WOUND HEALING

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- **Wound repair** is the effort of injured tissues **to restore their normal function and structural integrity after injury**

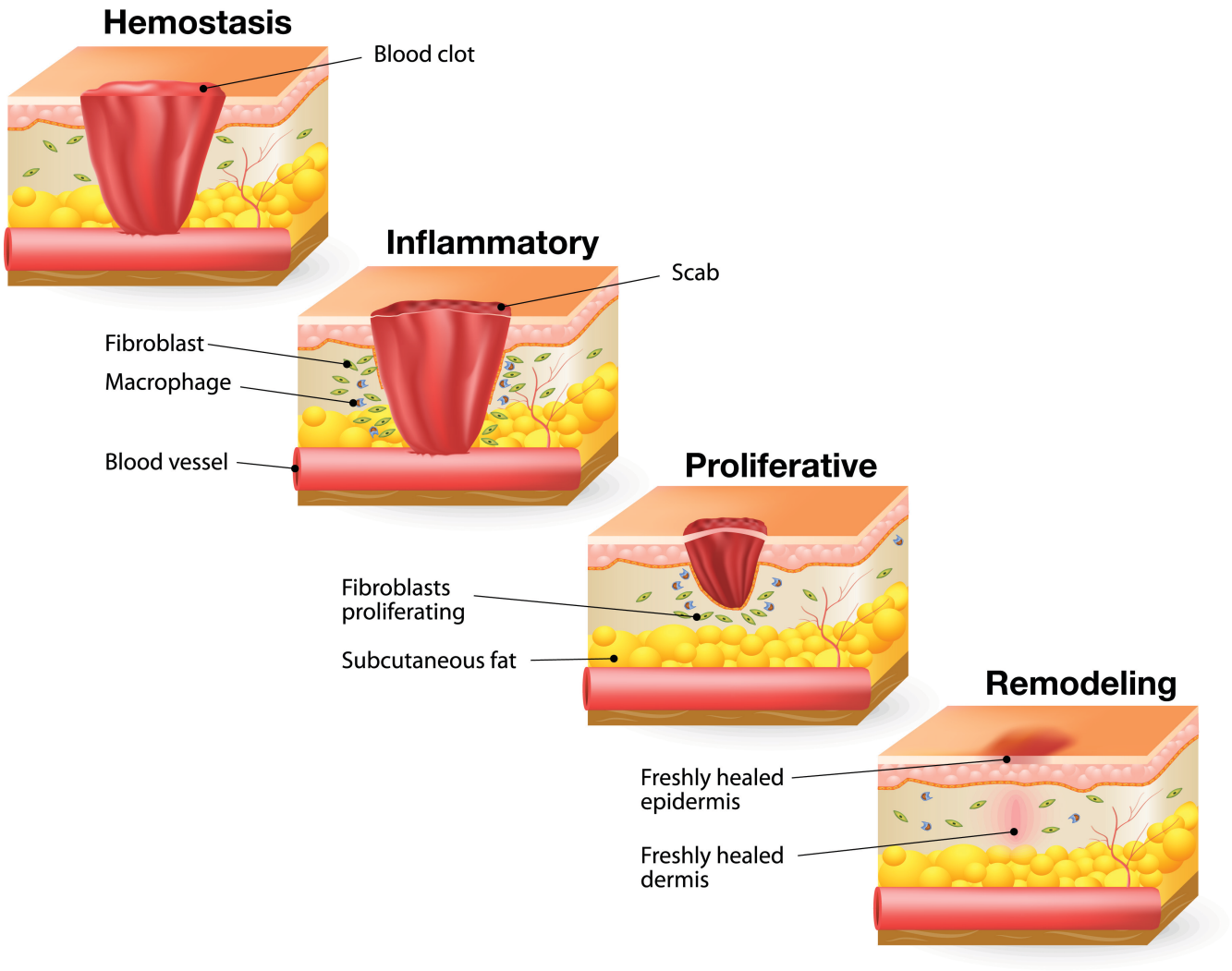
- **Surgical incisions** can be closed in two main ways

