Wound Healing & Regeneration

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Current Life Science Topics

1. Fundamentals in Basic Biochemistry & Cell Biology
2. Introduction to Tissue Engineering & Regenerative Medicine
3. Developmental Tissue Reconstruction
4. **Wound Healing & Regeneration**
5. Natural Tissue Composition & Cell-ECM Interaction
6. Stem Cells & Cell-Based Therapy
7. Biomaterials
8. *Mid-Term Exam*
9. Mechano-transduction & Bioreactors
10. Discussions on Tissue Reconstruction
11. Regulation & Ethics
12. AI in Current Life Science
13. Machine Learning & Github
14. Deep Neural Network
15. Convolutional Neural Network
16. *Final Exam*

Study Materials

Principles of Regenerative Medicine by A. Atala A, Chapter 6

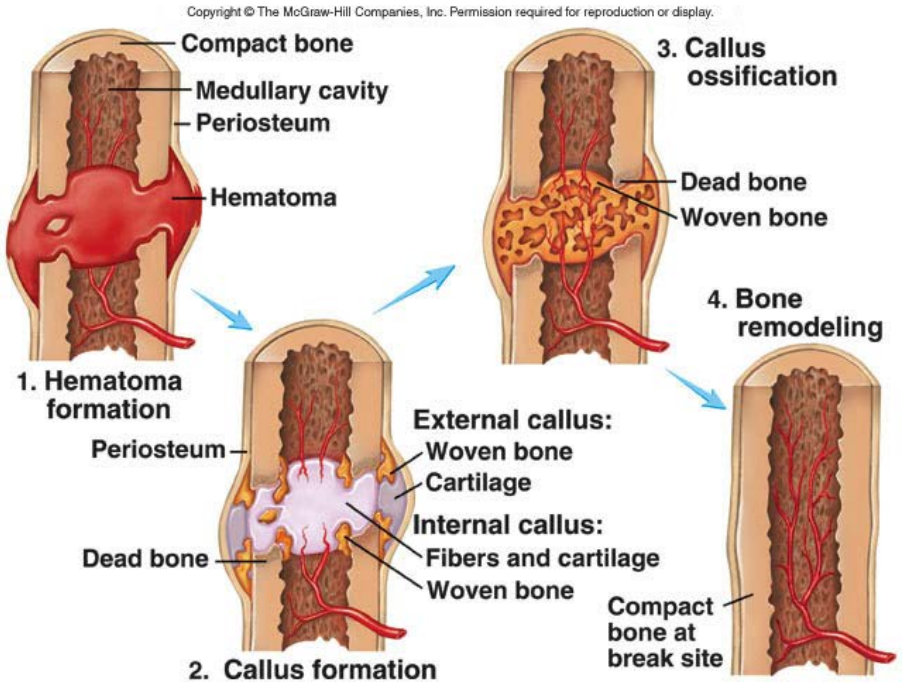
Eming SA 2014 Sci Transl Med

Keeley EC 2010 Int J Biochem Cell Biol

Bucala R 2012 Q J Med

- Scar in various tissues
- Gastrointestinal surgery
- Ligaments & tendons
- Nervous system
- Cornea
- Skin

- Exception: bone and liv



Clinical Burden of Scar

- **Adult normal scar is preferable to the two extreme outcomes**
- **Non-healing chronic ulcer**
  - **Underlying conditions:**
    - ***Diabetes mellitus***
    - **Vascular insufficiency**
    - **Paraplegia (Local pressure effects)**
    - **Systemic factors: Compromised nutrition, Immunological status, Age**
- **Excessive fibro-proliferative scarring**
  - **Hypertrophic scar**
  - **Keloids**

- **Mechanisms underlying pathological scarring are elusive**
  : Efficient treatment options are currently missing

Chronic Wound

Venous Leg Ulcer (VLU)

Mechanism remain unknown

Theories include…

- **Persistent inflammation**
- **Interruption of keratinocyte migration**
- **Mis-regulated signaling**

Arterial Ulcer

Consequence of reduced arterial blood supply

- **Tissue hypoxia**
- **Tissue damage**

Chronic Wound

Diabetic foot ulcer

Most common metabolic disease

Impaired wound healing condition

Mechanism unknown

- Insulin deficiency??
- Hyperglycemia??
- Hyperlipidemia??
- Peripheral neuropathy??
- Obesity??

5-year mortality rate for patients with diabetic-related amputation is about 50%.

Pressure sore

Tissue necrosis caused by unrelieved pressure to soft tissue for a prolonged period of time

Etiologic factors

- Biomechanical force
- Moisture
- Local ischemia

Advanced stage pressure ulcer shows high mortality (no efficacious therapy)

Diabetic foot ulcer

- Common in diabetes
- Hyperglycemia
- Micro-/macroangiopathy
- Neuropathy
- Infection
- Foot deformities

Pressure sore

- Area of tissue necrosis
- Caused by prolonged soft tissue compression
- Local ischemia, moisture
- Multi-morbid and elderly

Chronic skin ulcers

- Frequency increases as the population ages

- Biologic marker: excessive neutrophil infiltration
- Neutrophil (-> chronic inflammation)
- >release elastase
- >destroy PDGFs & TGF-beta
  (important for normal wound healing)

Scarring

- Scar: damaged tissue replaced by a pathological connective tissue
- Heterogeneity among diverse organisms
- In human, perfect tissue regeneration has only been described in fetal skin

Hypertrophic scar

- Rapid growth
- Generally regress <6 months
- αSMA⁺ myofibroblasts
- Collagen fibers parallel to skin surface
- Vertically oriented blood vessels

Keloid

- Constant growth
- No spontaneous regression
- Extend beyond margins of tissue damage
- Genetic predisposition
- Thick, haphazardly oriented collagen bundles

Hypertrophic Scars

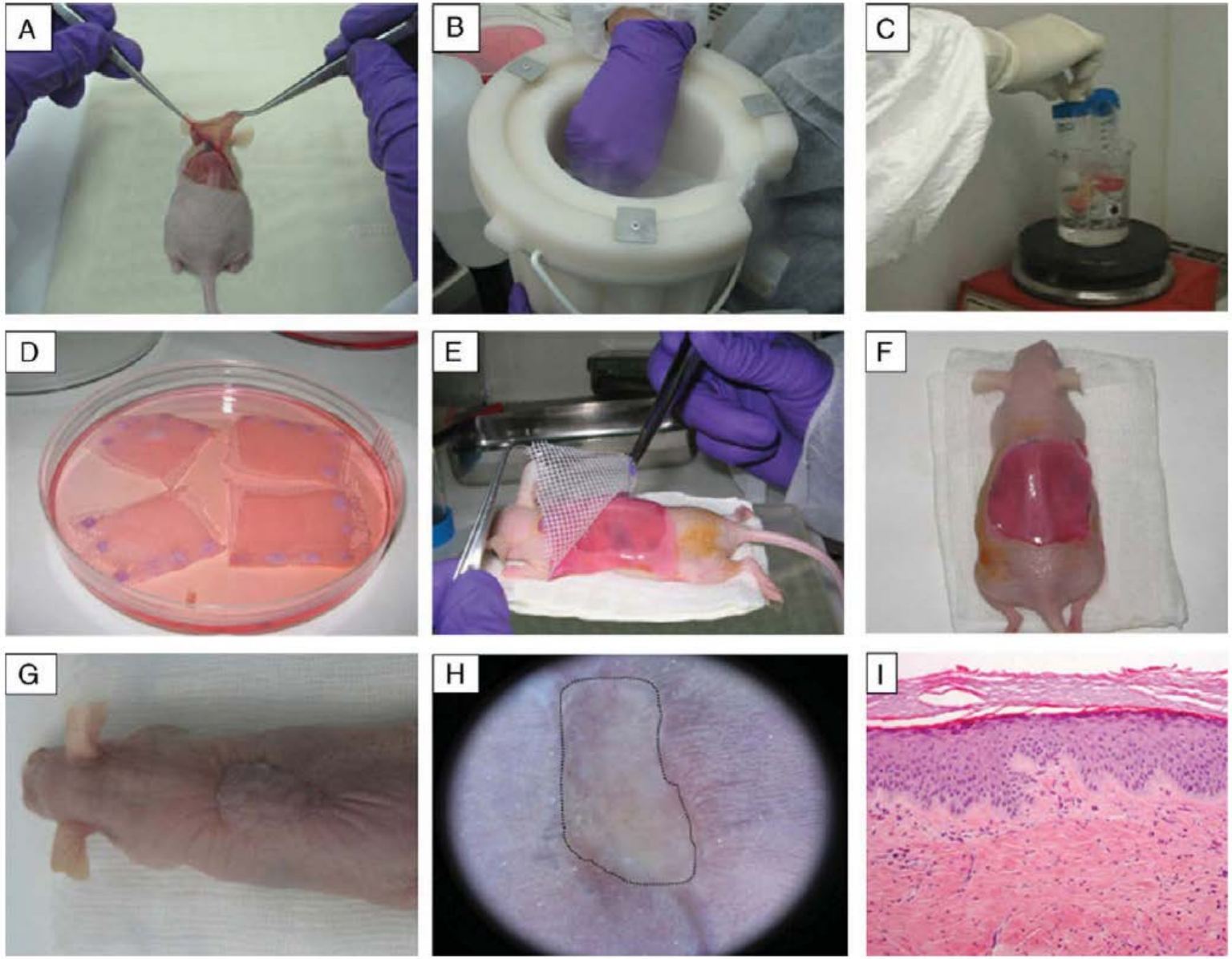
- Develop after…
- Surgery
- Trauma
- Burns
- spontaneously in predisposed patients
- Higher incidence compared with keloids
- Erythmatous, brownish-red in color
  (can become pale with age)
- Tends to be less nodular than keloids
  (do not raise more than 4 mm above skin)
- Histology: characterized by collagen bundles that are fine, well organized, and parallel to the epidermis
- Traits different from Keloids:
- Myoblasts are present
- Alpha-SMS is expressed in nodular pattern
- Mucin is absent
- Hyaluronic acid is a major component of papillary dermis

Keloids

- Keloids: benign tumors that develop at sites of skin injury over a period of months to years (disfiguring, nodular appearance tends to be dark and erythmatous)
- Can cause pain, burning, and itching
- Tend not to regress spontaneously
- Can continue to slowly grow over many years
- Histology: thick, large, closely packed bundles of disorganized collagen

- **Tissue damages can result from multiple acute or chronic stimuli**
- Autoimmune reactions
- Infections
- Mechanical injury
- **Difficulty due to paucity of animal models for wound healing research:**
- Paucity of animal models that precisely correlate with the human condition
- Current state of knowledge is in its infancy and is based on integration of data resulting from analysis of human wound samples
  (predominantly from VLUs and DFUs)
- Pig models of wound healing were used in the early days because of similarities to human skin
  : major drawback is poor genetic tractability and complicated procedure
- Recent model – "Humanized skin on mice" take over the predominant model

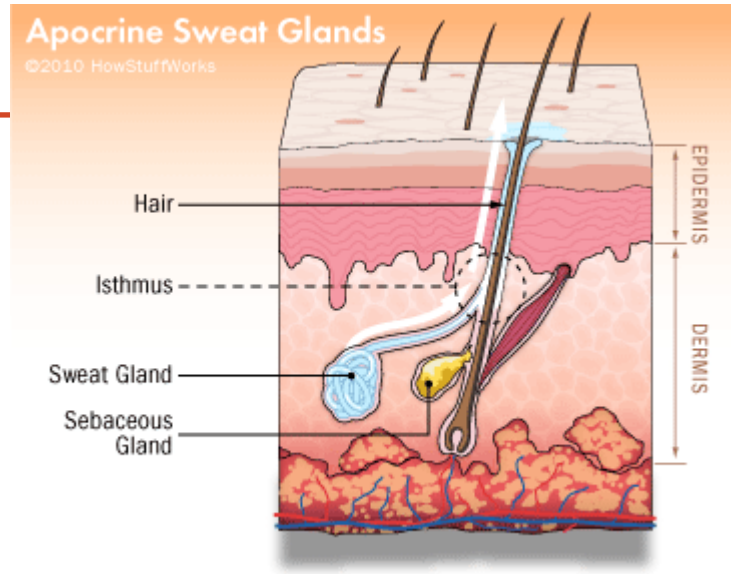
Skin-humanized mouse model

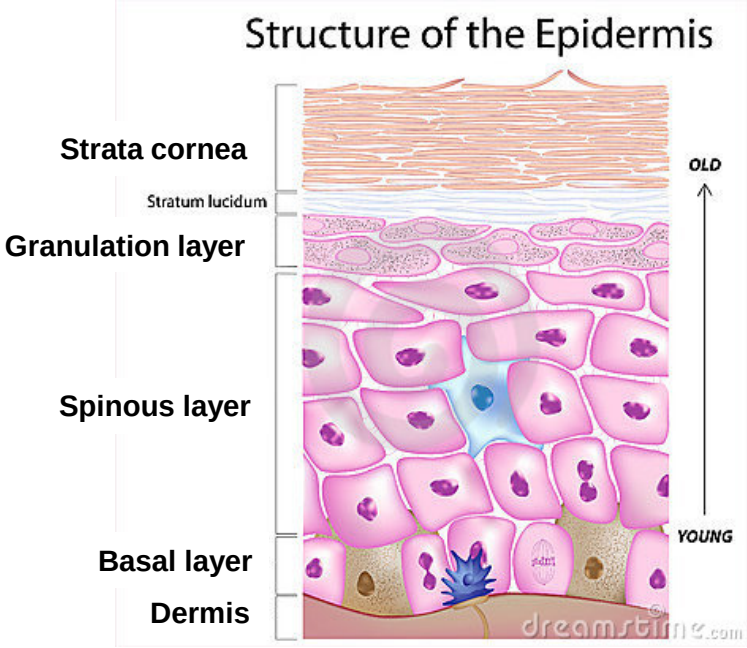


Anatomy of Adult Skin

- **Epidermis**
- 5 distinct layers: characterized by keratinocyte maturation
- Epidermal appendages:
  - sebaceous gland
  - Apocrine sweat glands
  - hair follicles

