CHS 2413
Pathology and Physiopathology

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Summary

This talk has covered….

- Wound
- Wound Healing
- Phases of healing
- Factors Impaired Wound Healing
- Wound Healing and the Presence of Biomaterials
- Mechanisms of Disease: Regeneration and Fibrous Repair
- Special features of healing in specific organs
- Cellular response in Regeneration or Healing (repair)

WOUND HEALING

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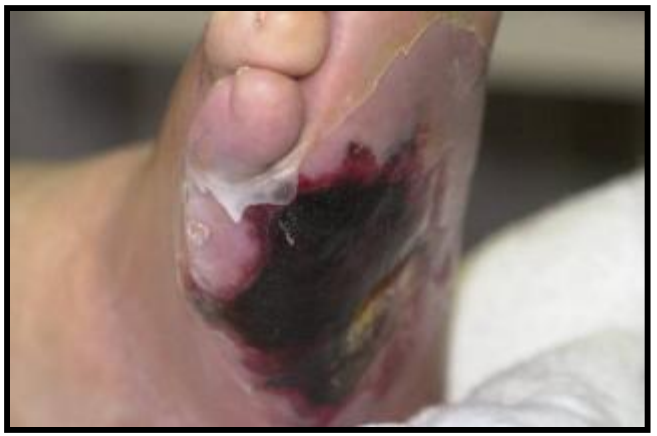
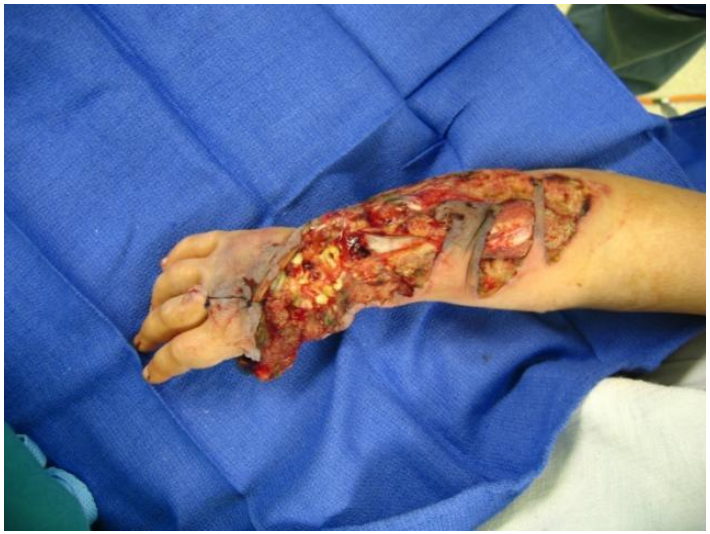
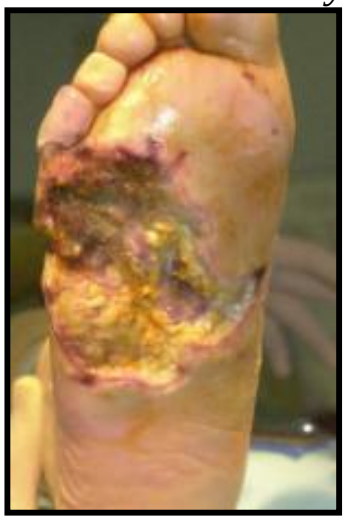
Wounds

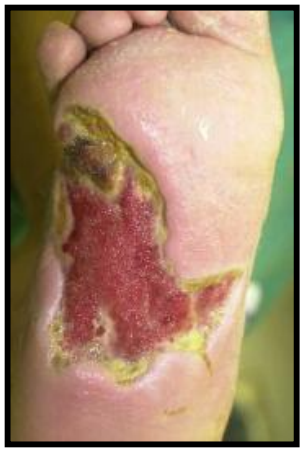
No two patients OR wounds are identical

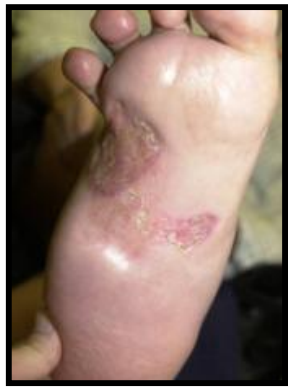


58y DM, Neuropathy: unaware of R foot gangrene









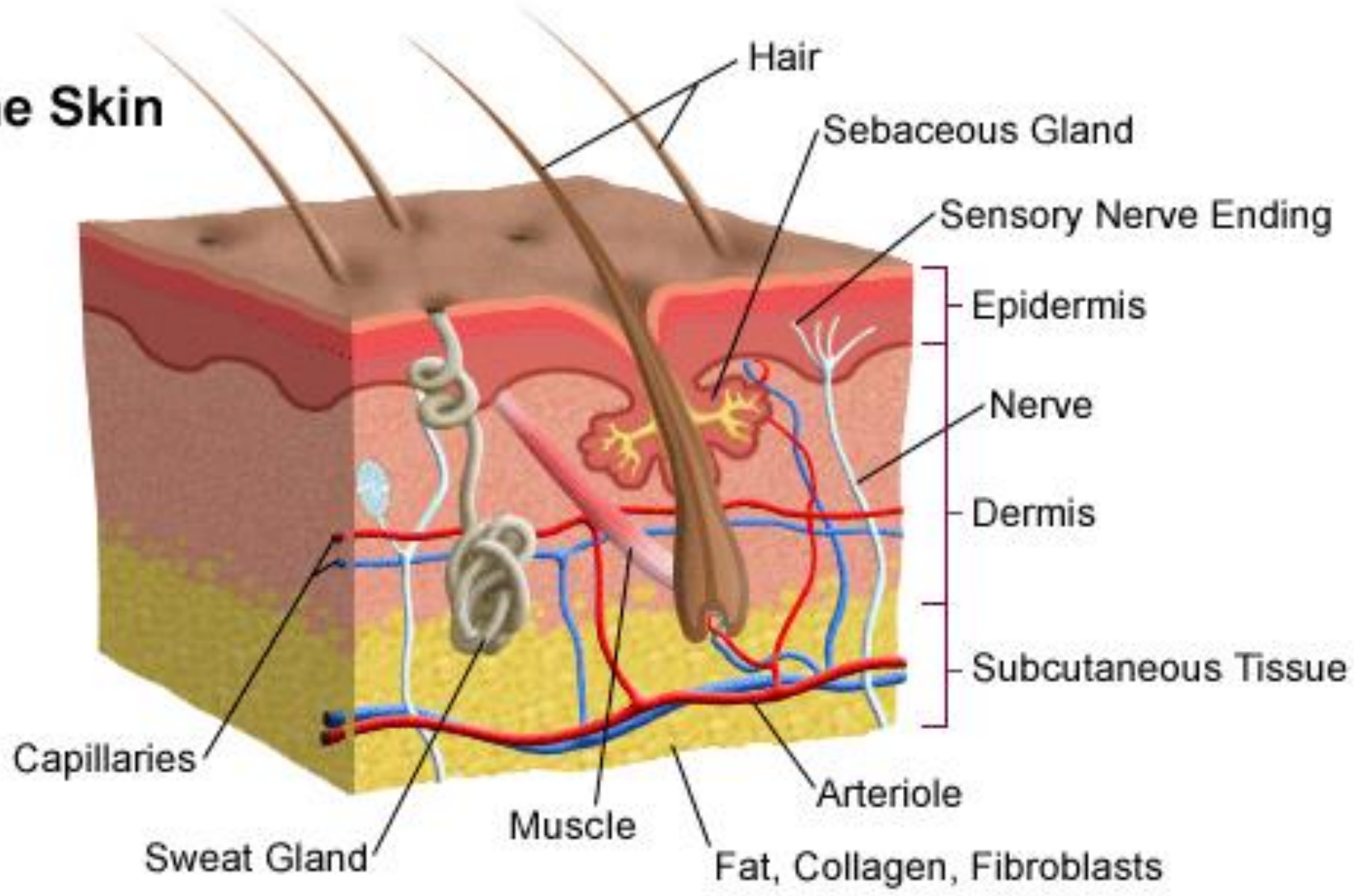
Anatomy of Skin

- **Epidermis:**
  - composed of several thin layers: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, stratum corneum
  - the several thin layers of the epidermis contain the following:

    a) melanocytes, which produce melanin, a pigment that gives skin its color and protects it from the damaging effects of ultraviolet radiation.