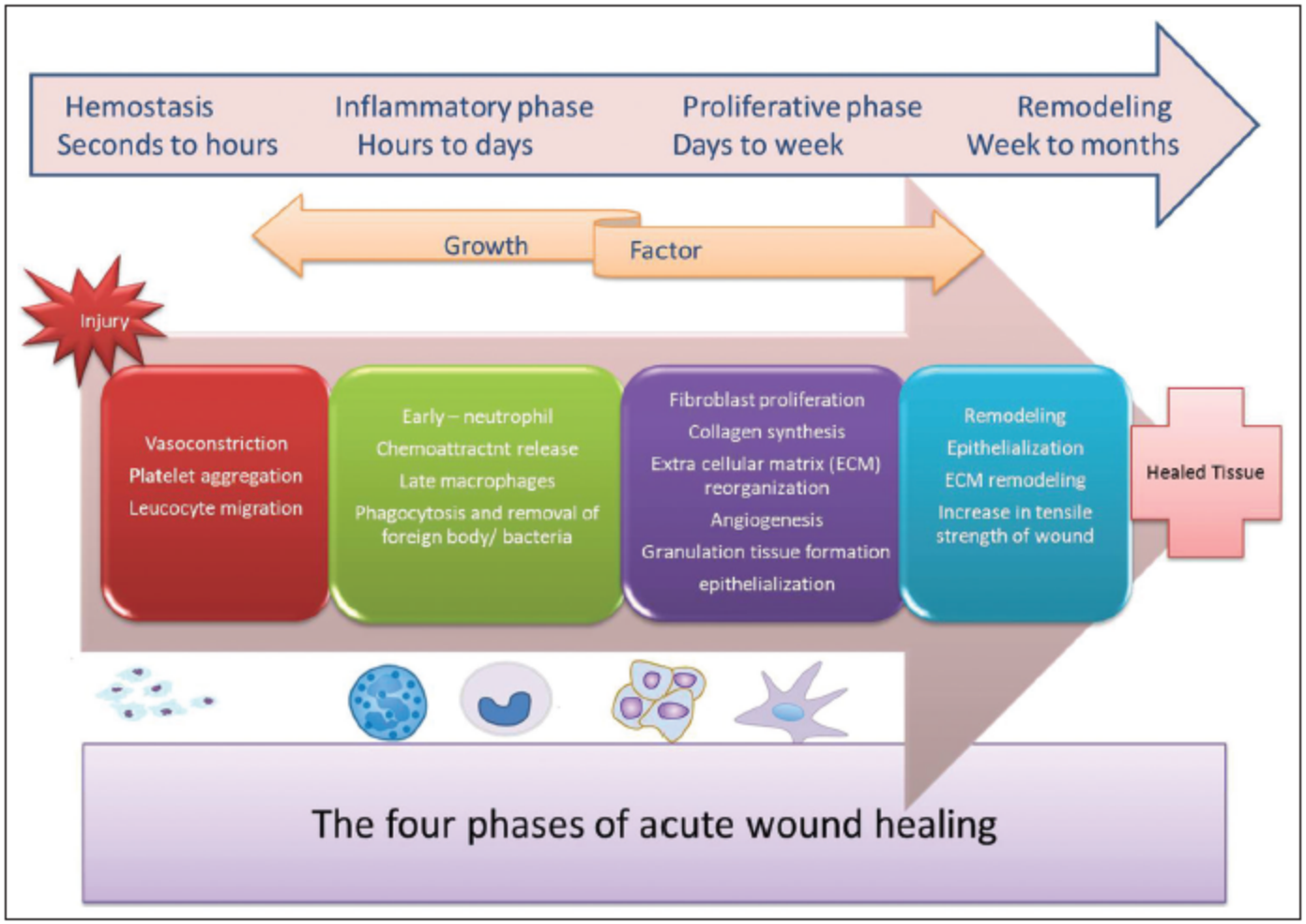
  - **Primary intention** – the edges of the wound are brought together and the wound is close with sutures, staples, adhesives or tape. Epithelialization occurs within 24–48 h.
  - **Secondary intention** – the wound is left open and allowed to granulate.

The stages of wound healing are three

- **1. Haemostasis and Inflammation** : inflammation is divided in two phases:
- **Early phase (24–48 h)** – the **complement** cascade is activated, and **granulocytes** produce **free radicals** and antibacterial proteases. Epithelial cell migration and proliferation then begins.
- **Late phase (2–3 days)** – **macrophages** replace granulocytes, remove dead cells and aid in wound debridement, and produce growth factors that stimulate angiogenesis. Epithelization is complete
- **2. Proliferation (or regeneration)**: takes place 3–20 days after a wound is sustained. **Fibroblasts** migrate to the wound site and lay down collagen, helping to create a **new extracellular matrix**. Angiogenesis continues.
- **3. Maturation (or remodelling)** takes place weeks after a wound is sustained. Consists of the **dynamic deposition and degradation of type III collagen**. A scar with 80 % of the original strength of the wound site forms by 12 weeks.

