Molecular Mechanisms in Fibrosis: the role of ECM

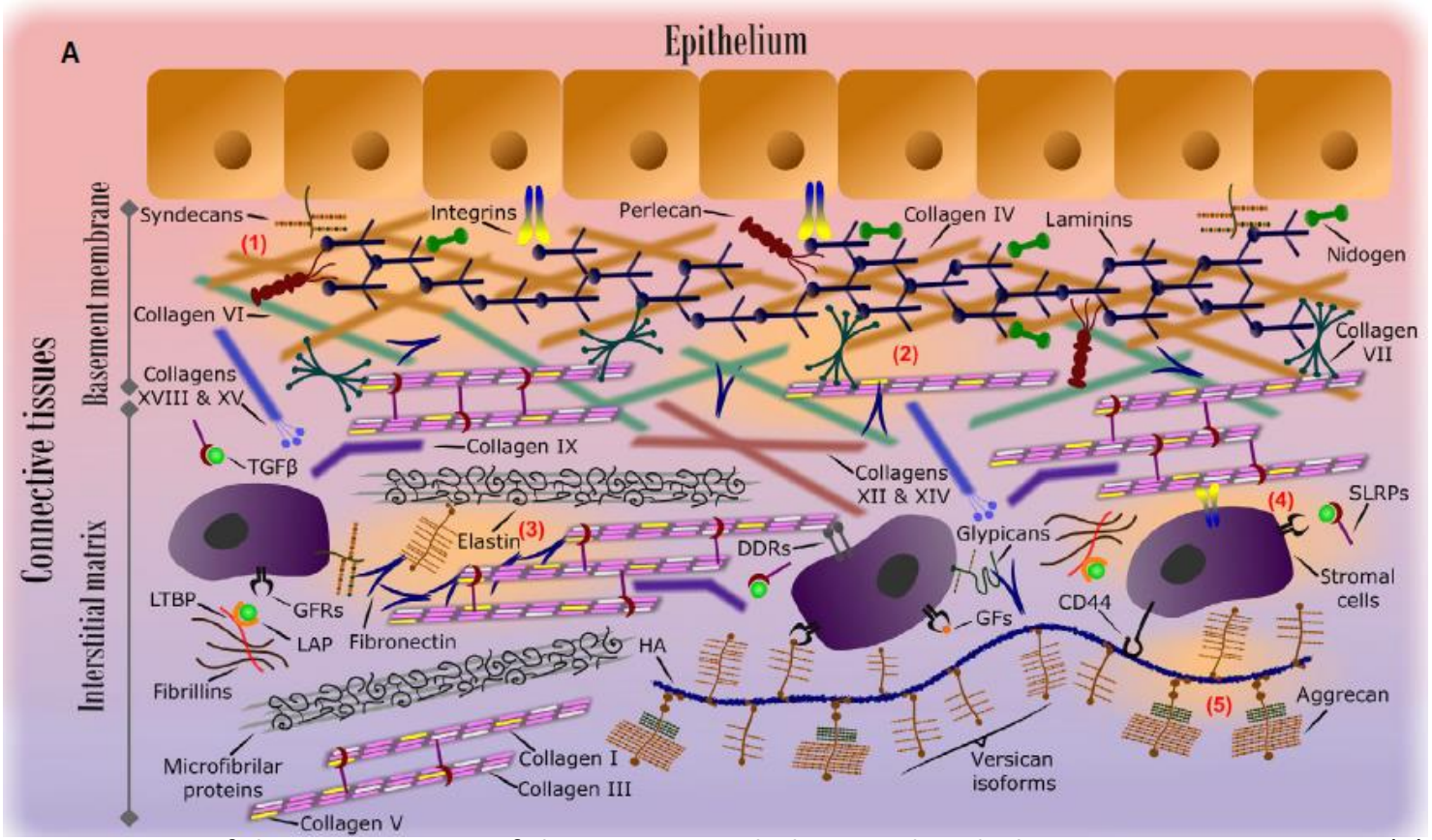
Fibrosis

Fibrosis is scarring and tissue hardening caused by the excess deposition of extracellular matrix (ECM) proteins by myofibroblasts in response to chronic inflammation.

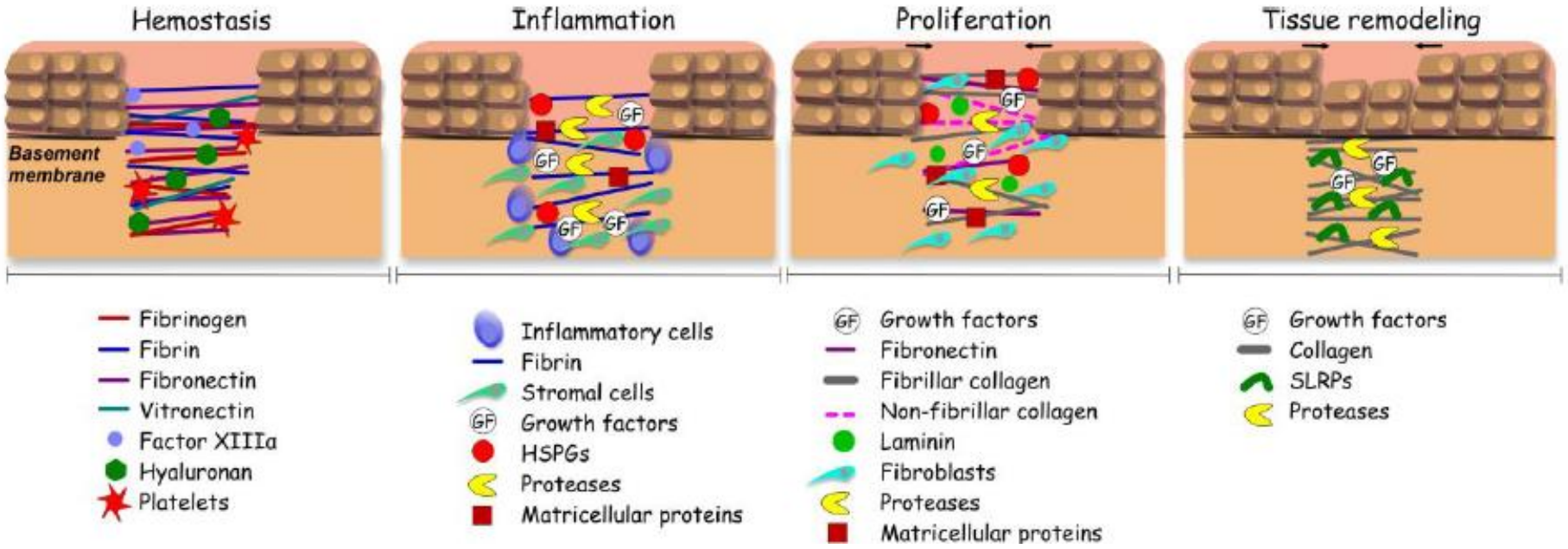
A variety of noxious stimuli—including toxins, infectious pathogens, autoimmune reactions, and mechanical stress—are able to induce a fibrotic cellular response.

Fibrosis can affect all tissues of the body, and left unchecked, can result in organ failure and death.

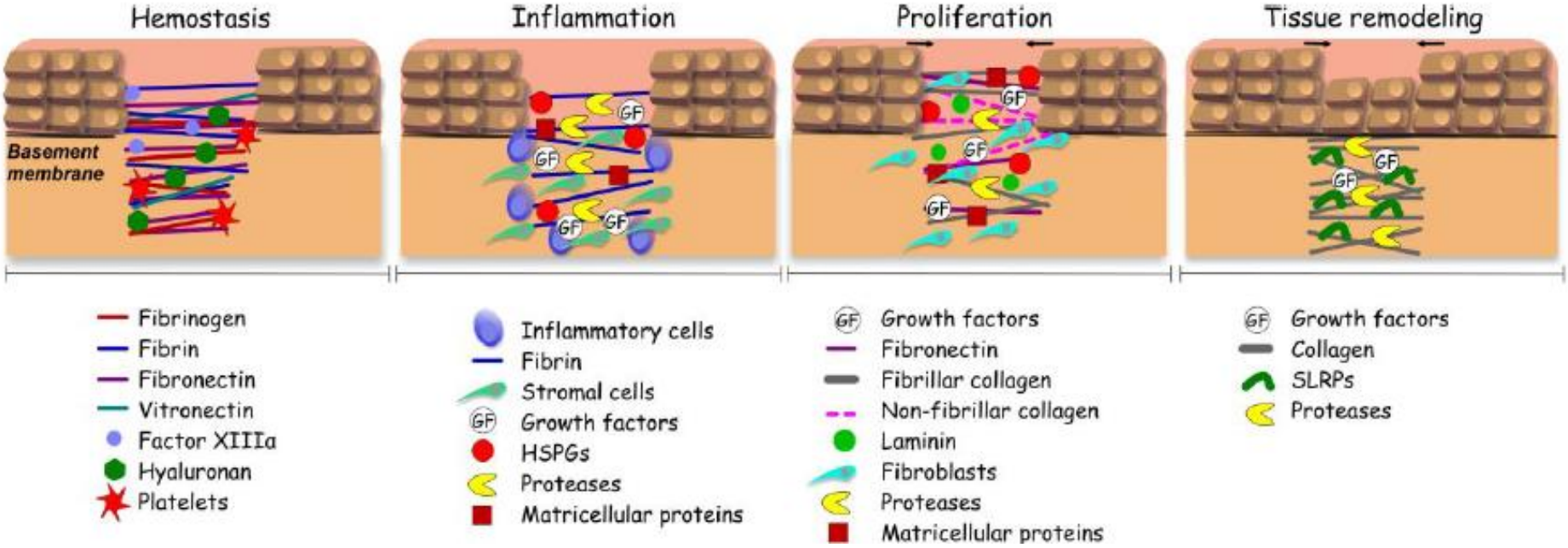
Current research on key signaling pathways that regulate fibrogenesis has identified potential therapeutic targets of interest to stem the progression of fibrosis and restore cellular function.



Schematic representation of the organization of the ECM in epithelium and underlying connective tissues . (1) Basement membrane is a unique pericellular matrix, defined as an amalgam of two networks: the scaffold of self-associating laminins and the polymer-like network of collagen IV. Perlecan and nidogens further bridge these networks. Epithelial cells are connected to the basement membrane through hemidesmosomes, where laminins are bound to integrins. (2) Basement membrane anchors to interstitial matrix through a variety of collagen fibrils, including collagens VI and VII. The former interacts with collagen IV and perlecan, whereas the latter with collagen IV, laminins, and collagen I fibrils. (3) The main collagen type in the interstitial matrix are the heterotypic fibrils of collagens I, III, and V. SLRPs and fibronectin participate in collagen assembly and fibrillogenesis. Many other components contribute in interstitial matrix organization including elastin, proteoglycans and HA. (4) Cell surface receptors of stromal cells such as integrins, DDRs, syndecans, glypicans, and growth factor receptors (GFR) interact with ECM components and growth factors (GF). The bioavailability and binding of growth factors such as TGFb, is regulated by SLRPs and the LAP and LTBP. (5) HA forms large aggregates with hyalectans, contributing to the rigidity of the ECM. It also interacts with cell surface receptors, such as CD44.



Schematic representation of wound healing phases. In each phase, the participation of ECM proteins is essential and its composition, mainly in the connective tissue, alters the hemostatic, inflammatory, proliferative and tissue remodeling phases of healing.
Fibrin, fibronectin, platelets and HA bound to fibrinogen, vitronectin, factor XIIIα and other clotting proteins are major constituents during the hemostatic phase of the wound.
As leukocytes migrate into the wound site, they release proteases to degrade the fibrin-rich ECM. Immune cells secrete a broad range of inflammatory cytokines and growth factors to attract stromal cells to migrate into the wound. Several matrix-stored growth factors (i.e. PDGF, EGF, TGF-β) and PGs released and secreted from endothelial cells (i.e. syndecans, decorin, lumican) are also displaced.



In the next phase, stromal fibroblasts migrating into the wound area (arrows) produce a provisional ECM characterized by the presence of fibronectin, matricellular proteins, decreased protease activity, fibrillar (type I, III) and non-fibrillar (type IV, VI, VII) collagen, and growth factors (i.e. EGF, FGF, TGF-β). The secreted matrix PGs (i.e. decorin, lumican) and collagen that are associated with mature healing wounds are secreted by keratinocytes and leukocytes to promote proliferation and migration of vascular components, endothelial and epithelial cells.

During the tissue remodeling phase, the stiffer matrix and several growth factors (i.e. TGF-β) trigger stromal cells to induce wound contraction by adopting a myofibroblast phenotype. The supportive ECM is enriched in matrix PGs, proteases and realigned collagen type I cross-linked fibers. The final step in wound repair involves the formation of overly aligned collagen fibers that regain almost 80% of the primary tissue functionality.

Inflammation and Wound Healing

What is Inflammation?

- Response to injury (including infection)
- Reaction of blood vessels leads to:
  - Accumulation of fluid and leukocytes in extravascular tissues
- Destroys, dilutes, or wash off the injurious agent
- Initiates the repair process
- Fundamentally a protective response
- May be potentially harmful
  - Hypersensitivity reactions to insect bites, drugs, contrast media in radiology
  - Chronic diseases: arthritis, atherosclerosis
  - Disfiguring scars, visceral adhesions
- Consists of two general components
  - Vascular reaction
  - Cellular reaction
- Controlled by a variety of chemical mediators
  - Derived from plasma proteins
  - Derived from cells inside and outside of blood vessels

Historical Highlights

- Celsus, a first century A.D. Roman, listed four cardinal signs of acute inflammation:
  - *Rubor* (erythema [redness]): vasodilatation, increased blood flow
  - *Tumor* (swelling): extravascular accumulation of fluid
  - *Calor* (heat): vasodilatation, increased blood flow
  - *Dolor* (pain)

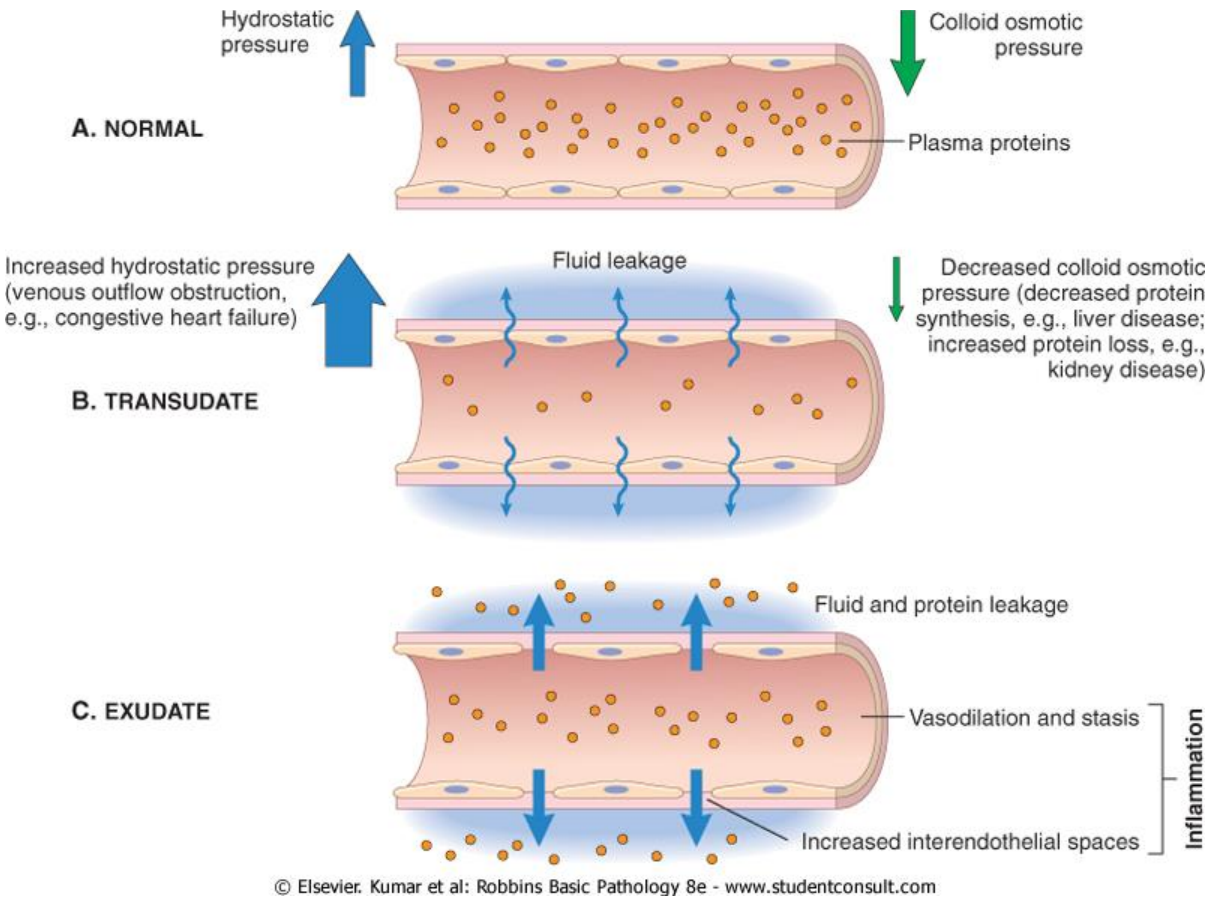
Types of Inflammation

- Acute inflammation
  - Short duration
  - Edema
  - Mainly neutrophils
- Granulomatous inflammation
  - Distinctive pattern of chronic inflammation
  - Activated macrophages (epithelioid cells) predominate
  - +/- Multinucleated giant cells
- Chronic inflammation
  - Longer duration
  - Lymphocytes & macrophages predominate
  - Fibrosis
  - New blood vessels (angiogenesis)

Acute Inflammation

- Three major components:
  - Increase in blood flow (redness & warmth)
  - Edema results from increased hydrostatic pressure (vasodilation) and lowered intravascular osmotic pressure (protein leakage)
  - Leukocytes emigrate from microcirculation and accumulate in the focus of injury
- Stimuli: infections, trauma, physical or chemical agents, foreign bodies, immune reactions

Edema in inflammation



Edema is a general term for swelling (usu. due to fluid)