    b) keratinocytes, which produce keratin, a water Repellent protein that gives the epidermis its tough, Protective quality.
- **Dermis:**
  - composed of a thick layer of skin that contains collagen and elastic fibers, nerve fibers, blood vessels, sweat and sebaceous glands, and hair follicles.
- **Subcutaneous Tissue:**
  - composed of a fatty layer of skin that contains blood vessels, nerves, lymph, and loose connective tissue filled with fat cells

The Skin


Hair
Sebaceous Gland
Sensory Nerve Ending
Epidermis
Nerve
Dermis
Subcutaneous Tissue
Capillaries
Sweat Gland
Muscle
Arteriole
Fat, Collagen, Fibroblasts

ExtraCellular Matrix (ECM)

- **Collagen(s) I-XXVII**
  - Type I - Bone, tendon, scars.
  - Type II - cartilage.
  - Type III - 'tissue scaffold'.
  - Type IV - non-fibrous, basement membranes ...
- **Elastin**
- **Fibrillin**
- **CAMs (Cell Adhesion Molecules)**
  - **Immunoglobulins, adherins, integrins, selectins**
- **Proteoglycans:** Heparan sulphate proteoglycan, ...
- **Glycoproteins**
  - Fibronectin, Osteonectin, Tenascin,...
- **Hyaluronic Acid**

Function:

- **Maintain cell differentiation**
- **Establish microenvironment**
- **Storage of GF's**

Function of Integument

1. Protection:

intact skin prevents invasion of the body by bacteria

2. Thermoregulation:

- intact skin facilitates heat loss and cools the body when necessary through the following processes:
  - production of perspiration which assists in cooling the body through evaporation
  - production of vasodilatation which assists in facilitating heat loss from the body through radiation and conduction
  - production of vasoconstriction which assists in preventing heat loss from the body through radiation and conduction

3. Fluid and Electrolyte Balance:

intact skin prevents the escape of water and electrolytes from the body

4. Vitamin D Synthesis

5. Sensation

6. Psychosocial

Types of collagen: So far, 28 types of collagen have been identified and described. The five most common types are:

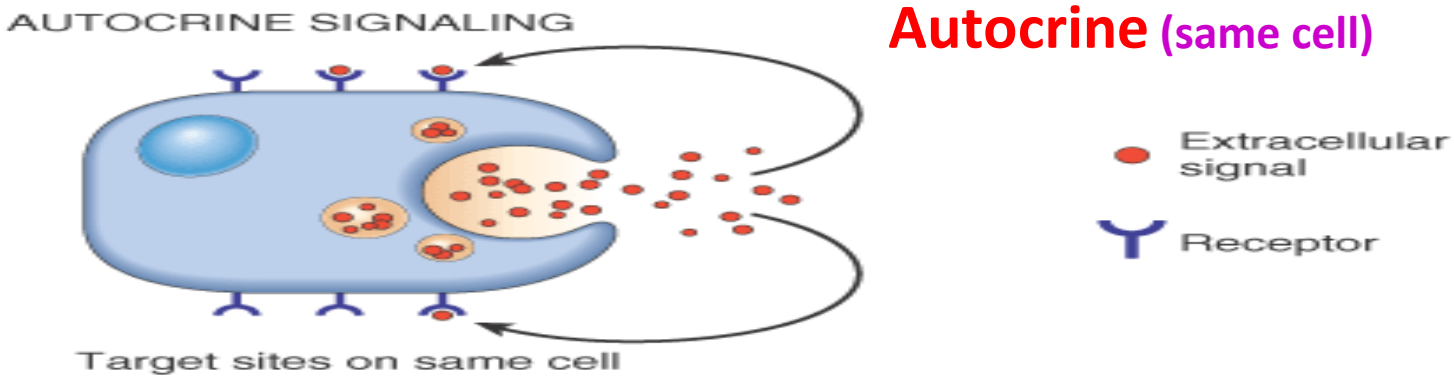
Fibril forming	Tissue	Function
Type 1 *(90%)*	Tendon, bone, ligaments and skin	Resistance to tension
Type 2	Hyaline and elastic cartilage	Resistance to pressure
Type 3	Skin, muscle, blood vessels	Structural framework for expanding tissues
Network forming	**Tissue**	**Function**
Type 4	Basement membrane	Filtration and support
Anchoring fibrils	**Tissue**	**Function**
Type 7	Epithelium	Anchors basal cells to underlying stroma

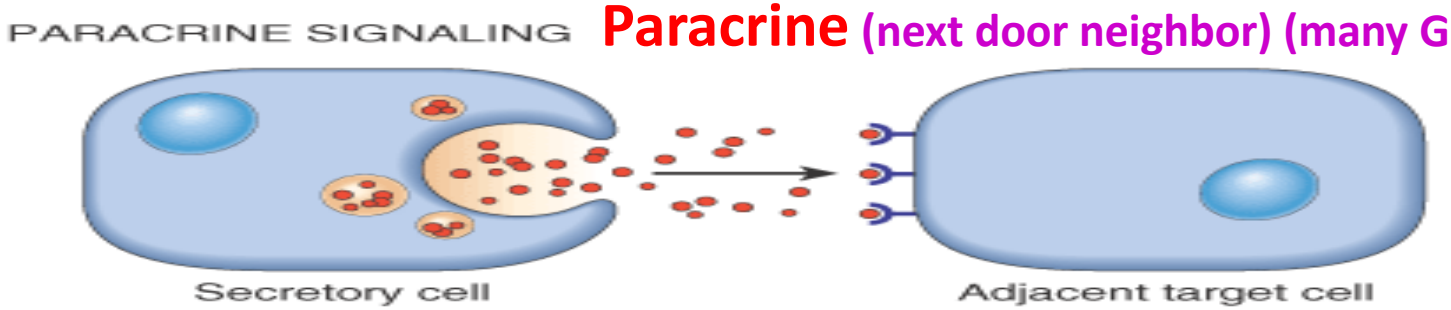
Type I collagen is stronger than steel !!

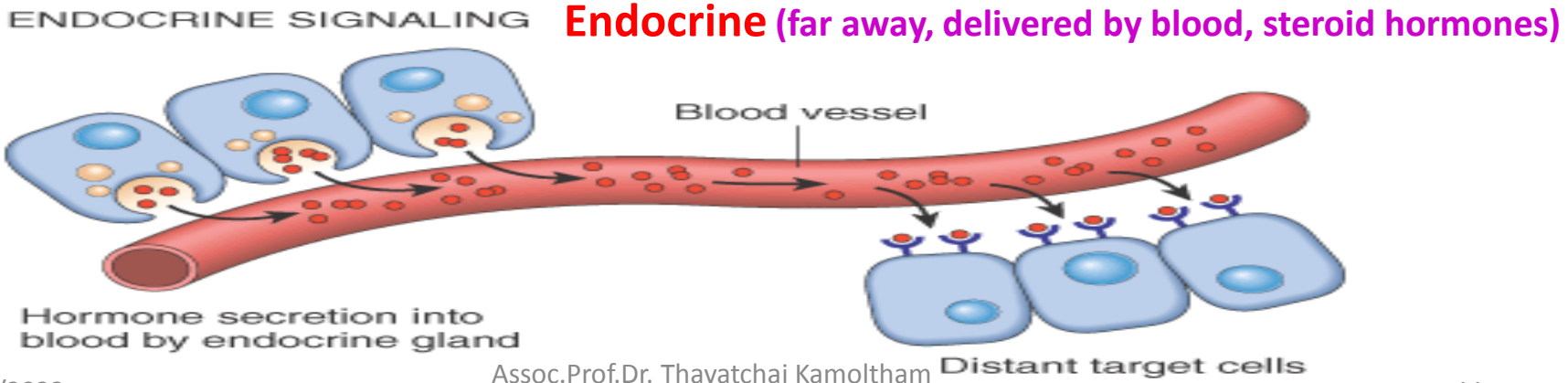
Tissue types

- permanent
  - nonproliferative in postnatal life
  - neurons , cardiomyocytes
- stable
  - regeneration as response to injury
  - parenchyma – liver, pancreas, renal tubules
  - mesenchymal cells, endothelium
- labile
  - continuous regeneration from stem cells (self-renewal)
  - hematopoietic cells in bone marrow
  - surface epithelia – skin, oral cavity, vagina, cervix
  - duct epithelia – salivary glands, pancreas, biliary tract
  - mucosas – GIT, uterus, fallopian tubes, urinary bladder