WOUND HEALING




Hemostasis
Seconds to hours
Inflammatory phase
Hours to days
Proliferative phase
Days to week
Remodeling
Week to months
Growth
Factor
Injury
Vasoconstriction
Platelet aggregation
Leucocyte migration
Early – neutrophil
Chemoattractnt release
Late macrophages
Phagocytosis and removal of foreign body/ bacteria
Fibroblast proliferation
Collagen synthesis
Extra cellular matrix (ECM) reorganization
Angiogenesis
Granulation tissue formation
epithelialization
Remodeling
Epithelialization
ECM remodeling
Increase in tensile strength of wound
Healed Tissue
The four phases of acute wound healing

- **Acute wounds** proceed in an orderly and timely reparative process to achieve sustained restoration of structure and function

- **A chronic wound**, in contrast, does not proceed to restoration of functional integrity. It is stalled in the inflammatory phase and does not proceed to closure

- **The three phases** of wound healing are **inflammation, proliferation, and maturation**

- In wound such as a **pressure sore**, **the eschar** or fibrinous exudate reflects the inflammatory phase, **the granulation tissue** is part of the proliferative phase, and the **contracting or advancing edge** is part of the maturational phase

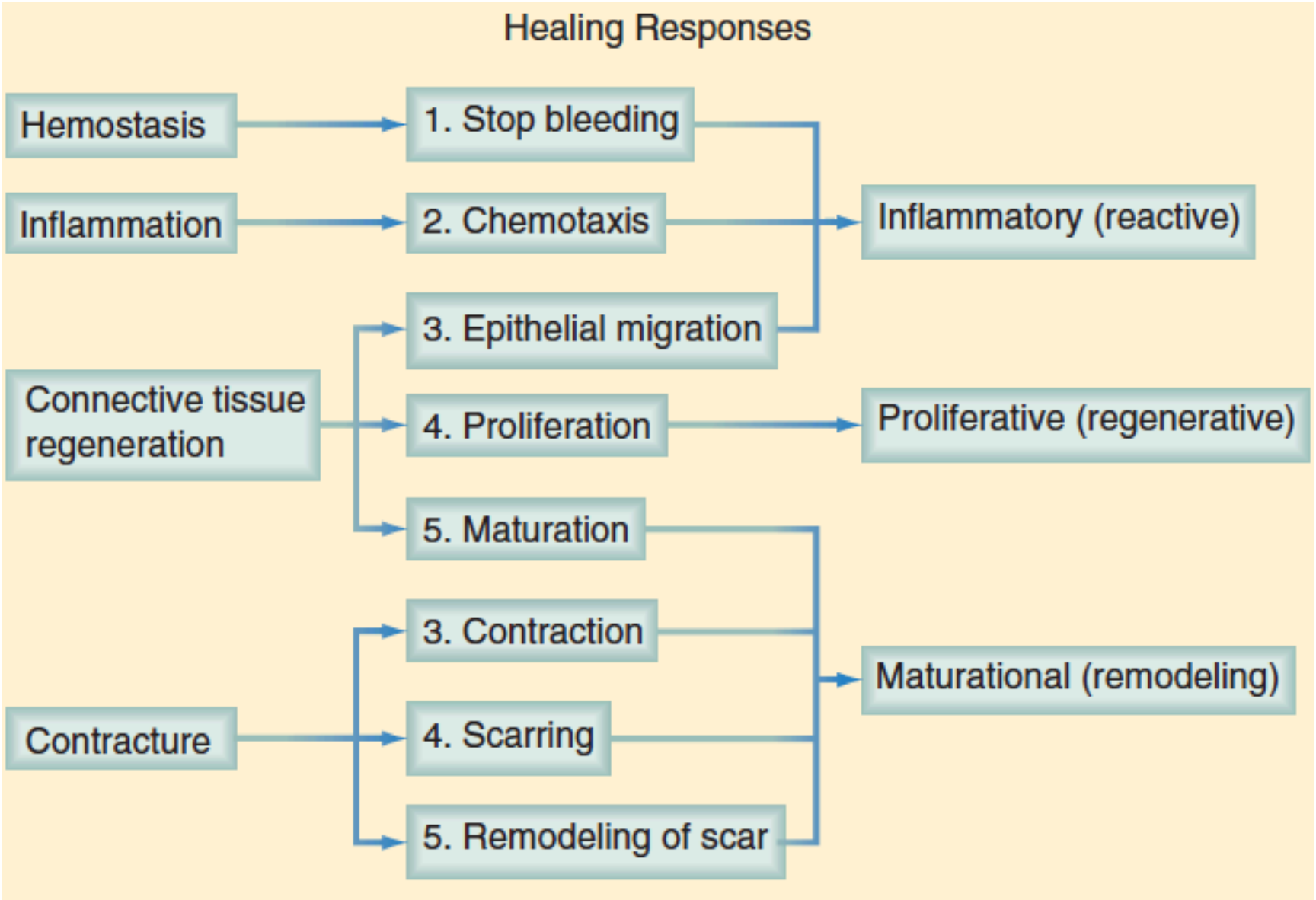
- All three phases may occur simultaneously, and the phases may overlap with their individual processes