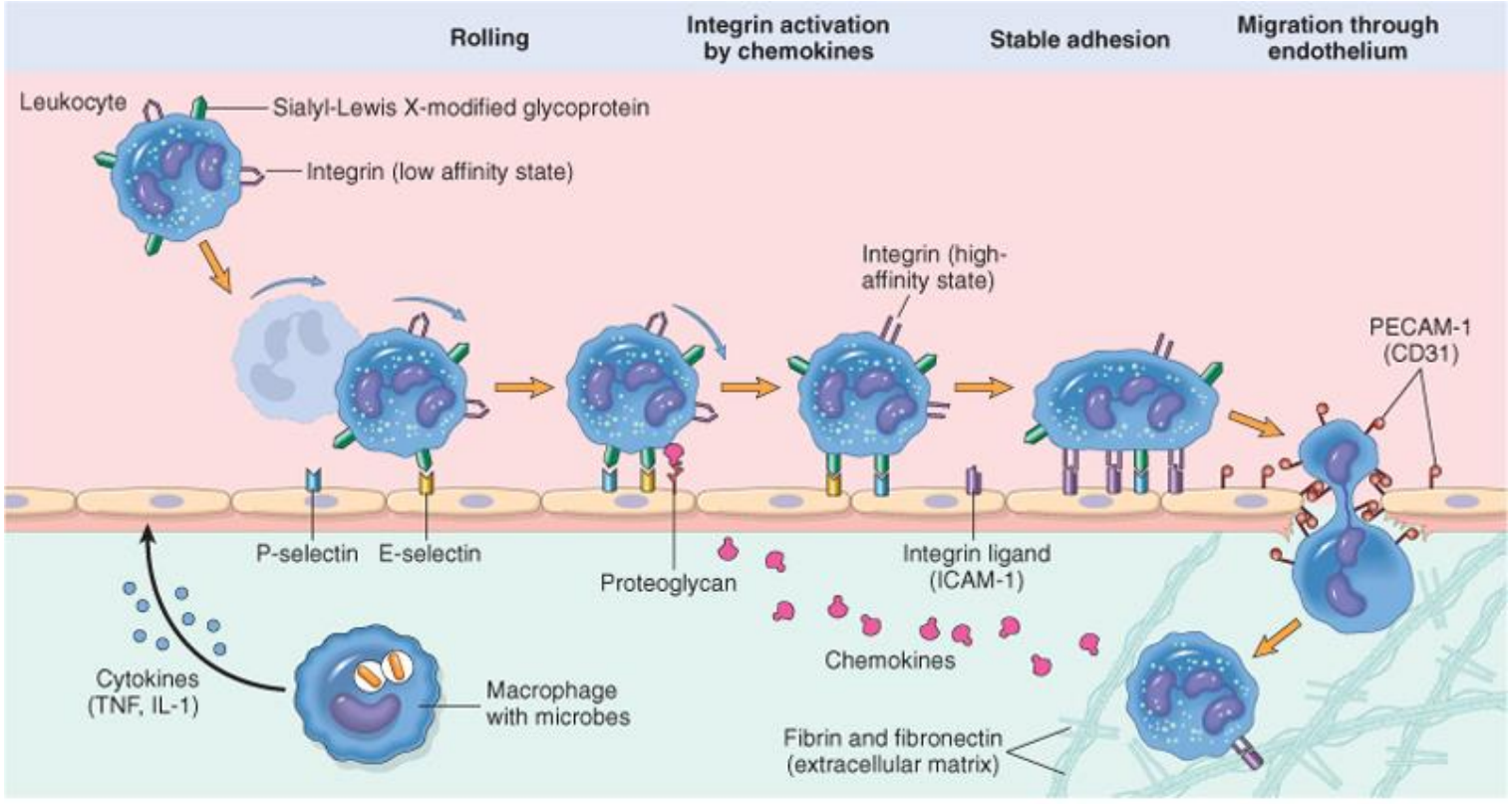
Plasma proteins in blood maintain a "colloid osmotic pressure" to help draw fluid that leaks out into tissue bed via hydrostatic pressure

Dysregulation of hydrostatic pressure (e.g. heart failure) and/or colloid pressure (decreased protein synthesis/retention) pushes out more fluid (transudate) into tissue bed

Inflammation causes endothelial cells to separate, thus allowing fluid + protein (exudate) to enter tissue bed.

Leukocyte Extravasation

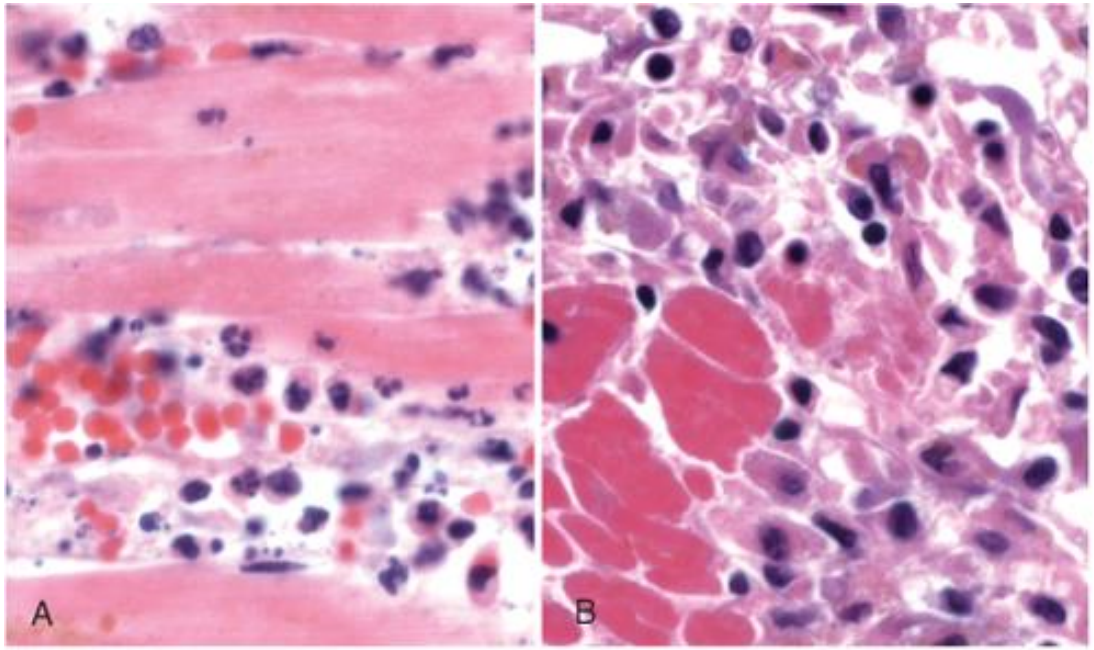
- Extravasation: delivery of leukocytes from the vessel lumen to the interstitium
  - In the lumen: margination, rolling, and adhesion
  - Migration across the endothelium (*diapedesis*)
  - Migration in the interstitial tissue (*chemotaxis*)
- Leukocytes ingest offending agents (phagocytosis), kill microbes, and degrade necrotic tissue and foreign antigens
- There is a balance between the helpful and harmful effects of extravasated leukocytes

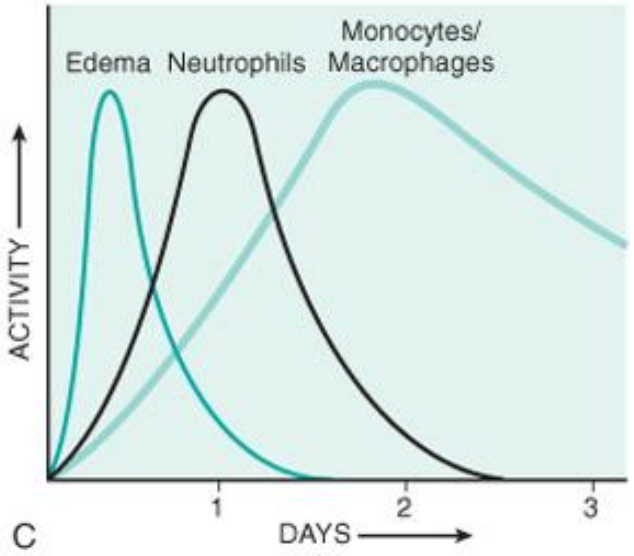


Sequence of Leukocyte Emigration

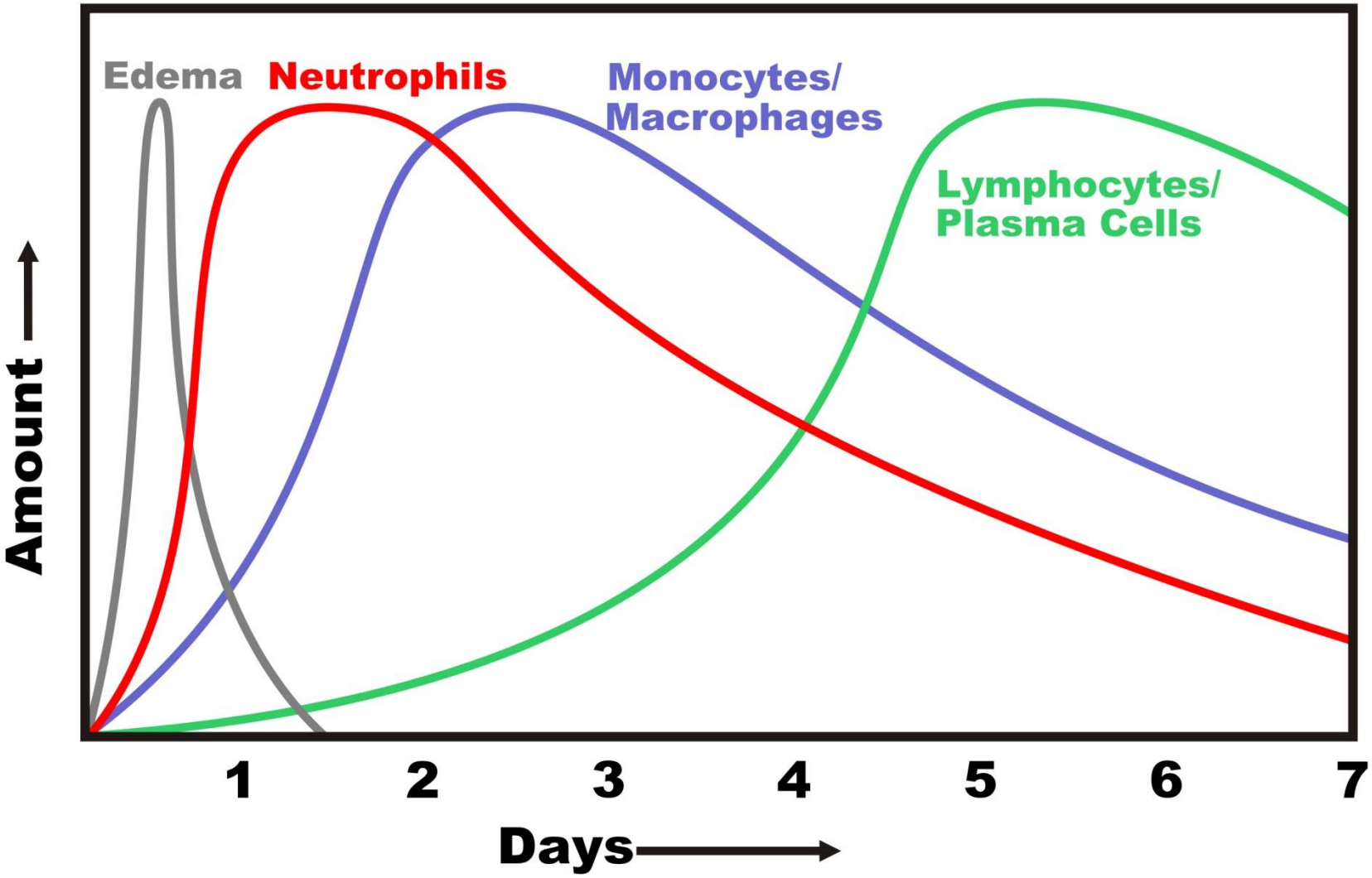
- Neutrophils predominate during the first 6 to 24 hours
- Monocytes in 24 to 48 hours
- Induction/activation of different adhesion molecule pairs and specific chemotactic factors in different phases of inflammation

Sequence of Events - Injury





Sequence of Events - Infection

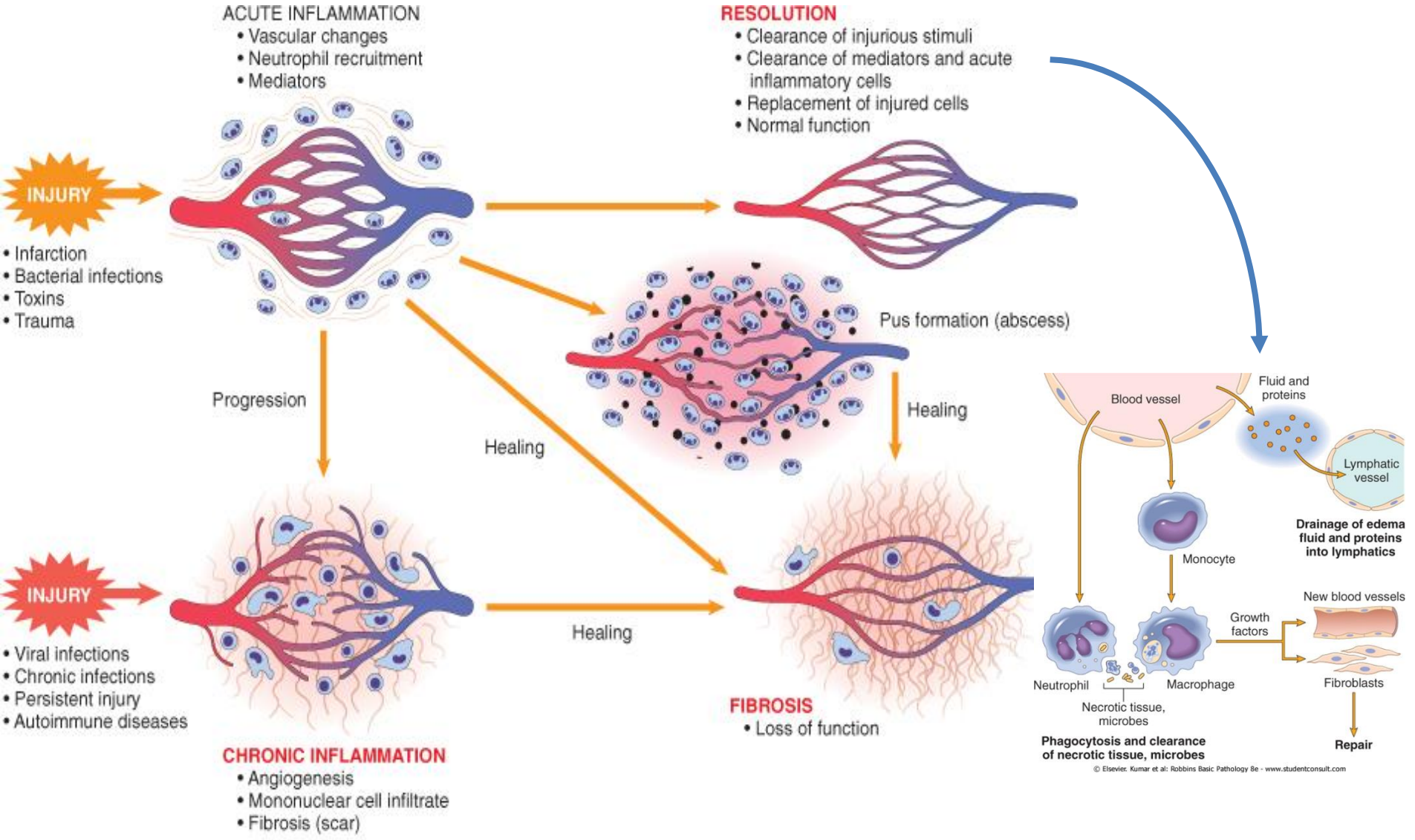


Outcomes of Acute Inflammation

- Complete resolution
- Abscess formation
- Fibrosis
  - After substantial tissue destruction
  - In tissues that do not regenerate
  - After abundant fibrin exudation, especially in serous cavities (pleura, peritoneum)
- Progression to chronic inflammation

Types of Inflammation: acute vs. chronic

Types of repair: resolution vs. organization (fibrosis)



Chronic Inflammation

- Inflammation of prolonged duration (weeks or months)
  - Active inflammation, tissue destruction, and attempts at repair are proceeding simultaneously
- May follow acute inflammation or begin insidiously and often asymptomatically
  - Persistent infections, exposure to toxic agents such as silica (silicosis), or by autoimmunity

Chronic Inflammation

- Persistent infections
  - *Treponema pallidum* [syphilis], viruses, fungi, parasites
- Exposure to toxic agents
  - Exogenous: silica (silicosis)
  - Endogenous: toxic plasma lipid components (atherosclerosis)
- Autoimmunity
  - Rheumatoid arthritis, systemic lupus erythematosus

Chronic Inflammation

- Histological features
  - Infiltration with mononuclear cells (macrophages, lymphocytes, and plasma cells)
  - Tissue destruction (induced by the inflammatory cells)
  - Healing by replacement of damaged tissue by connective tissue (fibrosis) and new blood vessels (angiogenesis)

Macrophages

- Monocytes begin to emigrate into tissues early in inflammation where they transform into the larger phagocytic cell known as the macrophage
- Macrophages predominate by 48 hours
  - Recruitment (circulating monocytes); division; immobilization
- Activation results in secretion of biologically active products

The products of activated macrophages serve to eliminate injurious agents such as microbes and to initiate the process of repair, and are responsible for much of the tissue injury in chronic inflammation.

Their impressive arsenal of mediators makes macrophages powerful allies in the body's defense against unwanted invaders, but the same weaponry can also induce considerable tissue destruction when macrophages are inappropriately activated.

It is because of the activities of these macrophages that tissue destruction is one of the hallmarks of chronic inflammation.

The ongoing tissue destruction can itself activate the inflammatory cascade, so that features of both acute and chronic inflammation may coexist in certain circumstances.