- **Dermis**
- Papillary layer – superficial, highly vascular
- Reticular layer – deeper, densely packed collagen fibers, less vascular

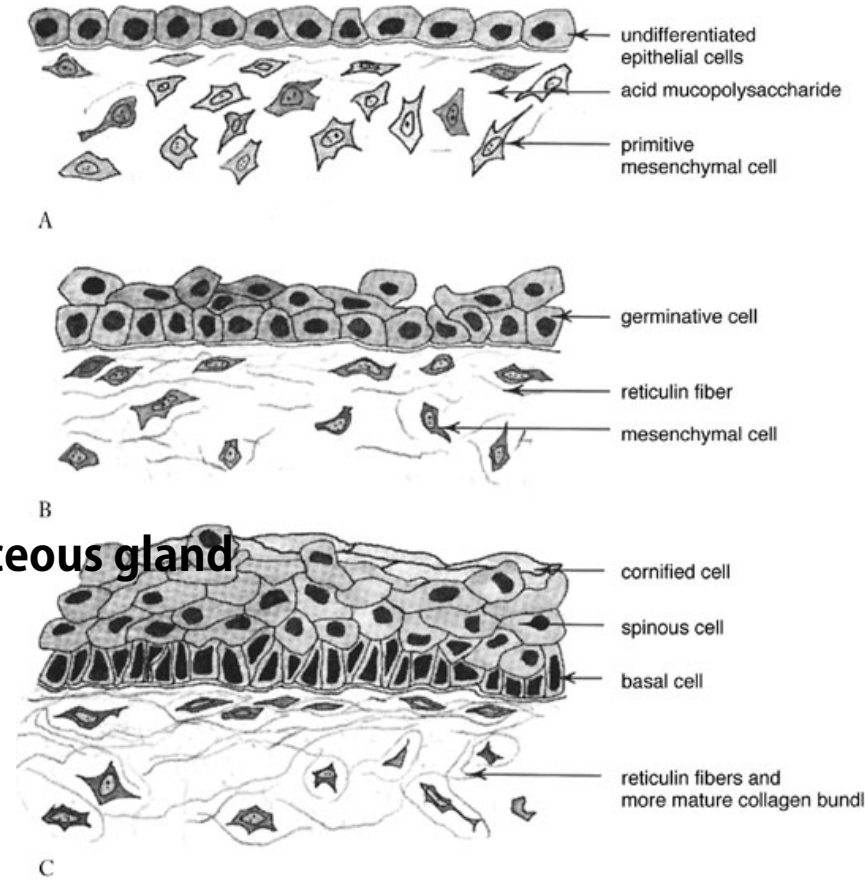




Development of Fetal Skin

- Developmental origin
- Epidermis – ectoderm
- Dermis – mesoderm

- Single layer stage
- Periderm stage (at 2 mo.)
- Outer layer of tissue
- Until 4 mo.:
  - epidermis becomes highly cellular
  - Continuously keratinized & shed
  - Vernix caseosa (greasy, white film): contains sebum from sebaceous gland
- 21 wks
- Periderm replaced by strata cornea
  -> epidermis stratifies into 4 layers
- Melanocytes of neural crest origin invade the epidermis



Difference between Fetal & Adult Skin ECM

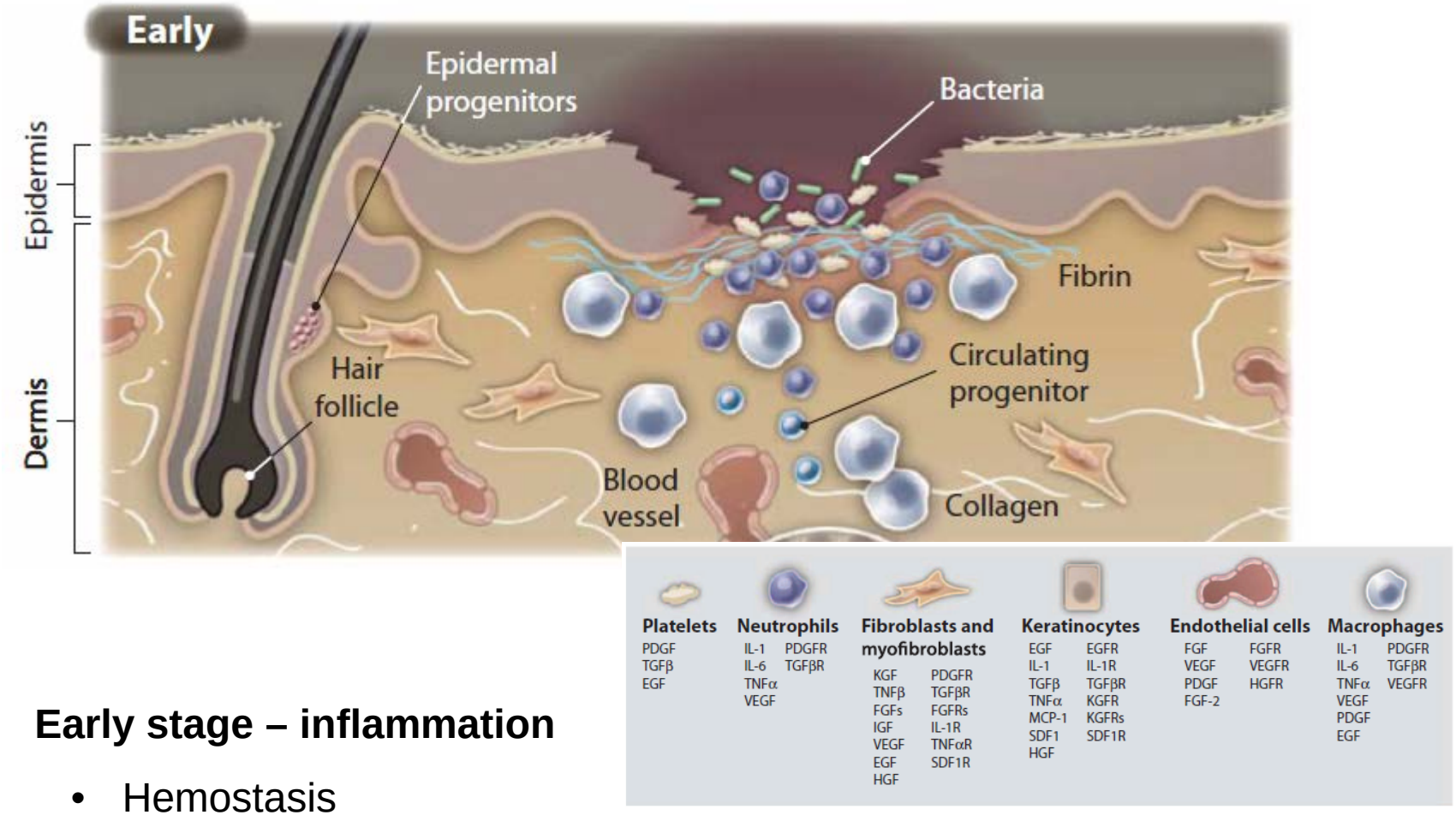
- **Fetal ECM is now known to be a reservoir of GFs essential to development**
- **Fetal ECM has a different structural protein composition**
- **Fetus – relatively high III/I collagen ratio**
  -> shift to adult phenotype in the post-natal period (less type III collagen)
- **Hyaluronic acid content initially increases dramatically during adult repair and decrease at Day5~10**
- **This hyaluronic acid profile is not the case in the fetal wound ECM (hyaluronic acid level remains high)**
- **Other substances**
  - **Decorin, lysyl oxidase, matrix metalloproteases (MMPs)- up-regulated**
  - **Fibromodulin – down-regulated**
  - **Those molecules play a role in the development and maturation of collagen.**

Fetal Scarless Repair Phenotype

- Adult wounds heal with fibrous tissue (scarring)
- Fetal wounds heal scarlessly
- Mechanism of scarless wound healing is...
- Intrinsic to fetal tissue
- Independent of environmental or systemic factors
  - Bathing in sterile amnionic fluid
  - Perfusion with fetal serum
  - Fetal immune system
    -> no influence on scarring
- Fetal human skin transplanted subcutaneously to athymic mice heals without a scar. (Intrinsic mechanism)
- Scarless wound repair outcome depends on 2 factors
- Gestation age
  - Transitional period of human (24 wks), rats (16.5 & 18.5 day)
- Size of the wound

Stages of wound healing

Molecular & Cellular Mechanisms in Normal Skin Repair



Early stage – inflammation

- Hemostasis
- Activation of keratinocytes & inflammatory cells

Inflammatory Phase

- **Hemostasis**
- **Under influence of platelet-derived inflammatory molecules**
- **Neutrophils & monocytes migrate to the wound**
- **Inflammation is prolonged in chronic wounds (trapped in chronic inflammatory state and fails to progress)**

- Injury -> immediate activation of the clotting cascade (Fibrin clot)
  - Provide basic matrix architecture to initiate the invasion & recruitment of inflammatory and other cells
- Platelet trapped in the clot
  - Release GFs & Chemokines
  - Preclinical model of platelet disorder : impaired healing