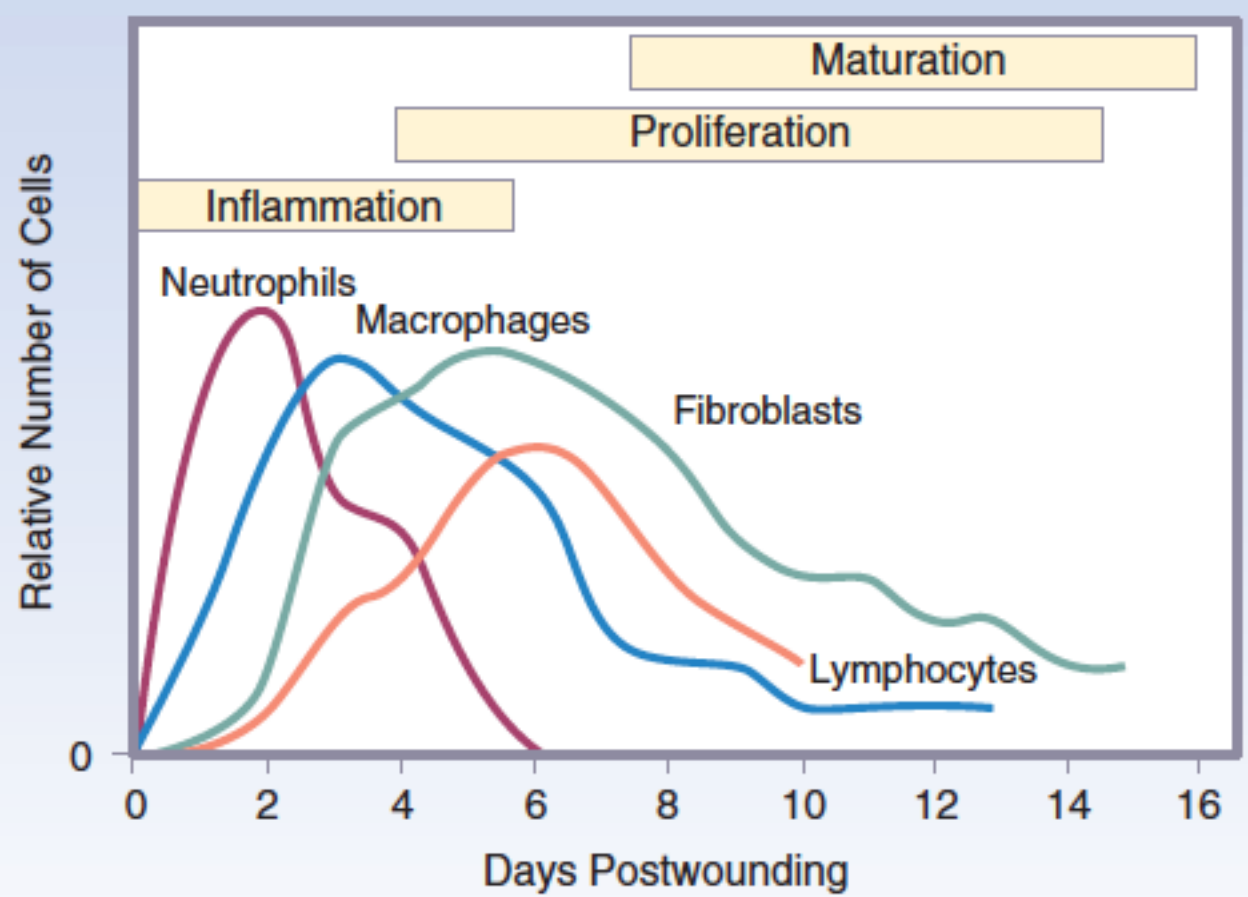
INFLAMMATORY PHASE

- During the immediate reaction of the tissue to injury, **hemostasis and inflammation** occur

- This phase represents an attempt to **limit damage** by stopping the bleeding, sealing the surface of the wound, and removing any necrotic tissue, foreign debris, or bacteria

- The **inflammatory phase** is characterized by
  - increased **vascular permeability**
  - **migration of cells** into the wound by chemotaxis
  - secretion of **cytokines and growth factors** into the wound
  - **activation of the migrating cells**


Healing Responses
Hemostasis
1. Stop bleeding
Inflammation
2. Chemotaxis
Inflammatory (reactive)
3. Epithelial migration
Connective tissue regeneration
4. Proliferation
Proliferative (regenerative)
5. Maturation
3. Contraction
Maturational (remodeling)
Contracture
4. Scarring
5. Remodeling of scar