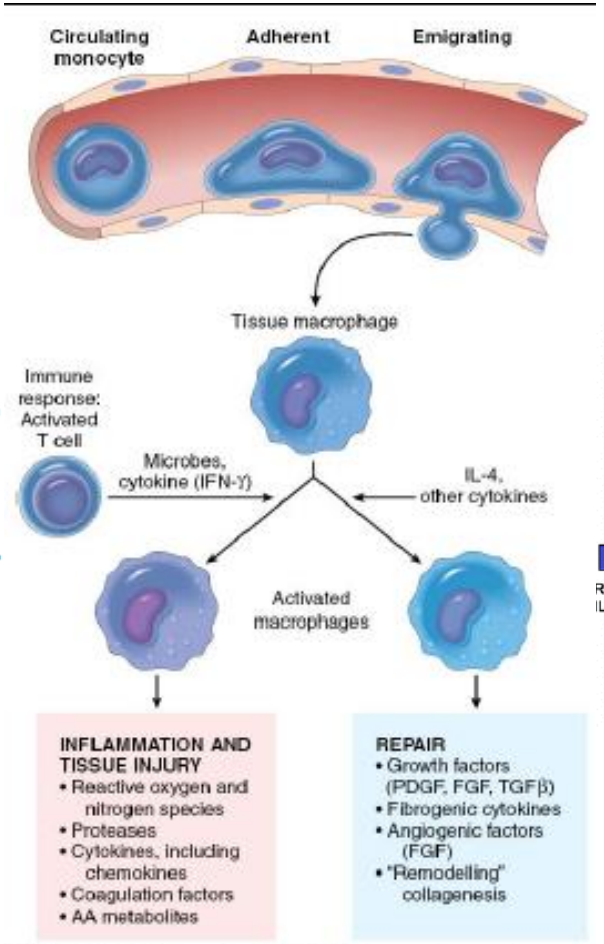
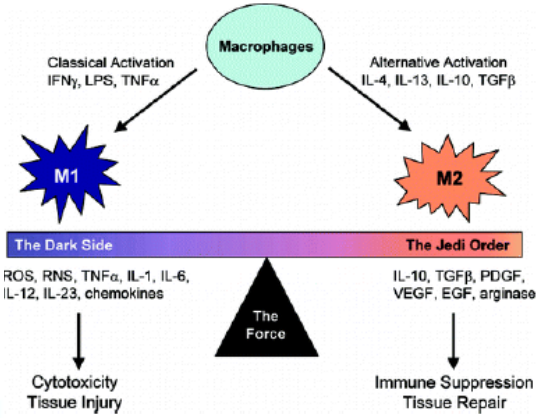


FIGURE 2–24 The roles of activated macrophages in chronic inflammation. Macrophages are activated by nonimmunologic stimuli such as endotoxin or by cytokines from immune-activated T cells (particularly IFN-γ). The products made by activated macrophages that cause tissue injury and fibrosis are indicated. AA, arachidonic acid; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; TGFβ, transforming growth factor β.



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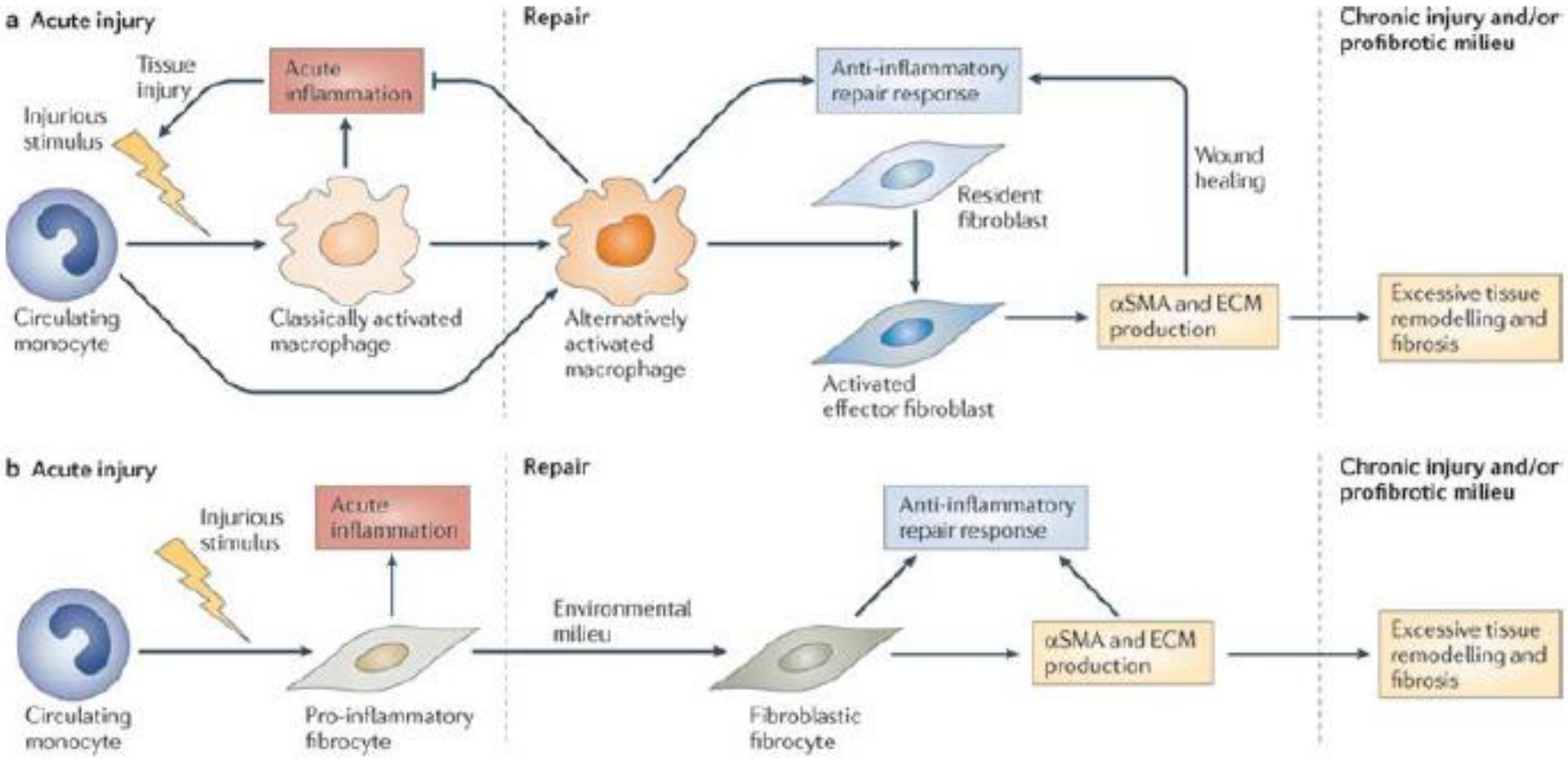

Fibrocytes are mesenchymal cells that arise from monocyte precursors. They are present in injured organs and have both the inflammatory features of macrophages and the tissue remodelling properties of fibroblasts. Chronic inflammatory stimuli mediate the differentiation, trafficking and accumulation of these cells in fibrosing conditions associated with autoimmunity, cardiovascular disease and asthma. This Opinion article discusses the immunological mediators controlling fibrocyte differentiation and recruitment, describes the association of fibrocytes with chronic inflammatory diseases and compares the potential roles of fibrocytes in these disorders with those of macrophages and fibroblasts. It is hoped that this information prompts new opportunities for the study of these unique cells.

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Other Cells in Chronic Inflammation

- Lymphocytes
  - Produce inflammatory mediators
  - Participate in cell-mediated immune reactions
  - Plasma cells produce antibody
  - Lymphocytes and macrophages interact in a bi-directional fashion

Other Cells in Chronic Inflammation

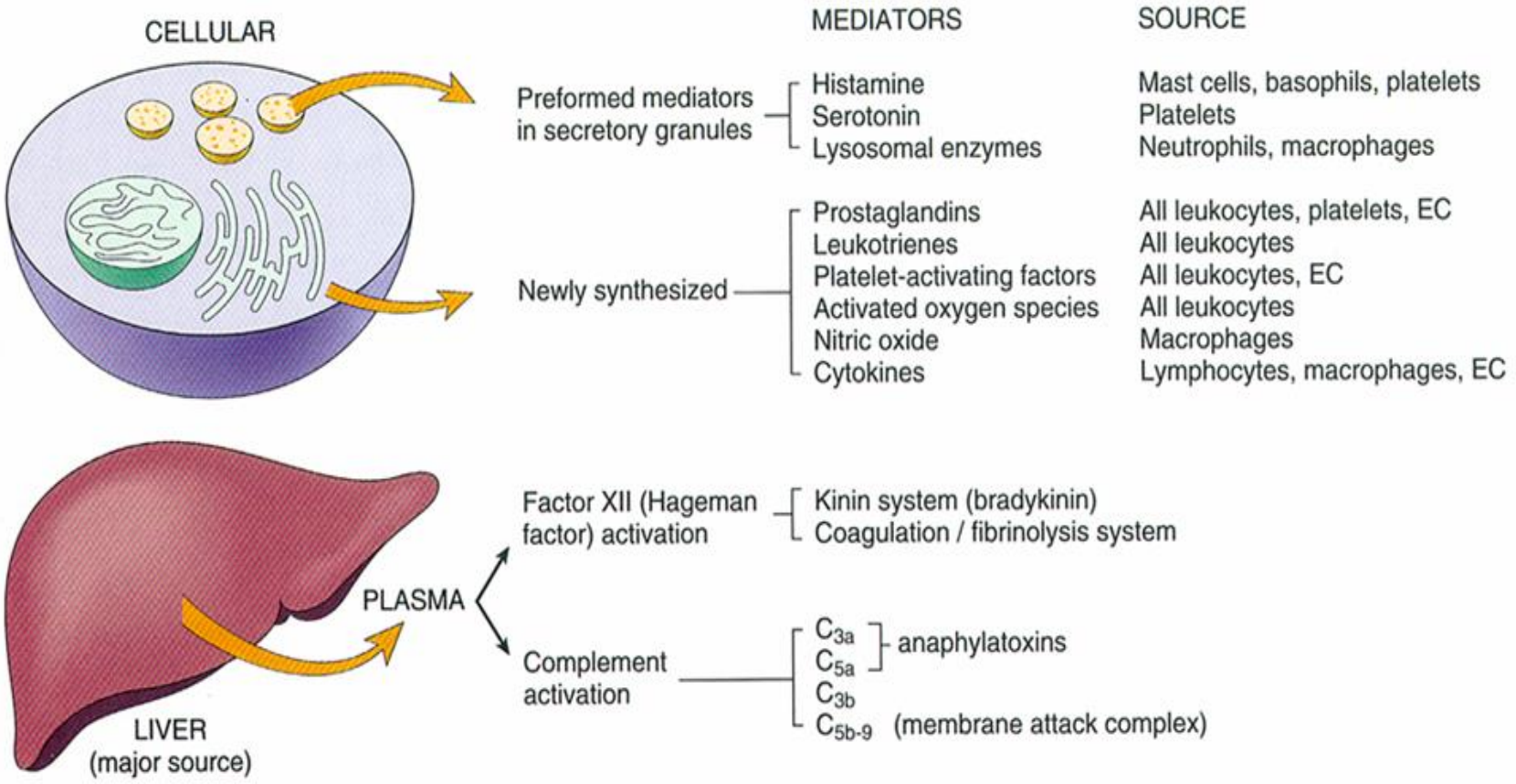
- Eosinophils
  - Immune reactions mediated by IgE
  - Parasitic infections
    - Eosinophil granules contain a protein that is toxic to parasites
- Mast cells
  - Release mediators (histamine) and cytokines

Granulomatous Inflammation

- Distinctive pattern of chronic inflammation
  - Predominant cell type is an activated macrophage with a modified epithelial-like (epithelioid) appearance
  - Giant cells may or may not be present
- Granuloma:
  Focal area of granulomatous inflammation

Chemical Mediators of Inflammation

- General principles of chemical mediators
  - May be derived from plasma or cells
  - Most bind to specific receptors on target cells
  - Can stimulate release of mediators by target cells, which may amplify or ameliorate the inflammatory response
  - May act on one or a few target cells, have widespread targets, and may have differing effects depending on cell and tissue types
  - Usually short-lived
  - Most have the potential to cause harmful effects



Chemical Mediators of Inflammation

- Vasoactive mediators
  - Histamine
  - Bradykinin
  - Complement (C3a, C5a)
  - Prostaglandins/leukotrienes
  - Platelet activating factor
  - Nitric oxide
- Chemotactic factors
  - Complement (C5a)
  - Leukotriene (B4)
  - Platelet activating factor
  - Cytokines (IL-1, TNF)
  - Chemokines
  - Nitric oxide

Cytokines

- Proteins produced by many cell types (principally activated lymphocytes & macrophages)
- Modulate the function of other cell types
- Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are the major cytokines that mediate inflammation

Chemokines

- Small proteins that act primarily as chemoattractants for specific types of leukocytes (approximately 40 known)
- Stimulate leukocyte recruitment in inflammation
- Control the normal migration of cells through tissues (organogenesis and maintenance of tissue organization)
- Examples: IL-8, eotaxin, lymphotactin

Renewal, Regeneration and Repair

Injury to cells and tissues sets in motion a series of events that contain the damage and initiate the healing process. This process can be broadly separated into regeneration and repair (Fig. 3-1).

Regeneration results in the complete restitution of lost or damaged tissue.

Repair may restore some original structures but can cause structural derangements.

In healthy tissues, healing, in the form of regeneration or repair, occurs after practically any insult that causes tissue destruction, and is essential for the survival of the organism.

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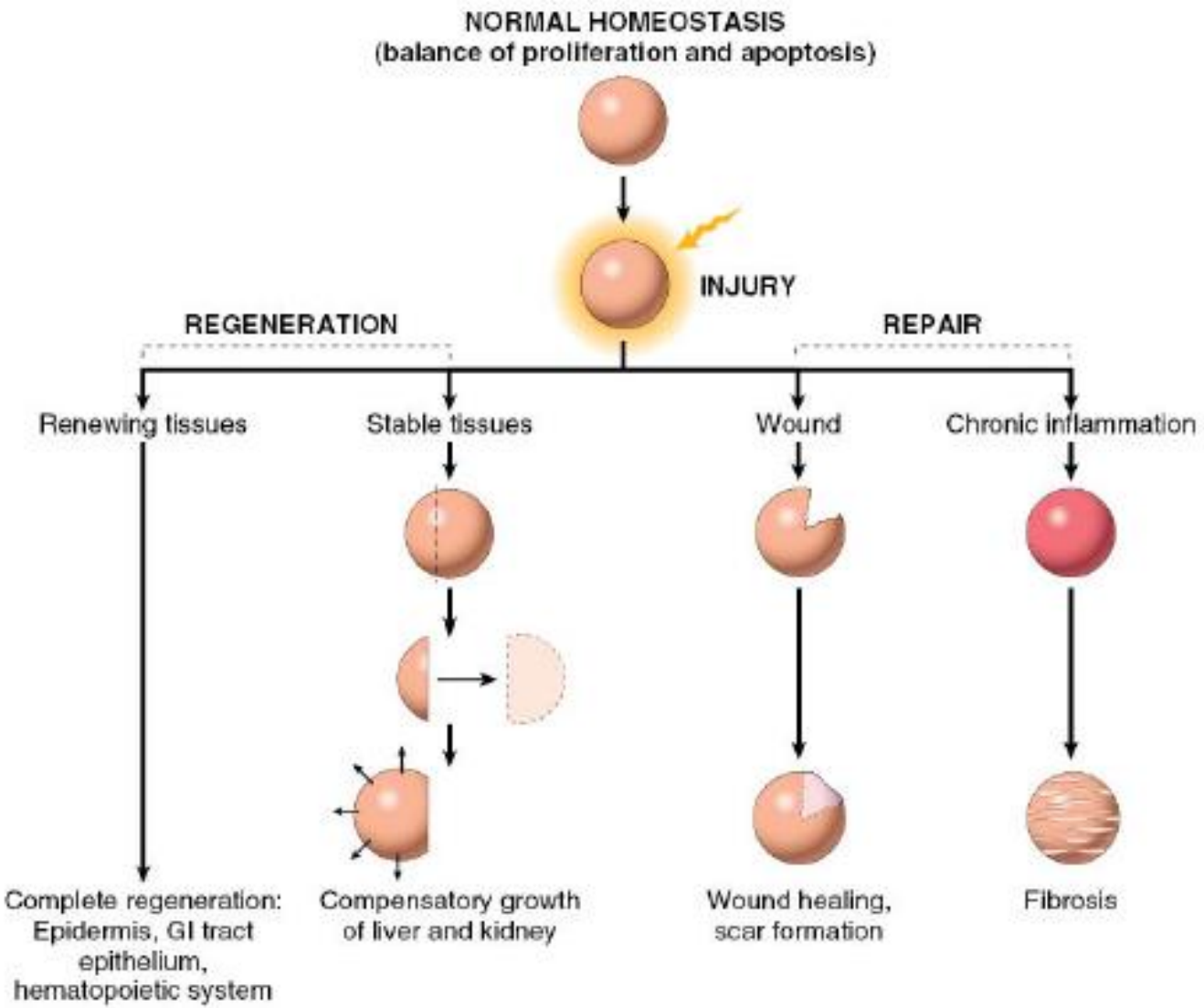


FIGURE 3–1 Overview of healing responses after injury. Healing after acute injury can occur by regeneration that restores normal tissue structure or by repair with scar formation. Healing in chronic injury involves scar formation and fibrosis. GI, gastrointestinal.

Regeneration

In mammals, whole organs and complex tissues rarely regenerate after injury, and the term is usually applied to processes such as liver growth after partial resection or necrosis, but these processes consist of compensatory growth rather than true regeneration.

Tissues with high proliferative capacity, such as the hematopoietic system and the epithelia of the skin and gastrointestinal (GI) tract, renew themselves continuously and can regenerate after injury, as long as the stem cells of these tissues are not destroyed.

Repair

Most often consists of a combination of regeneration and scar formation by the deposition of collagen.

Scar formation is the predominant healing process that occurs when the extracellular matrix (ECM) framework is damaged by severe injury (Fig. 3-2).

Chronic inflammation that accompanies persistent injury also stimulates scar formation because of local production of growth factors and cytokines that promote fibroblast proliferation and collagen synthesis.

The term fibrosis is used to describe the extensive deposition of collagen that occurs under these situations.