Inflammatory Phase

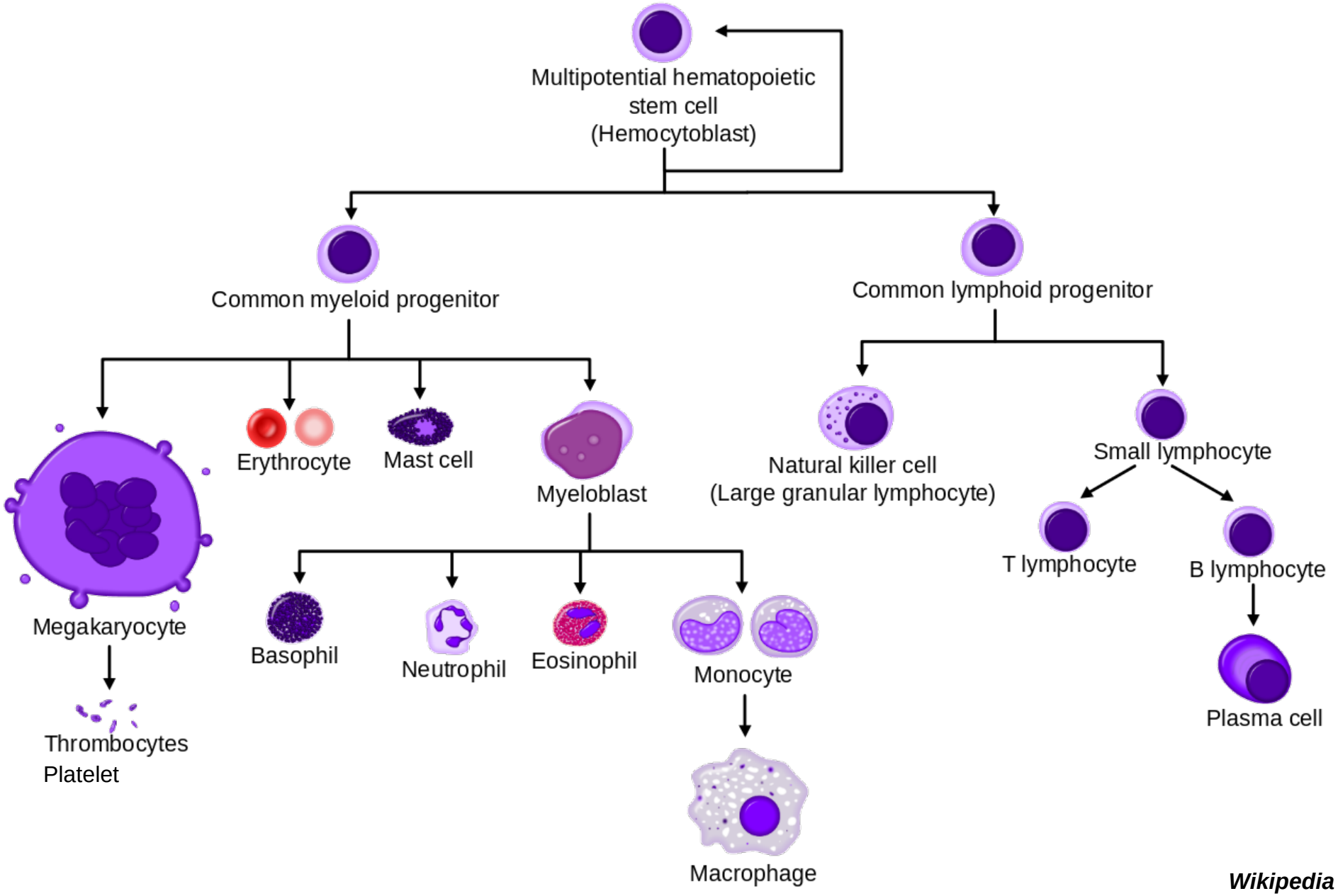
Cellular infiltrates

- **Neutrophils**
- Primarily produce degradative enzyme
- Phagocytose foreign & necrotic materials
- Produce VEGF, TNF-a, IL-1… and other GFs

- **Macrophages**
- Macrophages are essential to the normal wound healing process through their stimulation of collagen production, angiogenesis, and re-epithelialization
- However, similarly to the activity of neutrophils, if macrophages persist, the result is excess scar formation. (macrophages produce high amounts of cytokines that activate fibroblasts to deposit excessive amounts of collagen)

-> Contribute to delayed healing in chronic ulcers

- **Level of inflammation depends..**
- On the presence or absence of infection
- In the presence of infection:
  - Neutrophils continue to be active in high concentration leads to farther inflammation & fibrosis
- In the absence of infection:
  - Neutrophils greatly diminish activity at day 2 or 3
  - Monocyte increase in number in response to both extravascular and intravascular chemoattractants
- During the late inflammatory phase, monocytes transform into tissue macrophage -> release cytokines & scavenge dead neutrophils


Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Erythrocyte
Mast cell
Myeloblast
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
Megakaryocyte
T lymphocyte
B lymphocyte
Basophil
Neutrophil
Eosinophil
Monocyte
Plasma cell
Thrombocytes Platelet
Macrophage

Wikipedia

Continual presence of a high bacterial load in wounds results in a sustained influx of inflammatory cells and increased inflammation also leading to delayed healing

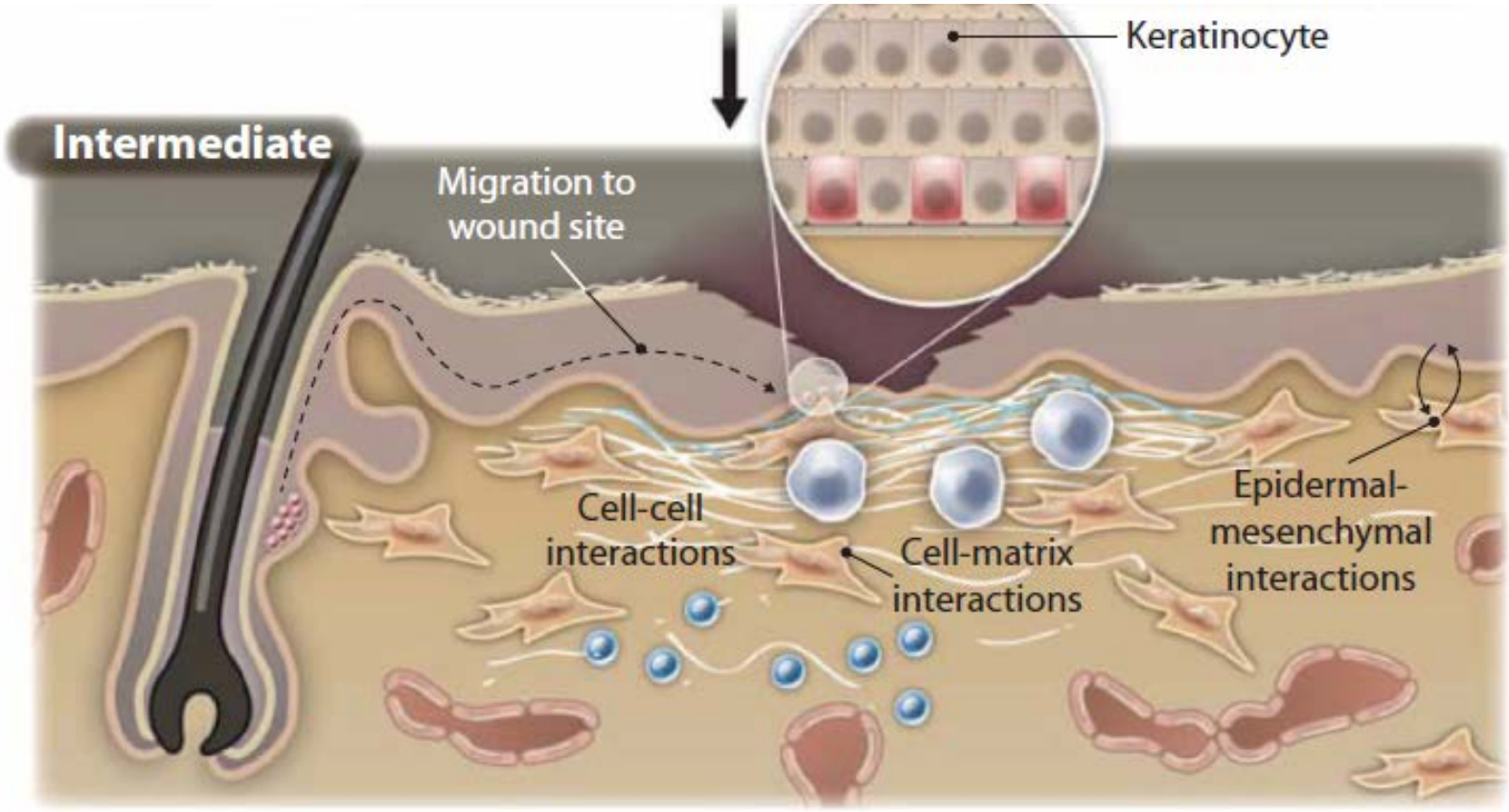
During wound healing, there is continual competition between inflammatory and anti-inflammatory signals

IL-1 β and TNF-α are increased in chronic wounds

- **> leads to elevated metalloproteinases**
- **> excessively degrade the local ECM**
- **> impair cell migration**

Proteases

- **Tissue proteases are normally under tight regulation during acute wound-healing process**
- **MMPs are elevated in chronic wounds**



Intermediate stage - proliferation

- Proliferation & migration of keratinocytes
- Proliferation of fibroblasts
- Matrix deposition
- Angiogenesis

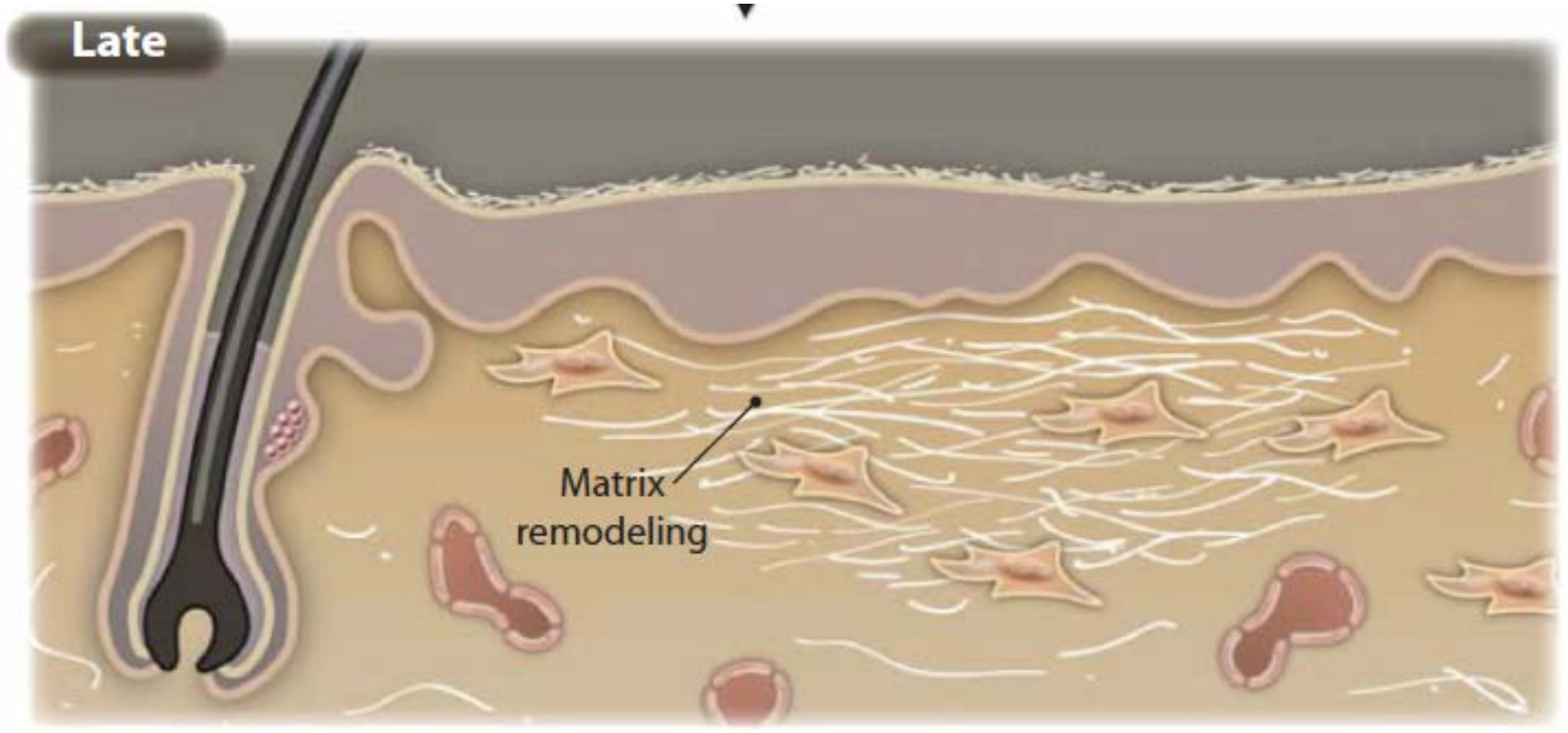
Proliferative Phase

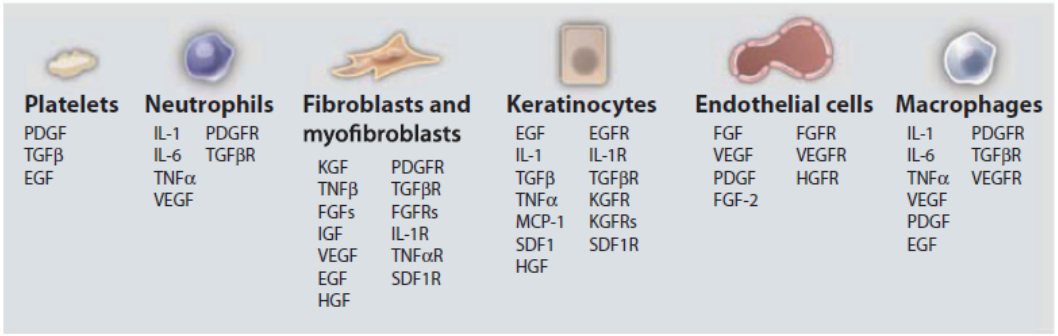
- Presence of macrophage in the wound marks the transition between the inflammatory phase and the proliferative phase of wound healing
- Begins around day 4~5 post injury. (in uninfected open wounds)
- Granulation tissue begins to form
- Start to form loose network of collagen, FN, hyaluronic acid… embedding a dense population of macrophages, fibroblasts, and newly formed blood vessels move into wound space.
- Fibroblasts at the wound site deposit collagen and a proteoglycan-rich provisional matrix (stimulated by TGF-β1 & β2)