Maturation
Proliferation
Inflammation
Neutrophils
Macrophages
Fibroblasts
Lymphocytes
Relative Number of Cells
0
0
2
4
6
8
10
12
14
16
Days Postwounding

INFLAMMATORY PHASE

Hemostasis and Inflammation

- Blood vessel damage → **vasoconstriction** → followed by **vasodilation and ↑ vascular permeability**

- **Erythrocytes and platelets adhere** to the damaged endothelium, resulting in **plugging** of capillaries → **STOP** of hemorrhage

- **Platelets** bind to **type IV and V collagen** from damaged endothelium → become activated → **platelet aggregation**

- The initial contact between platelets and collagen requires **von Willebrand factor (vWF) VIII**, synthesized by megakaryocytes and endothelial cells

- **Platelet adhesion** to the endothelium is mediated by interaction between **high-affinity glycoprotein receptors** and **integrin receptor GPIIb-IIIa ($\alpha_{IIb}\beta_3$)**

INFLAMMATORY PHASE

Increased Vascular Permeability

- **Platelet binding → intracellular signal transduction → platelet activation** and the release active proteins

- Platelet **alpha granules** are storage organelles that contain

  - platelet-derived growth factor (PDGF)
  - transforming growth factor-β (TGF-β)
  - insulin-like growth factor type I (IGF-I)
  - Fibronectin
  - Fibrinogen
  - Thrombospondin
  - vWF

- The dense bodies contain vasoactive amines, such as **serotonin**, **→ vasodilation** and **↑ vascular permeability**

INFLAMMATORY PHASE

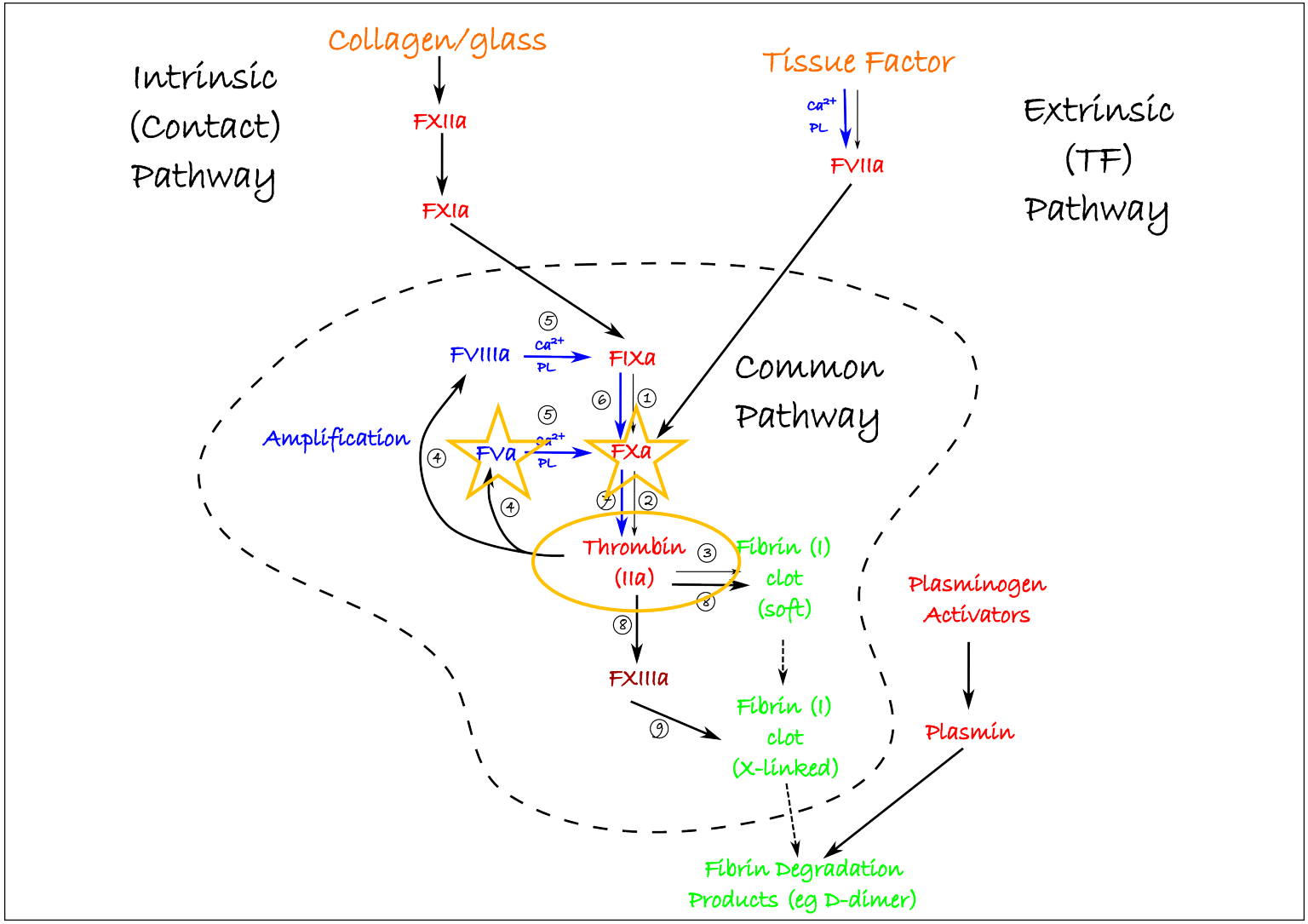
- **Mast cells** adherent to the endothelium release → **histamine and serotonin**, → **increased permeability** → leakage of **plasma**

