MMP's and Their Role in the Wound

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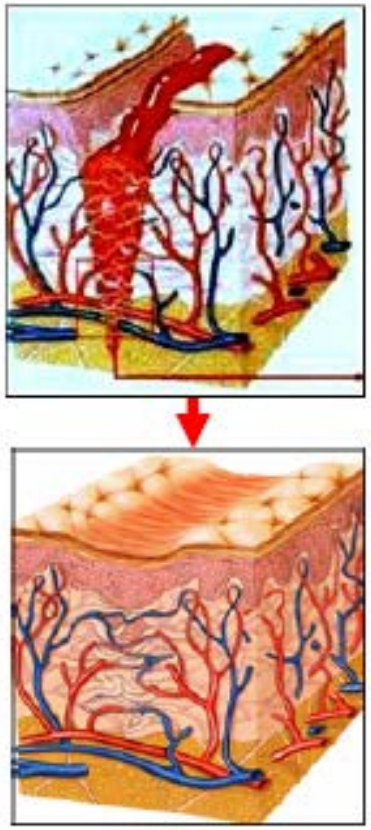
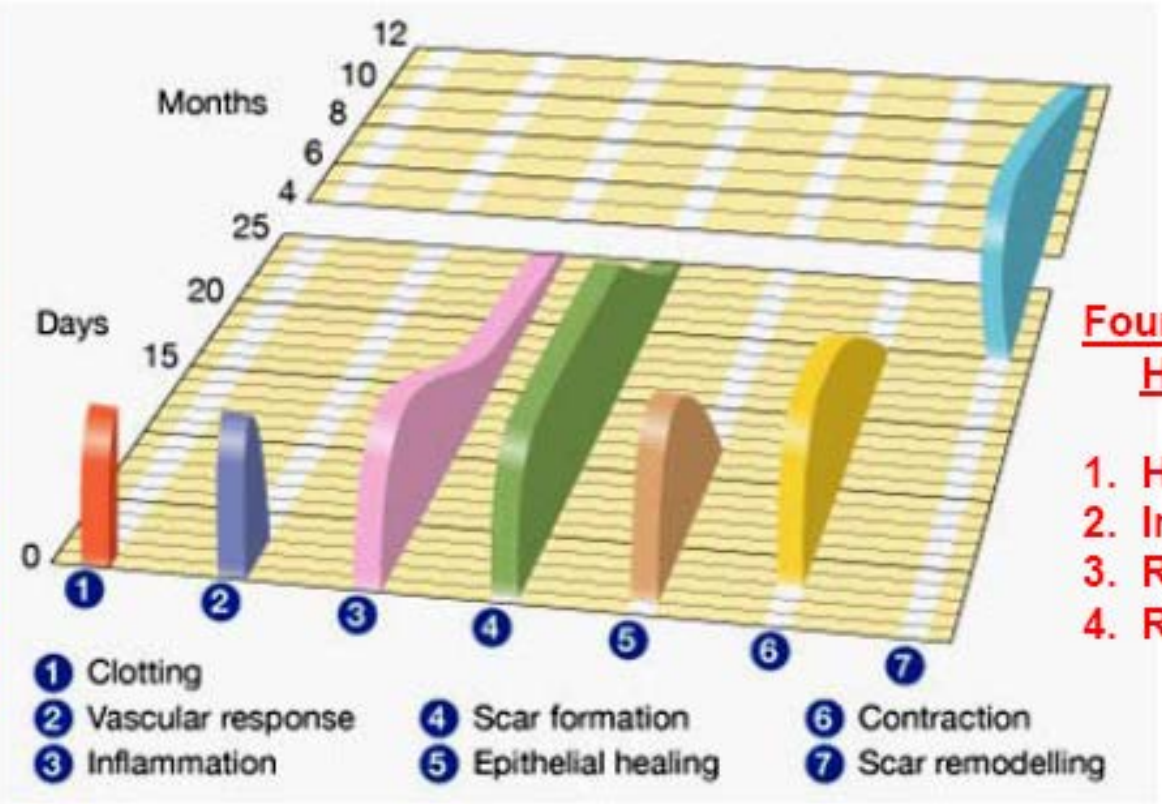
Sequence of Molecular and Cellular Events in Healing Wounds





Four Phases of Healing

1. Hemostasis
2. Inflammation
3. Repair
4. Remodeling

1	2	3	4	5	6	7
Clotting	Vascular Response	Inflammation	Scar Formation	Epithelial Healing	Contraction	Scar Remodeling

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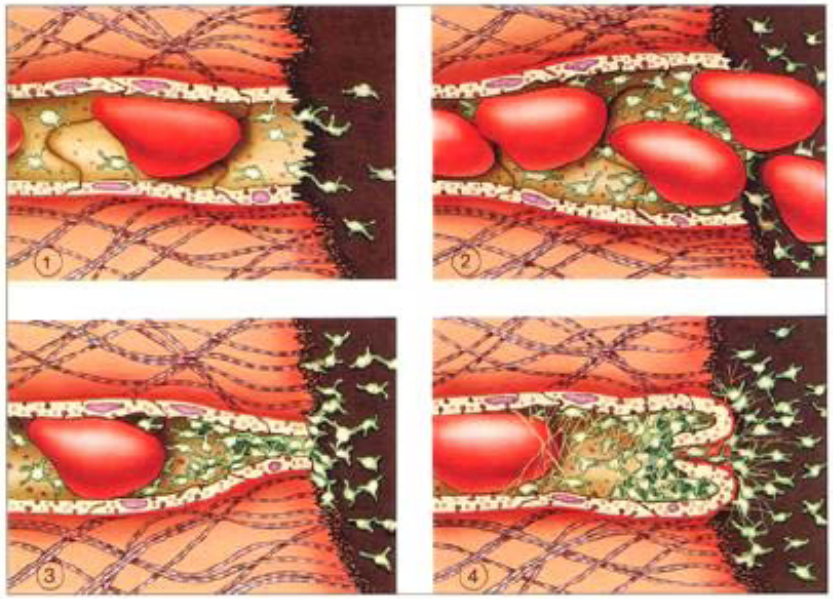
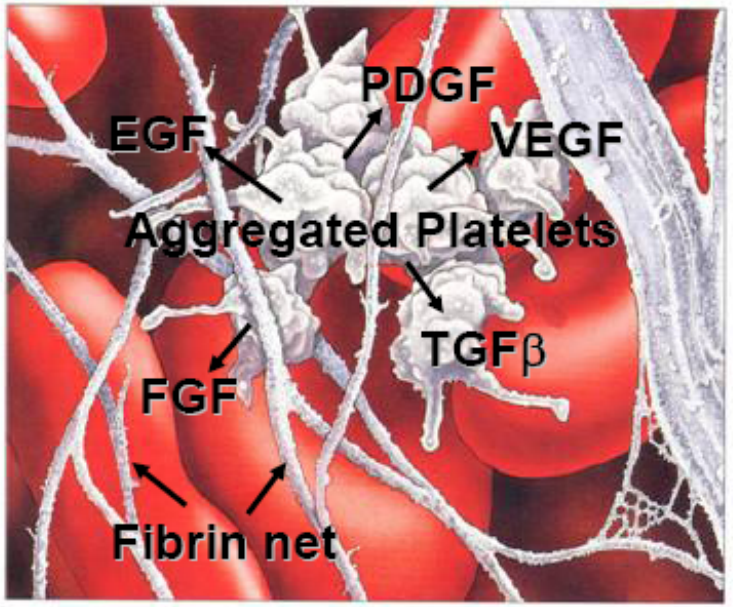
Hemostasis

Hemostasis: 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 ↑chemotaxis, proliferation, and matrix synthesis

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MAJOR FAMILIES OF GROWTH FACTORS

GROWTH FACTOR FAMILY	CELL SOURCE	ACTIONS
Transforming Growth Factor β TGF-β1 TGF-β2 TGF-β3	Platelets Fibroblasts Macrophages	Chemotatic for Fibroblast Promotes Extracellular Matrix Formation ↑ Collagen and TIMP Synthesis ↓ MMP Synthesis Reduces Scarring ↓ Collagen ↓ Fibronectin
Platelet Derived Growth Factor PDGF-AA, PDGF-BB VEGF	Platelets Macrophages Keratinocytes Fibroblasts	Activates Immune Cells and Fibroblasts Promotes ECM Formation ↑ Collagen and TIMP Synthesis ↓ MMP Synthesis ↑ Angiogenesis
Fibroblast Growth Factor Acidic FGF, Basic FGF KGF	Macrophages Endothelial Cells Fibroblasts	↑ Angiogenesis ↑ Keratinocyte Proliferation and Migration ↑ ECM Deposition
Insulin-like Growth Factor IGF-I, IGF-II Insulin	Liver Skeletal Muscle Fibroblasts Macrophages Neutrophils	↑ Keratinocyte & Fibroblast Proliferation ↑ Angiogenesis ↑ Collagen Synthesis ↑ ECM Formation ↑ Cell Metabolism
Epidermal Growth Factor EGF, HB-EGF, TGF-α, Amphiregulin, Betacellulin	Keratinocytes Macrophages	↑ Keratinocyte Proliferation and Migration ↑ ECM Formation
Connective Tissue Growth Factor CTGF	Fibroblasts Endothelial Cells Epithelial Cells	↑ Collagen Synthesis Mediates Action of TGF-βs on collagen synthesis

