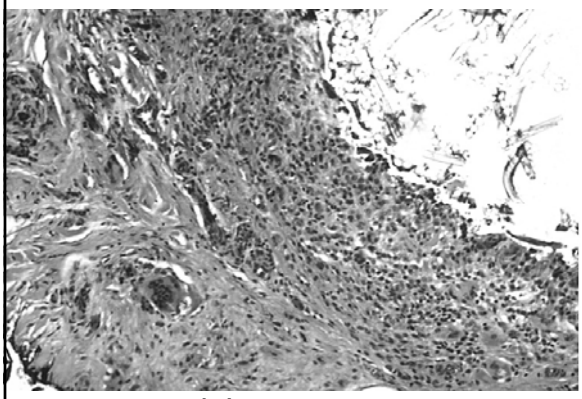




Inhibition of Repair

- Foreign body in wound

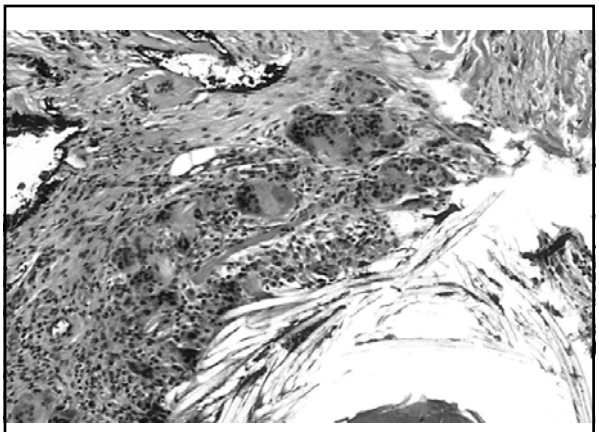


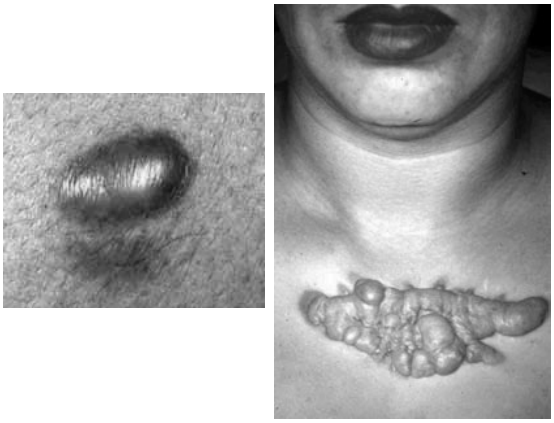


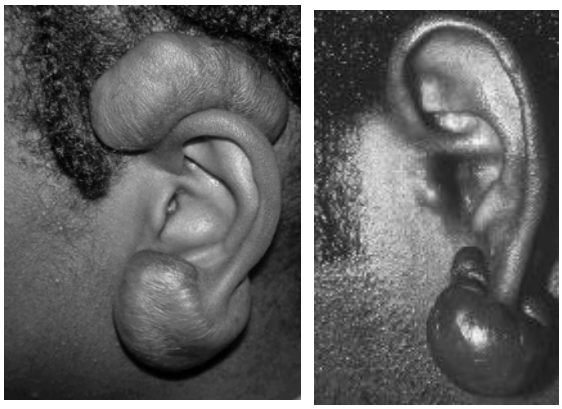
Foreign body - suture

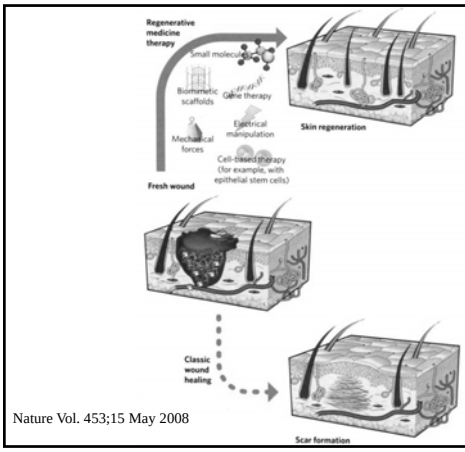
Abnormal Repair Processes

- 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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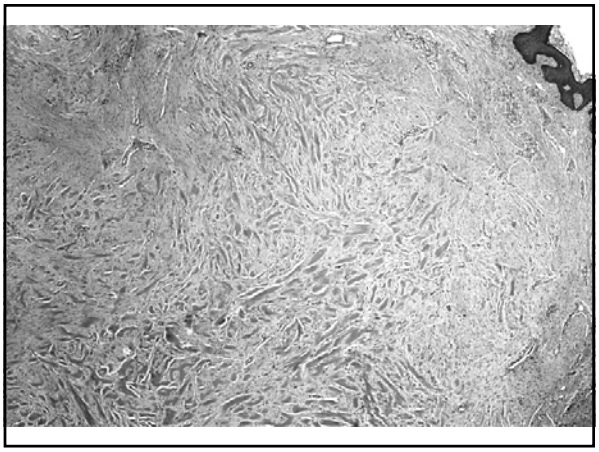
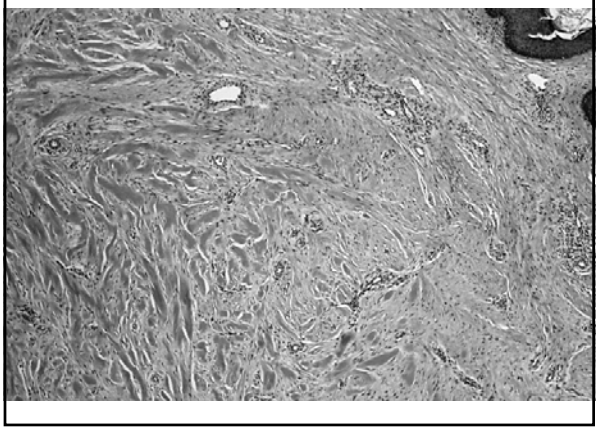
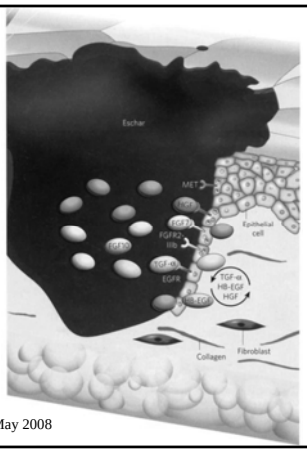


Extra Slides





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VEGF

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

EGF

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

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

FGF

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

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

Extra 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?