- The **clotting cascade** is initiated through the **intrinsic and extrinsic pathways**. **Activated platelets → their membrane phospholipids** bind **factor V,** which allows interaction with **factor X → prothrombinase activity → thrombin**

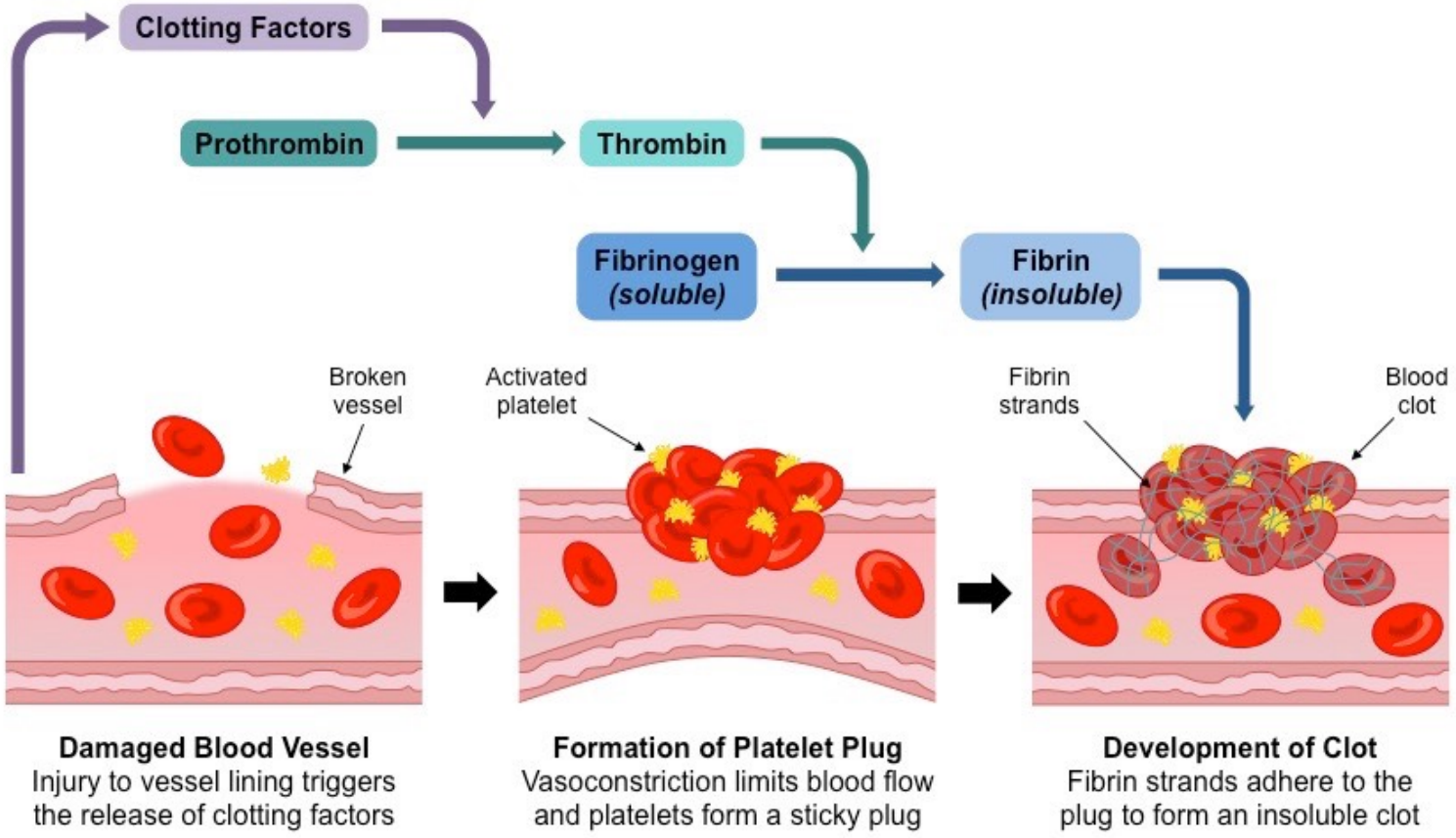
- The **thrombin** catalyzes the conversion of **fibrinogen** → **fibrin.** The **fibrin strands** trap red blood cells → **the clot**. This framework is the **scaffold for endothelial cells, inflammatory cells, and fibroblasts**