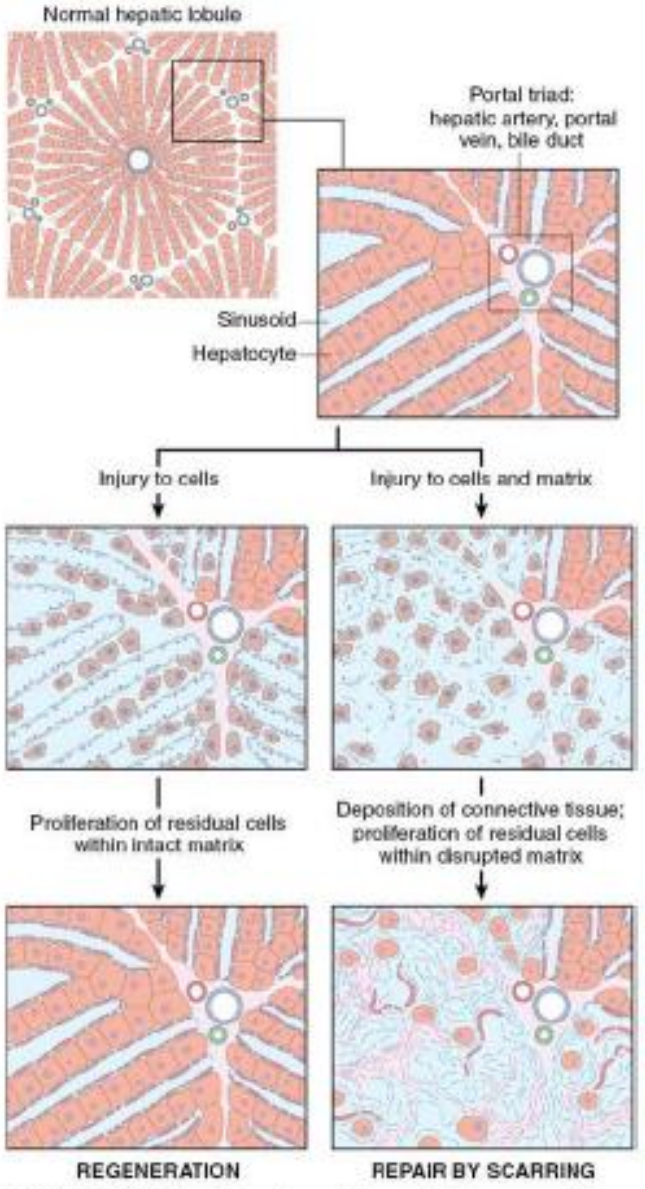
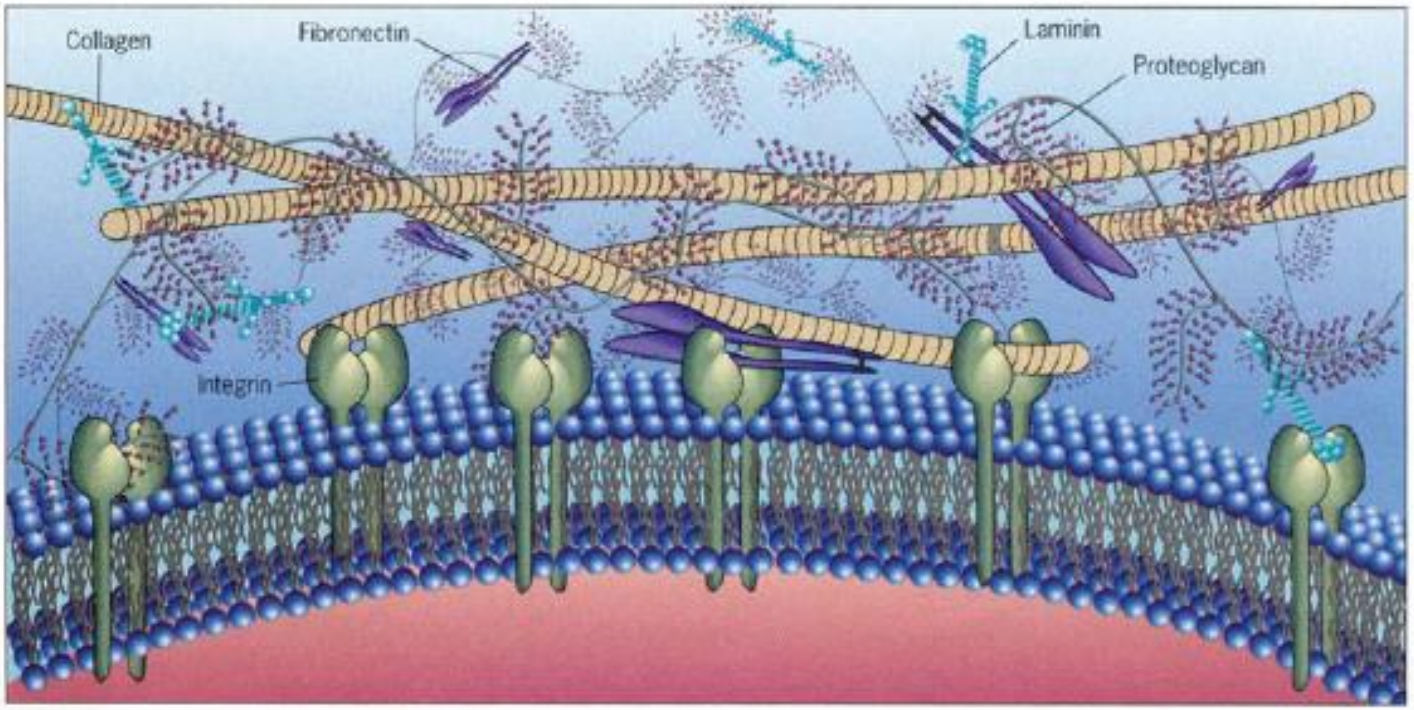


FIGURE 3–2 Role of the extracellular matrix in regeneration and repair. Liver regeneration with restoration of normal tissue after injury requires an intact cellular matrix. If the matrix is damaged the injury is repaired by fibrous tissue deposition and scar formation.

ECM components are essential for wound healing, because they provide the framework for cell migration, maintain the correct cell polarity for the re-assembly of multilayer structures, and participate in the formation of new blood vessels (angiogenesis).

Cells in the ECM (fibroblasts, macrophages, and other cell types) produce growth factors, cytokines, and chemokines that are critical for regeneration and repair. Although repair is a healing process, it may itself cause tissue dysfunction, as, for instance, in the development of atherosclerosis.



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Control of Normal Cell Proliferation and Tissue Growth

In adult tissues the size of cell populations is determined by the rates of cell proliferation, differentiation, and death by apoptosis (Fig. 3-3), and increased cell numbers may result from either increased proliferation or decreased cell death.

Differentiated cells incapable of replication are referred to as terminally differentiated cells.

The impact of differentiation depends on the tissue under which it occurs: in some tissues differentiated cells are not replaced, while in others they die but are continuously replaced by new cells generated from stem cells.

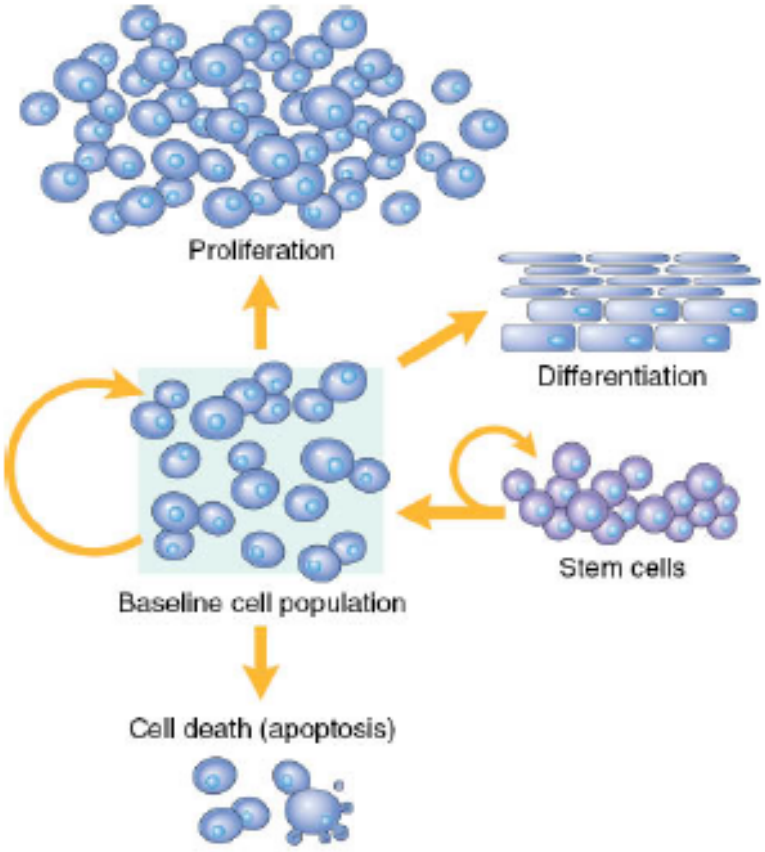


FIGURE 3–3 Mechanisms regulating cell populations. Cell numbers can be altered by increased or decreased rates of stem cell input, cell death due to apoptosis, or changes in the rates of proliferation or differentiation.

(Modified from McCarthy NJ et al: Apoptosis in the development of the immune system: growth factors, clonal selection and bcl-2. Cancer Metastasis Rev 11:157, 1992.)

Cell proliferation is largely controlled by signals (soluble or contact-dependent) from the microenvironment that either stimulate or inhibit proliferation. An excess of stimulators or a deficiency of inhibitors leads to net growth and, in the case of cancer, uncontrolled growth.

The tissues of the body are divided into three groups on the basis of the proliferative activity of their cells:

continuously dividing (labile tissues): Such as surface epithelium

quiescent (stable tissues): Such as liver

Non-dividing (permanent tissues): Such as neurons

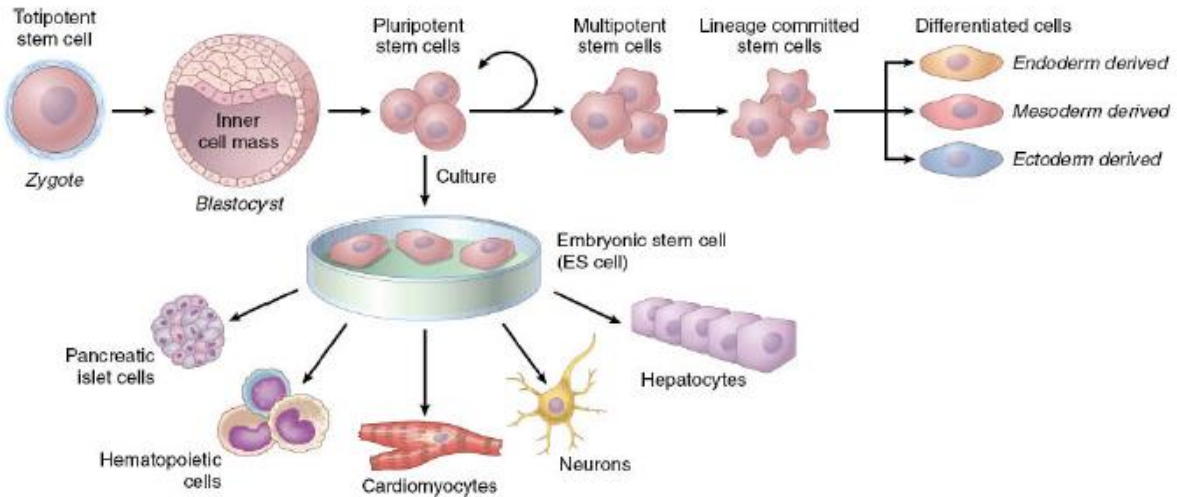
Stem cells are characterized by their self-renewal properties and by their capacity to generate differentiated cell lineages (Fig. 3-4).

To give rise to these lineages, stem cells need to be maintained during the life of the organism.

Such maintenance is achieved by two mechanisms:

(a) obligatory asymmetric replication, in which with each stem cell division, one of the daughter cells retains its self-renewing capacity while the other enters a differentiation pathway, and

(b) stochastic differentiation, in which a stem cell population is maintained by the balance between stem cell divisions that generate either two self-renewing stem cells or two cells that will differentiate.



In adults, stem cells (often referred to as adult stem cells or somatic stem cells) with a more restricted capacity to generate different cell types have been identified in many tissues.

Somatic stem cells for the most part reside in special microenvironments called niches (Fig. 3-5), composed of mesenchymal, endothelial, and other cell types.

It is believed that niche cells generate or transmit stimuli that regulate stem cell self-renewal and the generation of progeny cells.

Recent groundbreaking research has now demonstrated that differentiated cells of rodents and humans can be reprogrammed into pluripotent cells, similar to ES cells, by the transduction of genes encoding ES cell transcription factors. These reprogrammed cells have been named induced pluripotent stem cells (iPS cells).

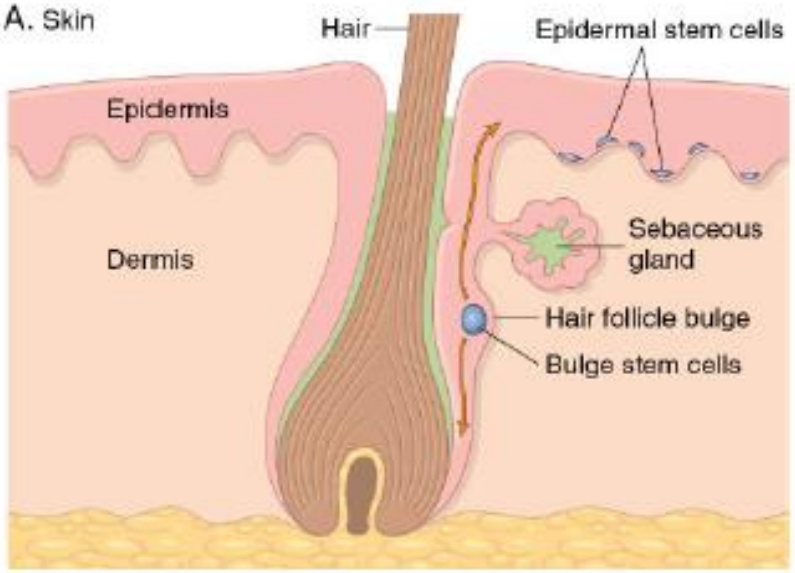


FIGURE 3–5A Stem cell niches in various tissues. **A,** Skin stem cells are located in the bulge area of the hair follicle, in sebaceous glands, and in the lower layer of the epidermis. **B,** Small intestine stem cells located near the base of a crypt, above Paneth cells (stem cells in the small intestine may also be located at the bottom of the crypt). **C,** Liver stem (progenitor) cells, known as oval cells, are located in the canals of Hering *(thick arrow)*, structures that connect bile ductules *(thin arrow)* with parenchymal hepatocytes (bile duct and Hering canals are stained for cytokeratin 7). **D,** Corneal stem cells are located in the limbus region, between the conjunctiva and the cornea.

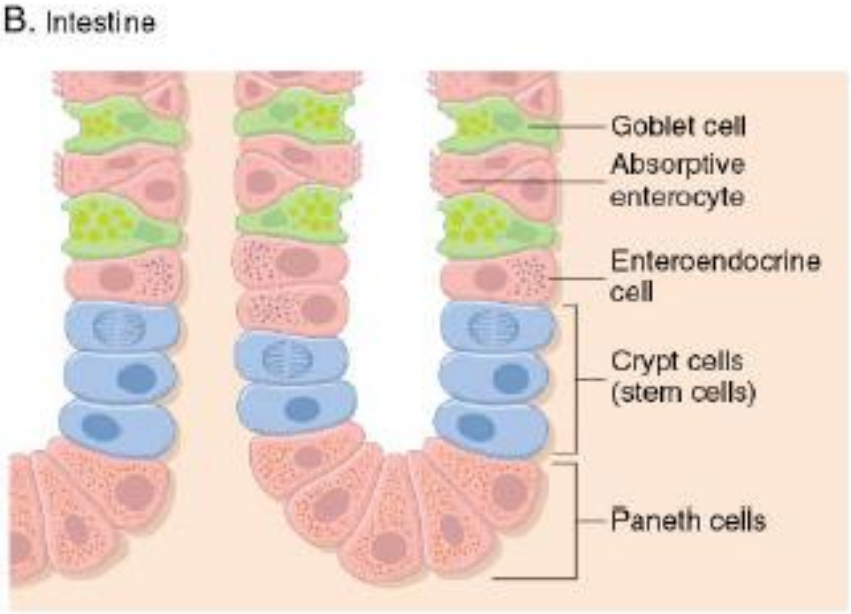


FIGURE 3–5B Stem cell niches in various tissues. **A,** Skin stem cells are located in the bulge area of the hair follicle, in sebaceous glands, and in the lower layer of the epidermis. **B,** Small intestine stem cells located near the base of a crypt, above Paneth cells (stem cells in the small intestine may also be located at the bottom of the crypt). **C,** Liver stem (progenitor) cells, known as oval cells, are located in the canals of Hering *(thick arrow)*, structures that connect bile ductules *(thin arrow)* with parenchymal hepatocytes (bile duct and Hering canals are stained for cytokeratin 7). **D,** Corneal stem cells are located in the limbus region, between the conjunctiva and the cornea.

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Healing by Repair, Scar Formation and Fibrosis

If tissue injury is severe or chronic, and results in damage of both parenchymal cells and the stromal framework of the tissue, healing can not be accomplished by regeneration.

Under these conditions, the main healing process is repair by deposition of collagen and other ECM components, causing the formation of a scar.

In contrast to regeneration which involves the restitution of tissue components, repair is a fibroproliferative response that "patches" rather than restores the tissue.

