Wound Healing

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Introduction

- Wound healing is a fundamental homeostatic event in response to injury.

- This process brings into play an exquisitely controlled cascade of molecular and cellular responses that are evolutionarily conserved across multicellular organisms.
Introduction

• Our understanding of the intricate crosstalk present between cells in the wound and other cells throughout the body has led to an appreciation that the process of tissue repair involves a coordinated interplay between metabolic, nervous, inflammatory, immune, and vascular systems

• Wound healing = systemic process
Inflammatory Phase
Inflammatory Phase

• Begins immediately after injury
• The initial response to the disruption of blood vessels is bleeding
• The homeostatic response to this is clot formation to stop hemorrhage
• Platelet plug formation initiates the hemostatic process along with recruitment of clotting factors activated by collagen and BM proteins exposed by the injury
Inflammatory Phase

- Fibrin, converted from fibrinogen by the clotting cascade, binds the platelet plug and forms the provisional matrix for the cellular responses that follow.

- After injury, transient vasoconstriction is mediated by catecholamines, thromboxane, and prostaglandin F$_{2\alpha}$
Degranulation

• Provides substances (PDGF and TGF-β) that initiate chemotaxis and proliferation of inflammatory cells, beginning the inflammatory response that will ultimately heal the wound.

• Transient vc is necessary to decrease blood loss at the time of the initial wounding and also to allow clot formation.
  - lasts for 5 to 10 minutes
Inflammatory Phase

- Once a clot has been formed and bleeding has stopped, blood flow increases locally, supplying cells and substrate necessary for further wound repair.
- The vascular endothelial cells also deform, increasing vascular permeability mediated by histamine, PGE$_2$, PGI$_2$, and VEGF released by injured endothelial cells and mast cells, enhancing the egress of cells and substrate into the wounded tissue.
Inflammatory Phase

• At this stage, the wound consists of a mixture of injured, devitalized tissue, clot, bacteria, extravasated serum proteins and foreign material introduced at the time of injury

• During the next several days, the wound is cleared of bacteria, devitalized tissue, and foreign material by recruited and activated phagocytic cells
PMNs

- Begin to arrive immediately, attaining large numbers within 24 hours
- The process of clearing the wound of debris usually takes several days
- Followed temporally by macrophages, which appear in wounds in significant numbers within 2 or 3 days
Macrophages

- Mononuclear phagocytic cells derived from circulating monocytes or resident tissue macrophages
- They complete the process of removing all material not necessary for the ensuing steps of wound healing
- In the absence of significant bacterial contamination, macrophages promptly replace PMNs as the dominant cell type during the inflammatory phase
Macrophages

- Their role is not limited to phagocytosis
- Source of > 30 different growth factors and cytokines
  - induce fibroblast and endothelial cell proliferation, EC matrix production, and recruit + activate additional macrophages
- Result = the induction of a wound healing amplification cycle as growth factors recruit macrophages and elicit additional growth factor release
Proliferative Phase
Proliferative Phase

- Begins with the formation of a provisional matrix of fibrin and fibronectin
- Initially, the provisional matrix is populated by macrophages
- By day 3, fibroblasts appear and initiate collagen synthesis
  - proliferate in response to growth factors to become the dominant cell type
Angiogenesis

- Induced by macrophage-derived growth factors produced by macrophages
  - induces the ingrowth and proliferation of endothelial cells, forming new capillaries

- Neovascularization is driven in large part by the local tissue hypoxia present in the wound center
  - orchestrated by the hypoxia-inducible factor-1 (HIF-1)
HIF-1

- Master transcription factor
- Functions as the switch
  - turns on angiogenic processes, metabolic adaptations to hypoxia, an increase in iron transport, and erythropoiesis
  - restores blood flow to an injured area
- Initially, the angiogenic cascade unleashed by HIF-1 results in a hypervascular network
Neovascularization

- Visible through the epithelium and gives the wound a pink or purple-red appearance
- Capillary ingrowth provides the fibroblasts with oxygen and nutrients to sustain cell proliferation and support the production of the permanent wound matrix
  - composed of collagen and proteoglycans or ground substance and replaces the provisional fibronectin-fibrin matrix
Collagen

- The dominant structural molecule in the wound matrix and in the final scar
- The principal structural protein in skin, bone, and all human tissues
- Synthesized into an organized cablelike network in a multistep process with both intracellular and extracellular components
Collagen