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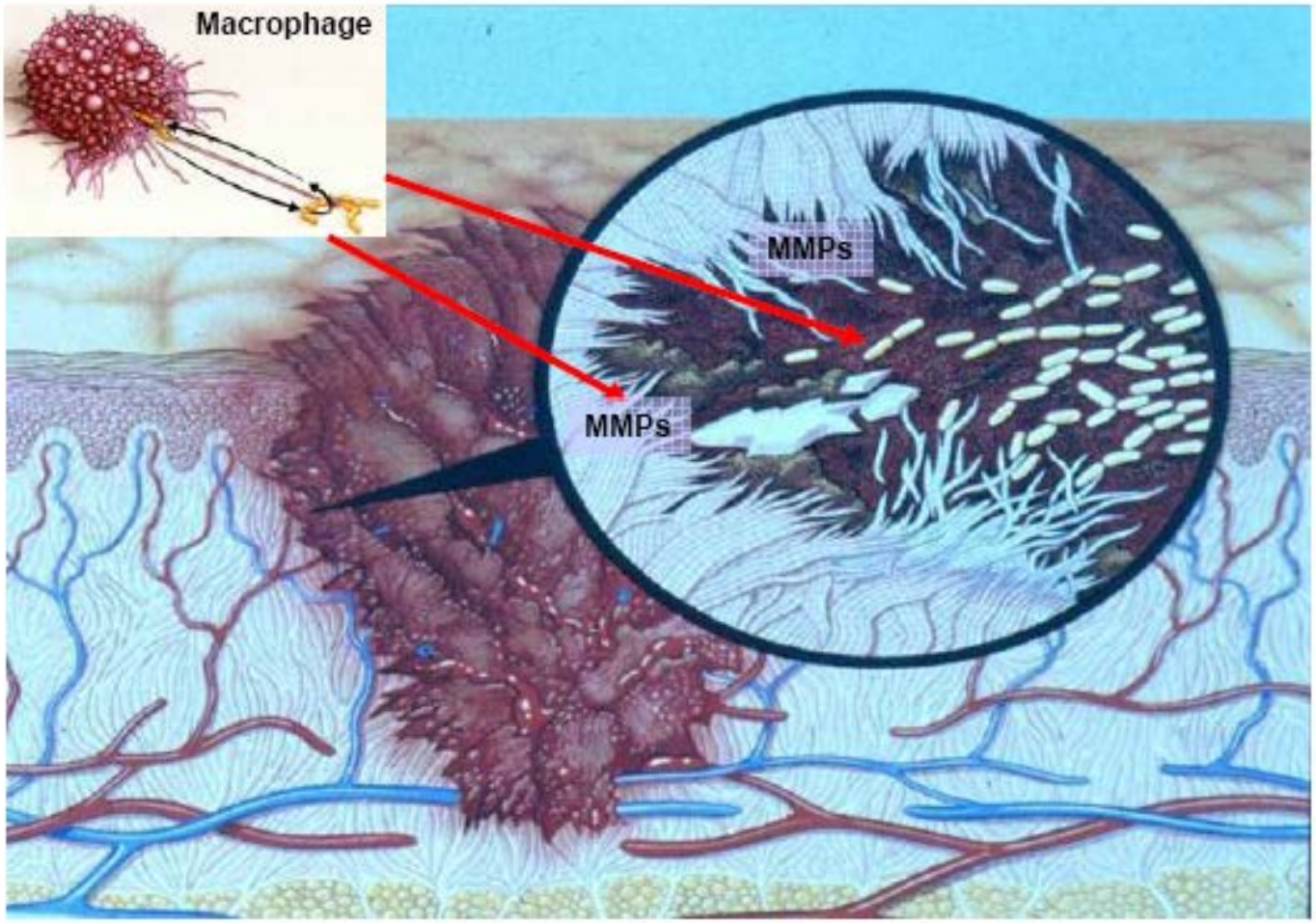
MMPs and Growth Factors

- Pathological dysregulation of MMP activity results in excessive breakdown of the extracellular matrix (ECM) and loss of growth factors, involved in normal healing, that are stored in the ECM.

- Growth factors such as PDGF-BB, TGF-β and VEGF are found in decreased amounts in chronic wounds, presumably due to the increase in MMPs (Loot et al., 2002; Lerman et al., 2003; Robson et al., 2004).

Inflammation

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.

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Major Cytokines Involved in Wound Healing

CYTOKINE	CELL SOURCE	BIOLOGICAL ACTIVITY
PRO-INFLAMMATORY CYTOKINES		
Tumor Necrosis Factor (TNF-α)	macrophages	↑ PMN margination and cytotoxicity ↑ MMP synthesis
Interleukin-1 (IL-1)	macrophages, keratinocytes	↑ fibroblast and keratinocyte chemotaxis, ↑ MMP synthesis
Interleukin-6 (IL-6)	macrophages, keratinocytes, PMNs	↑ fibroblast proliferation
Interleukin-8 (IL-8)	macrophages, fibroblasts	↑ macrophage and PMN chemotaxis ↓ collagen synthesis
Interferon-γ (INF-γ)	macrophages, T-lymphocytes	↑ macrophage and PMN activation ↓ collagen synthesis ↑ MMP synthesis
ANTI-INFLAMMATORY CYTOKINES		
Interleukin-4 (IL-4)	T-lymphocytes, basophils, mast cells	↓ TNF-α, IL-1, IL-6 synthesis ↑ fibroblast proliferation, collagen synthesis
Interleukin-10 (IL-10)	T-lymphocytes, macrophages, keratinocytes	↓ TNF-α, IL-1, IL-6 synthesis ↓ macrophage and PMN activation

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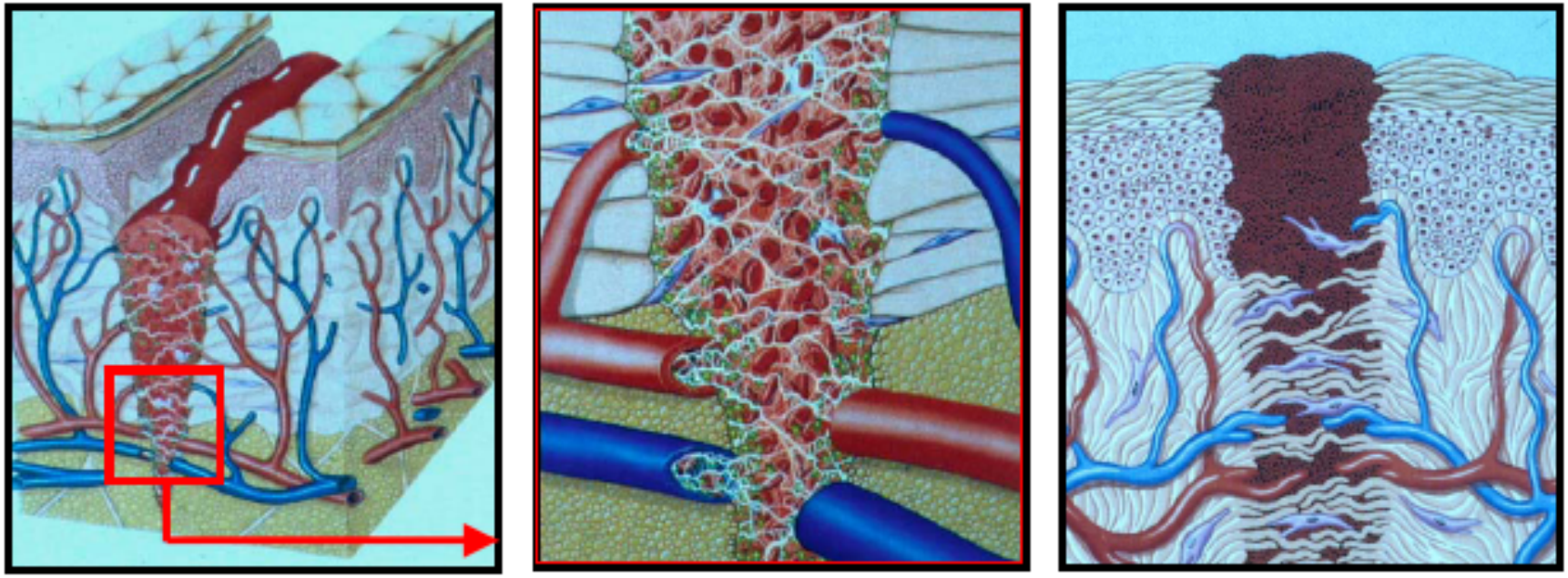
Repair

Provisional Wound Matrix is Replaced by Scar Tissue



Fibroblasts adjacent to the wound respond to chemotactic factors released by platelets, migrate into the provisional wound matrix (fibrin, fibronectin, and laminin). As healing proceeds, growth factors stimulate fibroblasts to synthesize new collagen, elastin and glycoproteins that replace the provisional wound and form the scar.