SIGNALING


AUTOCRINE SIGNALING
Autocrine (same cell)
Extracellular signal
Receptor
Target sites on same cell


PARACRINE SIGNALING
Paracrine (next door neighbor) (many GFs)
Secretory cell
Adjacent target cell


ENDOCRINE SIGNALING
Endocrine (far away, delivered by blood, steroid hormones)
Blood vessel
Hormone secretion into blood by endocrine gland
Distant target cells

Classification of Wounds

1) **Clean Wound:**

Operative incisional wounds that follow nonpenetrating (blunt) trauma.

2) **Clean/Contaminated Wound:**

uninfected wounds in which no inflammation is encountered but the respiratory, gastrointestinal, genital, and/or urinary tract have been entered.

3) **Contaminated Wound:**

open, traumatic wounds or surgical wounds involving a major break in sterile technique that show evidence of inflammation.

4) **Infected Wound:**

old, traumatic wounds containing dead tissue and wounds with evidence of a clinical infection (e.g., purulent drainage).

Three ways of Healing depending on the tissue involved & degree of injury

1. Resolution

- Damaged cells recover in short time
- Exp = mild sunburn

2. Regeneration

- Damaged cells replaced by identical cells via mitosis
- Only occurs in epithelia & connective tissue
- If complex organ, some damaged tissue replaced by regeneration & some by scar

3. Scar formation

- Key tissue = granulation tissue(highly vascular connective tissue)
  - » Collagen produced by fibroblasts makes granulation tissue into scar tissue
- Scar tissue is non-functional

Classification of Wounds Closure

1. Healing by Primary Intention:

1. 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.

2. Healing by Secondary Intention:

1. 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.

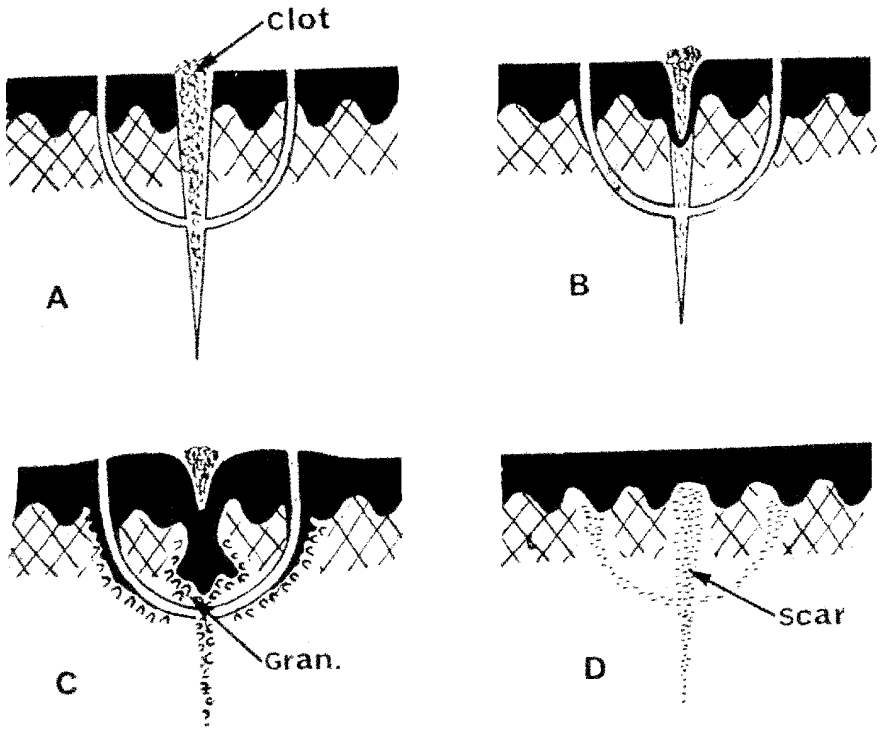
3. Healing by Tertiary Intention:

1. Also referred to as delayed primary closure.

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.



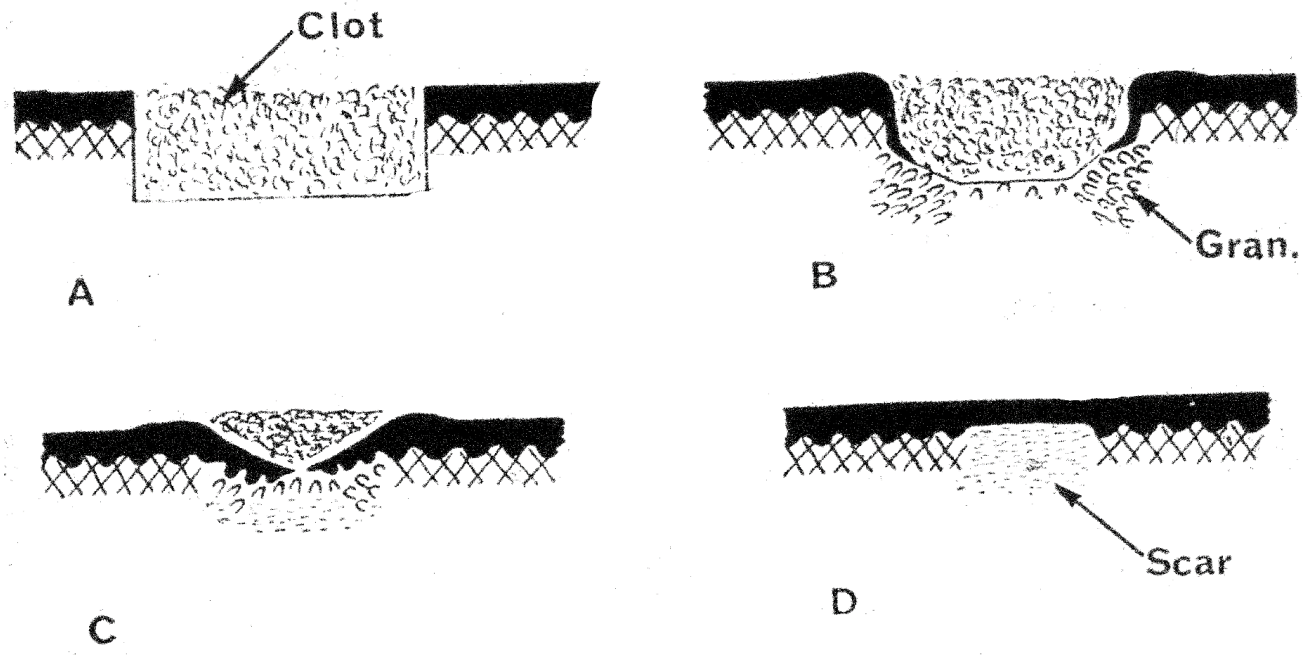
Healing by 'primary intention': A clean, sutured wound.