The term scar is most often used in connection to wound healing in the skin, but is also used to describe the replacement of parenchymal cells in any tissue by collagen, as in the heart after myocardial infarction. Repair by connective tissue deposition includes the following basic features:

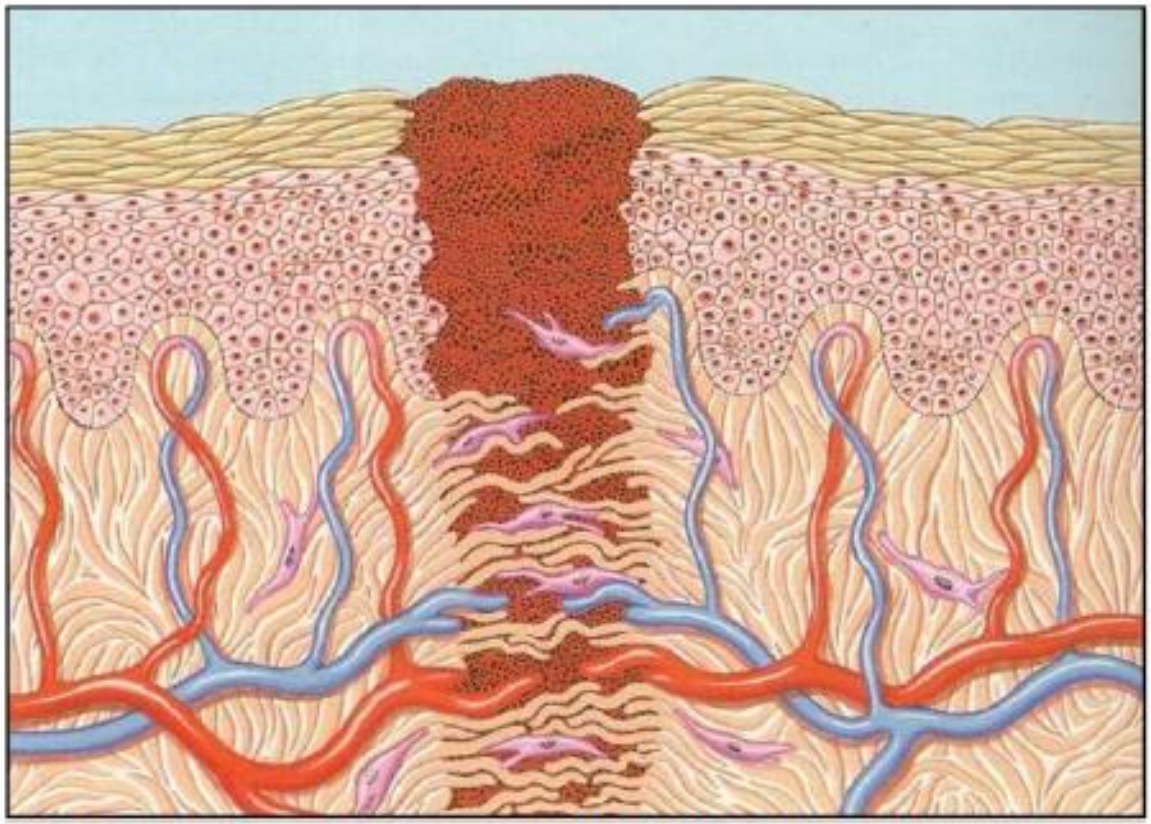
- inflammation
- angiogenesis,
- migration and proliferation of fibroblasts,
- scar formation
- connective tissue remodeling

The relative contributions of repair and regeneration are influenced by:

(1) the proliferative capacity of the cells of the tissue

(2) the integrity of the extracellular matrix

(3) the resolution or chronicity of the injury and inflammation



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Angiogenesis is a fundamental process that affects physiologic reactions (e.g. wound healing, regeneration, the vascularization of ischemic tissues, and menstruation), and pathologic processes, such as tumor development and metastasis, diabetic retinopathy, and chronic inflammation.

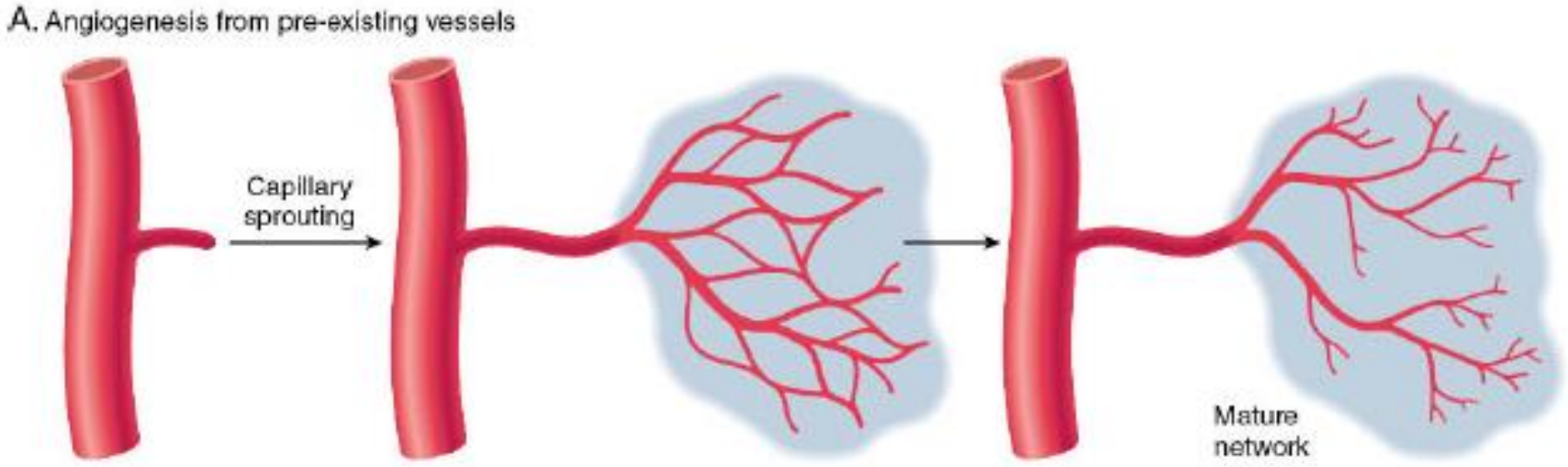


FIGURE 3–15A Angiogenesis by mobilization of endothelial precursor cells (EPCs) from the bone marrow and from preexisting vessels (capillary growth). **A**, In angiogenesis from preexisting vessels, endothelial cells from these vessels become motile and proliferate to form capillary sprouts. Regardless of the initiating mechanism, vessel maturation (stabilization) involves the recruitment of pericytes and smooth muscle cells to form the periendothelial layer. **B**, EPCs are mobilized from the bone marrow and may migrate to a site of injury or tumor growth. At these sites, EPCs differentiate and form a mature network by linking to existing vessels.

(Modified from Conway EM et al: Molecular mechanisms of blood vessel growth. Cardiovasc Res 49:507, 2001.)

Angiogenesis from Preexisting Vessels.

In this type of angiogenesis there is vasodilation and increased permeability of the existing vessels, degradation of ECM, and migration of endothelial cells. The major steps are listed below.

Vasodilation in response to nitric oxide, and VEGF-induced increased permeability of the preexisting vessel

Proteolytic degradation of the basement membrane of the parent vessel by matrix metalloproteinases (MMPs) and disruption of cell-to-cell contact between endothelial cells by plasminogen activator

Migration of endothelial cells toward the angiogenic stimulus

Proliferation of endothelial cells, just behind the leading front of migrating cells

Maturation of endothelial cells, which includes inhibition of growth and remodeling into capillary tubes

Recruitment of periendothelial cells (pericytes and vascular smooth muscle cells) to form the mature vessel

Angiogenesis from Endothelial Precursor Cells (EPCs)

EPCs can be recruited from the bone marrow into tissues to initiate angiogenesis (Fig. 3-15). The nature of the homing mechanism is uncertain.

The number of circulating EPCs increases greatly in patients with ischemic conditions, suggesting that EPCs may influence vascular function and determine the risk of cardiovascular diseases.

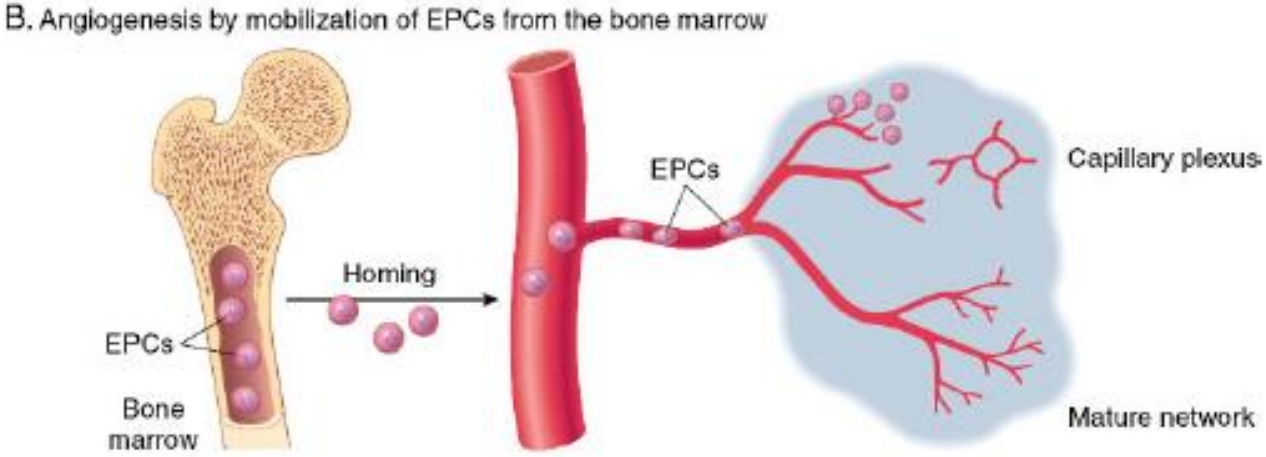


FIGURE 3–15B Angiogenesis by mobilization of endothelial precursor cells (EPCs) from the bone marrow and from preexisting vessels (capillary growth). **A,** In angiogenesis from preexisting vessels, endothelial cells from these vessels become motile and proliferate to form capillary sprouts. Regardless of the initiating mechanism, vessel maturation (stabilization) involves the recruitment of pericytes and smooth muscle cells to form the periendothelial layer. **B,** EPCs are mobilized from the bone marrow and may migrate to a site of injury or tumor growth. At these sites, EPCs differentiate and form a mature network by linking to existing vessels.

(Modified from Conway EM et al: Molecular mechanisms of blood vessel growth. Cardiovasc Res 49:507, 2001.)

Growth Factor-mediated Proliferation

- Platelet Derived Growth Factor (PDGF)
  - promotes the chemotactic migration of fibroblasts and smooth muscles
  - chemotactic for monocytes
  - competence factor that promotes the proliferative response of fibroblasts and smooth muscles upon concurrent stimulation with progression factors
- Epidermal Growth Factor (EGF)
  - promotes growth for fibroblasts, endothelial and epithelial cells
  - is a progession factor - promotes cell-cycle progression.
- Fibroblast Growth Factor (FGF)
  - promote synthesis of fibronectin and other extracellular matrix proteins
  - chemotactic for fibroblast and endothelial cells
  - promotes angiogenesis
  - links extracellular matrix components (collagen, proteoglycans) and macromulocules (fibrin, heparin) to cell-surface integrins.
- Transforming Growth Factors (TGFs)
  - TGF-α - similar to EGF
  - TGF-β - mitosis inhibitor that aids in modulating the repair process. May be responsible for hypertrophy by preventing cell division. Chemotactic for macropahges and fibroblasts
- Macrophage-derived cytokines (IL-1 and TNF)
  - promote proliferation of fibroblasts, smooth muscle and endothelial cells

VEGF is the most important growth factor in adult tissues undergoing physiologic angiogenesis (e.g., proliferating endometrium) as well as angiogenesis occurring in chronic inflammation, wound healing, tumors, and diabetic retinopathy.

ECM Proteins as Regulators of Angiogenesis

A key component of angiogenesis is the motility and directed migration of endothelial cells, required for the formation of new blood vessels. These processes are controlled by several classes of proteins, including:

(1) integrins, especially αvβ3, which is critical for the formation and maintenance of newly formed blood vessels

(2) matricellular proteins, including thrombospondin 1, SPARC, and tenascin C, which destabilize cell-matrix interactions and therefore promote angiogenesis

(3) proteinases, such as the plasminogen activators and MMPs, which are important in tissue remodeling during endothelial invasion. Additionally, these proteinases cleave extracellular proteins, releasing matrix-bound growth factors such as VEGF and FGF-2 that stimulate angiogenesis. Proteinases can also release inhibitors such as endostatin, a small fragment of collagen that inhibits endothelial proliferation and angiogenesis. αVβ3 Integrin expression in endothelial cells is stimulated by hypoxia and has multiple effects on angiogenesis: it interacts with a metalloproteinase (MMP-2, discussed below), it binds to and regulates the activity of VEGFR-2, and it mediates adhesion to ECM components such as fibronectin, thrombospondin, and OPN.[72]

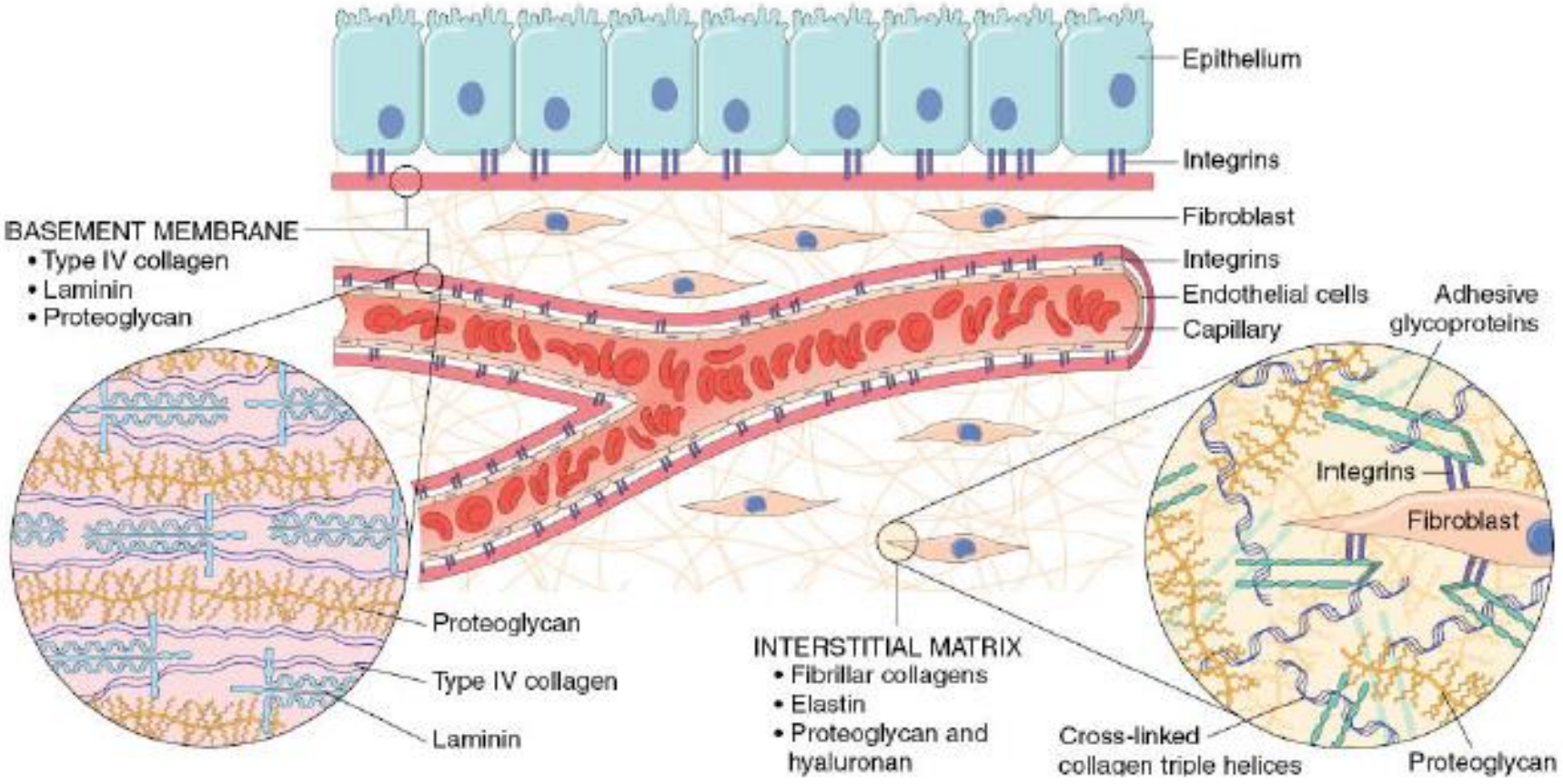
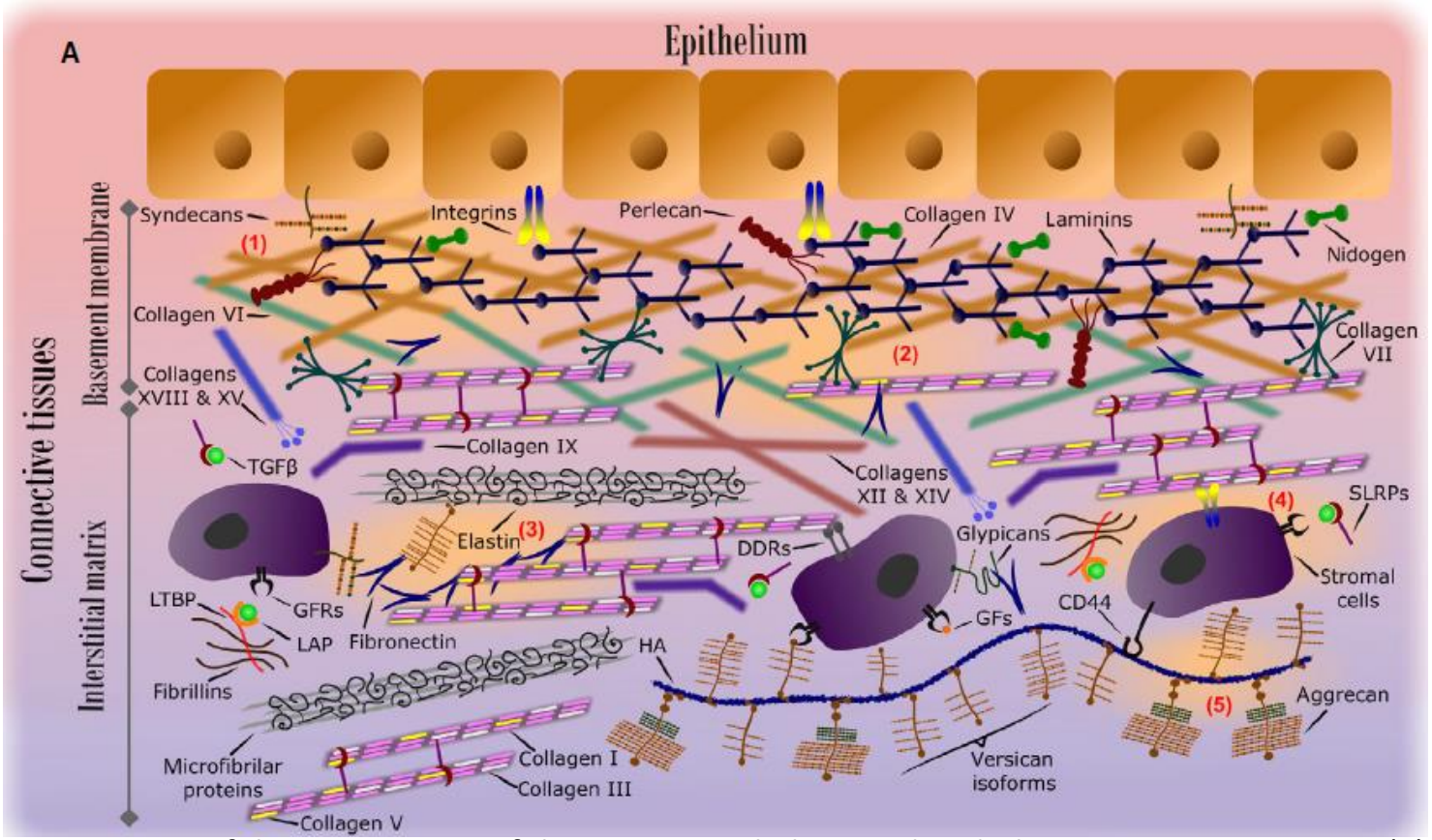


FIGURE 3–12 Main components of the extracellular matrix (ECM), including collagens, proteoglycans, and adhesive glycoproteins. Both epithelial and mesenchymal cells (e.g., fibroblasts) interact with ECM via integrins. Basement membranes and interstitial ECM have different architecture and general composition, although there is some overlap in their constituents. For the sake of simplification, many ECM components (e.g., elastin, fibrillin, hyaluronan, and syndecan) are not included.