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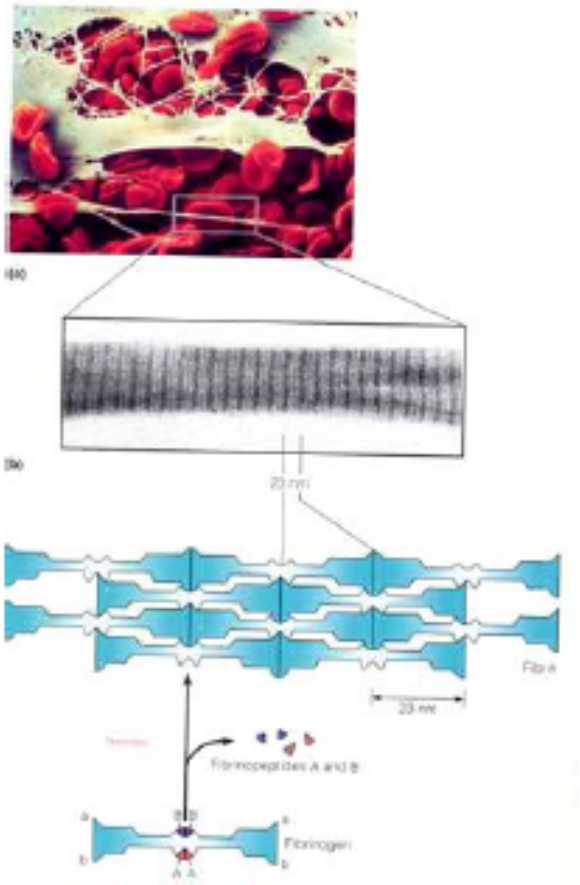
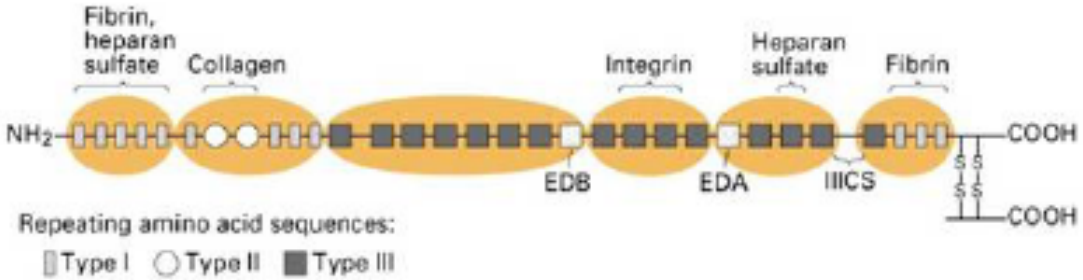
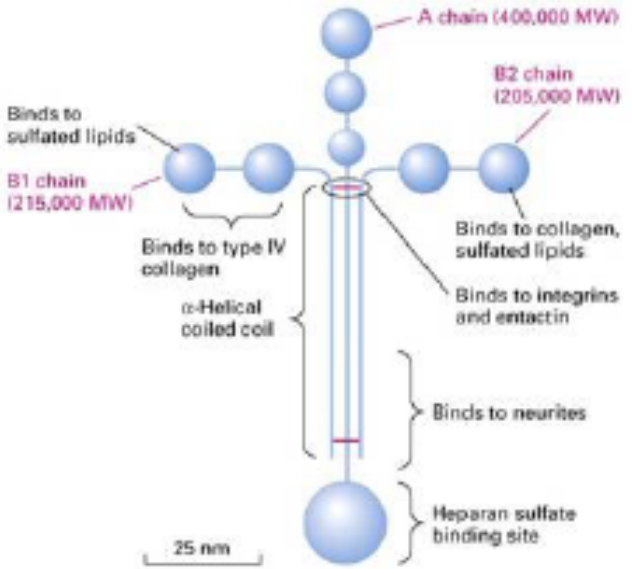
Fibrin, Fibronectin, and Laminin



Fibrin self associates into long fibers after thrombin cleaves off peptides



Fibronectin dimers have multiple binding sites for ECM components and integrin receptors on cells

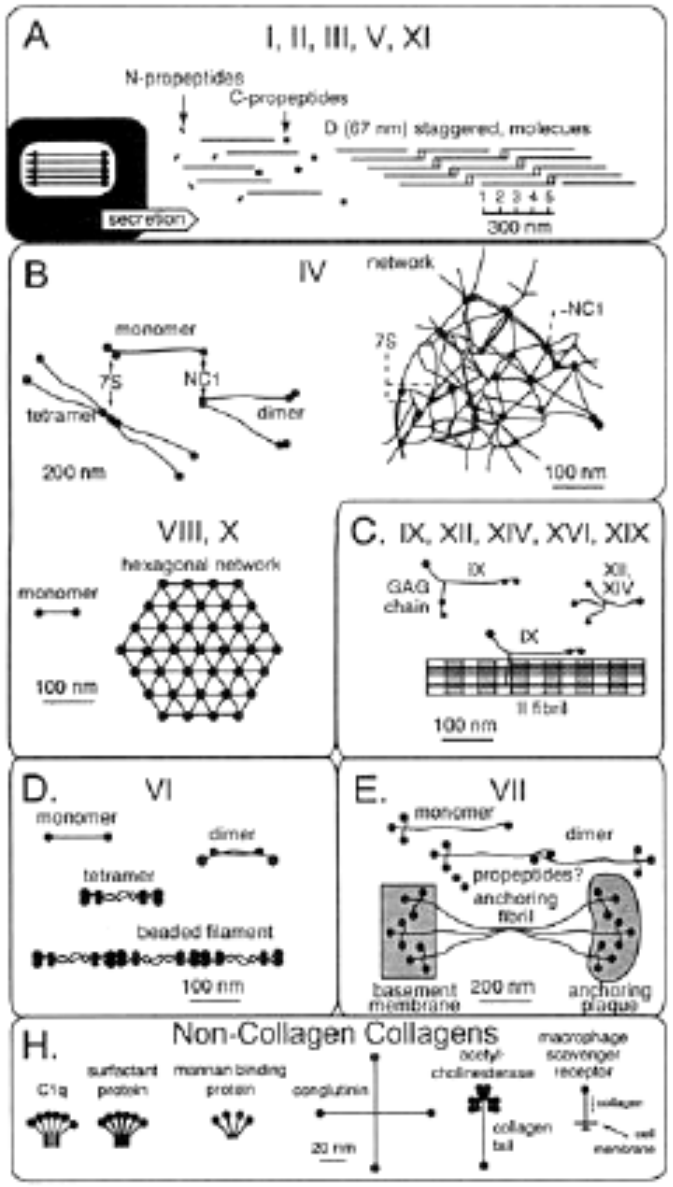


Laminin forms a cross with arms that bind ECM components and integrin receptors on cells

Proteins of the Initial Scar Stimulate Cell Migration and Binding

- Collagen molecules
  - Type III fibrillar collagen expressed early in scar formation
- Glycosaminoglycans
  - Linear chains of 20-100 sulfated disaccharides one of which is either N-acetylglucosamine or N-acetylgalactosamine
  - All are acidic and contain either sulfate or carboxylate groups
  - Heparin, heparan sulfate, dermatan sulfate, chrondroitin sulfate
  - Hyaluronic acid is not covalently linked to a core protein
  - All bind large amounts of water and provide resilience and lubrication
- Proteoglycans
  - Multiple glycosaminoglycan chains that branch from a linear protein core. Extracellular PGs are large hydrated molecules that cushion cells and cell-surface proteoglycans (syndecan) bind growth factors (FGF, TGFβ)
  - Perlecan (basal lamina), Syndecan (cell membranes) Aggrecan (cartilage)

Different Types of Collagen Proteins



A. Fibrillar collagens (form rods)

B. Network-forming collagens

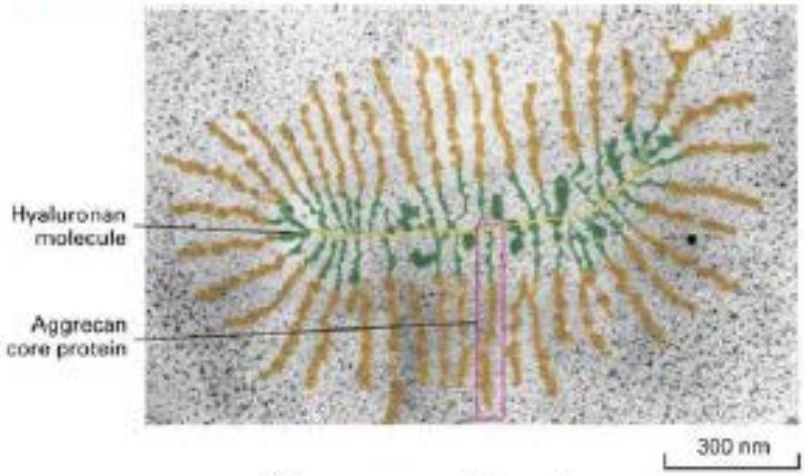
C. Fibril-associated collagens with interrupted triple helices (FACITs)