Healing by "Secondary Intention"

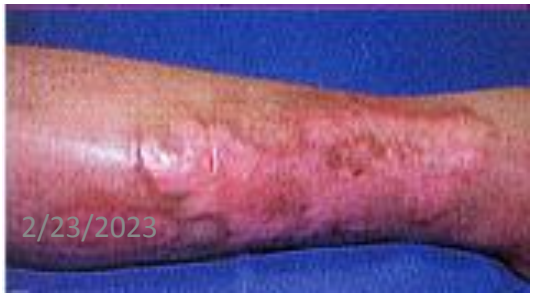
- Quantitative differences.
  - Initial contraction (in furry animals!)
  - Clot dries to form a 'scab' or ESCHAR
  - Epidermis regenerates beneath.
  - Repair process produces GRANULATION TISSUE

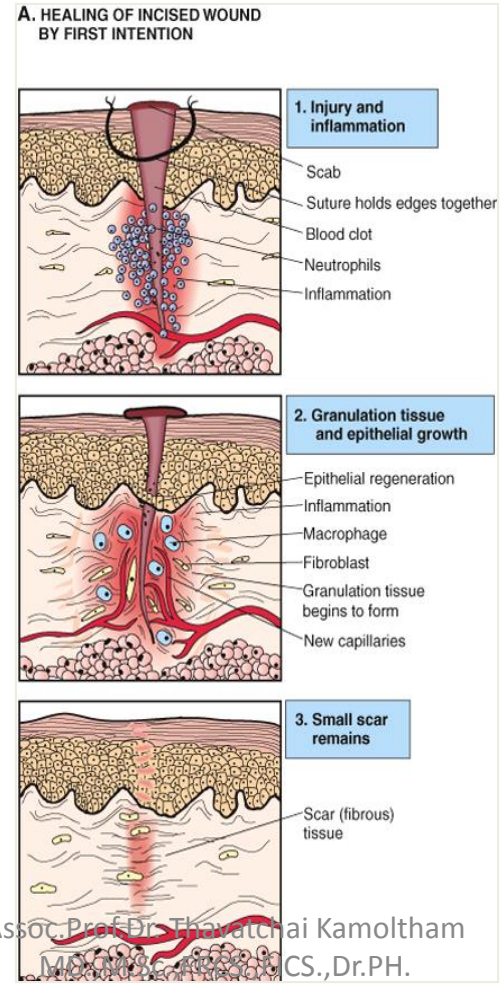
Comparison with primary intention:

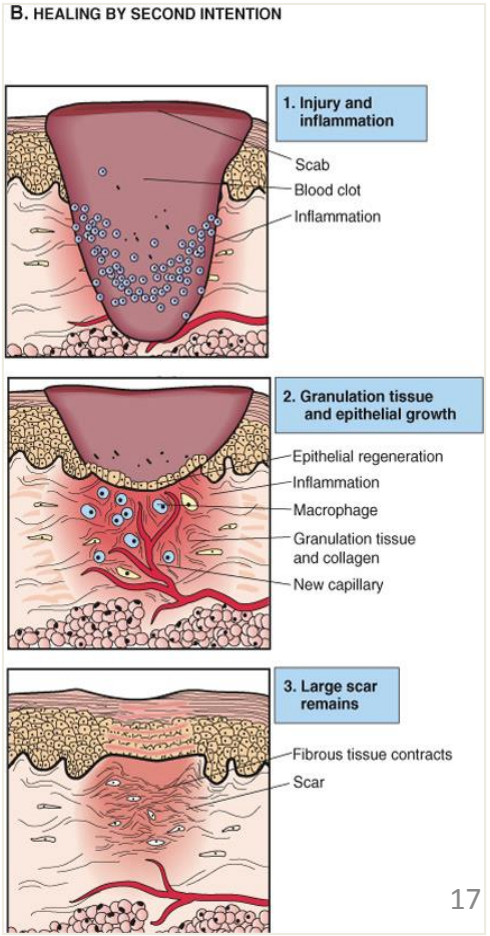
- Takes longer
- Produces a larger scar; not necessarily weaker
- Produces more late contraction



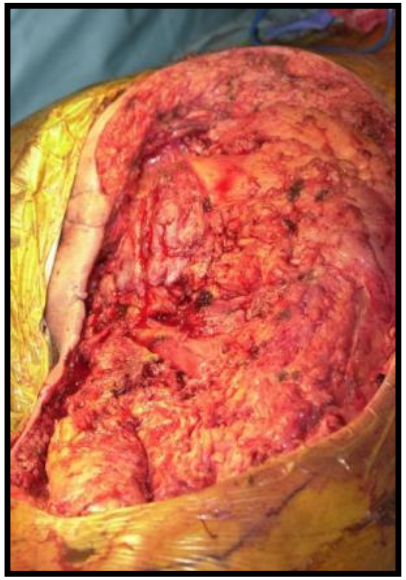
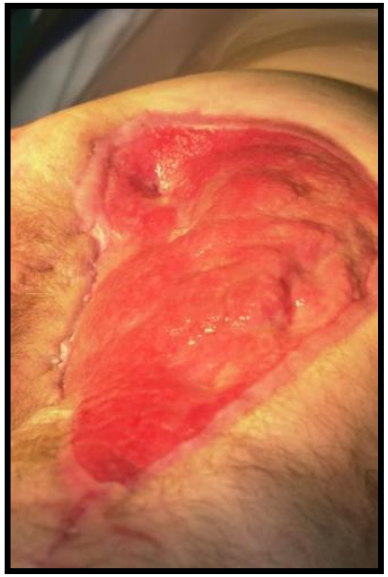
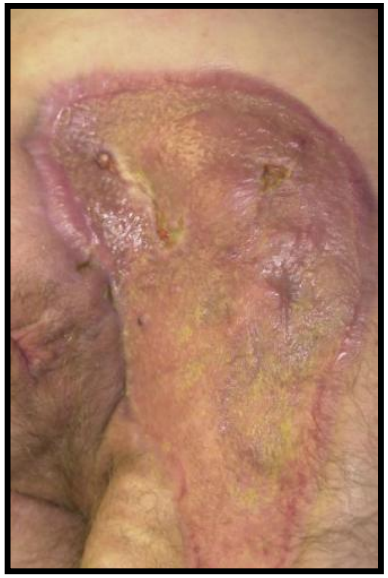
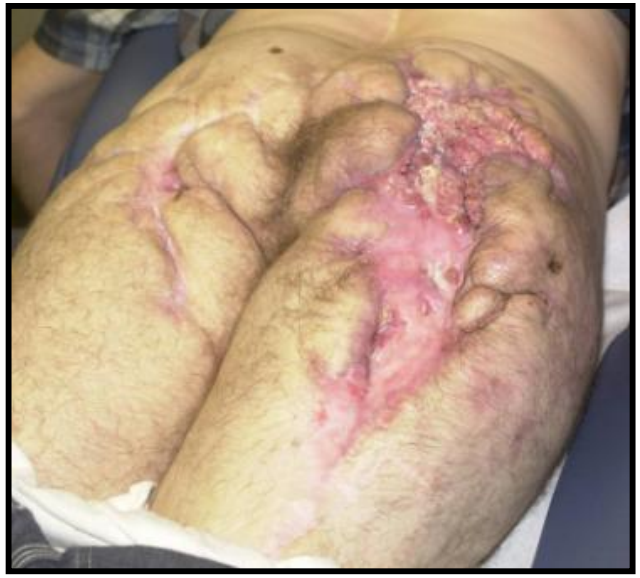
- **Healing by primary or secondary intention**
  - Depend weather edges of lesion can be brought together
  - **Primary (first) Intention** gives small scar formation
  - **Secondary Intention** gives large scar formation
  - **Tertiary Intention** Heals via granulation tissue