- Hydroxyproline and hydroxylysine
- The hydroxylation process that forms these aa requires vitamin C and is necessary for its subsequent stabilization and cross-linkage
- Procollagen is formed within the fibroblasts as a long linear aa segment with regular repeats of hydroxyproline every third aa and with terminal extension peptides
Procollagen

- Aggregates as three α chains to form a triple-helical complex = type I
  - maintained by intramolecular disulfide bonds between specific cystine residues.
- Secreted in its triple-helical form
- Extracellular peptidases cleave the extension peptides at the amino and carboxy termini, leaving the central collagen molecule
Maturation

- Collagen cross-linking then occurs in the extracellular space
- This produces stable, cross-linked collagen fibrils
- Intra- and intermolecular bonds provide strength and stability
- As the wound matures, fibrils cross-link – form Ig collagen cables – tensile strength
  - the wound = scar
Remodeling Phase
Remodeling

- The transition from the proliferative phase to the remodeling phase is defined by the achievement of collagen equilibrium.
- Collagen accumulation reaches a **Maximum** within **2 to 3 Post-Injury**.
- Although supra-normal rates of synthesis and degradation continue throughout remodeling, there is no further change in total collagen content.
Remodeling

• Tensile strength gradually increases as random collagen fibrils are replaced by organized fibrils with more intermolecular bonds

• During the initial phase of wound healing, there is a relative abundance of type III collagen in the wound

• With remodeling, the normal adult ratio of 4:1 (type I to type III) collagen is restored
Remodeling

- Under normal wound healing conditions, the capillary density gradually diminishes, and the number of fibroblasts is reduced
  - loses its pink or purple vascular color and becomes progressively pale
- The collagen undergoes constant remodeling
- New collagen is formed, and collagen degradation by specific collagenases is ongoing
Remodeling

- Collagenase activity is balanced against new production of collagen to produce a steady state.
- As equilibrium is achieved, the collagen fibrils align themselves in a longitudinal arrangement as dictated by stress placed on the wound.
Remodeling

- Scars never achieve the degree of order achieved by collagen in normal skin or tendons
  - increase in strength for 6 months or longer
  - eventually reaching 70% of the strength of unwounded skin
Proteoglycans

• The other important component of the extracellular matrix is the ground substance or proteoglycans

• Composed of a protein backbone with long hydrophilic carbohydrate side chains

• Their hydrophilic nature accounts for much of the water content of scars
Proteoglycans

- In the early immature wound, there is a disproportionately large amount of proteoglycans
  - hyaluronic acid
- During the maturation phase, the proteoglycan content returns to a level that closely approximates that of normal skin.
Epithelialization

- The skin is composed of two layers
  - the epidermis and the dermis
- The outermost layer = the epidermis
  - protective barrier
  - forms the external interface between the body and the environment
  - protects against water loss
  - allows cells to live in a liquid environment
  - forms a barrier to bacteria and other environmental factors
Epithelialization

- Reconstruction of the epithelial barrier (epithelialization) begins within hours of the initial injury

- As an initial step, epithelial cells from the basal layer at the wound edge flatten and migrate across the wound,
  - completing wound coverage within 18 to 24 hours in a coapted surgical wound
Epithelialization

• The cells along the margin are also dividing to re-form the characteristic basilar to apical differentiation of multilayered mature epithelium
• Epithelial cells exhibit contact inhibition
• Migration occurs by a process in which the epithelial cells send out pseudopods, attaching to the underlying extracellular matrix by integrin receptors
Delayed Epithelialization

- Bacteria, large amounts of protein exudate from leaky capillaries, and necrotic tissue all compromise this process, delaying epithelialization.

- Delayed epithelialization results in a more profound and prolonged inflammatory process.
Epithelialization

• Re-establishment of a mature epithelium with a multilayered keratin layer (stratum corneum), which completely restores the normal water barrier, is an important component of the scar resolution process and downregulation of the inflammatory cascade

• Establishment of an intact epithelial cover begins the process of wound resolution
Scar

- Visible scarring occurs only when the injury extends deeper than the superficial dermis.
- Scar formation at the molecular level is due to disorganized collagen.
- In response to injury, dermal integrity is restored by collagen synthesis and deposition.
Scar

- Over the time of scar maturation (6 months to several years), scar remodeling results in progressive collagen linking and increased organization

- The parallel organized collagen layers of normal skin are never completely reconstituted
  - result = visible scar
Open Wounds
Open Wounds - Acute

• Open wounds, whether ulcers or surgical incisions closing by secondary intention, heal with the same sequence of inflammation, matrix deposition, epithelialization, and scar maturation.