D. Beaded filament –forming collagens

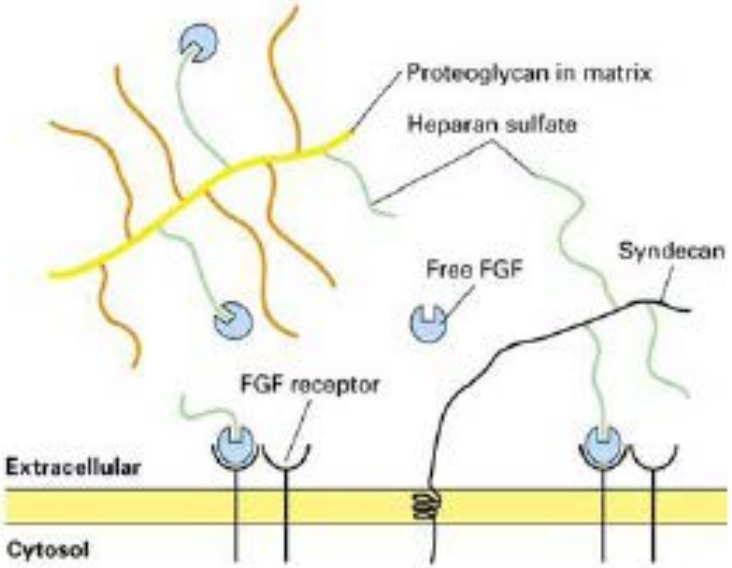
E. Membrane anchoring collagens

H. Non-collagen proteins containing collagen-like sequences

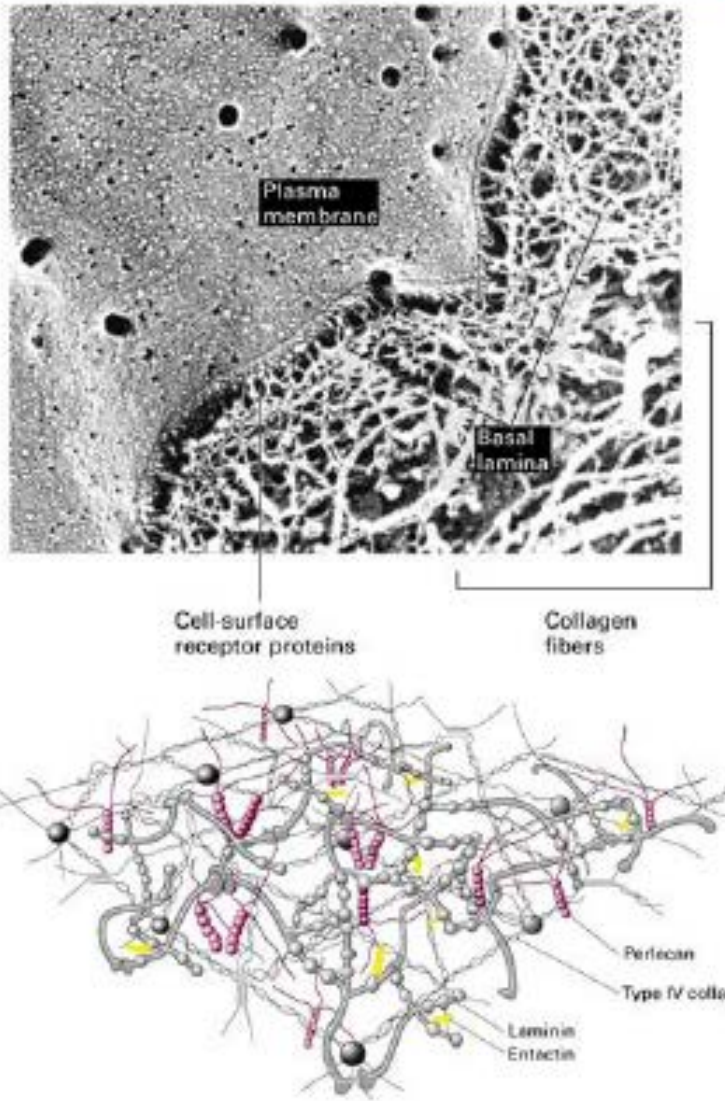
Glycosaminoglycans, Proteoglycans, and Basement Membranes



Glycosoaminoglycans



Proteoglycans



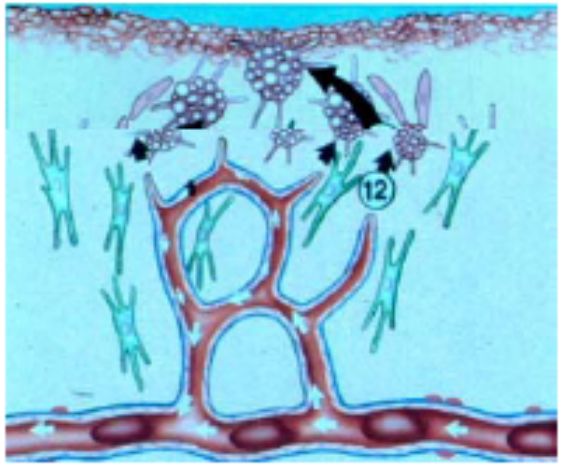
Basement membranes

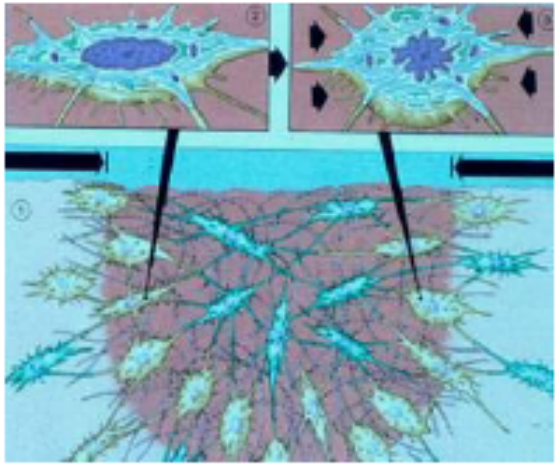
Matrix Metalloproteinases and their Substrates

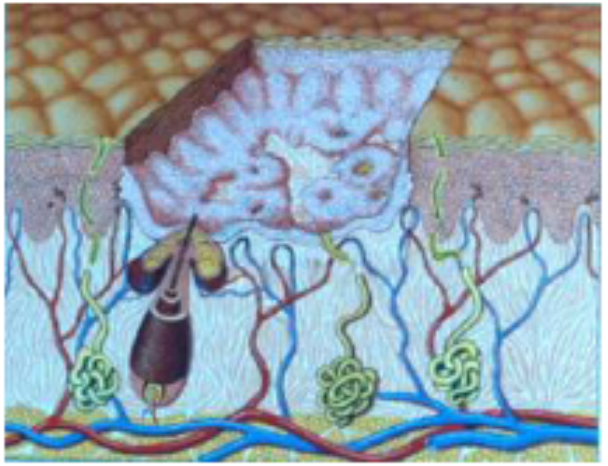
MMP	Synonyms	EC Classification	Latent M.W. (kDa)	Active M.W. (kDa)	Substrates
MMP-1	Interstitial Collagenase	3.4.24.7	55	45	Fibrillar collagens, casein, gelatin, proteoglycan
MMP-2	Gelatinase A, 72 kDa Type IV Collagenase	3.4.24.24	72	66	Denatured collagens, collagens IV, V, VII, X, gelatin
MMP-3	Stromelysin-1	3.4.24.17	57	45	Proteoglycan, collagens X, XI, procollagens, gelatin, laminin, collagenase, gelatinase B
MMP-7	Matrilysin ; PUMP	3.4.24.23	28	19	As stromelysin 1, elastin
MMP-8	Neutrophil collagenase	3.4.24.34	75	58	Fibrillar collagens, gelatin, proteoglycan
MMP-9	Gelatinase B, 92 kDa Type IV Collagenase	3.4.24.35	92	86	Denatured collagens, collagens IV, V, VII, X
MMP-10	Stromelysin 2	3.4.24.22	57	44	As Stromelysin 1
MMP-11	Stromelysin 3		62	47	Unknown
MMP-12	Macrophage metalloelastase	3.4.24.65	54	22	Elastin, gelatin, collagen IV, fibronectin, laminin, vitronectin, proteoglycan
MMP-13	Collagenase 3	--	66	48	Fibrillar collagens, gelatin, aggrecan

MMP	Other names
MMP-1*	Interstitial Collagenase, Fibroblast Collagenase
MMP-2	Gelatinase A, 72 kDa Gelatinase, 72 kDa Type IV Collagenase
MMP-3	Stromelysin-1, Transin
MMP-7	Matrilysin, Pump-1
MMP-8	Neutrophil Collagenase
MMP-9	Gelatinase B, 92 kDa Gelatinase, 92 kDa Type IV Collagenase
MMP-10	Stromelysin-2, Transin-2
MMP-11	Stromelysin-3
MMP-12	Metalloelastase, Macrophage Elastase (commonly confused with Neutrophil Elastase)
MMP-13	Collagenase-3
MMP-14	Membrane-type-1 MMP, MT1-MMP**
MMP-15	Membrane-type-2 MMP, MT2-MMP
MMP-16	Membrane-type-3 MMP, MT3-MMP
MMP-17	Membrane-type-4 MMP, MT4-MMP
MMP-18	*Xenopus* Collagenase
MMP-19	No trivial name assigned
MMP-20	Enamelysin
MMP-21	No trivial name assigned
MMP-22	No trivial name assigned
MMP-23	Cysteine Array MMP, CA-MMP
MMP-24	Membrane-type-5 MMP, MT5-MMP
MMP-25	Membrane-type-6 MMP, MT6-MMP
MMP-26	Matrilysin-2, Endometase
MMP-28	Epilysin

Growth Factors, Extracellular Matrix Proteins and Matrix Metalloproteinases Are Necessary for Angiogenesis, Contraction, and Epithelial Migration







Angiogenesis

Endothelial cells and inflammatory cells secrete angiogenic growth factors that simulate synthesis of MMPs which degrade the basement membrane surrounding capillaries, allowing endothelial cells to proliferate and migrate toward ischemic areas.

Contraction

Fibroblasts transform into myofibroblasts, which express contractile actin fibers and MMPs, and when myofibroblasts contract, force is applied to collagen fibers, which reduces the size of the wound.

Epithelial Healing

Growth factors stimulate epidermal cells to proliferate and migrate over and through provisional wound matrix. Actions of MMPs are important for migration of epidermal cells.