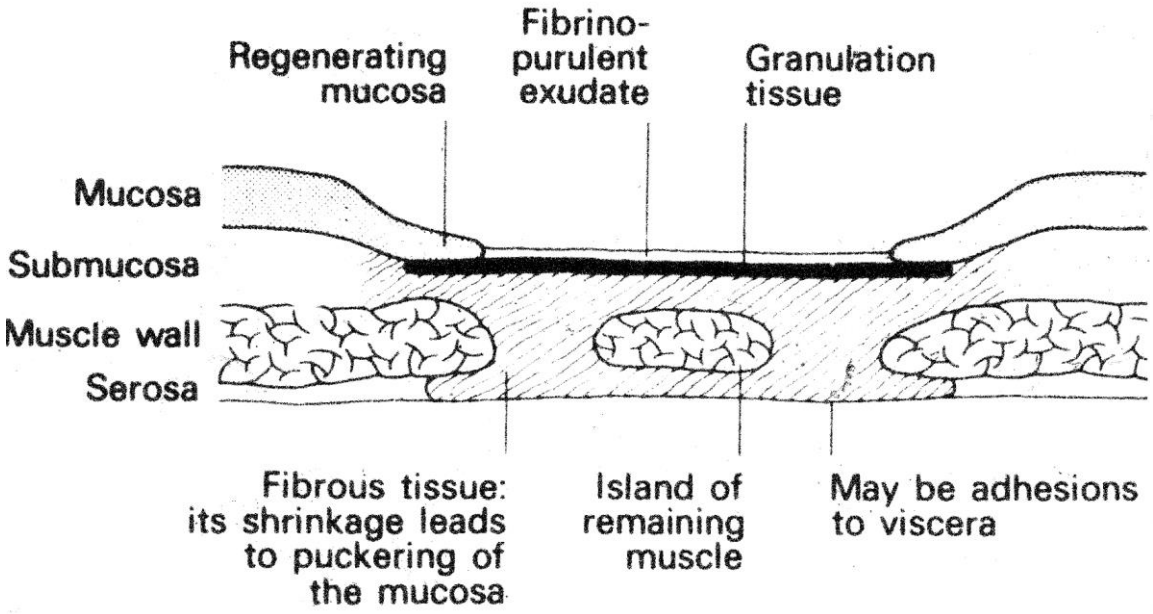


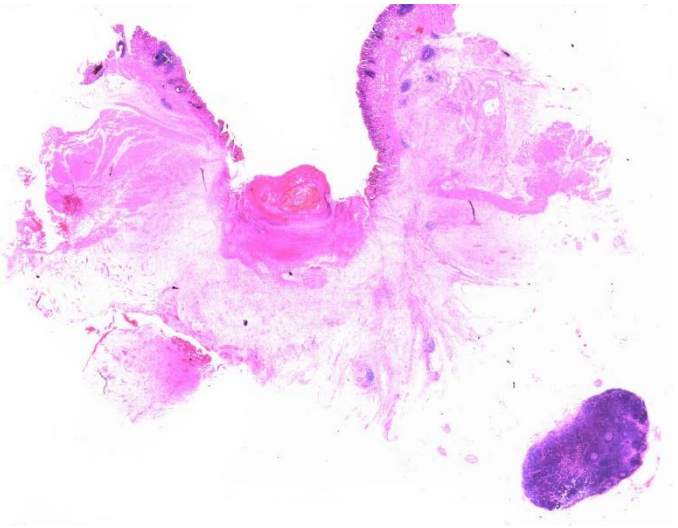


Secondary Wound Healing

Regeneration and repair combined: A chronic peptic ulcer





Three Phases of Wound Healing

1. Inflammatory Phase
2. Proliferative Phase
   - Begins when wound is covered by epithelium
   - Production of **collagen** is hallmark
   - 7 days to 6 weeks
3. Remodeling Phase (Maturation Phase)

Wound Healing

1. Inflammation occurs when the damaged endothelial cells release cytokines that increase expression of integrands in circulating lymphocytes.

– Histamine, serotonin, and kinins cause vessel contraction (thromboxane), decrease in blood loss, and act as chemotactic factors for neutrophils, the most abundant cells in the initial 24 hour period.

Wound Healing

2. Proliferative phase occurs next, 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.

Wound Healing

3. 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.

1.Inflammatory Phase

Hemostasis and Inflammation

- This phase takes 4 - 6 days
- Exposed collagen activates clotting cascade and inflammatory phase
- Fibrin clot = scaffolding and concentrate cytokines and growth factors

Granulocytes

- First 48 hours
- Attracted by inflammatory mediators
- Oxygen-derived free radicals
- Non-specific

Macrophages: Monocytes attracted to area by complement

Activated by:

- fibrin
- foreign body material
- exposure to hypoxic and acidotic environment

Reached maximum after 24 hours

Remain for weeks

- Activated Macrophage:
- Essential for progression onto Proliferative Phase
- Mediate:
  - Angiogenesis: FGF, PDGF, TGF-a&b and TNF-a
  - Fibroplasia: IL's, EGF and TNF
  - Synthesize: Nitric Oxide catalytic enzymes
  - Secrete collagenases

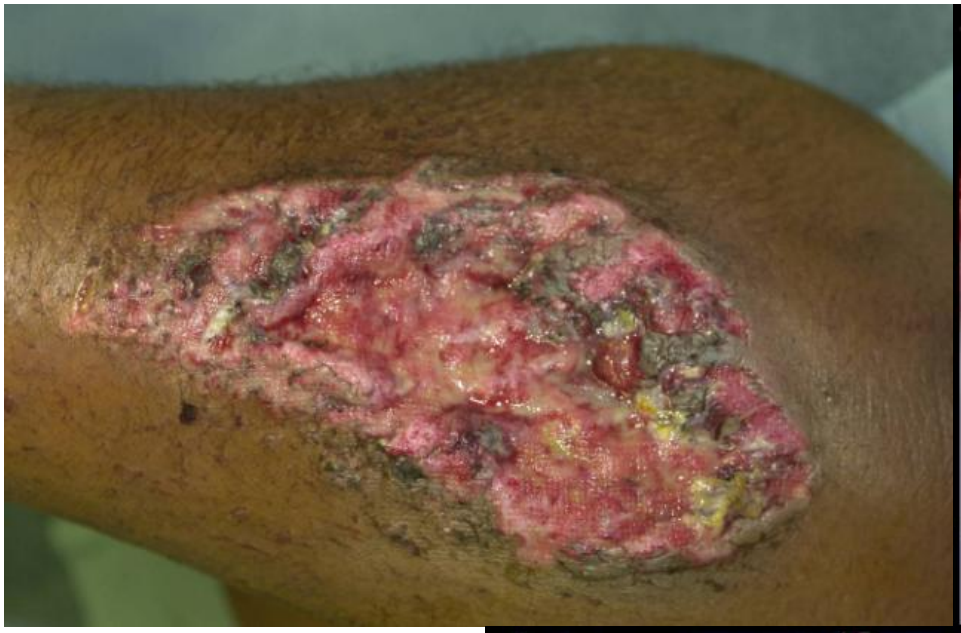
Table 1. Summary of Inflammatory Cytokines*