• In the closed (sutured) incisional wound, the healing process progresses through an orderly temporal sequence.
Open Wounds - Acute

• In an open wound, the healing events are spatially separated

• In the healing wound, a bed of granulation tissue forms over the exposed subq tissue
  - composed of new capillaries, proliferating fibroblasts and an immature matrix of collagen, proteoglycans, substrate adhesion molecules and acute and chronic inflammatory cells
Open Wounds - Acute

- There are variable amounts of bacteria and protein exudate, depending on the condition of the wound.
- At the advancing edge of epithelium, the process of acute inflammation is present.
- Behind the advancing edge, there is a proliferative area.
- Further behind, the scar is maturing and remodeling.
Healing

- The most important clinical factor in the healing of surgical incisions = the \textit{gain in tensile strength} of the wound
  - collagen deposition

- The \textit{rate of collagen synthesis} determines the initial wound strength

- Ultimate wound strength is determined by the \textit{degree of collagen organization and cross-linking}
Healing

- Defined primarily by epithelialization
- Successful healing is related more to the maintenance of epithelial integrity than to the tensile strength of the scar
Healing

- Rapid epithelialization is dependent on an optimal matrix, synthesized by the underlying granulation tissue, as well as on optimal delivery of nutrients and oxygen by an adequate blood supply.
- The rate of epithelialization is also inversely related to the degree of bacterial presence in the wound, a variable that is directly related to the quality of the granulation tissue present.
Healing

- Inflammatory cells in open wounds release growth factors
  - enhance migration and proliferation of fibroblasts and endothelial cells in wounds
  - leads to the formation of granulation tissue – the cobblestone-like pink surface of healthy new tissue
The ability of an open wound to form granulation tissue is governed by the blood supply to the tissue and the relative absence of devitalized tissue and bacteria.

Débridement
Débridement

• Frequently underutilized in clinical care
• Simple technique
• Critical to ensuring healing in a contaminated surgical wound
• Removes bacteria and necrotic, devitalized tissue – serve to propagate a counterproductive hyperinflammatory phase and divert metabolic resources away from the healing wound
Contraction

- Important event that contrasts the healing of open wounds with closed incisions
- The surrounding skin is pulled over the wound to reduce its size
- Can occur faster than epithelialization
- Increases the speed of wound closure
- The open wound is resurfaced by the normal sensate skin
- Driven by fibroblasts
Clinical

• It takes at least 3 weeks for collagen to undergo sufficient remodeling and cross-linking to attain moderate strength

• At 1 to 2 weeks, the time when most sutures are removed, a wound has a small fraction of its eventual strength

• Deep sutures are placed in collagen-containing structures to hold prolonged tension
• Dermis, intestinal submucosa, muscular fascia, tendon, ligament, Scarpa fascia, and blood vessel wall represent a partial list of tissues with high collagen content

• Sutures used for abdominal fascia should ideally retain their tensile strength for close to 6 weeks
After 6 weeks, wounded tissue has gained about 50% of its eventual strength.

To prevent hernia formation, heavy lifting is avoided after major abdominal surgery for 6 weeks.
Clinical

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Factors

- Nutrition
- Aging
- Ischemia
- Smoking
- Immunosuppression
- Radiation
- Edema
- DM
What You Need to Know

• The inflammatory phase of acute wound healing begins immediately after injury
• The initial response to the disruption of blood vessels is bleeding
• The proliferative phase of acute wound healing begins with the formation of a provisional matrix of fibrin and fibronectin as part of initial clot formation
What You Need to Know

• The transition from the proliferative phase to the remodeling phase of acute wound healing is defined by reaching collagen equilibrium.

• Reconstruction of the epithelial barrier (epithelialization) begins within hours of the initial injury.
What You Need to Know

- It takes at least 3 weeks for collagen to undergo sufficient remodeling and cross-linking to attain moderate strength.
- There are numerous modifiable factors that negatively affect wound healing.