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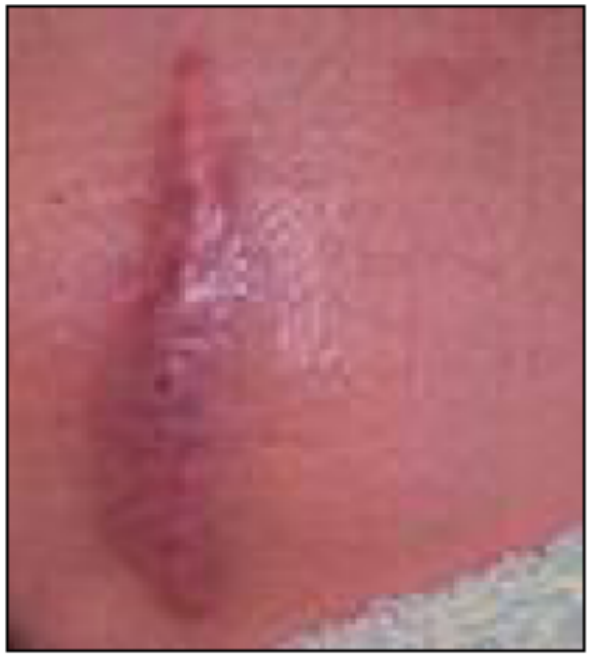
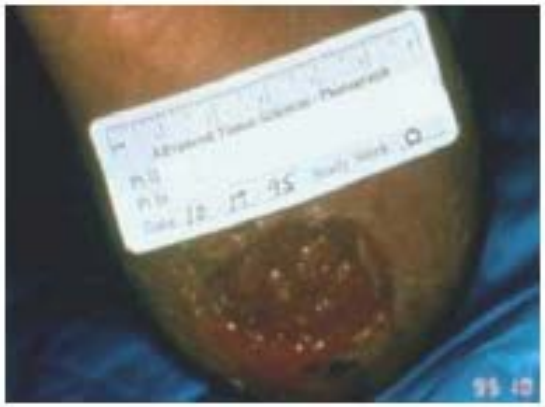
Remodeling

Remodeling of Scar Tissue

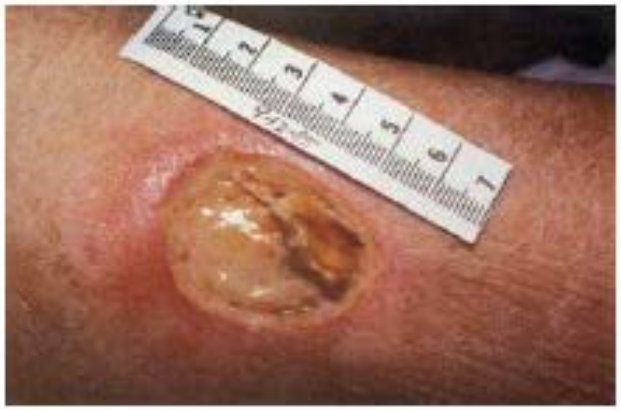
- The high numbers of inflammatory cells, fibroblasts and vascular endothelial cells decrease through apoptosis
- The balance between deposition (replacement) of ECM and removal of ECM shifts slightly in favor of removal by proteases (MMPs, elastase)
- New collagen is deposited in the scar at a low level for a year, but slowly the matrix remodels to a structure more closely resembling normal dermis (but it is never fully regenerates the normal dermal architecture)



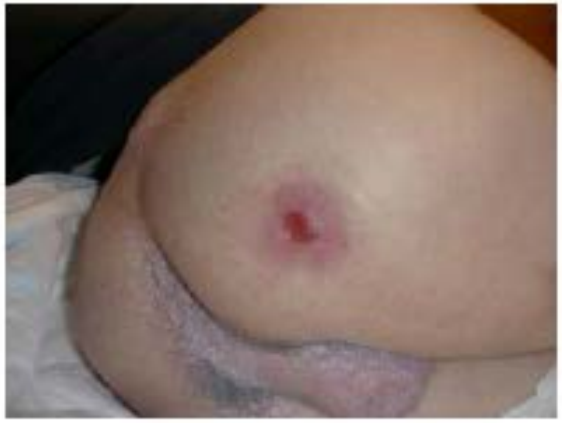
What Is The Molecular Pathology Of Chronic Wounds??



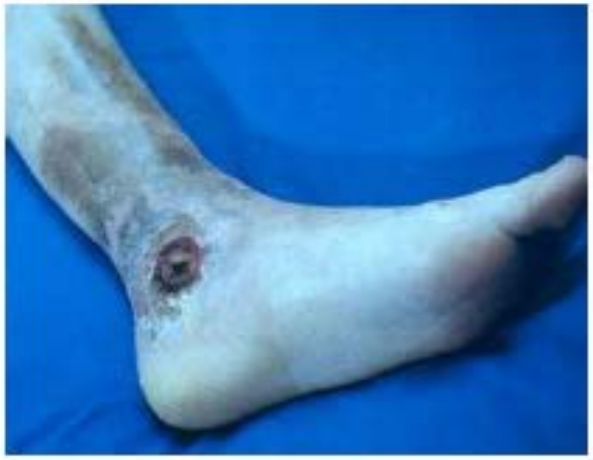
Diabetic foot ulcer



Arterial ulcer



Pressure ulcer



Venous ulcer

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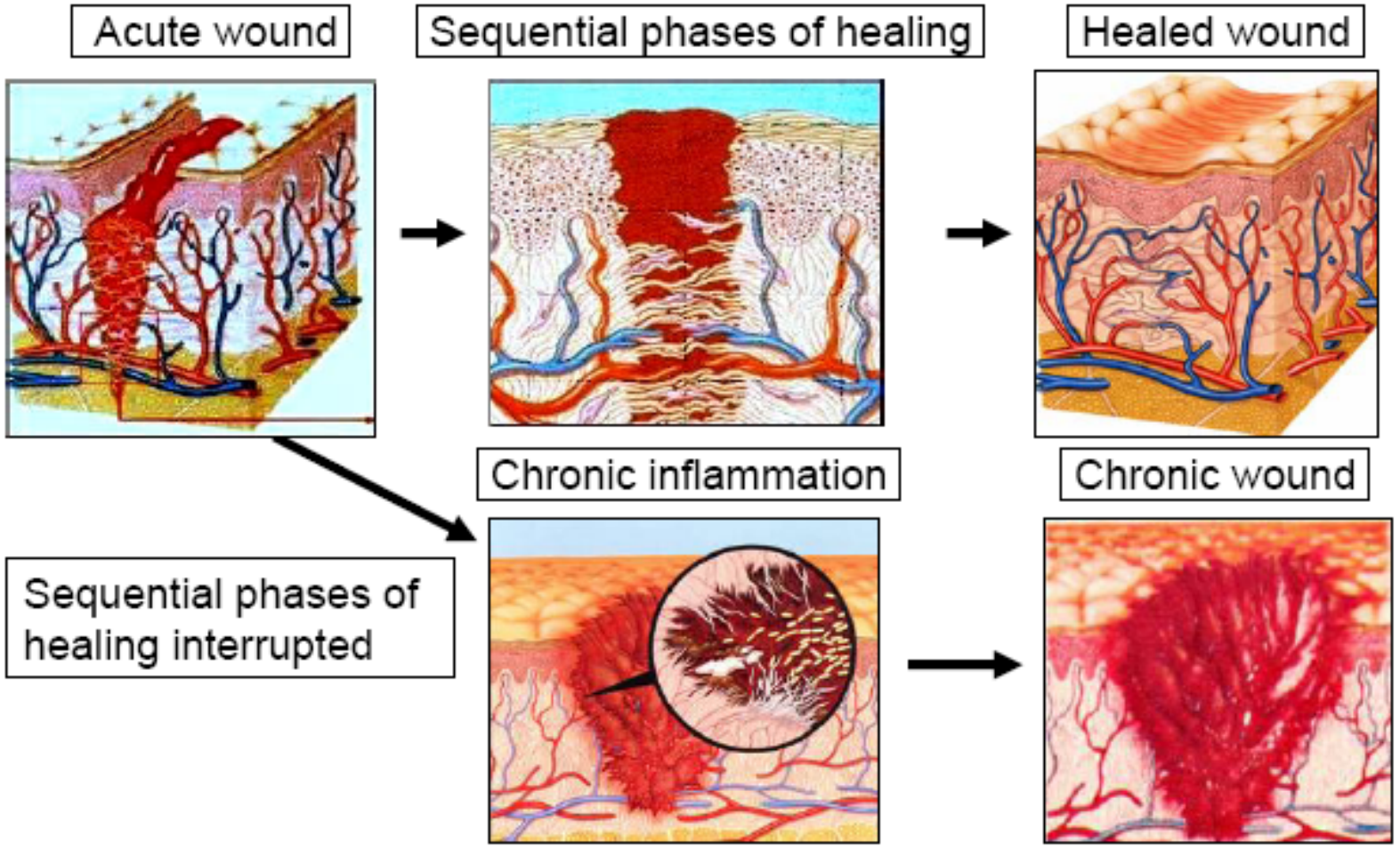
Chronic Wounds Do Not Follow The Normal Sequential Phases Of Wound Healing