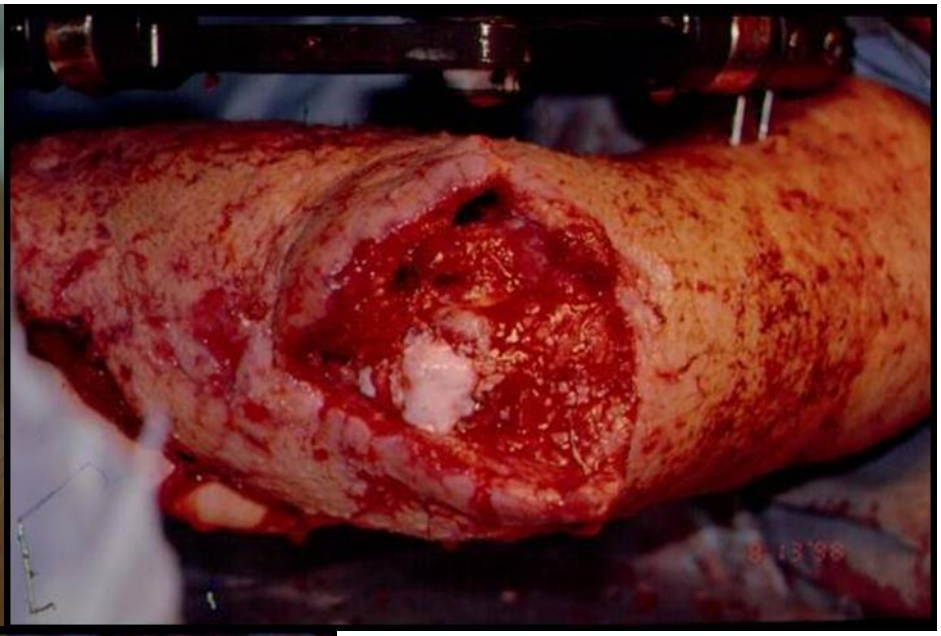
Cytokine	Cell of Origin	Function
EGF	Platelets, macrophages	Mitogenic for keratinocytes and fibroblasts, stimulates keratinocyte migration
FGF	Macrophages, mast cells, T lymphocytes, endothelial cells	Chemotactic and mitogenic for fibroblasts and keratinocytes, stimulates angiogenesis
IFNs (α, β, and γ)	Lymphocytes, fibroblasts	Activate macrophages, inhibit fibroblast proliferation
ILs (1, 2, 6, and 8)	Macrophages, mast cells, keratinocytes, lymphocytes	IL-1: induces fever and adrenocorticotrophic hormone release; enhances TNF-α and IFN-γ, activates granulocytes and endothelial cells; and stimulates hematopoiesis IL-2: activates macrophages, T cells, natural killer cells, and lymphokine-activated killer cells; stimulates differentiation of activated B cells; stimulates proliferation of activated B and T cells; and induces fever IL-6: induces fever and enhances release of acute-phase reactants by the liver IL-8: enhances neutrophil adherence, chemotaxis, and granule release
KGF	Fibroblasts	Stimulates keratinocyte migration, differentiation, and proliferation
PDGF	Platelets, macrophages, endothelial cells	Cell chemotaxis, mitogenic for fibroblasts, stimulates angiogenesis, stimulates wound contraction
TGF-α	Macrophages, T lymphocytes, keratinocytes	Mitogenic for keratinocytes and fibroblasts, stimulates keratinocyte migration
TGF-β	Platelets, T lymphocytes, macrophages, endothelial cells, keratinocytes	Cell chemotaxis stimulates angiogenesis and fibroplasia
Thromboxane A2	Destroyed wound cells	Potent vasoconstrictor
TNF	Macrophages, mast cells, T lymphocytes	Activates macrophages, mitogenic for fibroblasts, stimulates angiogenesis

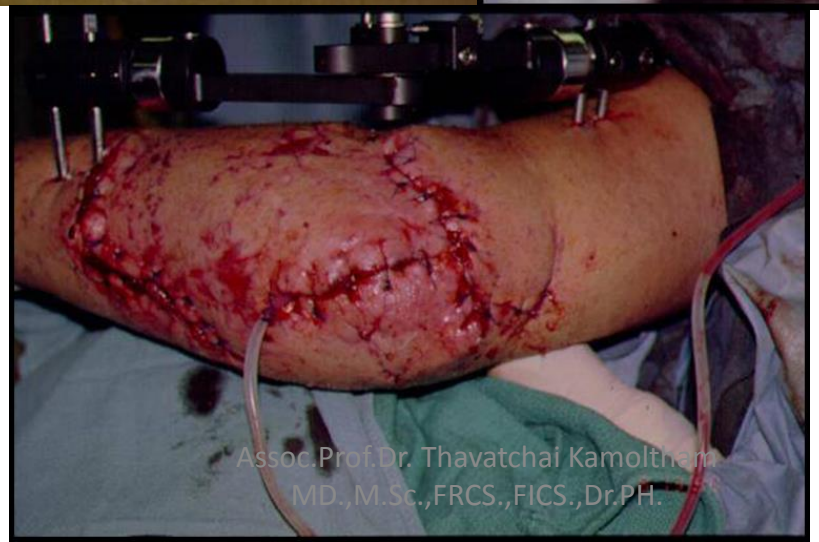
EGF, epidermal growth factor; FGF, fibroblast growth factor; IFN, interferon; IL, interleukin; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor.

*From Henry, G., and Garner, W. Inflammatory mediators in wound healing. *Surg. Clin. North Am.* 83: 483, 2003; and Lawrence, W., and Diegelmann, R. Growth factors in wound healing. *Clin. Dermatol.* 12: 157, 1994.

Inflammatory Phase







Inflammatory-Response Phase

- After injury, healing process begins immediately
  - Destruction of tissue produces direct injury to cells of various soft tissue
  - Characterized by redness, swelling, tenderness and increased temperature
  - Critical to entire healing process
- Leukocytes and other phagocytic cells delivered to injured tissue
  - Dispose of injury by-products through phagocytosis
- Entire phase last 2-4 days
- Greater tissue damage longer inflammatory phase

- NSAIDS may inhibit inflammatory response thus delaying healing process
- Will assist with pain and swelling

Inflammatory-Response Phase

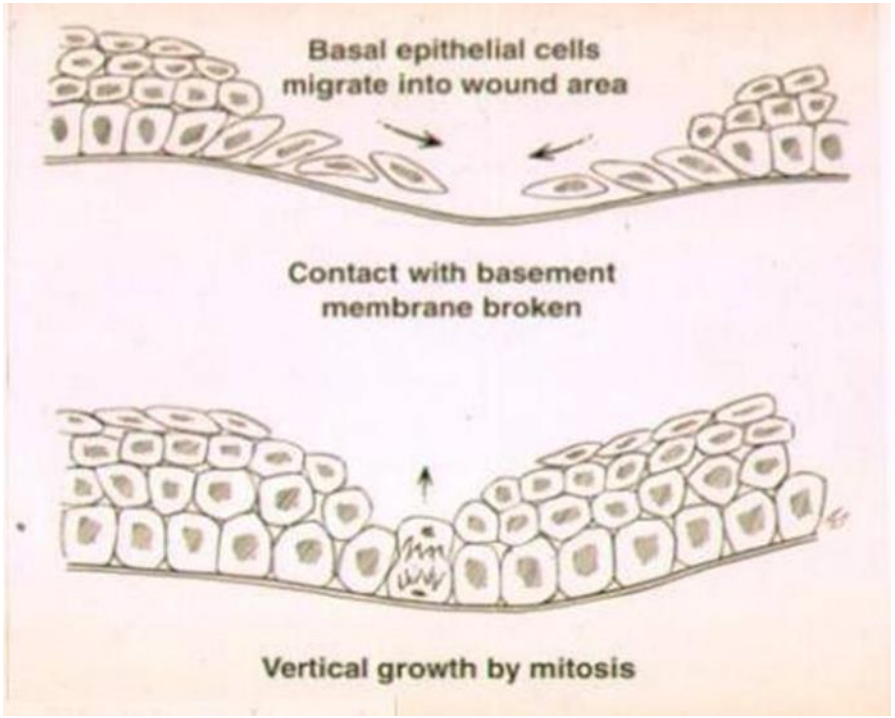
- Vascular reaction
  - Blood coagulation and growth of fibrous tissue occurs
  - First 5-10 minutes vasoconstriction occurs
    - Best time to evaluate
    - Followed by vasodilation
    - Effusion of blood and plasma last 24 to 36 hours
- Chemical mediators
  - Released from damaged tissue, white blood cells and plasma
  - Histamine, leukotrienes and cytokines assist in limiting exudate/swelling
  - Amt of swelling directly related to extent of vessel damage