- **Collagen deposition**
- Day5~day14 post-wounding
- Collagen is deposited at the wound site
  -> later, suppressed by negative feedback

- **Re-epithelialization**
- Begins in the first 24 hours after wounding
- Basal keratinocyte detach from ECM and migrate laterally to fill the void in the epidermis
  (In this process, keratinocytes are exposed to serum for the first time)

- **Neo-vascularization**
- Formation of blood vessels is induced by lactic acid, plasminogen activator, collagenases, & low oxygen tension (hypoxia)





Late stage - remodeling

- Remodeling of ECM
- Results in scar formation
- Restoration of barrier

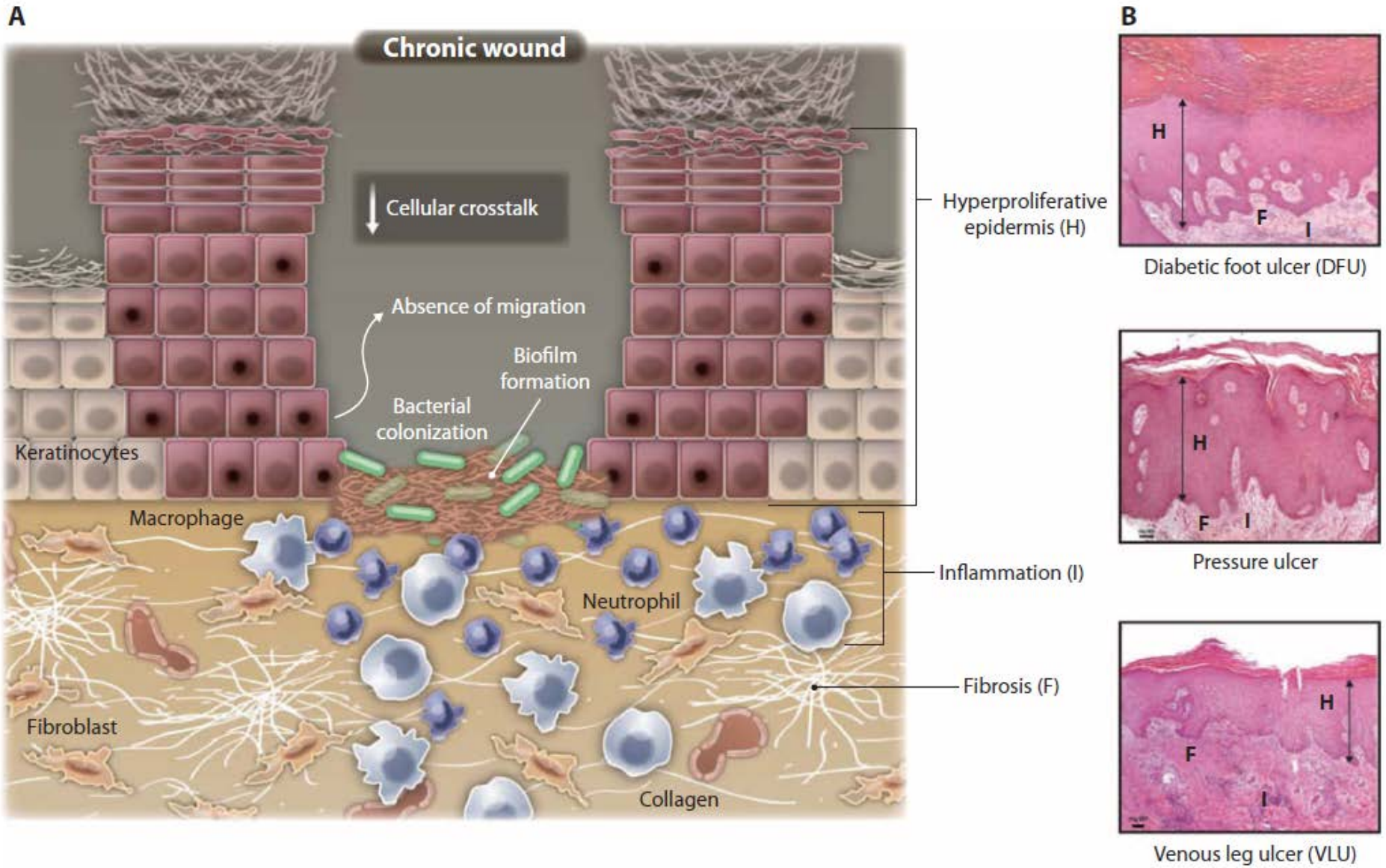
Collagen Remodeling

- Maturation stage of wound healing
- Begins during the 2nd week of healing
- Fibroblasts become myofibroblasts
- Scar tissue gains strength as collagen cross-links increase during remodeling.
  However, never reach the original strength of unwounded skin
- Initial, randomly oriented type I & type III collagen
  -> replaced by type I collagen
  -> organized along the lines of tension

Natural wound healing proceeds through…

- **Inflammatory response**
- **Cell recruitment**
- **Angiogenesis**
- **Proliferation**
- **Matrix deposition**
- **Tissue remodeling**

➢ Disruption of deregulation of one or more phases of the would-healing process leads to nonhealing (chronic) wounds


A
Chronic wound
Cellular crosstalk
Absence of migration
Biofilm formation
Bacterial colonization
Keratinocytes
Macrophage
Neutrophil
Fibroblast
Collagen
Hyperproliferative epidermis (H)
Inflammation (I)
Fibrosis (F)
B
H
F
I
Diabetic foot ulcer (DFU)
Pressure ulcer
Venous leg ulcer (VLU)

Angiogenesis & vasculogenesis

- Angiogenesis: sprouting of capillaries from existing blood vessels
- Vasculogenesis: mobilization of BM-derived endothelial progenitors
- Impaired healing of DFU is due to inadequate local angiogenesis
- Reduced angiogenesis -> elevated cell death
- Modulation of fine balance is needed between pro- and anti- angiogenic molecules for proper would healing

Cell recruitment

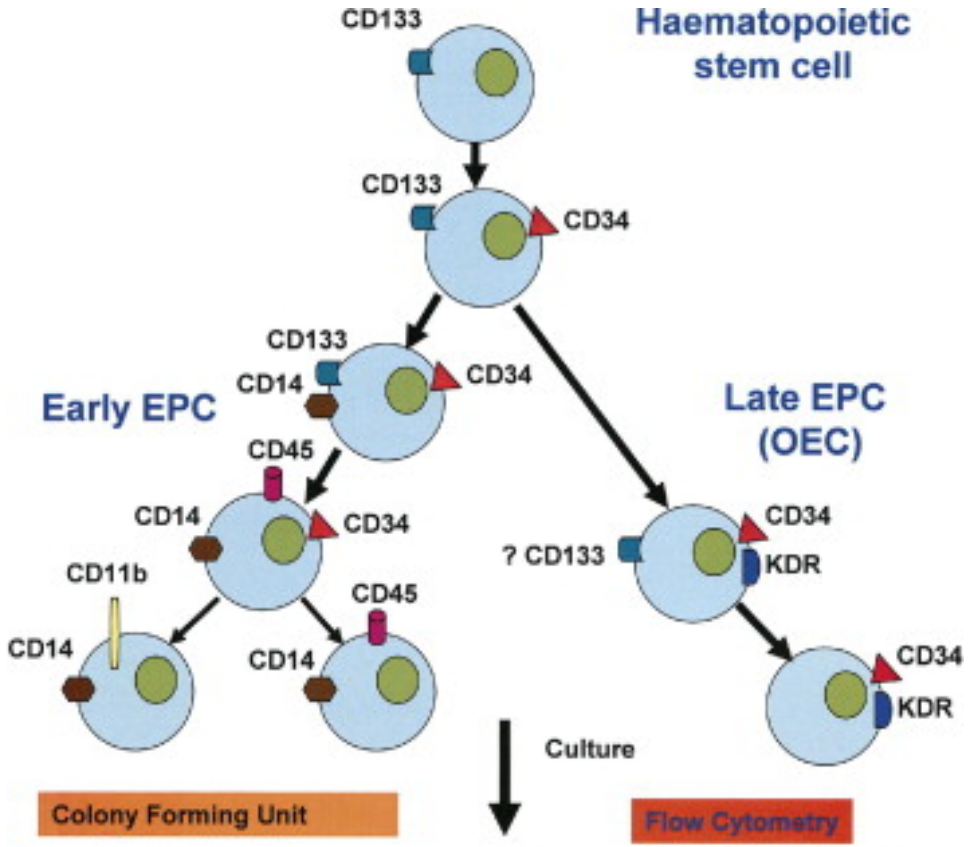
- Activated fibroblasts transform into α-SMA-expressing myofibroblasts that promote wound contraction
- Fibroblasts can be derived from two additional source
- From epithelial cells in a process known as epithelial-mesenchymal transition (EMT)
- From bone marrow-derived circulating progenitor cells (fibrocytes)
- Recruitment of bone marrow and endothelial progenitors to the site of injury is coordinated by specific chemokines
  : depletion of recruitment -> compromised healing response
- Stem cell modulation is becoming one the of most explored potential therapeutic strategies

Fibro-proliferative Scarring

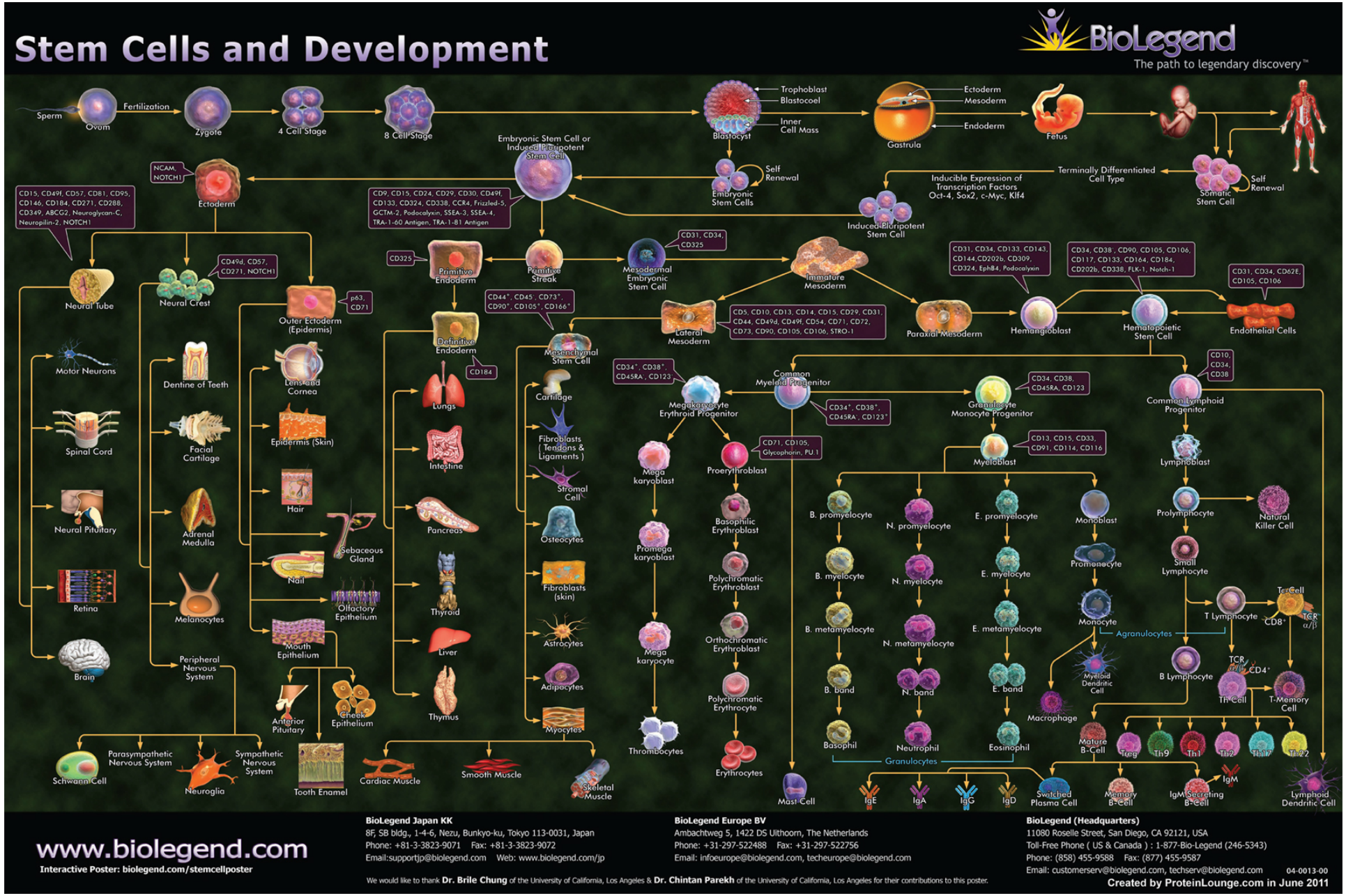
- **Fibrosis: the replacement of the normal structural elements of the tissue by distorted, non-functional, and excessive accumulation of scar tissue**
- Many medical problems are linked to excessive fibrosis
  : keloids & hypertrophic scars are clinical examples of excessive cutaneous fibrosis
- Excessive fibro-proliferative scarring occurs when the mechanisms of wound healing go into overdrive.
  (Abnormal scar =excess accumulation of an unorganized collagenous ECM)
- **Factors that influence the severity of scarring tissue site, gender, race, age, magnitude of injury, wound contamination**
- Thicker dermis tend to scar greater
- Estrogen is believed to promote scarring
- Darkly pigmented skin are more prone to thicker scarring
- Young people are prone to thicker scarring
- Larger, deeper, and more contaminated wounds also tends to produce increased scar formation