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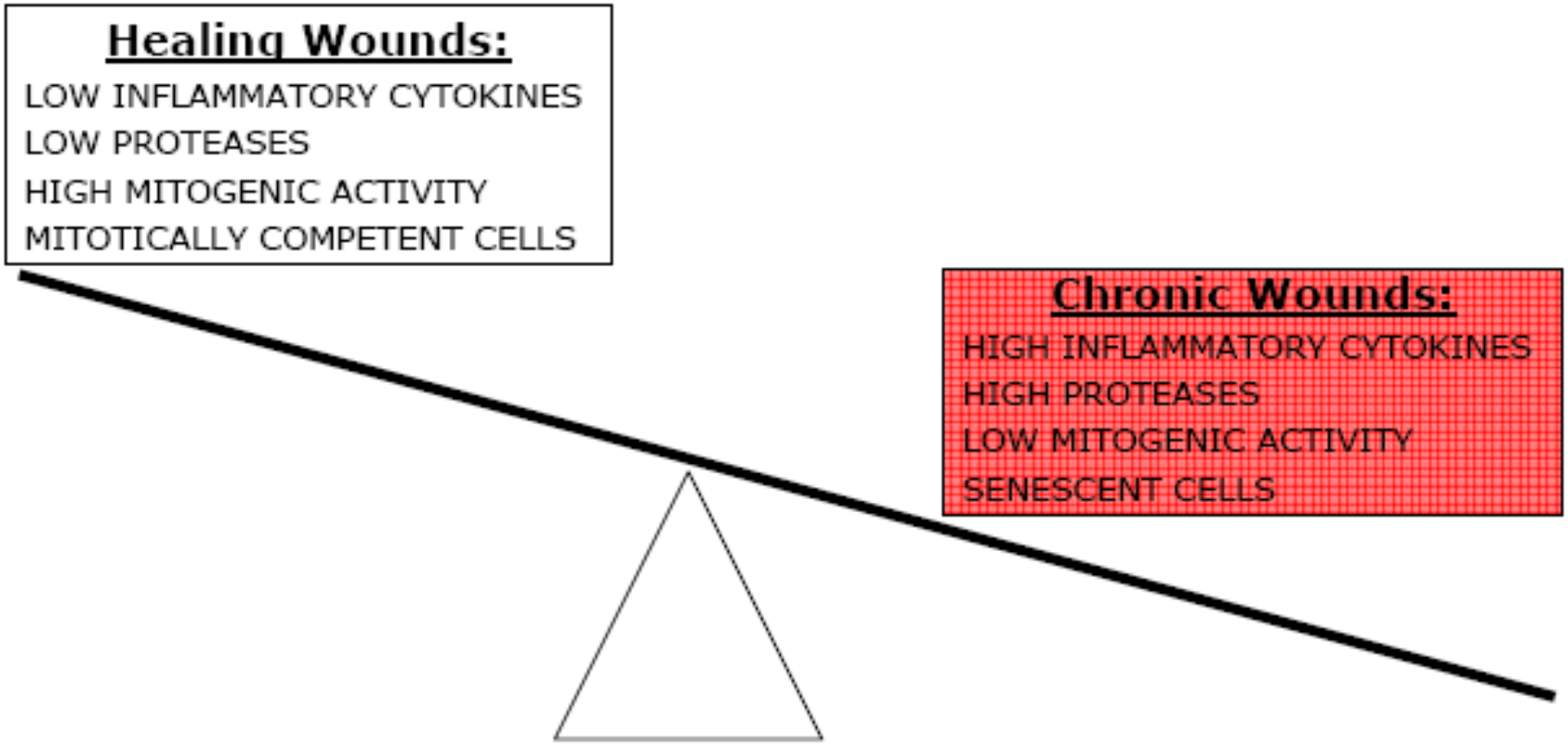
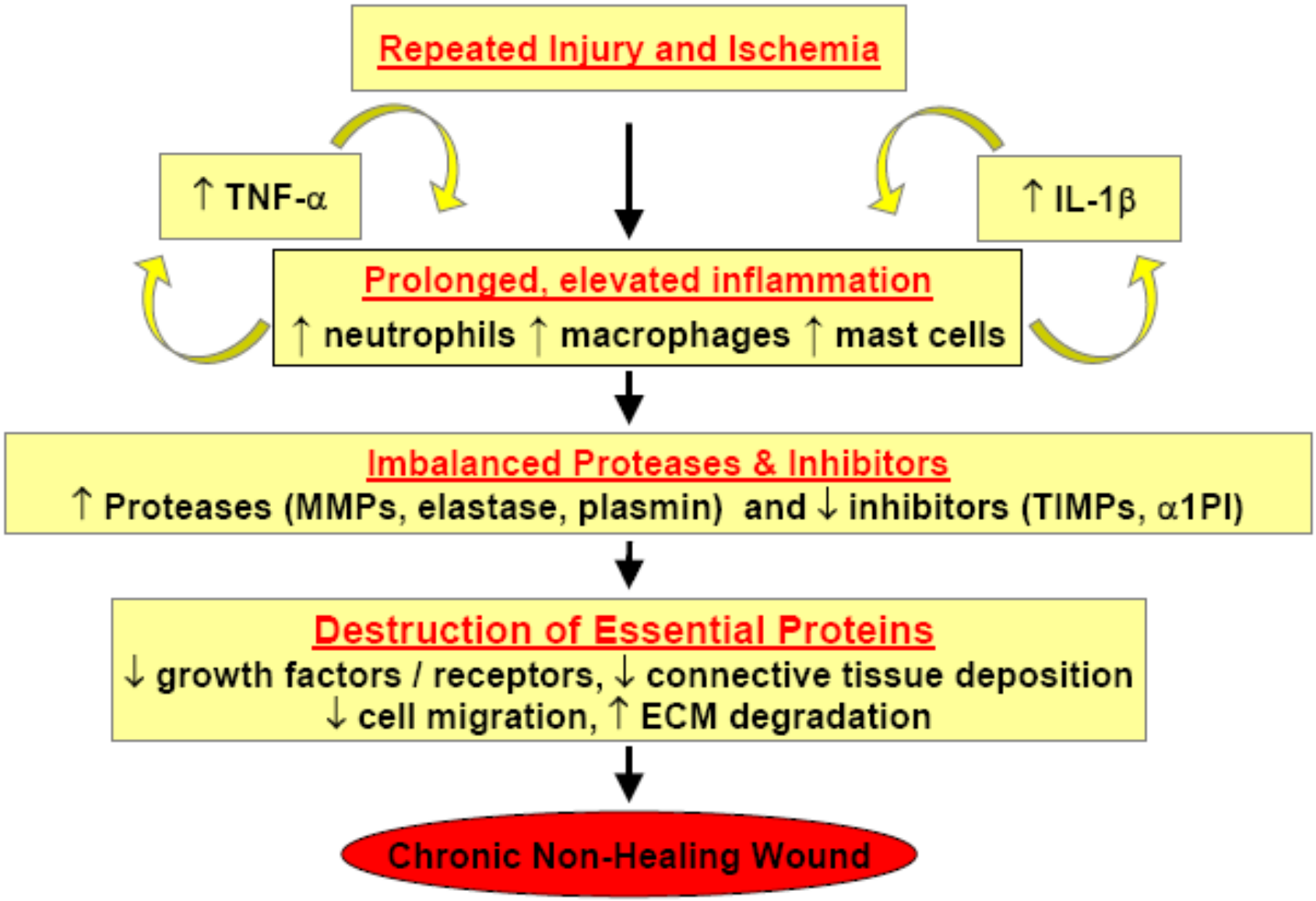
Imbalanced Molecular Environments Of Healing And Chronic Wounds



Schultz, Sibbald, Falanga, Ayello, Dowsett, Harding, Romanelli, Stacey, Teot, Vansheidt. Wound Rep Reg 11:1-28, 2003

Hypothesis Of Chronic Wound Pathophysiology



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Tissue Inhibitor of Metalloproteinases-1 Is Decreased and Activated Gelatinases Are Increased in Chronic Wounds

Elizabeth C. Bullen, Michael T. Longaker,* Dawn L. Updike, Richard Benton, Daniel Ladin,† Zizheng Hou,† and Eric W. Howard

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J Invest Dermatol 104: 236–240, 1995

Wound Fluids from Human Pressure Ulcers Contain Elevated Matrix Metalloproteinase Levels and Activity Compared to Surgical Wound Fluids

Dorne R. Yager, Liang-Y. Zhang, Hui-Xiu Liang, Robert F. Diegelmann, and I. Kelman Cohen

The Wound Healing Center, Division of Plastic and Reconstructive Surgery, Department of Surgery, Medical College of Virginia, Richmond, Virginia, U.S.A.

J Invest Dermatol 107:743–748, 1996

Diabetologia (2002) 45:1011–1016
DOI 10.1007/s00125-002-0868-8

Diabetologia

Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients

R. Lobmann[1], A. Ambrosch[2], G. Schultz[3], K. Waldmann[1], S. Schiweck[1], H. Lehnert[1]

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[3] Institute of Wound Research, Department of Obstetrics and Gynecology, University of Florida, Gainesville, Florida, USA

NITRIC OXIDE: Biology and Chemistry
Vol. 4, No. 6, pp. 572–582 (2000)
doi:10.1006/niox.2000.0307, available online at http://www.idealibrary.com on IDEAL®

Enhancement of Fibroblast Collagen Synthesis by Nitric Oxide[1]

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The American Journal of Surgery 183 (2002) 406–412