Schematic representation of the organization of the ECM in epithelium and underlying connective tissues . (1) Basement membrane is a unique pericellular matrix, defined as an amalgam of two networks: the scaffold of self-associating laminins and the polymer-like network of collagen IV. Perlecan and nidogens further bridge these networks. Epithelial cells are connected to the basement membrane through hemidesmosomes, where laminins are bound to integrins. (2) Basement membrane anchors to interstitial matrix through a variety of collagen fibrils, including collagens VI and VII. The former interacts with collagen IV and perlecan, whereas the latter with collagen IV, laminins, and collagen I fibrils. (3) The main collagen type in the interstitial matrix are the heterotypic fibrils of collagens I, III, and V. SLRPs and fibronectin participate in collagen assembly and fibrillogenesis. Many other components contribute in interstitial matrix organization including elastin, proteoglycans and HA. (4) Cell surface receptors of stromal cells such as integrins, DDRs, syndecans, glypicans, and growth factor receptors (GFR) interact with ECM components and growth factors (GF). The bioavailability and binding of growth factors such as TGFb, is regulated by SLRPs and the LAP and LTBP. (5) HA forms large aggregates with hyalectans, contributing to the rigidity of the ECM. It also interacts with cell surface receptors, such as CD44.

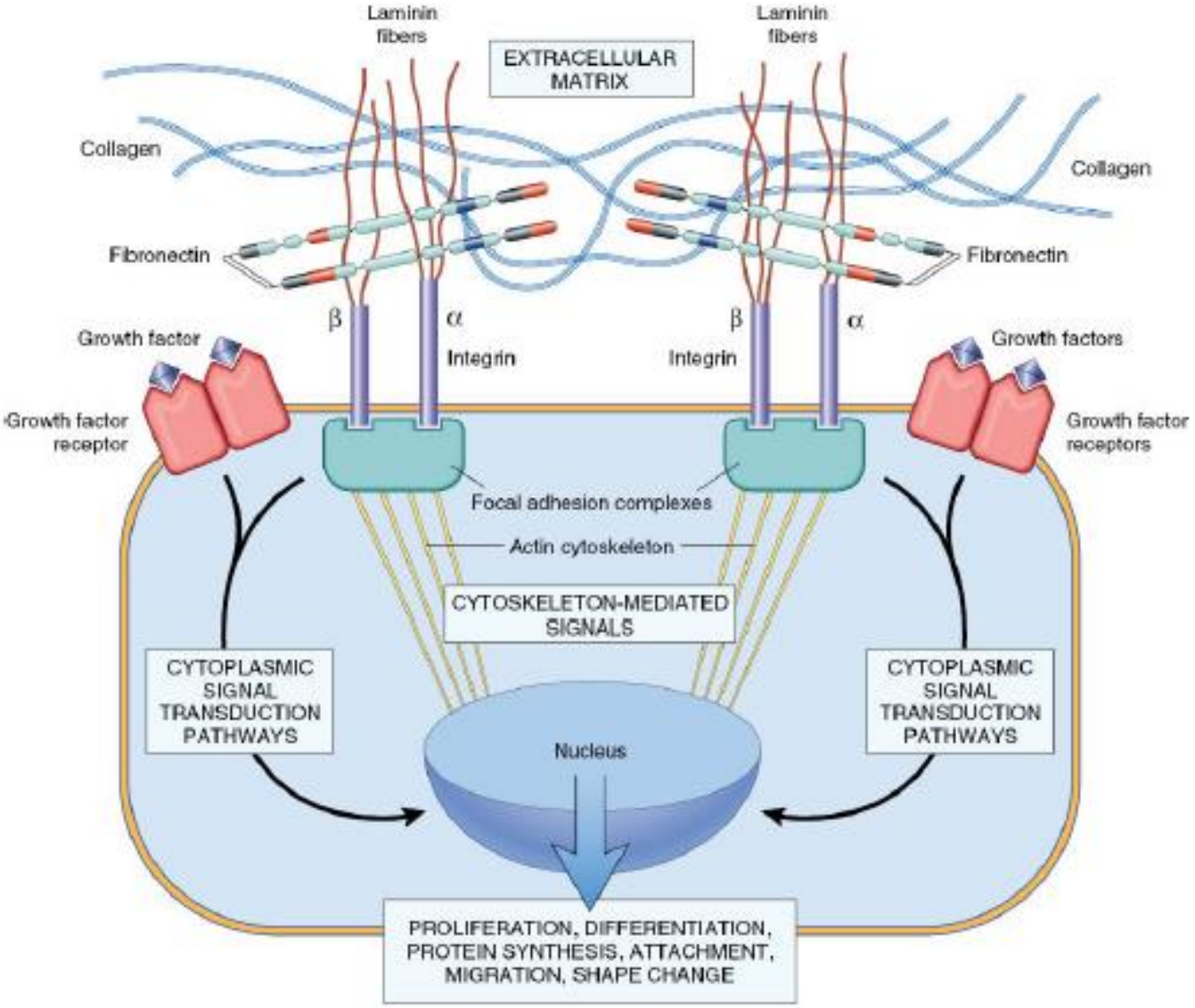


FIGURE 3–13 Mechanisms by which ECM components and growth factors interact and activate signaling pathways. Integrins bind ECM components and interact with the cytoskeleton at focal adhesion complexes (protein aggregates that include vinculin, α-actin, and talin). This can initiate the production of intracellular messengers or can directly mediate nuclear signals. Cell surface receptors for growth factors may activate signal transduction pathways that overlap with those activated by integrins. Signaling from ECM components and growth factors is integrated by the cell to produce various responses, including changes in cell proliferation, locomotion, and differentiation.

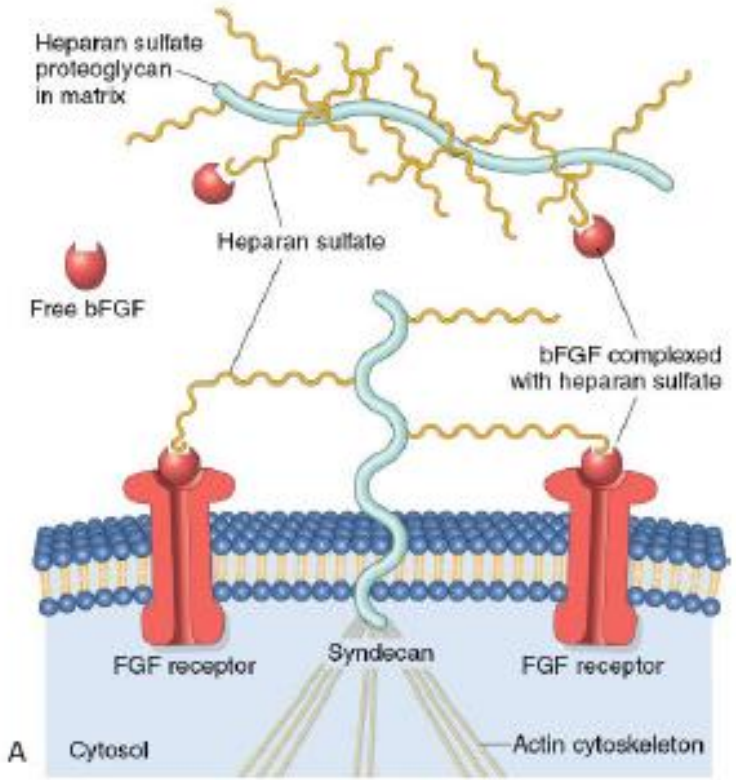


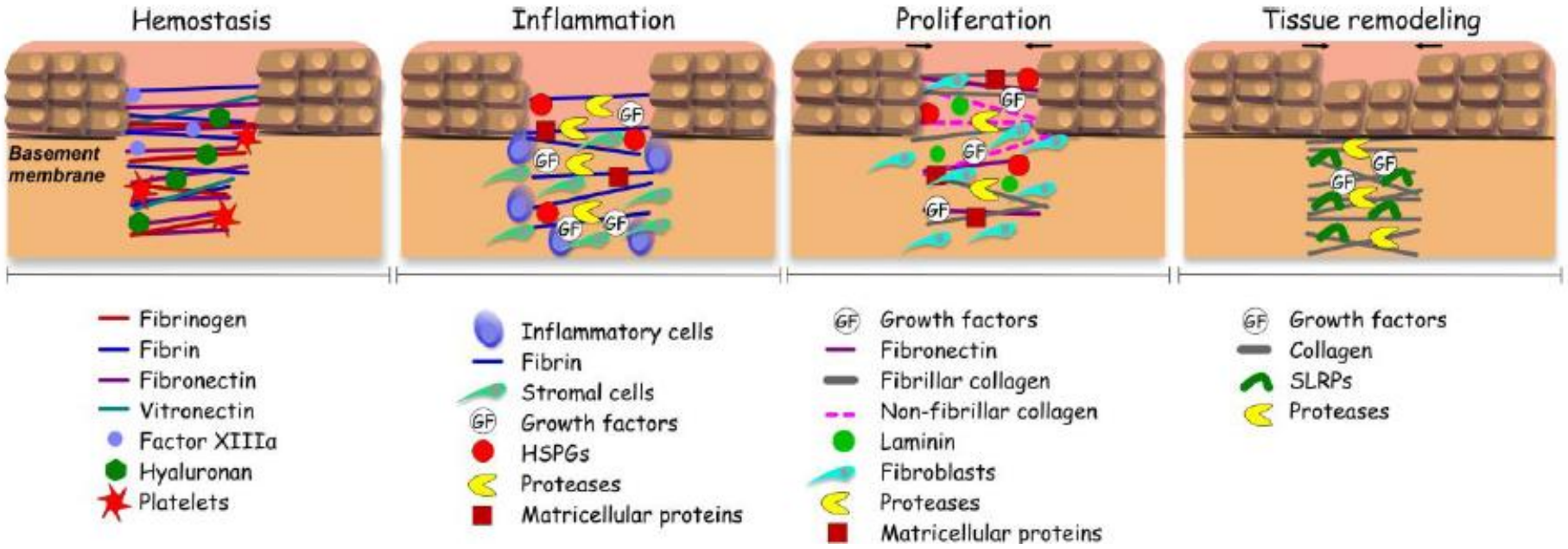
FIGURE 3–14A Proteoglycans, glycosaminoglycans (GAGs), and hyaluronan. **A,** Regulation of FGF-2 activity by ECM and cellular proteoglycans. Heparan sulfate binds FGF-2 (basic FGF) secreted into the ECM. Syndecan is a cell surface proteoglycan with a transmembrane core protein, extracellular GAG side chains that can bind FGF-2, and a cytoplasmic tail that binds to the actin cytoskeleton. Syndecan side chains bind FGF-2 released by damage to the ECM and facilitate the interaction with cell surface receptors. **B,** Synthesis of hyaluronan at the inner surface of the plasma membrane. The molecule extends to the extracellular space, while still attached to hyaluronan synthase. **C,** Hyaluronan chains in the extracellular space are bound to the plasma membrane through the CD44 receptor. Multiple proteoglycans may attach to hyaluronan chains in the ECM.

CUTANEOUS WOUND HEALING

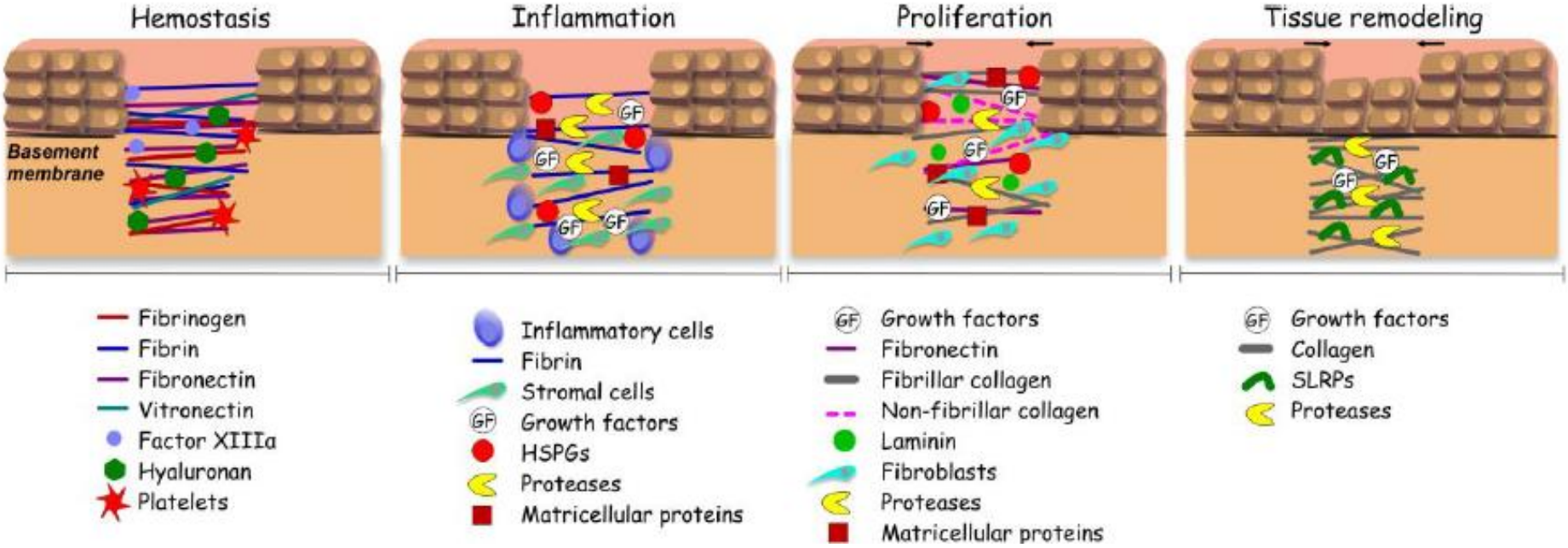
Cutaneous wound healing is divided into three phases: inflammation, proliferation, and maturation (Fig. 3-18).

These phases overlap, and their separation is somewhat arbitrary, but they help to understand the sequence of events that take place in the healing of skin wounds. The initial injury causes platelet adhesion and aggregation and the formation of a clot in the surface of the wound, leading to inflammation.

In the proliferative phase there is formation of granulation tissue, proliferation and migration of connective tissue cells, and re-epithelialization of the wound surface. Maturation involves ECM deposition, tissue remodeling, and wound contraction

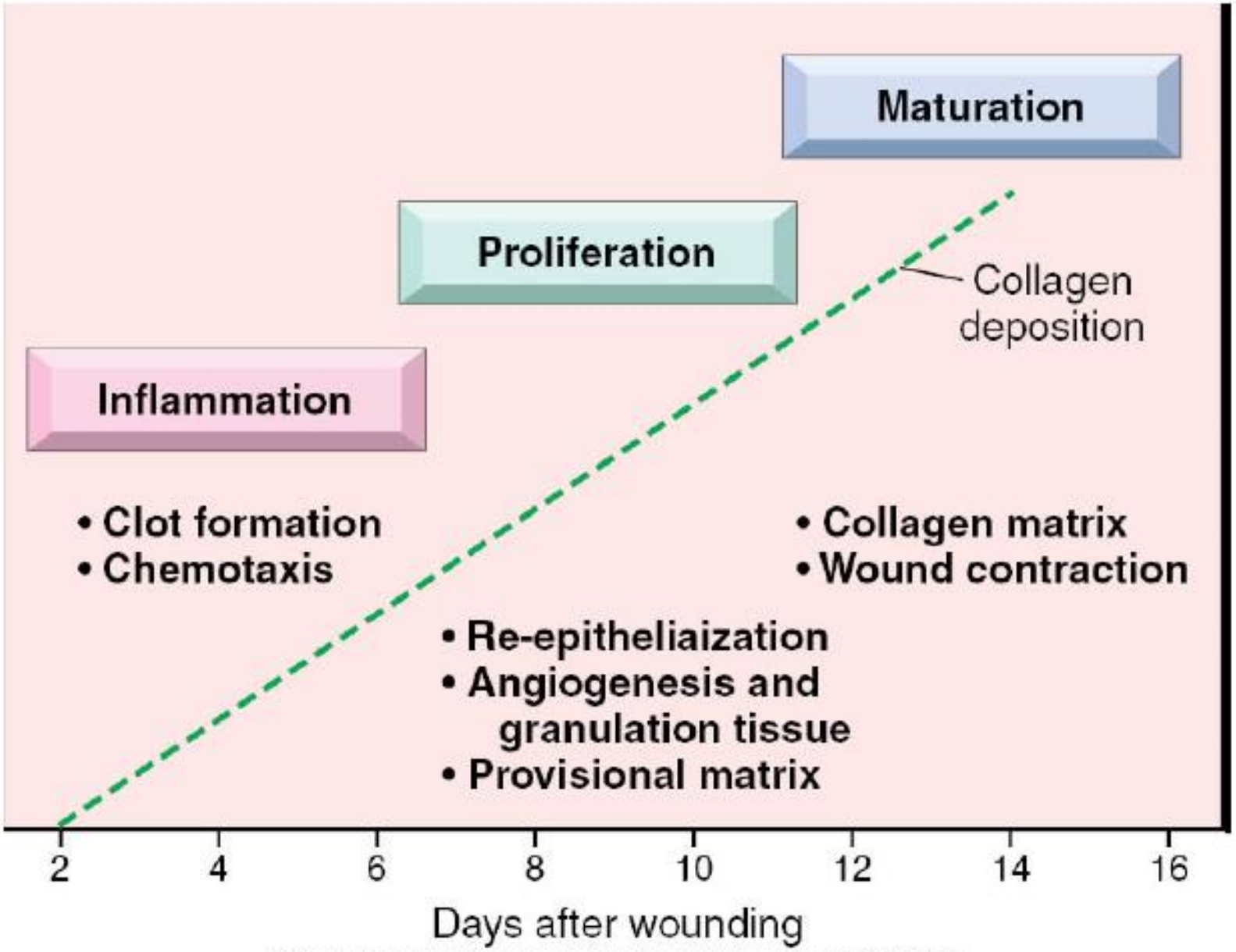


Schematic representation of wound healing phases. In each phase, the participation of ECM proteins is essential and its composition, mainly in the connective tissue, alters the hemostatic, inflammatory, proliferative and tissue remodeling phases of healing.
Fibrin, fibronectin, platelets and HA bound to fibrinogen, vitronectin, factor XIIIα and other clotting proteins are major constituents during the hemostatic phase of the wound.
As leukocytes migrate into the wound site, they release proteases to degrade the fibrin-rich ECM. Immune cells secrete a broad range of inflammatory cytokines and growth factors to attract stromal cells to migrate into the wound. Several matrix-stored growth factors (i.e. PDGF, EGF, TGF-β) and PGs released and secreted from endothelial cells (i.e. syndecans, decorin, lumican) are also displaced.