Inflammatory Response Con't

- Formation of Clot
  - Platelets adhere to collagen fibers and create sticky matrix
    - Platelets and leukocytes adhere to matrix to form plug
    - Clot formation occurs 12 hours after injury and is complete w/in 48 hrs
    - Set stage for fibroblastic phase
- Chronic inflammation
  - Acute phase dos not respond sufficiently to eliminate injury agent and restore tissue to normal physiologic state
  - Damage occurs to connective tissue and prolongs healing and repair process
  - Response to overuse and overload

2. Proliferative Phase

1. Epithelialization, Angiogenesis and Provisional Matrix Formation
   - Begins when wound is covered by epithelium
- Day 4 through 14
- Production of **collagen** is hallmark
- 7 days to 6 weeks
- Basal epithelial cells at the wound margin flatten (mobilize) and migrate into the open wound
- Basal cells at margin multiply (mitosis) in horizontal direction
- Basal cells behind margin undergo vertical growth (differentiation)



Proliferative Phase:

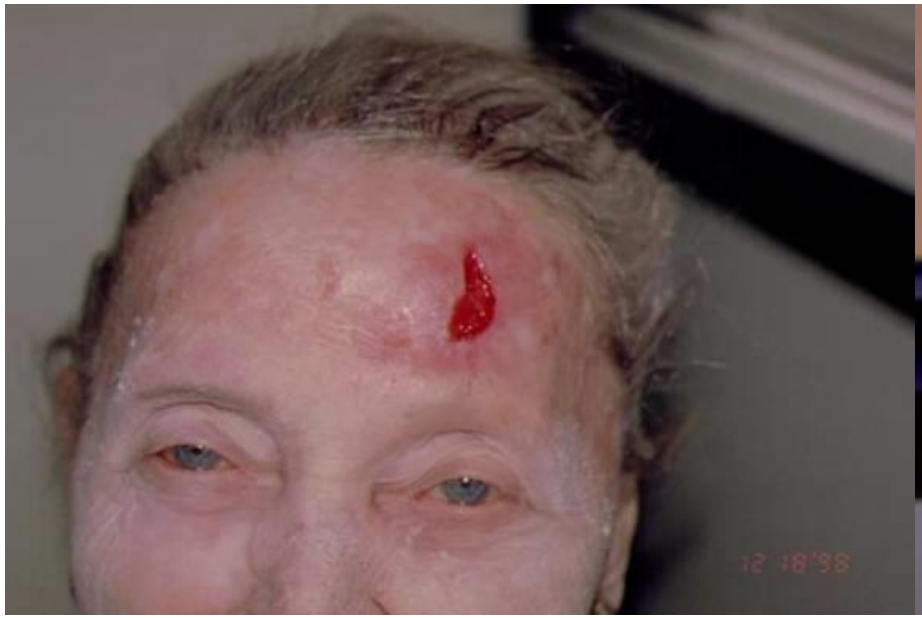
2. Lay down Fibroblast

- Work horse of wound repair
- Produce Granulation Tissue:
  - Main signals are PDGF and EGF
  - Collagen type III
  - Glycos-aminoglycans
  - Fibronectin
  - Elastin fibers
- Tissue fibroblasts become myofibroblasts induced by TGF-b1

3. Wound Contraction

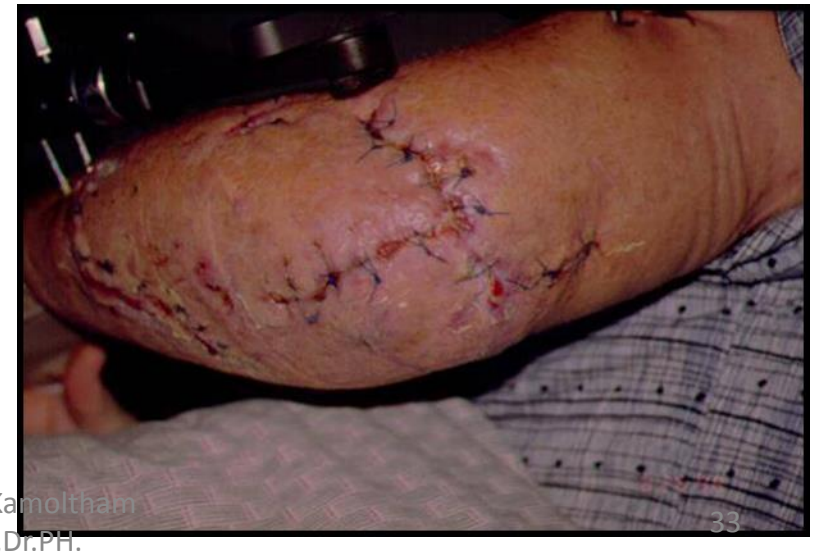
- Actual contraction with pulling of edges toward center making wounds smaller
- Myofibroblast: contractile properties
- Surrounding skin stretched, thinned
- Original dermal thickness maintained
- No hair follicles, sweat glands

Epithelialization/Contraction



Collagen Homeostasis

- After Wounding (Optimal Healing)
  - Days 3 - 7 week
    - Collagen production begins
  - Days 7 – 42
    - Synthesis with a **net GAIN** of collagen
    - Initial increase in tensile strength due to increase amount of collagen
  - Days 42+
    - Remodeling with **No net** collagen gain
- Normal Skin
  collagen ratio 4 : 1 Type I/III
- Hypertrophic Scar
  collagen ratio 2 : 1 Type I/III



Fibroblastic-Repair Phase

- Proliferative and regenerative activity leads to scar formation
  - Begins w/in 1st few hours after injury and can last as long as 4-6 weeks
  - Signs and Symptoms of inflammatory phase subside
  - Increased O2 and blood flow deliver nutrients essential for tissue regeneration

Fibroblastic-Repair Phase

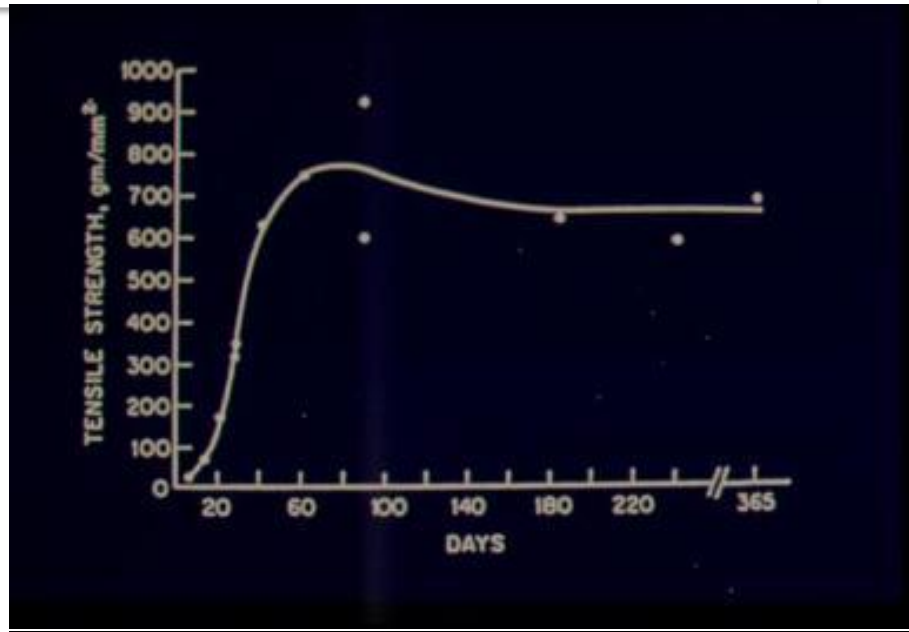
- Break down of fibrin clot forms connective tissue called granulation tissue
  - Consist of fibroblast, collagen and capillaries
    - Fills gap during healing process
      - Unorganized tissue/fibers form scar
    - Fibroblast synthesize extracellular matrix consisting of protein fibers (Collagen and Elastin)
      - Day 6 –7 collagen fibers are formed throughout scar
      - Increase in tensile strength increases with rate of collagen synthesis