Role of nitric oxide in wound repair

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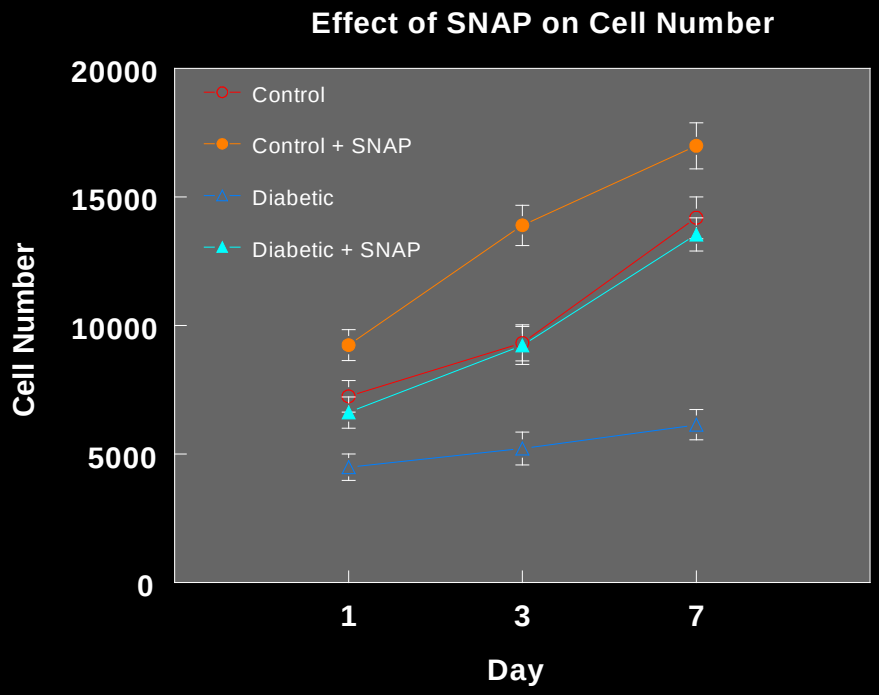
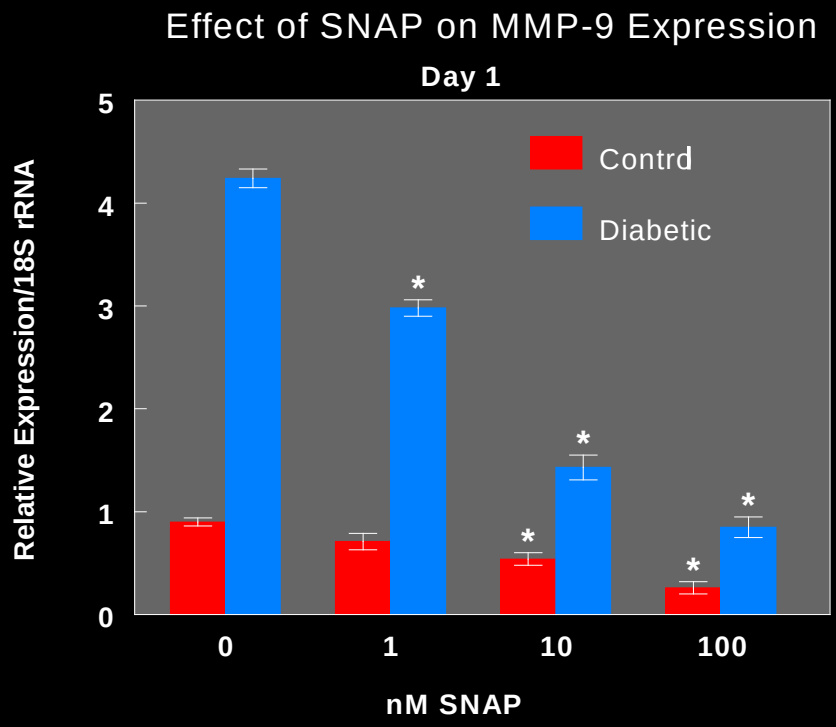
Diabetes-impaired healing and reduced wound nitric oxide synthesis: A possible pathophysiologic correlation

Michael R. Schäffer, MD,[a] Udaya Tantry, PhD, Philip A. Efron, BS, Gretchen M. Ahrendt, MD, Francis J. Thornton, MD, *and* **Adrian Barbul, MD, FACS,** *Baltimore, Md.*

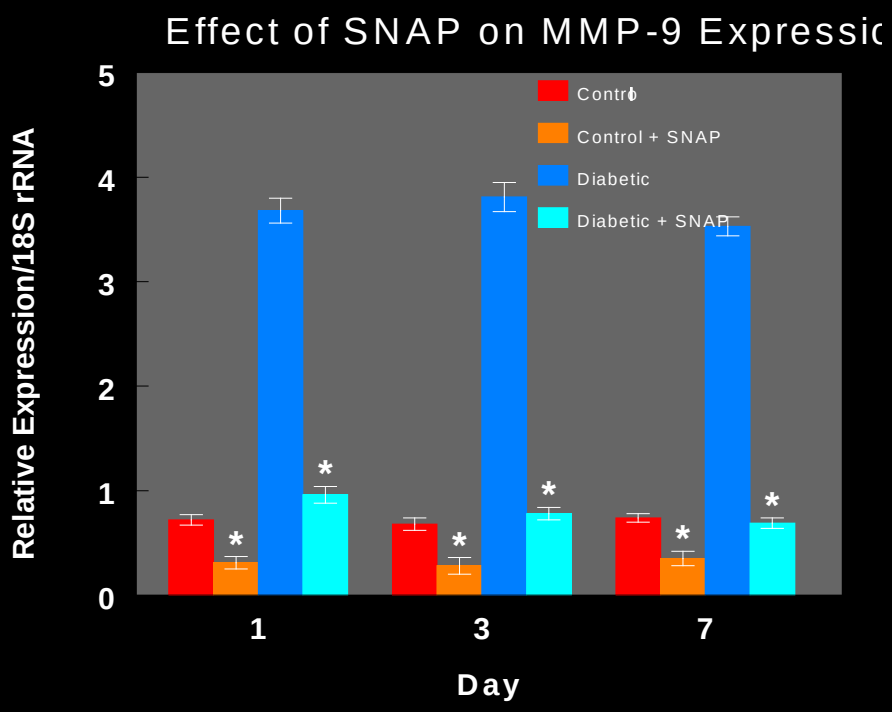
(Surgery 1997;121:513-9.)

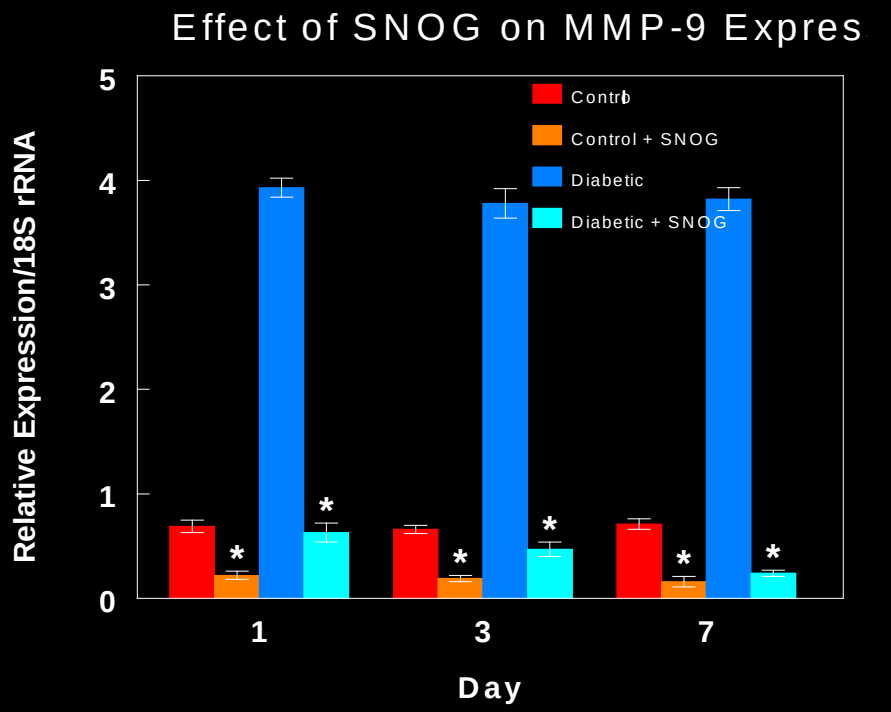
Effect of Nitric Oxide Donor SNAP on Human Skin Fibroblasts



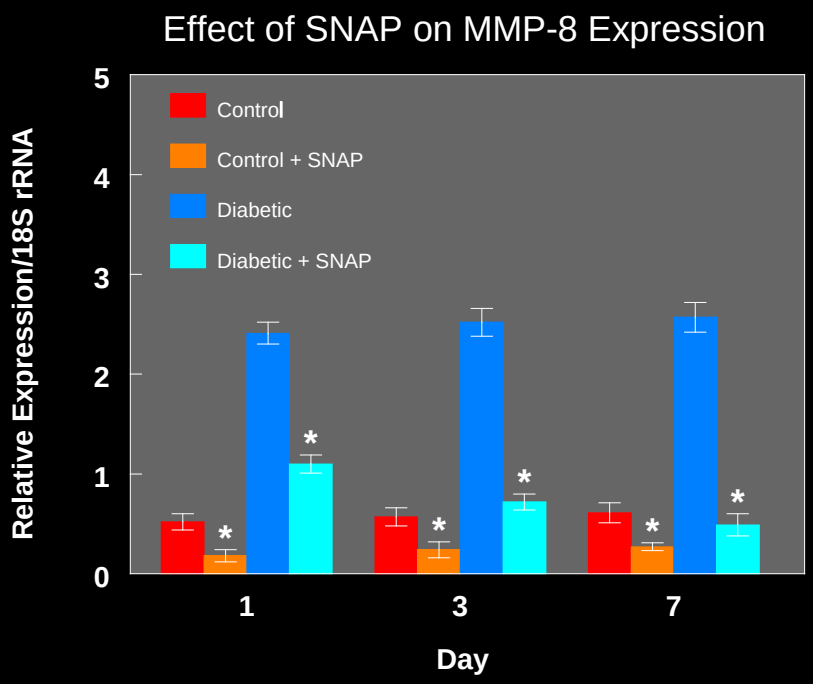


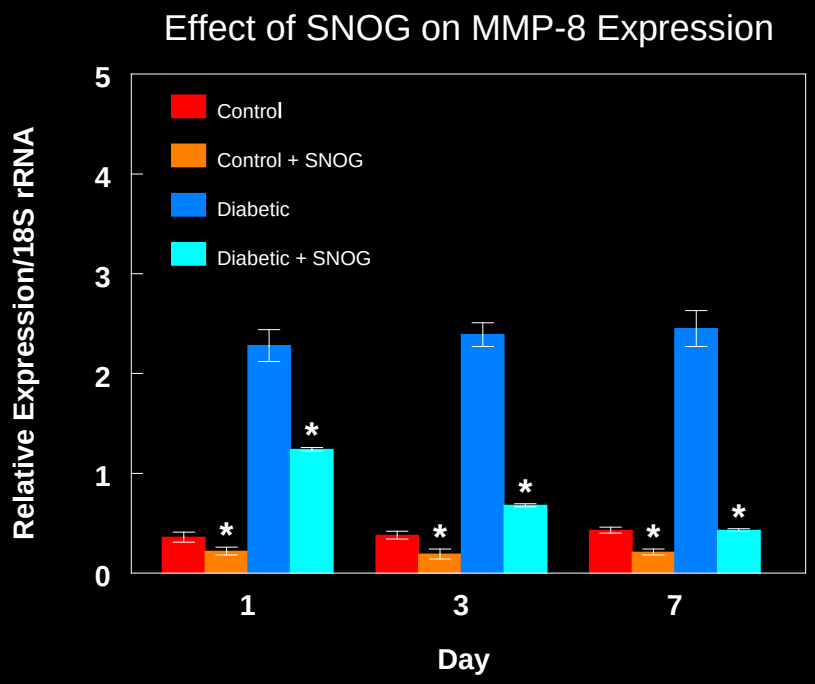
Effect of Nitric Oxide Donors on MMP-9 Expression