In the next phase, stromal fibroblasts migrating into the wound area (arrows) produce a provisional ECM characterized by the presence of fibronectin, matricellular proteins, decreased protease activity, fibrillar (type I, III) and non-fibrillar (type IV, VI, VII) collagen, and growth factors (i.e. EGF, FGF, TGF-β). The secreted matrix PGs (i.e. decorin, lumican) and collagen that are associated with mature healing wounds are secreted by keratinocytes and leukocytes to promote proliferation and migration of vascular components, endothelial and epithelial cells.

During the tissue remodeling phase, the stiffer matrix and several growth factors (i.e. TGF-β) trigger stromal cells to induce wound contraction by adopting a myofibroblast phenotype. The supportive ECM is enriched in matrix PGs, proteases and realigned collagen type I cross-linked fibers. The final step in wound repair involves the formation of overly aligned collagen fibers that regain almost 80% of the primary tissue functionality.



(Modified from Broughton G et al. The basic science of wound healing. Plast Reconstr Surg 117:12S–34S, 2006.)

Stages of Normal Cutaneous Wound Healing

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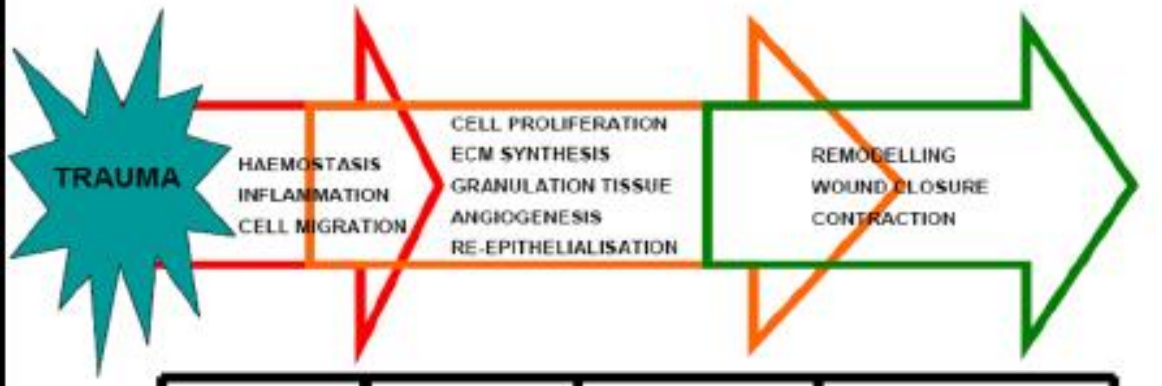
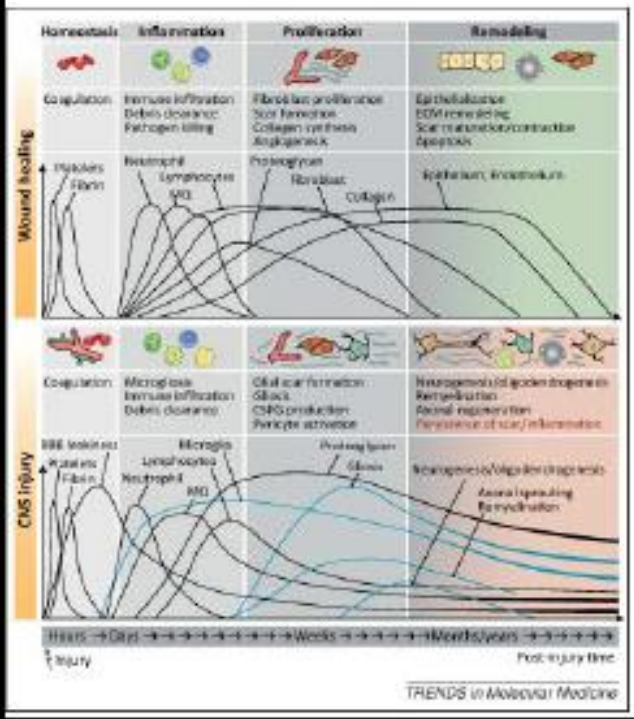


CELL TYPE			
	PLATELETS NEUTROPHILS LYMPHOCTYES MACROPHAGES EPCs	KERATINOCYTES ENDOTHELIAL CELLS FIBROBLASTS MACROPHAGES EPCS	MYOFIBROBLASTS MACROPHAGES



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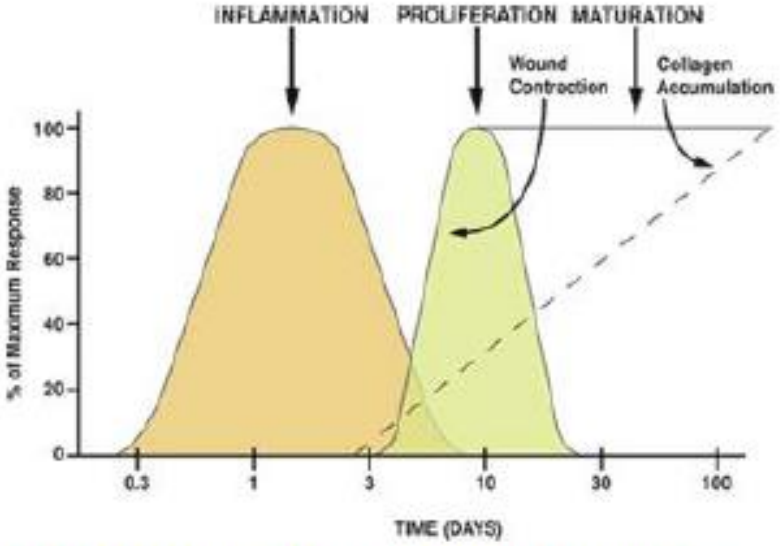


http://www.clinimed.co.uk/Wound-Care/Education/Wound-Essentials/Phases-of-Wound-Healing.aspx

Repair Process

- Removal of Debris
  - begins early and initiated by liquefaction and removal of dead cells and other debris
- Formation of Granulation Tissues
  - connective tissue consisting of capillaries and fibroblasts that fills the tissue defect created by removal of debris
- Scarring
  - fibroblasts produce collagen until granulation tissue becomes less vascular and less cellular
  - progessive contraction of the wound occurs, resulting in deformity of original structure

Factors that Impede Repair

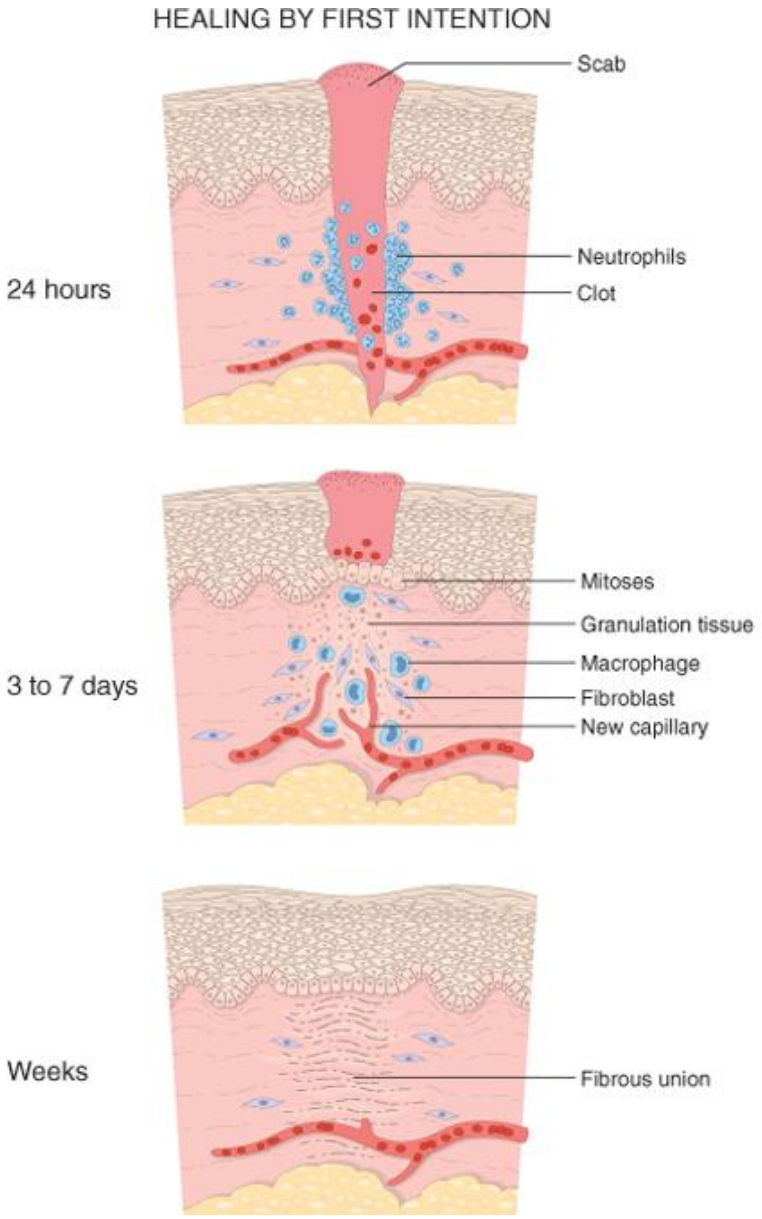
- Retention of debris or foreign body
- Impaired circulation
- Persistent infection
- Metabolic disorders
  - diabetes
- Dietary deficiency
  - ascorbic acid
  - protein

Healing and granulation

- Fibroplasia is a response to
  - Damaged connective tissue
  - Parenchymal damage exceeds regenerative capacity
- Hyperplasia of connective tissue
- Neovascularization
- Granulation
  - coordinated proliferation of fibroblasts with a rich bed of capillaries
  - intensely hyperemic with a roughened or granular, glistening surface
  - healthy granulation tissue resists secondary infections

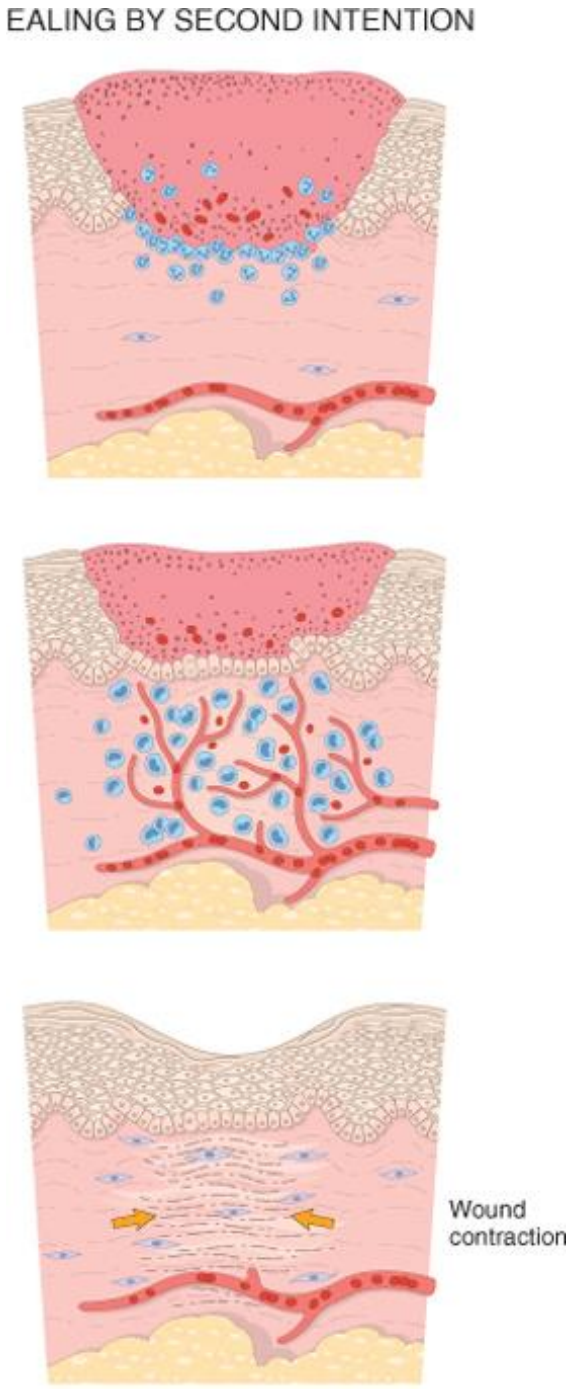
Healing by First Intention

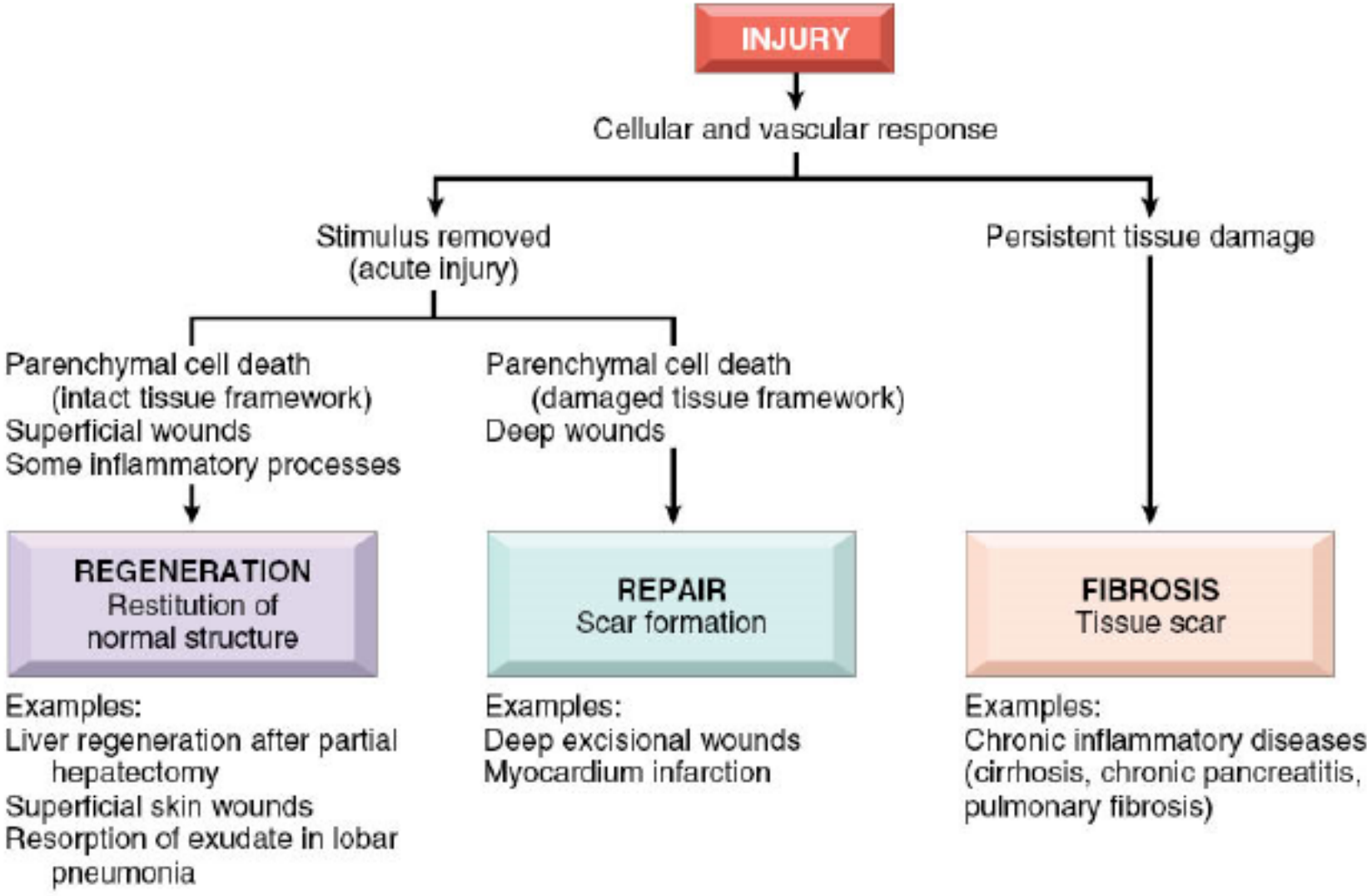
- Clean, surgical incision or other clean narrow cut
- Focal disruption of epithelial basement membrane with little cell damage
- Regeneration dominates fibrosis
- Scabbing with fibrin-clotted blood
- Neutrophils migrate to edges
- Epidermis becomes mitotic and deposits ECM
- Macrophages replace neutrophils
- Vascularization and collagen deposition fills gap
- Contraction of collagen minimizes epidermal regeneration



Healing by Second Intention

- Larger area of tissue injury such as abcess, ulcer, infarction that destroys ECM
- Large clot or scab with fibrin and fibronectin fills gap
- Larger volume of necrotic debris must be removed by more neutrophils and macrophages
  - Opportunity for collateral damage by phagocytes
- Scar tissue formed from vascular cells, fibroblasts, and myofibroblasts
- Contraction of myofibroblasts distorts tissue
- More prone to infection