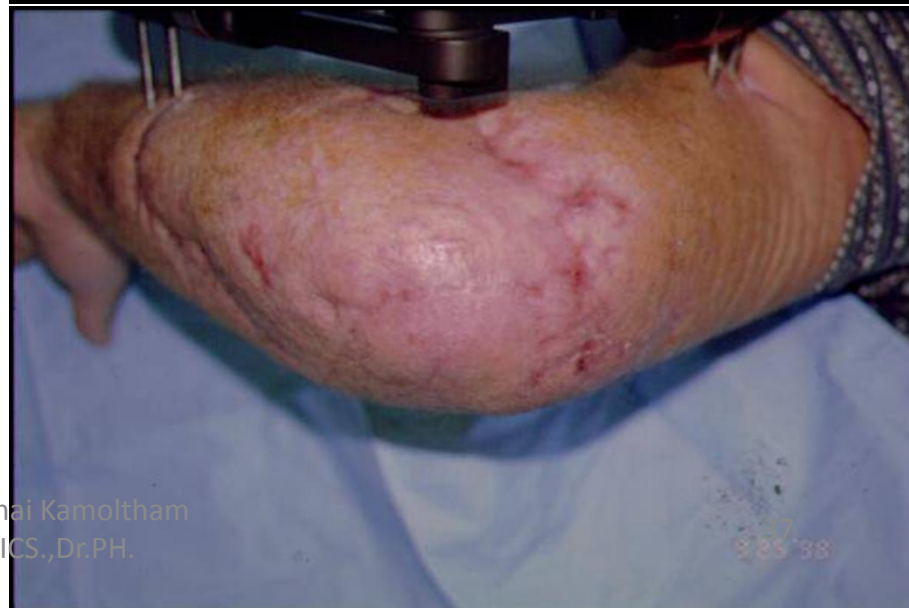
Fibroblastic-Repair Phase

- Importance of Collagen
  - Major structural protein that forms strong, flexible inelastic structure
  - Type I, II & III
    - Type I found more in fibroblastic repair phase
    - Holds connective tissue together and enables tissue to resist mechanical forces and deformation
      - Direction of orientation of collagen fibers is along lines of tensile strength
  - Mechanical properties
    - Elasticity
      - Capability to recover normal length after elongation
    - Viscoelasticity
      - Allows slow return to normal length and shape after deformation
    - Plasticity
      - Allows permanent change and deformation

3. Remodeling (Maturation) Phase

- Random to organized fibrils
- Day 8 through years
- Type III replaced by type I
- Wound may increase in strength for up to 2 years after injury
  - Collagen organization
  - Cross linking of collagen





Maturation-Remodeling Phase

- Long term process that involves realignment of collagen fibers that make up scar
  - Increased stress and strain causes collagen fibers to realign to position of maximum efficiency
    - Parallel to lines of tension
    - Gradually assumes normal appearance and function
    - Usually after 3 weeks a firm, contracted, nonvascular scar exist
      - Total maturation phase may take years to be totally complete

Maturation-Remodeling Phase

- Wolf's law
  - Bone and soft tissue will respond to physical demands placed on them
    - Remodel or realign along lines of tensile force
    - Critical that injured structures are exposed to progressively increasing loads throughout rehab process
      - As remodeling phase begins aggressive active range of motion and strengthening
      - Use pain and tissue response as a guide to progression
- Controlled mobilization vs. immobilization
  - Animal studies show Controlled mob. Superior to Immobilization for scar formation
    - However, some injuries may require brief period of immob. During inflammatory phase to facilitate healing process

Factors Influencing Wound Healing

- To treat the wound, you have to treat the patient
- Optimize the patient
  - Circulatory
  - Pulmonary
  - Nutrition
  - Associated diseases or conditions

Fig. 1. Local factors affecting wound healing.

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

FACTORS INFLUENCING WOUND HEALING

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

Factors retarding wound healing

LOCAL

- DECREASED Blood supply
- Denervation
- Local Infection
- FB
- Hematoma
- Mechanical stress
- Necrotic tissue

SYSTEMIC

- DECREASED Blood supply
- Age
- Anemia
- Malignancy
- Malnutrition
- Obesity
- Infection
- Organ failure

Oxygen

- Fibroblasts are oxygen-sensitive
- PO2 < 40 mmHg collagen synthesis cannot take place
- Decreased PO2: most common cause of wound infection
- Healing is Energy Dependent
- Proliferative Phase has greatly increased metabolism and protein synthesis

Hypoxia

- Endothelium responds with vasodilation
- Capillary leak
- Fibrin deposition
- TNF-a induction and apoptosis

Edema

- Increased tissue pressure
- Compromise perfusion
- Cell death and tissue ulceration

Infection

- Decreased tissue PO2 and prolongs the inflammatory phase
- Impaired angiogenesis and epithelialization
- Increased collagenase activity

Nutrition

- Low protein levels prolonged inflammatory phase
- impaired fibroplasia
- Of the essential amino
- Methionine is critical

Hydration

- A well hydrated wound will epithelialize faster than a dry one
- Occlusive wound dressings hasten epithelial repair and control the proliferation of granulation tissue

Temperature

- Wound healing is accelerated at environmental temperatures of 30°C
- Tensile strength decreases by 20% in a cold (12°C) wound environment

Denervation

- Denervation has no effect on either wound contraction or epithelialization

Diabetes Mellitus

- Larger arteries, rather than the arterioles, are typically affected
  - Sorbitol accumulation
  - Increased dermal vascular permeability and pericapillary albumin deposition
  - Impaired oxygen and nutrient delivery
- Stiffened red blood cells and increased blood viscosity
- affinity of glycosylated hemoglobin for oxygen contributing to low O2 delivery
- impaired phagocytosis and bacterial killing
- Neuropathy

Radiation Therapy

- Acute radiation injury
- stasis and occlusion of small vessels
- fibrosis and necrosis of capillaries
- decrease in wound tensile strength
- direct, permanent, adverse effect on fibroblast
- may be progressive
- fibroblast defects are the central problem in the healing of chronic radiation injury

Medications

- Steroids
  - Stabilize lysosomes and arrest of inflammation response
  - inhibit both macrophages and neutrophils
  - interferes with fibrogenesis, angiogenesis, and wound contraction
  - Also direct effect on Fibroblasts
    - Minimal endoplasmic reticulum
- Vitamin
  - **vitamin A**
    - oral ingestion of 25,000 IU per day pre op and 3d post op (not to pregnant women)
    - Restores inflammatory response and promotes epithelializaton
    - Does not reverse detrimental effects on contraction and infection
  - Vitamin C (Ascorbic Acid)
    - is an essential cofactor in the synthesis of collagen
    - excessive concentrations of ascorbic acid do not promote supranormal healing
  - Vitamin E
    - therapeutic efficacy and indications remain to be defined
    - large doses of vitamin E inhibit healing
    - increase the breaking strength of wounds exposed to preoperative irradiation

Nutritional Supplements

- Glutamine
  - Enhance actions of lymphocytes, macrophages and neutrophils
- Glycine
  - Inhibitory effect on leukocytes, might reduce inflammation related tissue injury
- Zinc
  - common constituent of dozens of enzymes
  - Influences B and T cell activity
  - epithelial and fibroblastic proliferation is impaired in patients with low serum zinc levels

Smoking

- 1ppd = 3x freq. of flap necrosis
- 2ppd = 6x freq. of flap necrosis
- Nicotine acts via the sympathetic system
  - vasoconstriction and limit distal perfusion
    - 1 cigarette = vasoconstriction > 90 min
  - Decrease proliferation of erythrocytes, macrophages and fibroblasts
- Smoke contains high levels of carbon monoxide
  - shifts the oxygen-hemoglobin curve to the left
  - decreased tissue oxygen delivery

Excessive Healing

Hypertrophic Scars

Keloids

Questions?