- **Thromboxane A2 and prostaglandin F2α** released by degraded cells → **platelet aggregation and vasoconstriction**

INFLAMMATORY PHASE



INFLAMMATORY PHASE



INFLAMMATORY PHASE

Chemokines

Chemokines stimulate the migration of **inflammatory cells** into the wound regulate wound healing

- **Macrophage chemoattractant** protein (MCP-1, or CCL2) is induced in **keratinocytes** after injury. It is a potent chemoattractant for monocytes/macrophages, T lymphocytes, and mast cells**.** Expression of this chemokine is sustained in chronic wounds and results in the prolonged presence of polymorphonuclear cells and macrophages

- **CXCL1 (GRO-α)** is a potent PMN chemotactic regulator and is increased in acute wounds

- **Interleukin-8 (IL-8, or CXCL8)** expression is increased in acute and chronic wounds. It is involved in *reepithelialization* and induces the *leukocyte expression of matrix metalloproteinases*

- **Stromal cell-derived factor-1 (SDF-1, or CXCL12)** is expressed by endothelial cells, myofibroblasts, and keratinocytes and is involved in inflammation by recruiting lymphocytes to the wound and promoting angiogenesis

Polymorphonuclear Cells

- **Histamine and serotonin → vascular permeability**
- **Complement C5a and leukotriene B4 → neutrophil adherence and chemoattraction**.
- **Monocytes and endothelial cells → IL-1 and tumor necrosis factor-α (TNF-α)**, **→ endothelial-neutrophil adherence**
- **Neutrophils begin their migration** and release **elastase** into the extracellular matrix (ECM), which facilitates neutrophil migration
- The combination of **intense vasodilation and increased vascular permeability** → clinical findings of inflammation, **rubor (redness), tumor (swelling), calor (heat), and dolor (pain)**

Polymorphonuclear Cells

- Migration of PMNs interactions between **β_1 and β_2 integrins** and ECM components
- Binding sites for integrins have been identified on **collagen, laminin, and fibronectin**
- **Four phases of integrin-mediated cell motility** have been described: **adhesion, spreading, contractility or traction,** and **retraction**
- **Neutrophils** also possess **receptors for IgG and the complement** (C3b and C3bi)
- **Bacteria** are opsonized by **complement**, → recognition by **the neutrophils** and phagocytosis

Polymorphonuclear Cells

- **Activated neutrophils scavenge** for **necrotic debris, foreign material, and bacteria and generate free oxygen radicals**

- **Superoxide anion (O_2^-)** is formed. Superoxide dismutase catalyzes the formation of **hydrogen peroxide (H_2O_2)**
  - Reaction between **H_2O_2 and O_2^-** forms **hydroxyl radicals (OH·)**. **This potent free radical is bactericidal**

- **Migration of PMNs stops** when wound contamination has been controlled, (first few days after injury)

- **PMNs do not survive longer than 24 hours**. After 24 to 48 hours, the predominance of cells in the wound cleft shifts to **mononuclear cells**.

Polymorphonuclear Cells

- **If wound contamination persists** or secondary infection occurs, **continuous activation of the complement system** results in a sustained influx of PMNs into the wound

- → **delay in healing,** prolonged inflammation → **destruction of normal tissue**, with progression to **tissue necrosis, abscess formation, and possibly systemic infection**

- The role of PMN in **phagocytosis and antimicrobial defense may be taken over by macrophages**. Sterile incisions will heal normally without the presence of PMNs.

Macrophages

- They are **crucial to wound healing:** they guide the release of **cytokines and stimulate wound healing**

- **Macrophages appear** at the same time that neutrophils disappear. They induce **apoptosis** of PMNs

- **Chemotaxis of migrating blood monocytes occurs within 24 to 48 hours**. Chemotactic for monocytes is facilitated by the interaction of integrin receptors on the monocyte surface with ECM (fibrin and fibronectin)

- The **β integrin receptor** also **transduces the signal** and **promotes trasformation of** monocytes into **wound macrophages**