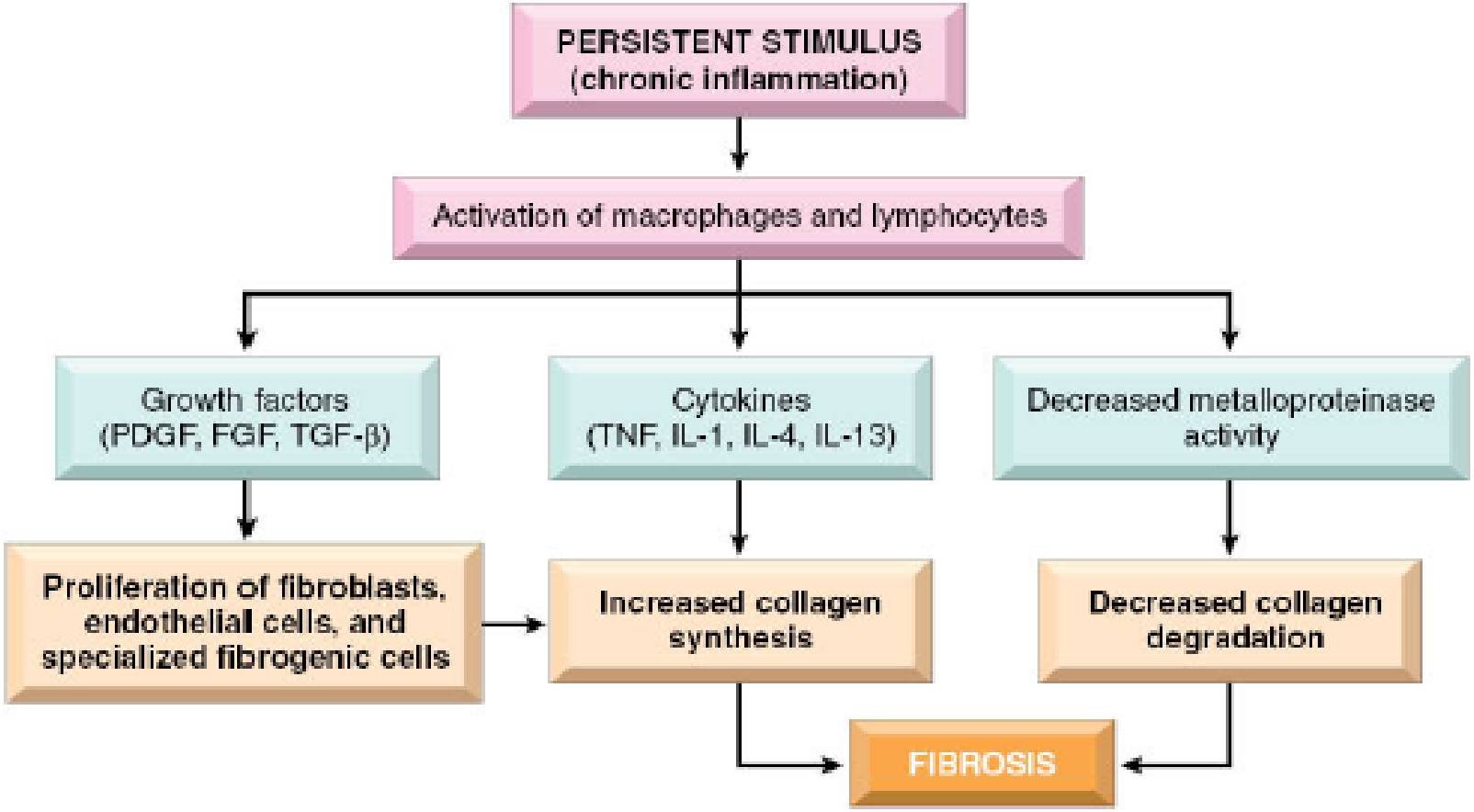
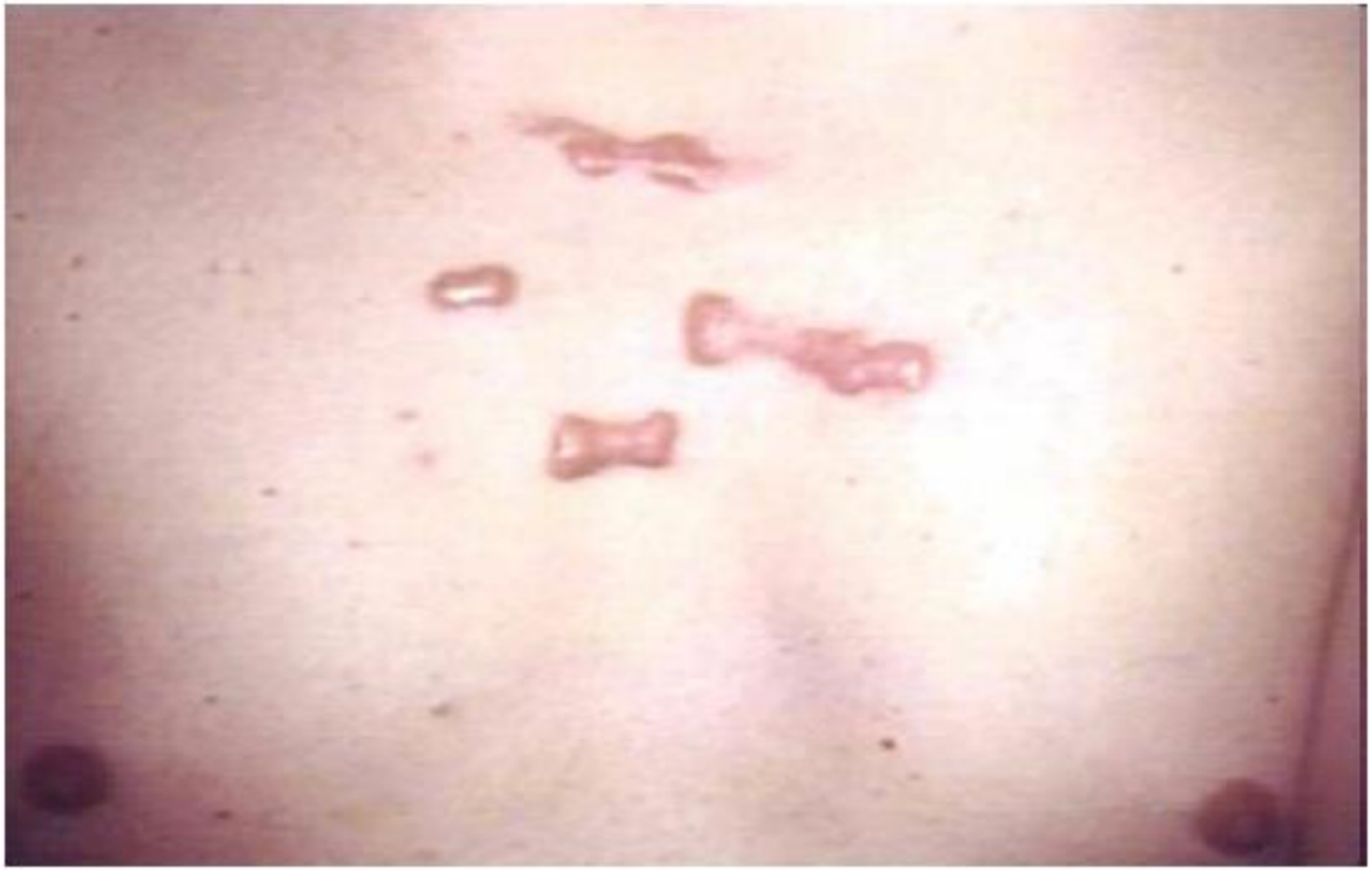
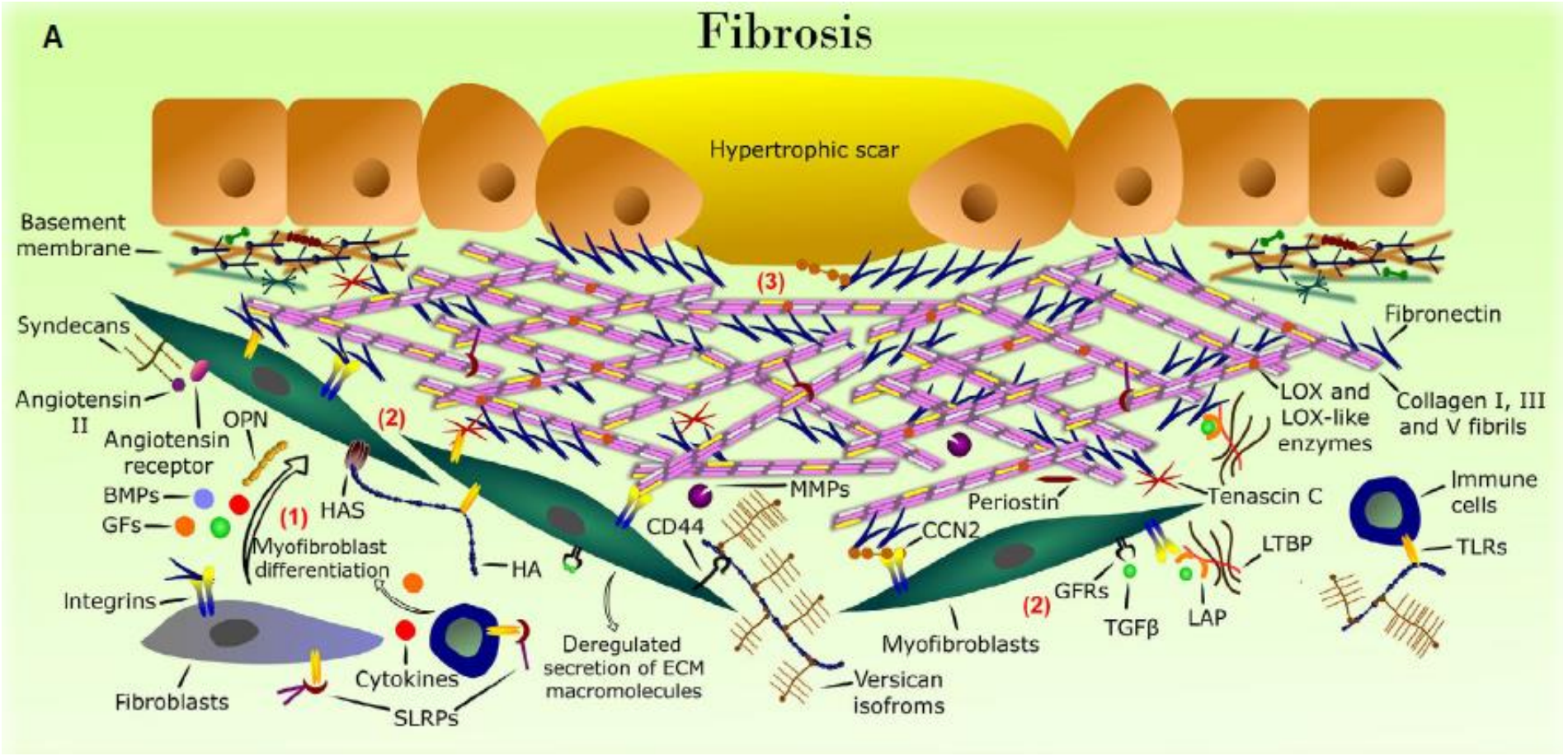


FIGURE 3–25 Development of fibrosis in chronic inflammation. The persistent stimulus of chronic inflammation activates macrophages and lymphocytes, leading to the production of growth factors and cytokines, which increase the synthesis of collagen. Deposition of collagen is enhanced by decreased activity of metalloproteinases.

Keloid—excessive cutaneous fibrosis





Schematic representation of ECM remodeling during fibrosis ((A) Abnormal tissue repair due to deregulated myofibroblasts results in a stiff and disorganized ECM. (1) Various persistent stimuli like growth factors (GFs) and cytokines from immune cells and injury-activated fibroblasts promote their differentiation to myofibroblasts. (2) Myofibroblasts secrete abnormal amounts of ECM macromolecules, whereas their interactions with the stiff matrix via cell surface receptors such as integrins further advance their activation and fibrous matrix accumulation. TLRs promote proinflammatory signaling via binding to fibronectin, TN C or HA. Integrins bind to matricellular proteins activating intracellular signals but also activate TGFb inducing potent profibrotic signaling. Syndecans also facilitate the profibrotic signaling of angiotensin II. (3) The fibrotic ECM is characterized by high amounts of heterotypic collagens I, III, and V fibrils, as well as fibronectin fibrils, versican and HA aggregates, matricellular proteins, and cross-linking enzymes, such as LOX and LOX-like enzymes.

Pro-fibrotic growth factors and cytokines at sites of tissue injury

TGFb1, PDGF, Wnts, BMPs, IL-4, and IL-17 have been shown to activate and maintain myofibroblasts contributing to progression of fibrosis

Anti-fibrotic growth factors and cytokines

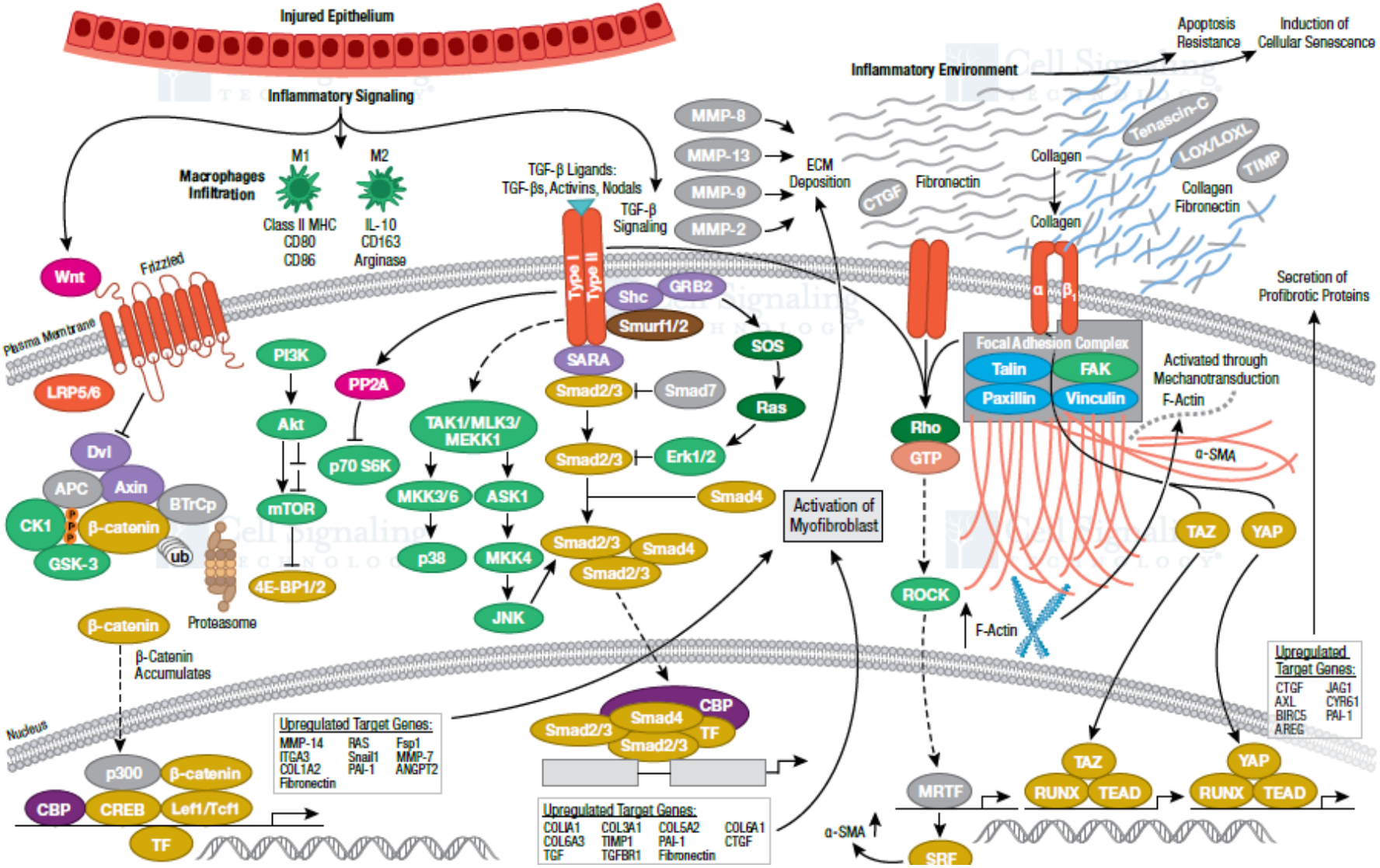
FGF2, interferon c, TGFb3 and IL-1 signaling suppresses myofibroblasts

Mechanical challenge is instructive for the progression of fibrosis for example by activating TGFb1 signaling

•Integrins such as avb1, avb3, avb5, avb6 and avb8 bind to RGD sequence of LAP of TGFb1 and TGFb3 and activate latent TGFb by applying cell-mediated mechanical forces on latent TGFb complexes and present TGFb on cell receptor

•Integrin avb8 activates latent TGFb by presenting this complex to MT1-MMP for proteolysis that leads to release of active TGFb1

Mechanisms of Fibrosis



In response to tissue damage, myofibroblasts—derived from a number of sources including resident fibroblasts, mesenchymal cells, circulating fibrocytes, and the transdifferentiation of other cell types—initiate a wound healing response by remodeling the extracellular environment to restore tissue integrity and promote the replacement of parenchymal cells. Normally, this pro-fibrotic program is turned off as the tissue heals. However, persistent insult and injury results in dysregulation of this process, leading to pathologically excessive deposition of ECM proteins and, in concert with upregulated myofibroblast activity, creates a chronic inflammatory environment with macrophage and immune cell infiltration. In this cellular milieu, cytokines and growth factors are abundantly released, including transforming growth factor-beta (TGF-β) family members and Wingless/Int-1 (Wnt1) which act as the principal effectors of the fibrotic process. TGF-β and Wnt1 bind to their cognate cell surface receptors and initiate downstream signaling—ultimately leading to the nuclear translocation of Smad2/3 and CBP/β-Catenin transcriptional modulators, respectively. This results in the upregulated expression of target genes that function to further enhance myofibroblast differentiation and the production and secretion of ECM proteins including collagen, laminin, and fibronectin.

As excessive ECM deposition progresses, the structure of the matrix alters and becomes stiff. ECM tension is sensed by cells through mechanotransduction via cell surface integrin receptors which activate the Hippo signaling pathway and its primary downstream effectors YAP and TAZ. In yet another feed forward loop, activated YAP and TAZ translocate to the nucleus and contribute to the upregulation of profibrotic genes—including CTGF and PDGF—which promote myofibroblast proliferation and activation via the PI3K/AKT/mTOR pathway.