Fibrocytes – a double-edged sword

- Originally described in 1994.
- Spindle shaped
- Express collagen and procollagen
- CD34+, CD45+ cell population that are distinct from monocytes, dendritic cells, T cells, fibroblasts, epithelial cells, and endothelial cells
- Fibrocytes increase upto 10% in wound chamber while they comprise 0.1-1% of peripheral blood in healthy donors
- Substantial information support the hypothesis that fibrocytes are derived from the bone marrow
- Differentiation of fibrocyte -> myofibroblast is augmented in the presence of TGF-β. (result in production of FN and Col and express α-SMA)
- Fibrocyte contribute to normal wound healing by several important mechanisms including…
- acting as antigen-presenting cells
- Serving as the contractile force of wound closure via α-SMA expression
- Promoting angiogenesis
- Producing cytokines, chemokines and GFs that induce fibroblast hyperplasia
- Secreting components of ECMs



Early EPC	Characteristics	Late EPC
3-5 days	Duration	7-14 days
Heterogeneous	Morphology	Homogenous
$CD14/CD45/KDR_{Low}$ CD31/vWf	Antigen markers	CD34/KDR CD31/vWf
+++	Secretion of angiogenic cytokines	+
Low	*In-vivo* angiogenic effects	High


Stem Cells and Development
BioLegend
The path to legendary discovery™
Sperm
Ovum
Fertilization
Zygote
4 Cell Stage
8 Cell Stage
Trophoblast
Blastocoel
Inner Cell Mass
Blastocyst
Ectoderm
Mesoderm
Endoderm
Gastrula
Fetus
Embryonic Stem Cell or Induced Pluripotent Stem Cell
Self Renewal
Embryonic Stem Cells
Inducible Expression of Transcription Factors Oct-4, Sox2, c-Myc, Klf4
Induced Pluripotent Stem Cell
Terminally Differentiated Cell Type
Somatic Stem Cell
Self Renewal
NCAM, NOTCH1
Ectoderm
CD15, CD49f, CD57, CD81, CD95, CD146, CD184, CD271, CD288, CD349, ABCG2, Neuroglycan-C, Neuropilin-2, NOTCH1
CD9, CD15, CD24, CD29, CD30, CD49f, CD133, CD324, CD338, CCR4, Frizzled-5, GCTM-2, Podocalyxin, SSEA-3, SSEA-4, TRA-1-60 Antigen, TRA-1-81 Antigen
CD325
Primitive Endoderm
Primitive Streak
CD31, CD34, CD325
Mesodermal Embryonic Stem Cell
Immature Mesoderm
CD31, CD34, CD133, CD143, CD144,CD202b, CD309, CD324, EphB4, Podocalyxin
CD34, CD38-, CD90, CD105, CD106, CD117, CD133, CD164, CD184, CD202b, CD338, FLK-1, Notch-1
CD31, CD34, CD62E, CD105, CD106
Neural Tube
Neural Crest
CD49d, CD57, CD271, NOTCH1
Outer Ectoderm (Epidermis)
p63, CD71
CD44+, CD45-, CD73+, CD90+, CD105+, CD166+
Definitive Endoderm
CD184
Lateral Mesoderm
CD5, CD10, CD13, CD14, CD15, CD29, CD31, CD44, CD49d, CD49f, CD54, CD71, CD72, CD73, CD90, CD105, CD106, STRO-1
Paraxial Mesoderm
Hemangioblast
Hematopoietic Stem Cell
Endothelial Cells
Mesenchymal Stem Cell
Motor Neurons
Spinal Cord
Neural Pituitary
Retina
Brain
Dentine of Teeth
Facial Cartilage
Adrenal Medulla
Melanocytes
Peripheral Nervous System
Lens and Cornea
Epidermis (Skin)
Hair
Sebaceous Gland
Nail
Olfactory Epithelium
Mouth Epithelium
Anterior Pituitary
Cheek Epithelium
Tooth Enamel
Lungs
Intestine
Pancreas
Thyroid
Liver
Thymus
Cartilage
Fibroblasts (Tendons & Ligaments)
Stromal Cell
Osteocytes
Fibroblasts (skin)
Astrocytes
Adipocytes
Myocytes
Cardiac Muscle
Smooth Muscle
Skeletal Muscle
Schwann Cell
Parasympathetic Nervous System
Sympathetic Nervous System
Neuroglia
CD34+, CD38+, CD45RA-, CD123-
Common Myeloid Progenitor
Megakaryocyte Erythroid Progenitor
CD34+, CD38+, CD45RA-, CD123+
Granulocyte Monocyte Progenitor
CD34, CD38, CD45RA, CD123
Common Lymphoid Progenitor
CD10, CD34, CD38
CD71, CD105, Glycophorin, PU.1
Mega karyoblast
Proerythroblast
Promega karyoblast
Basophilic Erythroblast
Polychromatic Erythroblast
Mega karyocyte
Orthochromatic Erythroblast
Polychromatic Erythrocyte
Thrombocytes
Erythrocytes
CD13, CD15, CD33, CD91, CD114, CD116
Myeloblast
Lymphoblast
B. promyelocyte
N. promyelocyte
E. promyelocyte
Monoblast
B. myelocyte
N. myelocyte
E. myelocyte
Promonocyte
B. metamyelocyte
N. metamyelocyte
E. metamyelocyte
Monocyte
B. band
N. band
E. band
Basophil
Neutrophil
Eosinophil
Granulocytes
Agranulocytes
Macrophage
Myeloid Dendritic Cell
Prolymphocyte
Natural Killer Cell
Small Lymphocyte
T Lymphocyte
T-Cell
CD8+
TCR α/β
B Lymphocyte
TCR γ/δ
CD4+
TH Cell
T-Memory Cell
Mast Cell
IgE
IgA
IgG
IgD
IgM
Mature B-Cell
Treg
Th9
Th1
Th2
Th17
Th22
Switched Plasma Cell
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IgM Secreting B-Cell
Lymphoid Dendritic Cell
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04-0013-00
We would like to thank Dr. Brile Chung of the University of California, Los Angeles & Dr. Chintan Parekh of the University of California, Los Angeles for their contributions to this poster.
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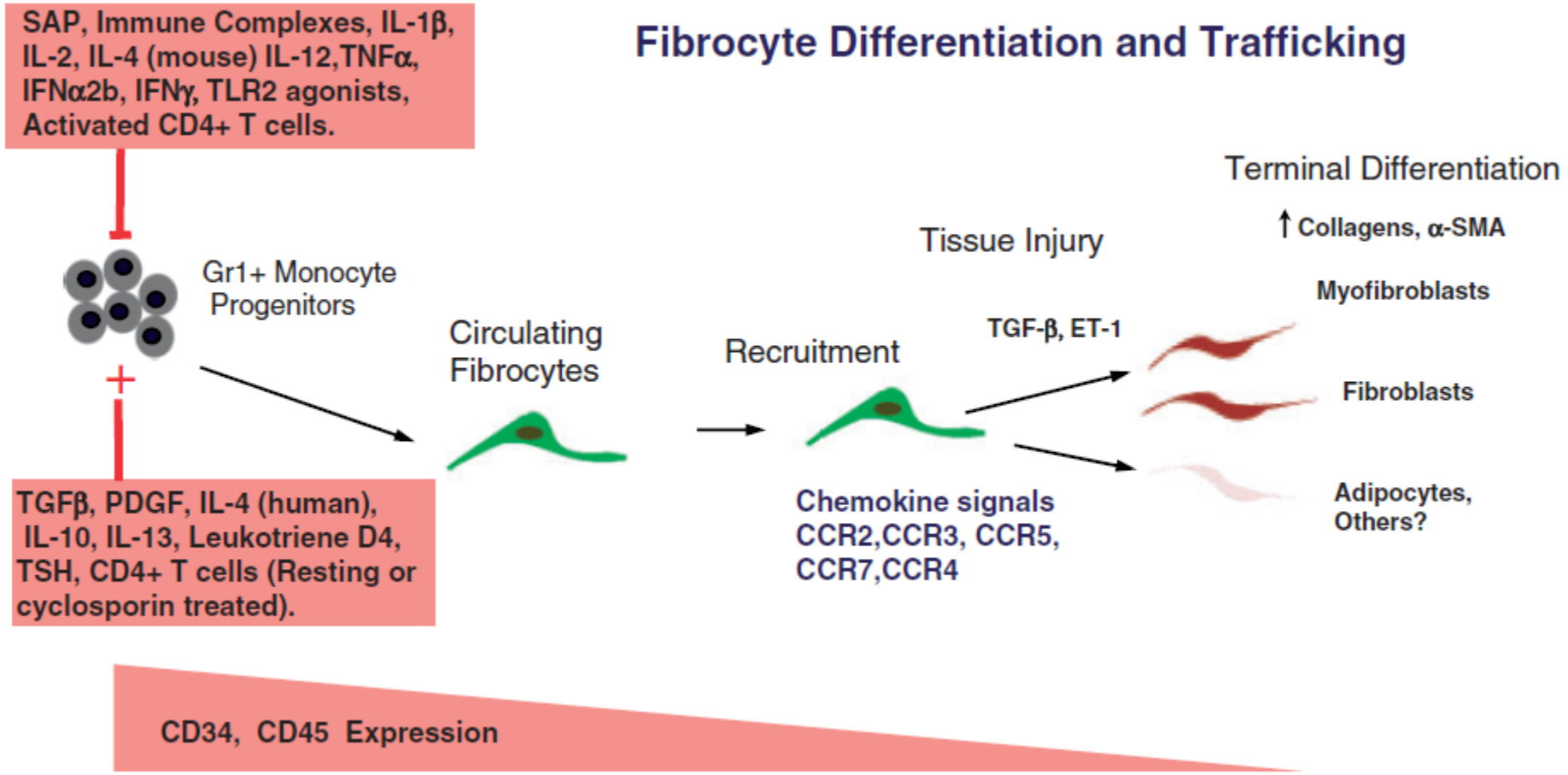
Fibrocytes


SAP, Immune Complexes, IL-1β, IL-2, IL-4 (mouse) IL-12,TNFα, IFNα2b, IFNγ, TLR2 agonists, Activated CD4+ T cells.
Fibrocyte Differentiation and Trafficking
Terminal Differentiation
↑ Collagens, α-SMA
Myofibroblasts
Tissue Injury
Gr1+ Monocyte Progenitors
Circulating Fibrocytes
Recruitment
TGF-β, ET-1
Fibroblasts
Chemokine signals CCR2,CCR3, CCR5, CCR7,CCR4
Adipocytes, Others?
TGFβ, PDGF, IL-4 (human), IL-10, IL-13, Leukotriene D4, TSH, CD4+ T cells (Resting or cyclosporin treated).
CD34, CD45 Expression