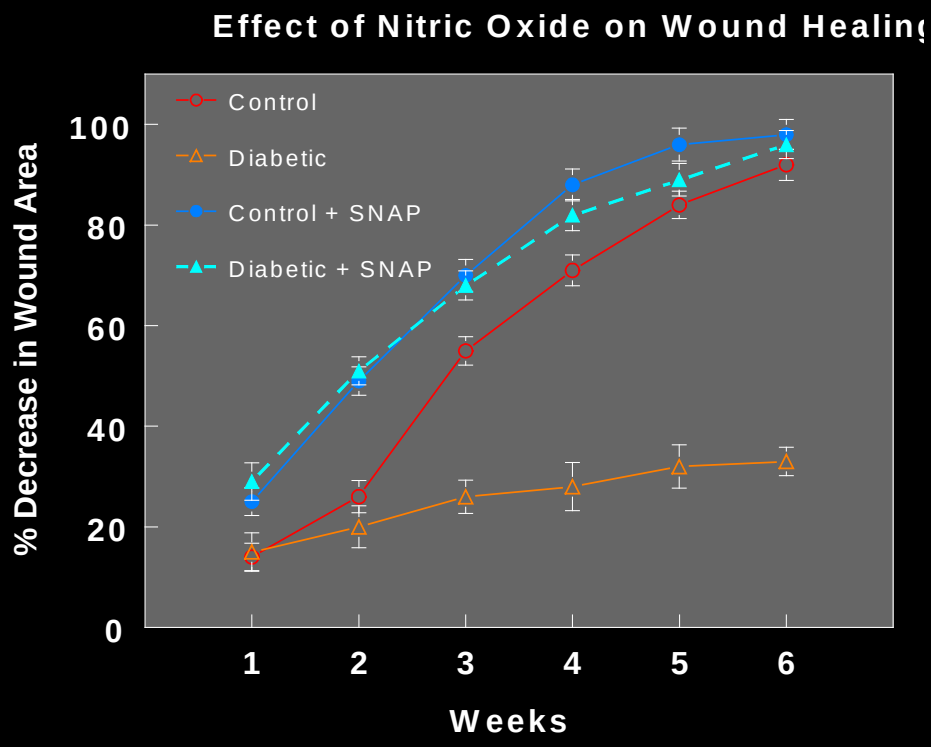


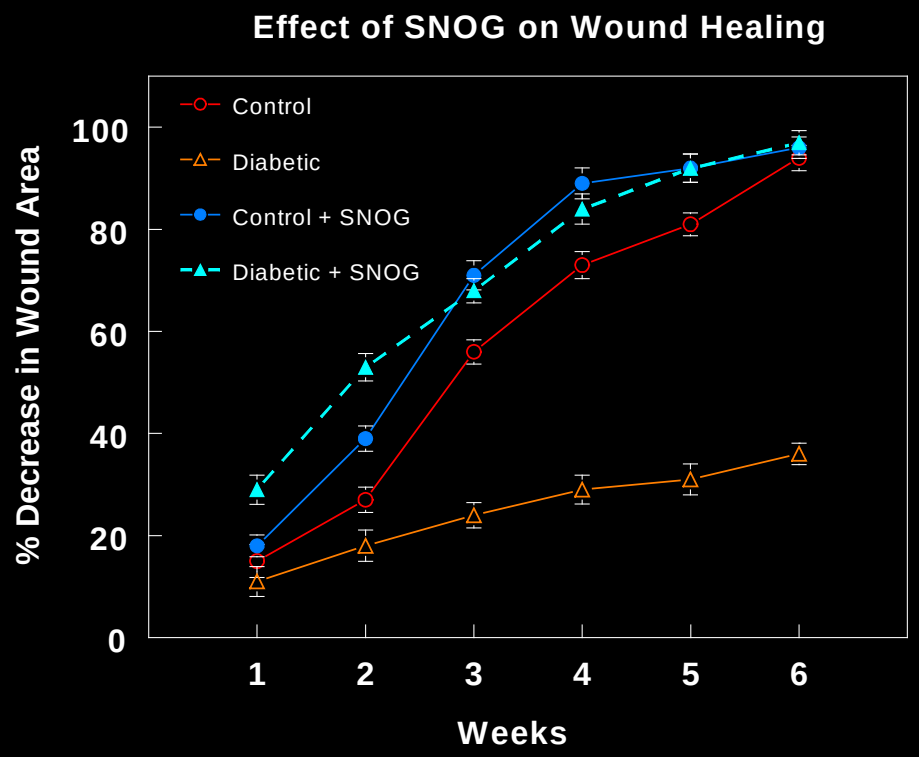
Effect of Nitric Oxide Donors on MMP-8 Expression



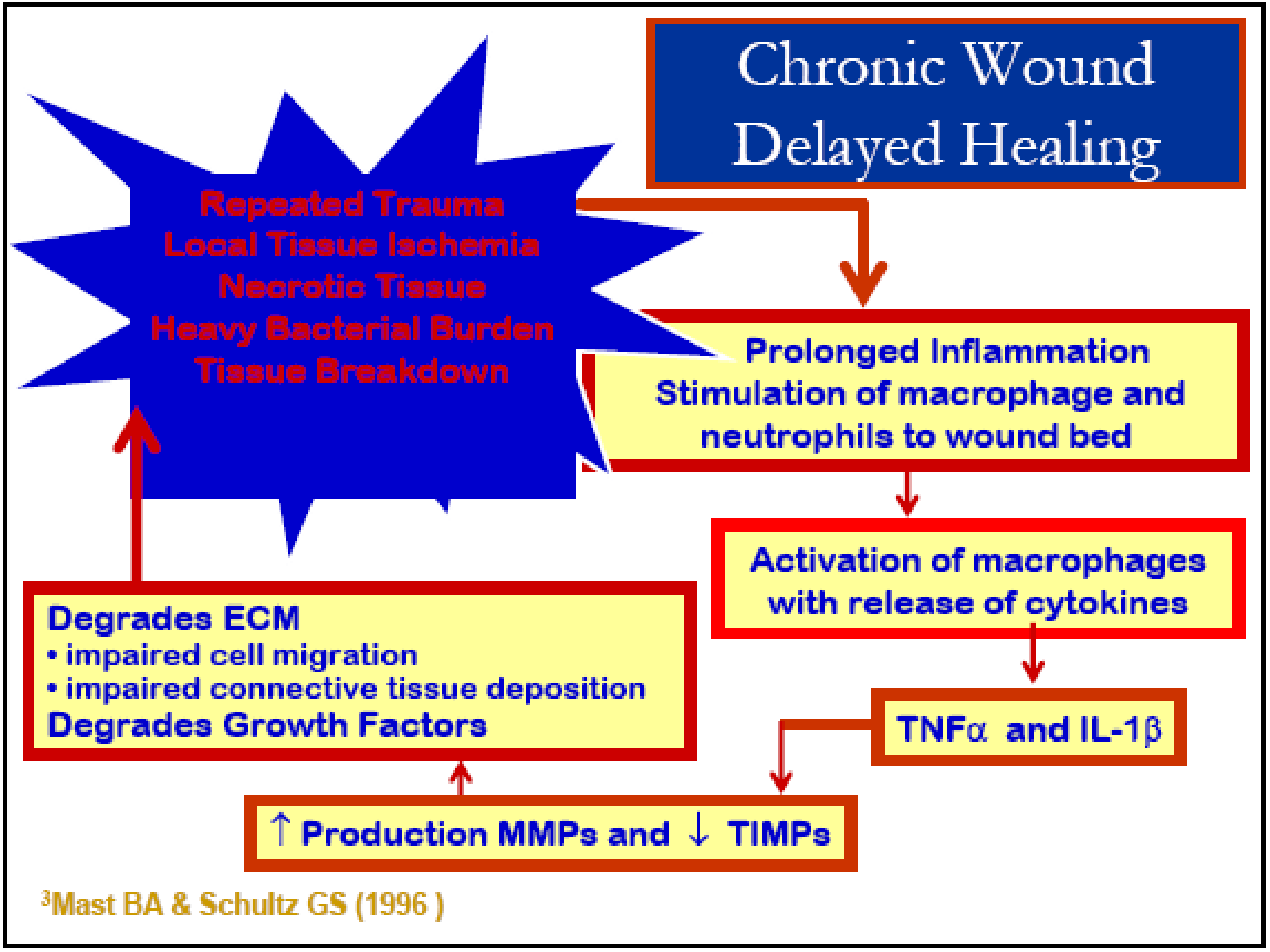


Effect of Nitric Oxide Donors on Wound Healing





Questions?



[3]Mast BA & Schultz GS (1996)