Overview of topics

- Consider wound healing as a spectrum of outcomes: normal scars, fibrotic scars, or chronic wounds
- Review sequential phases of normal wound healing and recognize the beneficial effects of controlled inflammation and protease activities
- Understand the detrimental effects on healing of chronic inflammation caused by planktonic and biofilm bacteria, which leads to elevated MMP activity in wounds that destroy proteins that are essential to healing (ECM, GFs, receptors)
- Learn about the key roles that TGFβ and CTGF play in stimulating excessive scar formation (fibrosis) and how to reduce pathological scar formation

Think of wound healing as a spectrum of clinical outcomes

Inadequate healing (Chronic)  Normal healing (Repair)  Excessive healing (Fibrosis)

- Venous leg ulcer
- Good skin scar
- Hypertrophic scar
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Sequence of molecular and cellular events in skin wound healing

Four phases of healing:
1. Hemostasis
2. Inflammation
3. Repair
4. Remodeling

1. Clotting
2. Vascular response
3. Inflammation
4. Scar formation
5. Epithelial healing
6. Contraction
7. Scar remodeling

Hemostasis
Fibrin clot & platelets

Vascular response, blood clotting, and platelet release of growth factors

Key points:
1. Fibrin clot forms a provisional wound matrix that promotes coagulation and migration of fibroblasts, vascular endothelial cells
2. Platelets release growth factors that initiate healing by stimulating chemotaxis, proliferation, and matrix synthesis

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Sequential phases of cytokine and growth factor expression

- **Day 0 to 1**: Platelets
- **Days 1 to 5**: Neutrophils, Macrophages
- **Days 5 to 21**: Wound tissue cells

### Major families of growth factors

<table>
<thead>
<tr>
<th>Growth Factor Family</th>
<th>Cell source</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transforming Growth Factor β</strong></td>
<td>Platelets, Fibroblasts, Macrophages</td>
<td>Chemotactic for fibroblast, Promotes extracellular matrix formation, ↑ Collagen and TIMP synthesis, ↓ MMP synthesis, Reduces scarring</td>
</tr>
<tr>
<td><strong>Platelet Derived Growth Factor</strong></td>
<td>Platelets, Macrophages, Keratinocytes, Fibroblasts</td>
<td>Activates immune cells and fibroblasts, Promotes ECM formation, ↑ Collagen and TIMP synthesis, ↓ MMP synthesis, Reduces scarring</td>
</tr>
<tr>
<td><strong>Insulin-like Growth Factor</strong></td>
<td>Insulin, Liver, Skeletal muscle, Fibroblasts, Macrophages, Neutrophils</td>
<td>↑ Keratinocyte proliferation, ↑ Angiogenesis</td>
</tr>
</tbody>
</table>

### Inflammation

- Cytokines, neutrophils, macrophages, proteases, and Reactive Oxygen Species
Controlled wound inflammation is beneficial

Inflammatory cells kill microorganisms and release proteases (MMPs, elastase) that remove denatured ECM components and permit wound healing to proceed. Wounds that are contaminated by bacteria and fungus must not be closed.

Respiratory burst in neutrophils & macrophages produces Reactive Oxygen Species (ROS) that kill bacterial & fungi

In the membranes of neutrophils, NADPH oxidase generates superoxide ($\text{O}_2^-$), which spontaneously dismutates to $\text{H}_2\text{O}_2$, and is converted to hypochlorous acid ($\text{HOCl}$) by myeloperoxidase (MPO).

Major cytokines involved in wound healing

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Cell source</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-inflammatory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor Necrosis Factor (TNF-α)</td>
<td>macrophages</td>
<td>↑ PMN margination and cytotoxicity</td>
</tr>
<tr>
<td>Interleukin-1 (IL-1)</td>
<td>macrophages, keratinocytes</td>
<td>↑ fibroblast and keratinocyte chemotaxis, ↑ MMP synthesis</td>
</tr>
<tr>
<td>Interleukin-4 (IL-4)</td>
<td>macrophages, keratinocytes</td>
<td>↑ fibroblast proliferation</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
<td>macrophages, fibroblasts</td>
<td>↑ fibroblast and PMN chemotaxis, ↑ collagen synthesis</td>
</tr>
<tr>
<td>Interferon-γ (INF-γ)</td>
<td>macrophages, T-lymphocytes</td>
<td>↑ macrophage and PMN activation</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-4 (IL-4)</td>
<td>T-lymphocytes, basophils, mast cells</td>
<td>↓ TNF-α, IL-1, IL-6 synthesis</td>
</tr>
<tr>
<td>Interleukin-10 (IL-10)</td>
<td>T-lymphocytes, macrophages, keratinocytes</td>
<td>↓ TNF-α, IL-1, IL-6 synthesis</td>
</tr>
</tbody>
</table>
Repair phase

