Tissue Repair: Regeneration and Fibrosis

Patrice Spitalnik, MD
Pfs2101@columbia.edu

Lecture Outline

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

Baseline cell population

Differentiation

Stem cells

Cell death
Apoptosis

Tissue Steady State

Epidermis

Stratum corneum

Stratum granulosum

Stratum spinosum

Stratum basale
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, angiogenesis, wound contraction and matrix production
Role of ECM

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

FGF stimulates keratinocyte migration, wound contracture and matrix deposition

Model of Leukocyte Transmigration
Macrophages in inflammation and repair

1. Blood flow

2. Lymph flow

3. Fluid and proteins

4. Growth factors

5. New blood vessels

6. Repair

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Wound Repair and Regeneration

Patch of fibroblasts with disorganized ECM

Scalp
Non-functional tissue

Injury to tissue

Lung
Kidney
Heart
Skin
Liver
Spleen

Functional tissue

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

A. Angiogenesis by mobilization of EPCs from the bone marrow

B. Angiogenesis from pre-existing vessels

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1. Proteolysis of ECM
2. Migration and chemotaxis
3. Proliferation
4. Lumen formation, maturation, and inhibition of growth
5. Increased permeability through gaps and transcytosis

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

3. Activation
   - Procollagenases
   - Prostromelysins
   - Collagenase
   - Stromelysin

4. Degraded ECM
   - ECM
   - TIMPs

ECM Degraded ECM

Plasmin
Plasminogen

Activators

- Plasminogen
- Activators

- Procollagenases
- Prostromelysins
- Collagenase
- Stromelysin
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

Injury → Response → Acute Inflammation

- Stimulus Promptly Destroyed
  - Minimal necrosis
    - Exudate resolved
      - Normal tissue
      - Mild burn
    - Exudate organized
      - Scarring
      - Fibrinopurulent Pericarditis, peritonitis
  - Exudate organized
  - Normal tissue
  - Lobar Pneumonia

- Stimulus Not Destroyed
  - Necrosis
    - Framework intact
      - Labile or stable cells
      - Normal tissue
      - Lobar Pneumonia
    - Framework destroyed
      - Scar
      - Bacterial abscess
      - Scar
      - Myocardial Infarction
    - Permanent cells
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
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Stimulus Not Destroyed

Necrosis

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Possible Outcomes after Injury

Injury

- Response

Acute Inflammation

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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

**PERSISTENT STIMULUS**
(chronic inflammation)

- Activation of macrophages and lymphocytes
  - Growth factors (PDGF, FGF, TGFβ)
  - Cytokines (TNF, IL-1, IL-4, IL-13)
  - Decreased metalloproteinase activity

- Proliferation of fibroblasts, endothelial cells, and specialized fibrogenic cells
- Increased collagen synthesis
  - FIBROSIS
  - Decreased collagen degradation
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
Cirrhosis
Cutaneous Wound Healing

Classic Stages of Wound Repair

- Inflammation – until 48 hrs. after injury
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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)
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

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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
Abnormal Repair Processes

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