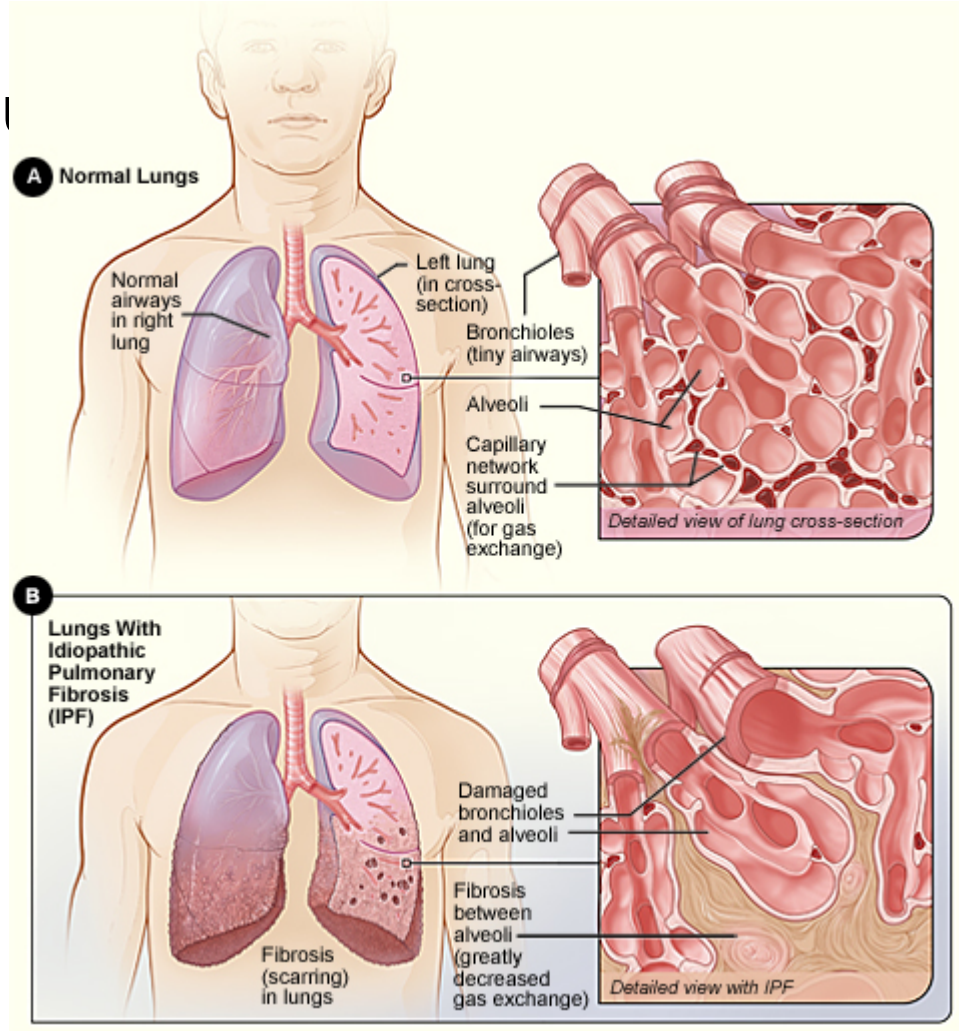
Main signal known to induce fibrosis

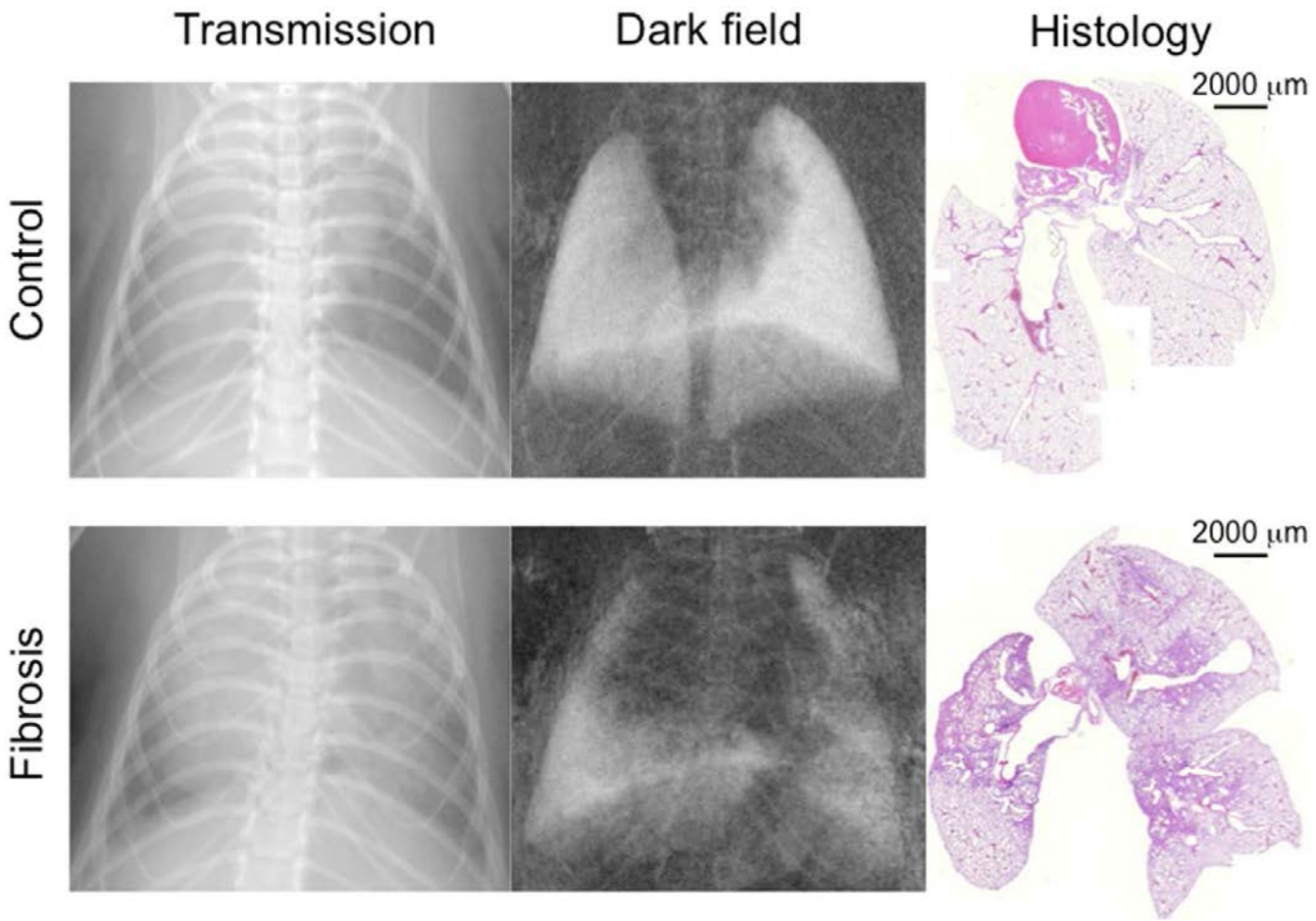
- Transforming Growth Factor-β (TGF-β)
- Connective Tissue Growth Factor (CTGF)
- Interleukin-4 (IL-4)
- IL-13
- Platelet-Derived Growth Factor (PDGF)
- Osteopontin (OPN)

Fibrotic diseases

Fibrotic Lung Disease

- Fibrosis of lung parenchyma
- Progressive replacement of lung tiss
- Lead to thickening of the wall
- Perpetual shortness of breath