Conversion of the provisional wound matrix into initial scar tissue

Provisional wound matrix is replaced by initial scar tissue

- Provisional wound matrix (fibrin clot) is replaced by new collagen, elastin, proteoglycans and glycoproteins synthesized by fibroblasts that migrate into the wound.
- TGFβ and CTGF are dominant growth factors that stimulate scar formation.

Angiogenesis is stimulated by hypoxia-induced VEGF and controlled MMP activity

A - Tissue injury (A) causes hypoxia - induces Hypoxia Inducible Factor (HIF) - stimulates release of angiogenic growth factors like VEGF - induces MMPs - erode holes in the basement membrane (B) surrounding capillaries.
B - Vascular endothelial cells proliferate & migrate to ischemic area (C) - creating new capillary area (D, E, F).
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Contraction of wounds by myofibroblasts

(A) Full thickness excision for melanoma on thigh cannot be closed by surgical means because of its extent and depth. It heals by secondary intention and closes mainly by the formation of new tissue and contraction (skin grafting was not performed at patient's request). (B) After 10 days, granulation tissue can be seen in the wound bed. (C) On the 21st day, the size of the wound bed has markedly decreased through contraction. (D) After 2 years the wound is closed and epithelialized. Tension lines caused by contraction are running towards the center of the wound. MMPs secreted by myofibroblasts are required for matrix contraction.

Epithelial healing of deep skin wounds

Epithelialization of deep partial thickness or full thickness wounds occurs predominantly from the edge of the wound by proliferation and migration of epithelial cells. Epithelialization is more rapid under moist conditions (left) than dry conditions (right) because migrating epithelial cells must penetrate under scab and desiccated matrix.

Remodeling phase

Slow removal of initial irregular scar tissue by proteases and replacement with extracellular matrix that is more normal structure and composition.
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Remodeling phase (2)

Controlled MMPS are necessary for wound healing
Debridement, angiogenesis, contraction, epithelial migration, remodeling

MMPS are necessary for several key processes in wound healing:
1. Removing denatured matrix
2. Degrading capillary basement membrane for angiogenesis
3. Contraction of ECM by myofibroblasts
4. Migration of epithelial cells
5. Remodeling of scar

Is there a common molecular pathology of chronic wounds?

Diabetic foot ulcer
Arterial ulcer
Pressure ulcer
Venous ulcer
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Hypothesis of chronic wound pathophysiology
- Repeated injury, ischemia and planktonic and biofilm bacteria
  - ↑ TNF-α
  - ↑ IL-1α, IL-6
- Prolonged, elevated inflammation
  - ↑ neutrophils, macrophages, mast cells
- Imbalanced proteases & inhibitors
  - ↑ Proteases (MMPs, elastase, plasmin) and ↓ inhibitors (TIMPs, α1PI)
- Destruction of essential proteins
  - ↓ growth factors / receptors, ECM degradation
  - ↓ cell migration, ↓ cell proliferation

Chronic non-healing wound

Imbalanced molecular environments of healing and chronic wounds

Healing wounds:
- Low levels of bacterial and inflammatory cytokines
- Low proteases, ROS, RNS
- Intact functional matrix
- High mitogenic activity
- Mitotically competent cells

Chronic wounds:
- High levels of bacteria – biofilm, MRSA
- High inflammatory cytokines
- High proteases, altered nitric oxide
- Degraded non-functional matrix
- Low mitogenic activity
- Senescent cells

High levels of MMP activity in chronic wounds decrease as wounds heal

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Low protease activity in chronic wound fluids of pressure ulcers predicts the rate and extent of healing

Ladwig, Robson, Liu, Kuhn, Muir, Schultz. Ratios of activated matrix metalloproteinase-9 to tissue inhibitor of matrix metalloproteinase-1 in wound fluids are inversely correlated with healing of pressure ulcers. Wound Repair Reg 10: 26-37, 2002

MMP levels in healthy skin versus venous ulcer tissues before and after compression therapy

Healthy skin
VU Before Tx
VU After Tx

Inverse correlation between percent wound closure and elevated MMP-1 levels in venous ulcers after compression therapy


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Fibronectin is rapidly degraded by chronic wound fluids. Fibronectin is absent in the base of chronic venous ulcers. Fibronectin reappears in ulcer base during healing.


Fibronectin is degraded in non-healing ulcer. Fibronectin reappears (stable) as ulcer heals. Fibronectin is degraded in non-healing ulcer. Fibronectin profile in plasma shows a single intact band at 250 kDa. In contrast, fibronectin is degraded to lower molecular weight fragments in venous stasis ulcers and in diabetic ulcers.

Wysocki and Grinnell. Lab Invest. 63, 825, 1990

Fibronectin is absent in the base of chronic venous ulcers. Fibronectin reappears in ulcer base during healing.

Fibronectin promotes cell attachment, spreading, and migration.

(A) Fibroblasts cultured on glass do not spread.
(B) In contrast, the fibroblasts cultured on fibronectin-coated slides attach and spread quickly within 1 hour.
(C) Addition of fibronectin (FN) promotes migration of vascular endothelial cells following scrape wound in vitro.

PDGF-AA immunostaining

Detection of Platelet-derived Growth Factor (PDGF)-AA in Actively Healing Human Wounds Treated with Recombinant PDGF-BB and Absence of PDGF in Chronic Nonhealing Wounds


- Low in normal skin
- High in acute healing wound
- Low in chronic wound

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If chronic wounds fail to heal because of hostile molecular environment, can it be corrected?

Yes, by applying the principles of wound bed preparation.

Wound bed preparation and ‘T I M E’
Tissue, Inflammation/Infection, Moisture, Edge

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Major developments in last 10 years

- New debridement techniques
  - Medical honey
  - Medical larvae
  - Anionic polymers
  - Ultrasonic debridement
- Better understanding of infection/inflammation
  - Roles of biofilms
  - DNA-based identification of bacteria
  - Diagnosis for proteases
  - Protease inhibiting dressings
- Better moisture control
  - NPWT + Instillation with microbicidal solutions - impacted "I" and "M"
  - Advanced super absorbent polymers
- Better agents to stimulate epithelial cell proliferation and migration
  - Amniotic membranes
  - Dermal matrix dressings

Pathological scarring - fibrosis

Signaling pathway in scar formation
Second generation antisense oligonucleotides
Highly evolved 2'MOE Antisense Oligos (ASO) drugs

Antisense oligonucleotide targeting CTGF
reduces scarring in rabbit ear wound model

Conclusions: The CTGF ASO demonstrated a significant reduction in skin scarring compared to saline control

Hypertrophic scar in skin


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Phase I/IIa/IIb/IIc clinical trials in skin Abdominoplasty – hypertrophic scar revision

- Average skin excision during abdominoplasty: 40 x 12 cm ellipse
- 32 patients received injection of ASO to one end of incision and scrambled ASO to other end – separated by 2 cm in all directions
- Patient returns 12 and 24 weeks later for assessment of scar
- Revision of hypertrophic scar following breast surgery – implants or reduction – one end (side) injected with ASO, the other injected with scrambled ASO – evaluated at 24 & 24 weeks post surgery

- Analysis of incision:
  - Histological
  - Molecular
  - Wound strength

ASO or vehicle treatment of incision

Unreated Treated

Physician responses on scar appearance

- Overall
- Surface area
- Pliability
- Relief
- Thickness
- Pigmentation
- Vascularity

EXC 001
No difference
Placibo

# of physician scores favoring

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### Summary

- Healing of acute healing wounds proceeds through four major sequential phases (hemostasis, inflammation, repair, and remodeling)
- Chronic wounds frequently have bacterial biofilms that cause elevated levels of pro-inflammatory cytokines, leading to chronic inflammation
- Elevated proteases from inflammatory cells destroy essential growth factors, receptors, and ECM proteins, which impairs healing
- Fibrosis (excessive healing) involves elevated levels of TGFβ and CTGF that increase synthesis of ECM molecules (collagen) and decreases proteases
- Reduction of CTGF gene by ASO decreases scarring