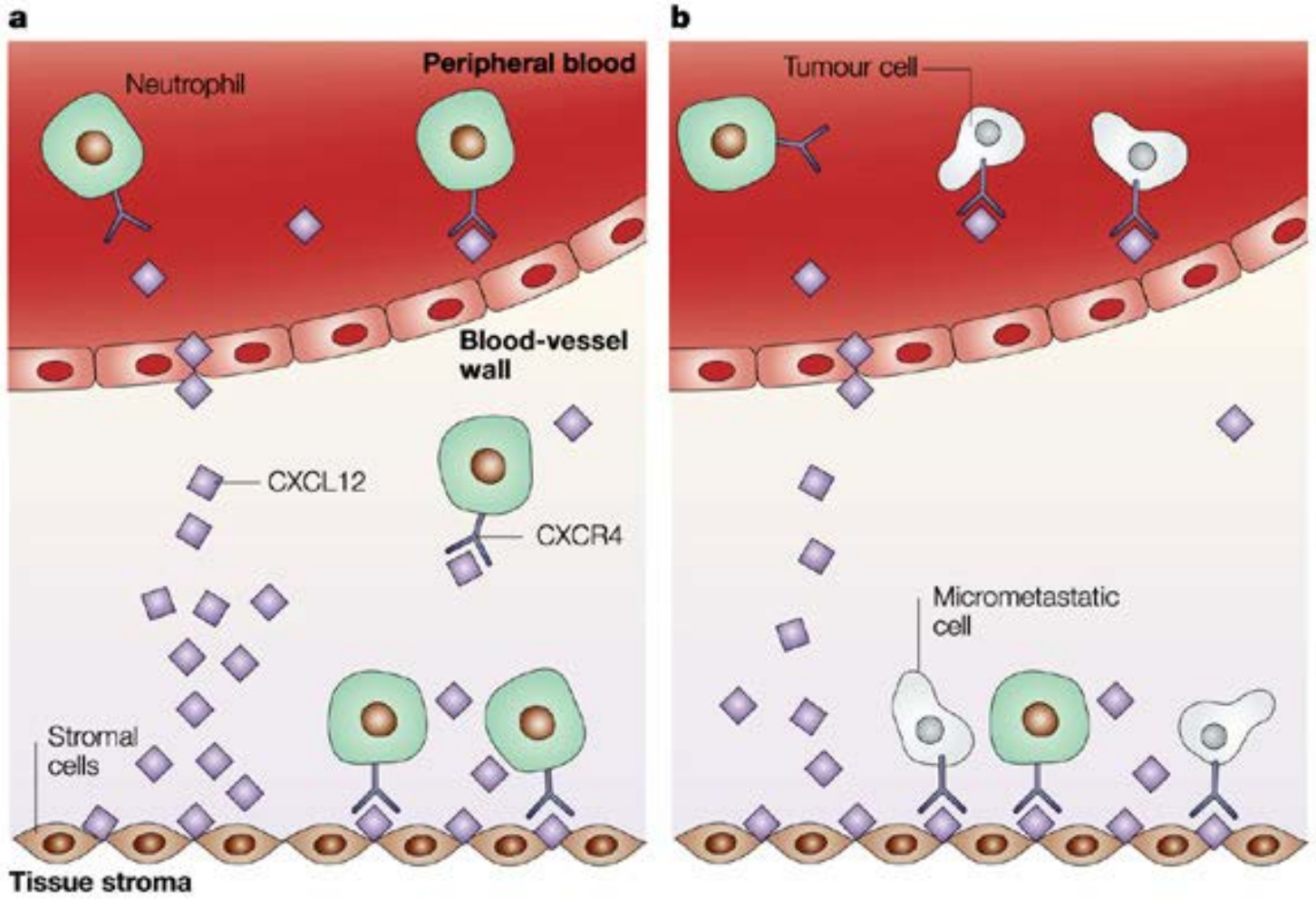




Helbach K 2017 Sci Rep

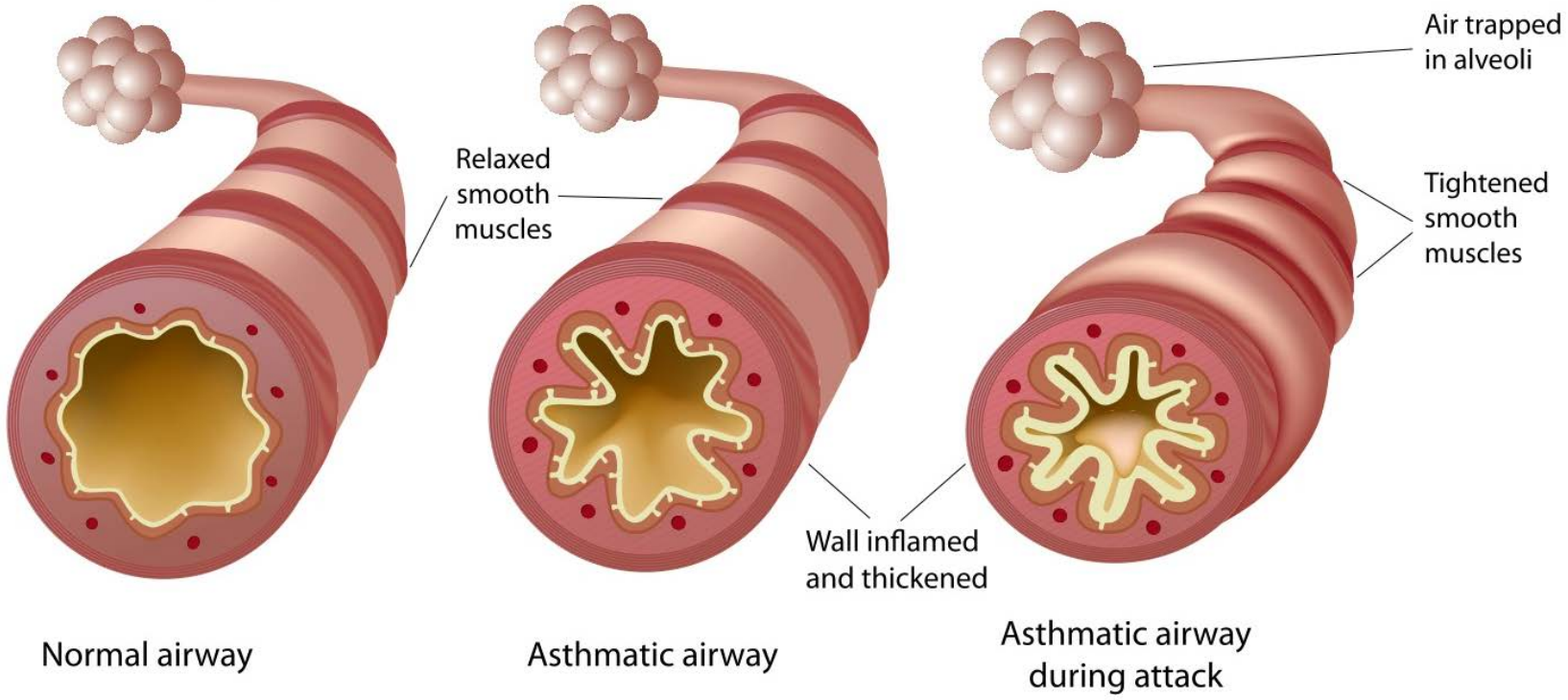
Idiopathic pulmonary fibrosis

- **Animal model: Bleomycin-induced pulmonary fibrosis**
- **Human fibrocytes that were administered intravenously to SCID mice preferentially homed to the lungs**
- **The magnitude of lung procollagen I and III upregulation correlated with the number of CD45+, Col I+, CXCR4+ fibrocytes in the BM, blood, and in the lung**
- **CXCL12 was significantly increased in bleomycin-treated lung**
- **Administration of neutralizing anti-CXCL12 andtibodies resulted in…**
  - **significantly reduced Fibrocyte extravasation into the lung**
  - **Reduced collagen deposition in the lungs**
  - **Reduced α-SMA expression**


a
Neutrophil
Peripheral blood
Blood-vessel wall
CXCL12
CXCR4
Stromal cells
Tissue stroma
b
Tumour cell
Micrometastatic cell
Nature Reviews | Cancer

Asthma

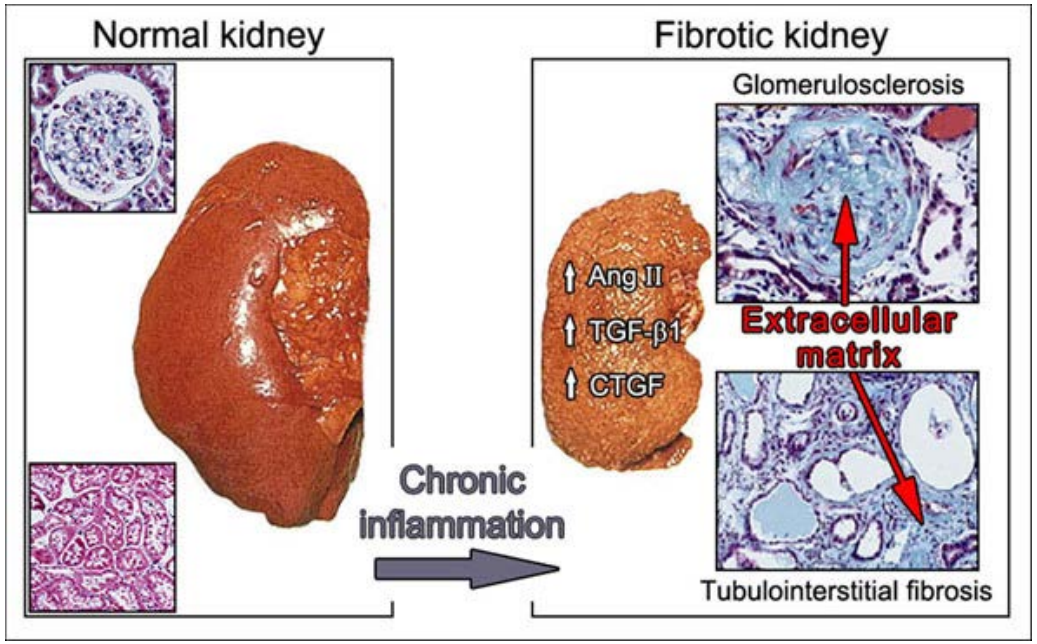
- **Characterized by persistent airway inflammation and structural aberrant remodeling of the airways**
- **Extensive deposition of ECM**
- **Fibrocytes expressing CD34, procollagen I, and α-SMA (myofibroblasts resulted in increased thickness of the lamina reticularis)**

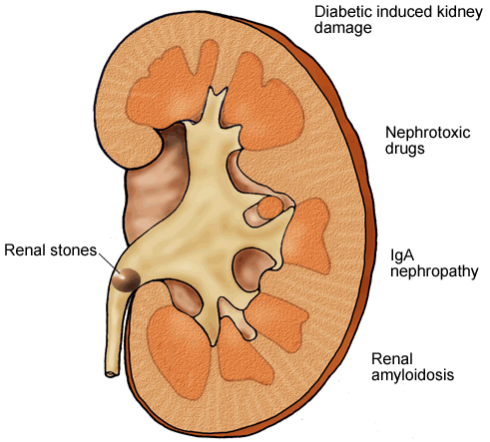


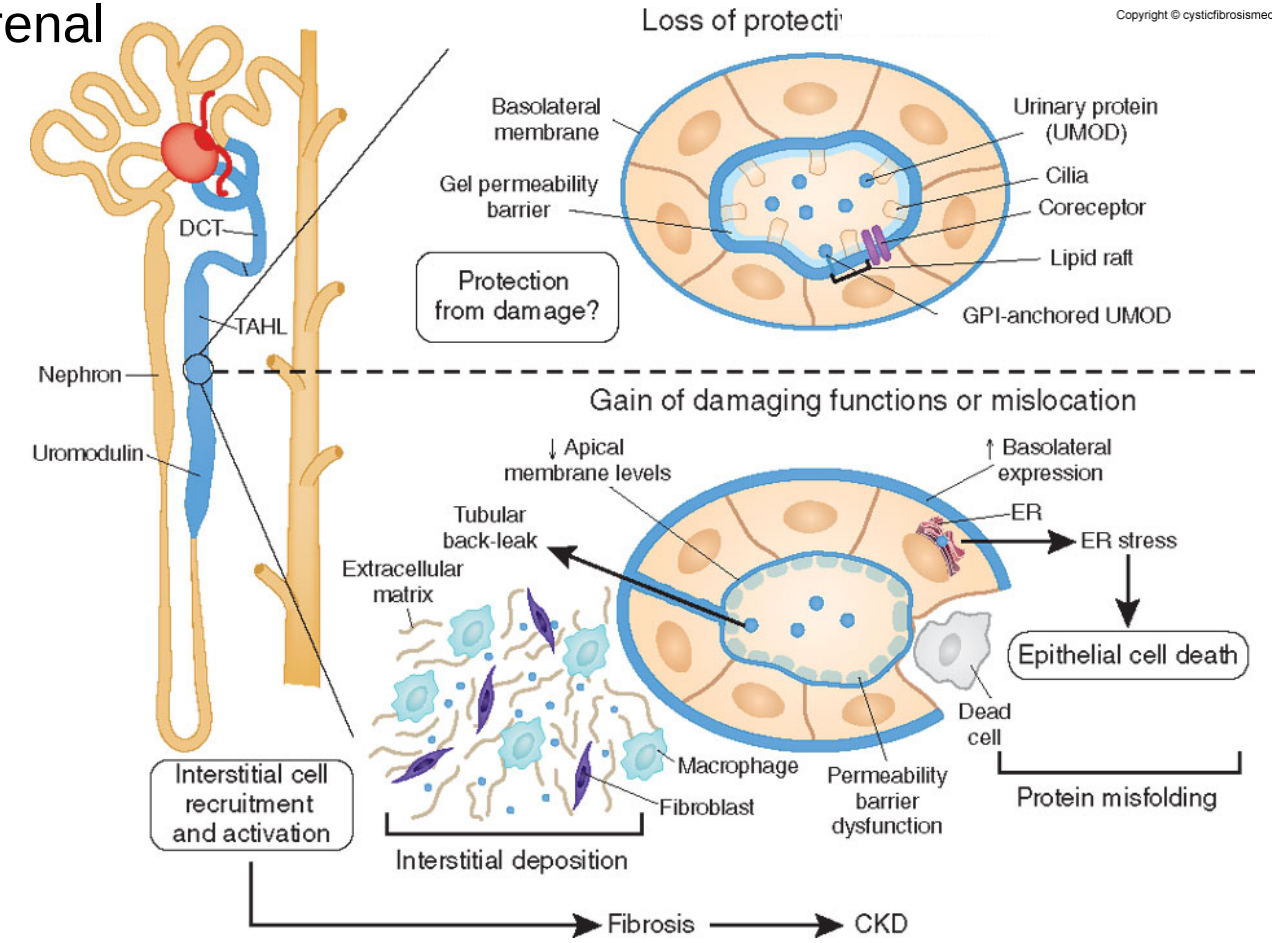
Chronic Kidney Disease (CKD)

Renal fibrosis is defined by…

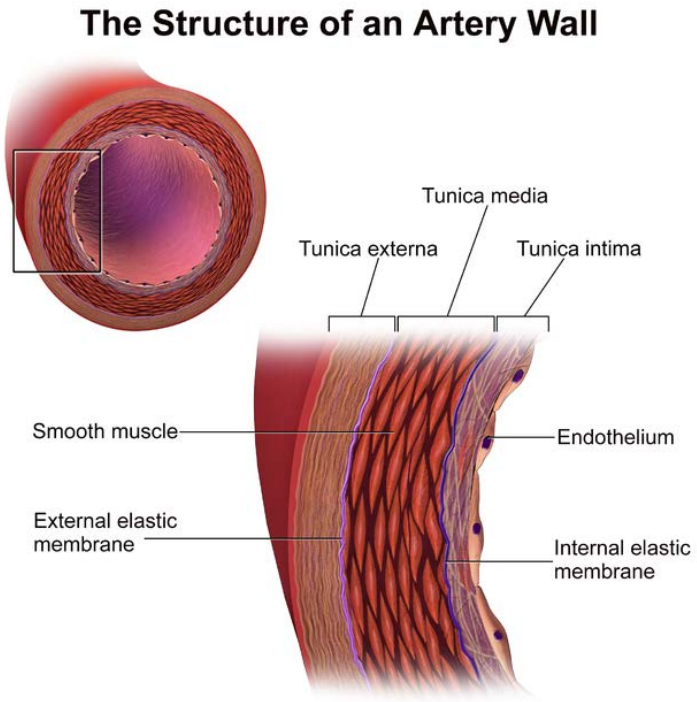
- Tubular atrophy and dilation
- Interstitial leukocyte infiltration
- Increased interstitial matrix deposition
- Upto 15% of myofibroblasts contributing to renal fibrosis are of BM origin



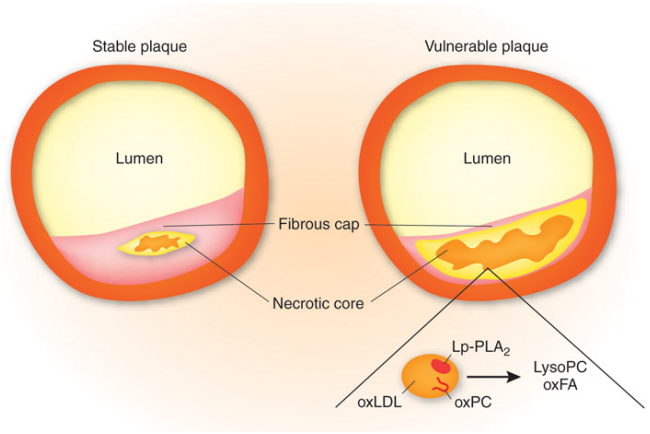


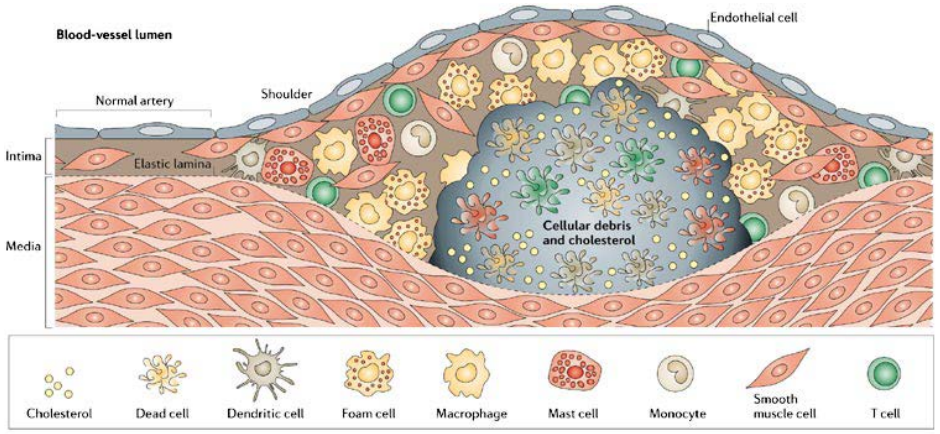


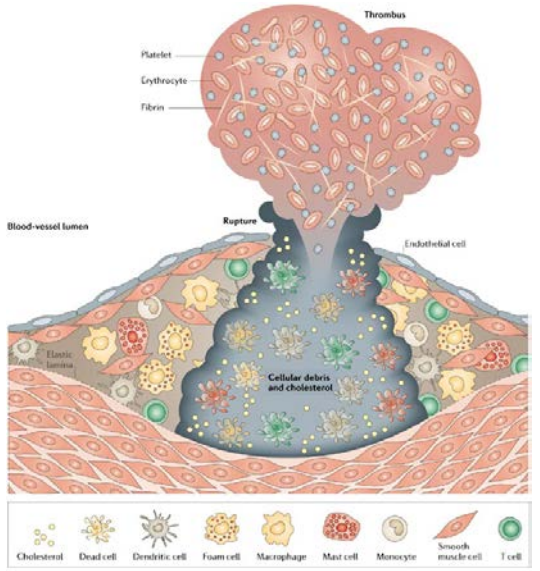
Atherosclerosis



- Thickening of tunica intima of blood vessels is a universal response to vessel injury
- Atherosclerotic plaque contains cells positive for CD34, CD45, and α-SMA







Hansson *et al.* 2006 *Nature Reviews Immunology*

Regenerative Healing & Scar Reduction Theory

Targeting the Inflammatory Response

- **Regenerative wound healing is replace by scarring as the immune system in the embryo develops (Martin, 1997)**
- **Reduction of inflammation in post-natal skin wounds correlates with reduced scarring**
- **Mice devoid of functional neutrophils & macrophages**
  - **healed wounds over a similar time course**
  - **Exhibited scar-free healing similar to embryonic wound healing**
- **Wound inflammatory cells produce signals that either directly or indirectly induce collagen deposition and granulation tissue formation -> increase scarring**

Recent studies

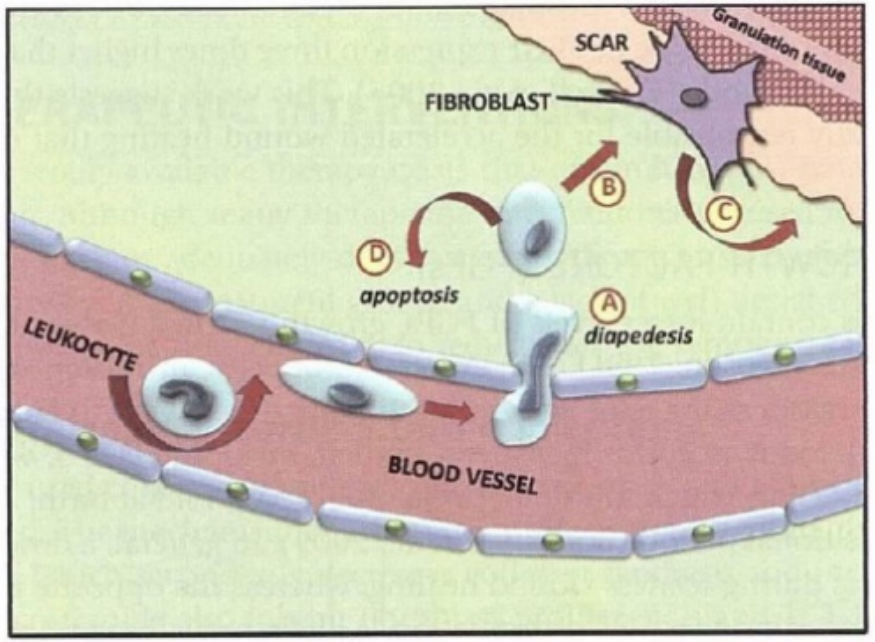
- The inflammatory phase & scarring might not be as directly linked
  - <EX> Cox-2
    (enzyme involved in prostaglandin production)
  - Effect of Cox-2 inhibition is controversial (reduction vs no difference)

Fibrocytes

- Have a role in granulation tissue deposition and scar formation (neutrophils & macrophages might not be the only mediators implicated)
- Sub-population of circulating leukocytes, fibroblast-like characteristics
  - Express both leucocyte marker (CD34) and ECM protein (Collgen)
- Increase the intensity of the inflammatory response
- Secretion of PDGF & TGF-beta
  -> Guide the action of fibroblast at the wound site

Major target - Inflammatory response

- Leucocytes (at any point as they migrate….)
  - Through the vessel wall
  - To the wound
  - Transmit a signal to fibroblasts
- Fibroblasts (block their response to leucocyte signaling)



Targeting Cytokines and GFs

- TGF-beta superfamily
- Connective tissue growth factor (CTGF)
- Vascular Endothelial Growth Factor (VEGF)
- Fibroblast Growth Factors (FGFs)
- Platelet-derived Growth Factor (PDGF)
- Wnt signaling
- Interleukins

TGF-beta Superfamily

- Secreted by keratinocytes, fibroblasts, platelets, and macrophages
- Influence the migration of keratinocytes and fibroblasts to the wound bed
- Matrix remodeling
- Collagen synthesis
- Myofibroblast differentiation
- Fetus TGF-beta isoform profile
- TGF-beta3 - relatively high
- TGF-beta1 & beta2 - relatively low
- Those profile experimentally proved to reduce scar

Connective tissue growth factor

- Pro-fibrotic
  - Mechanism related to TGF-beta3
  - Activated by Smad proteins
- Stimulates the deposition of ECM
- Higher expression in adult fibroblasts

VEGF

- Produced by keratinocyte, fibroblasts, macrophages
- Scarless wound healing shows an increase of VEGF expression 3x higher
  - Increased VEGF expression is partially responsible for the accelerated wound healing

FGFs

- In general, down regulation of the FGF isoforms occurs during scarless wound healing (embryonic)
- The opposite is true during adult wound healing
- FGF up-regulation is likely partially responsible for scar formation

PDGF

- Pro-fibrotic growth factor
- Adult wounds contain very high amount of PDGF
- PDGF is virtually absent in embryonic wounds
- Administration of PDGF to fetal wound
  → increased inflammation, fibroblast recruitment, collagen deposition
  → induce scarring

Wnt signaling

- With wounding, fetal wnt expression remains stable at its high basal level
- In adult skin, increases during repair

Interleukins

- Class of cytokines involved in activation of the inflammatory cascade
- With insult to skin integrity IL-6 & IL-8 rapidly increase expression
- IL-6
  - Produced by fibroblasts in response to stimulation by PDGF
  - Activate macrophage
  - Stimulate monocyte chemotaxis
- IL-8
  - Stimulates neovascularization
  - Attracts neutrophils
- Elevated expression is..
- Maintained over a period of 72 hours during adult repair
- Suppressed after 12 hours during carless fetal repair
- Early fetal fibroblasts express lower level of both IL-6 & IL-8

- IL-10 is thought to be anti-inflammatory
- Antagonism of IL-6 & IL-8
- Fetal skin grafts from IL-10 KO mice
  - Grafted to syngeneic mice -> showed scar formation (proves intrinsic mechanism)
- Administration of IL-10 over-expression Adenoviral vector…
  - Reduced inflammation
  - Induced scarless wound healing in adult mouse wounds (Gordon et al., 2008)

Senescence

- Cellular senescence is implicated in pathological tissue repair
- Fibroblasts become prematurely senescent within a chronic wound setting

Current Therapeutic Intervention

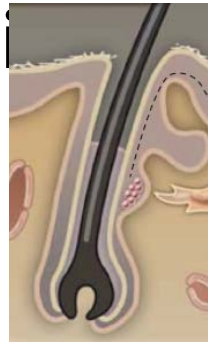
- **Corticosteroid injections**
- **5-flurorouracil (5-FU)**
- **Imiquinod**
- **Laser therapy**
- **Bleomycin**
- **Silicone gel sheeting**
- **Pressure dressings**
- **Radiation therapy**
- **Cryotherapy**
- **surgery**
  - Non of these are optimal and effective
  - Therapy based on molecular targets remain elusive

Future Therapeutic Intervention

- TGF-beta associated therapies
- PDGF (approved by US FDA for treatment of DFUs)
- Targeting gap junctions and connexins
  (to stop propagation of a signal from one cells to an adjacent cells)
- Other drugs & biologics
- Stem cells
  - ES cells
  - MSCs
  - Epidermal stem cells
  - iPS cells
- Wound dressing incorporated with GFs and live cells

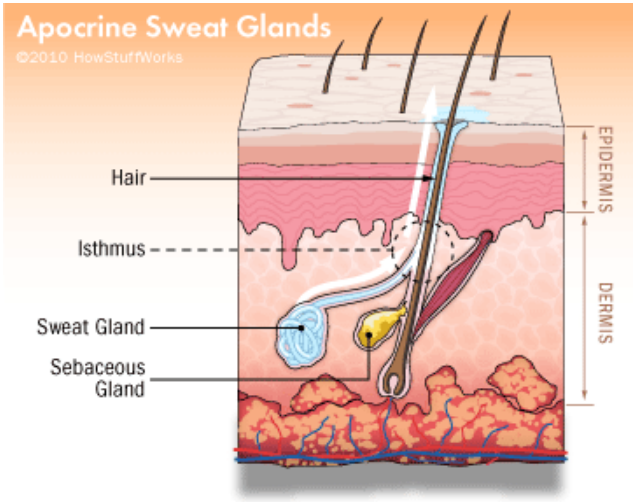
Epithelial stem cell niche

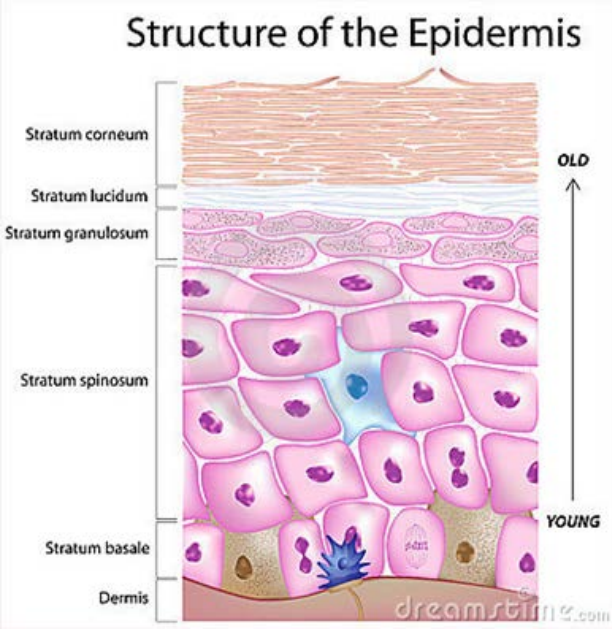
- The bulge of the hair follicle
- The base of the sebaceous gland
- The basal layer of the epidermi



- Progenitors:
- Local adipocyte progenitor cells
- Local melanocyte progenitor cells

- Recruitment of bone marrow and endothelial progenitors





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