Central role of macrophages

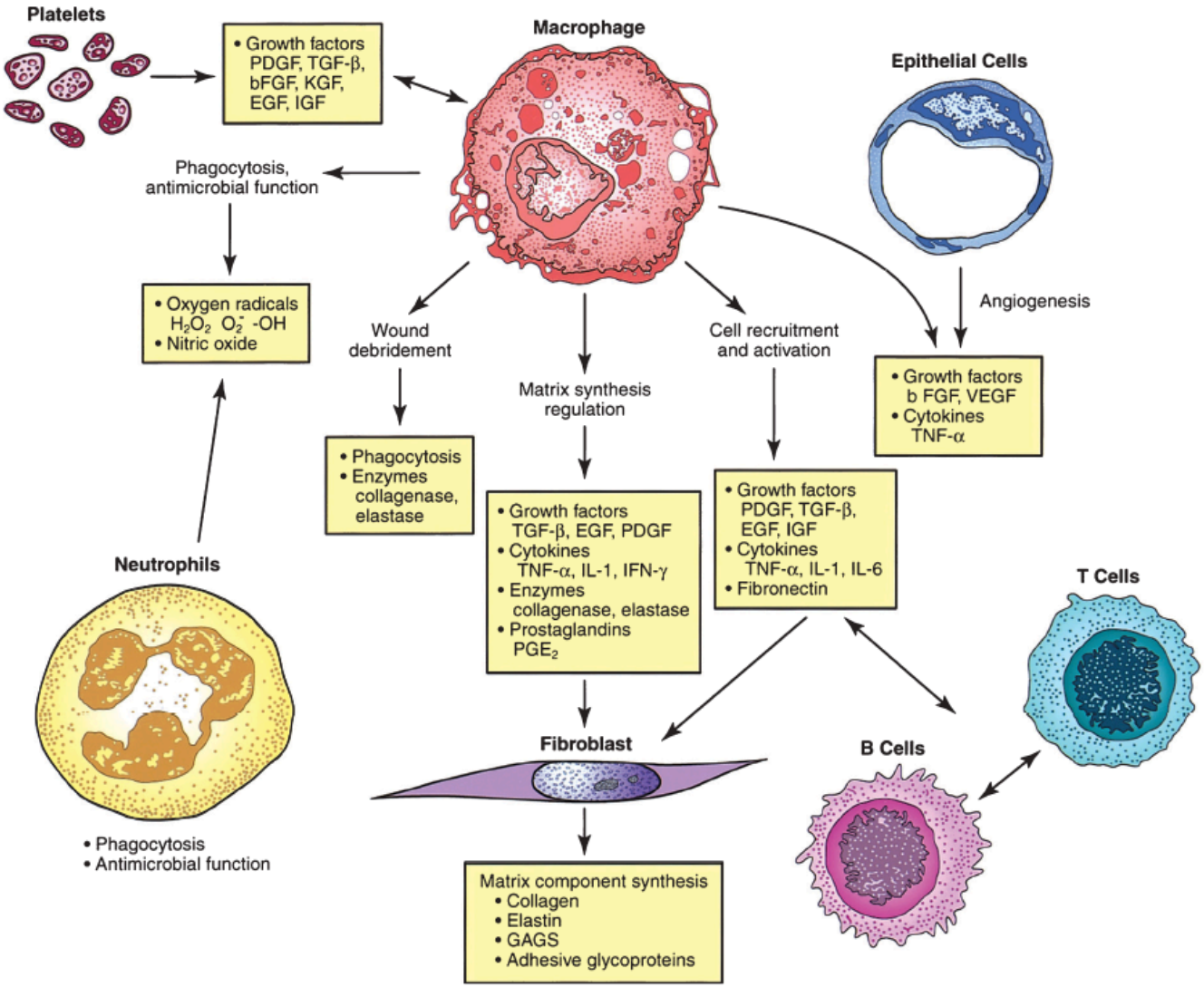


FIGURE 6-3 Interaction of cellular and humoral factors in wound healing. Note the key role of the macrophage. *bFGF,* basic fibroblast growth factor; *EGF,* epidermal growth factor; *GAGs,* glycosaminoglycans; H_2O_2, hydrogen peroxide; *IFN-γ,* interferon-γ; *IGF,* insulin-like growth factor; *IL-1,* interleukin-1; *IL-6,* interleukin-6; *KGF,* keratinocyte growth factor; O_2^-, superoxide; *–OH,* hydroxyl radical; *PDGF,* platelet-derived growth factor; PGE_2, prostaglandin E2; *TGF-β,* transforming growth factor-β; *TNF-α,* tumor necrosis factor-α; *VEGF,* vascular endothelial growth factor. (Adapted from Witte MB, Barbul A: General principles of wound healing. *Surg Clin North Am* 77:509–528, 1997.)

Lymphocytes

- **T lymphocytes** appear at the **5th day**, with a peak at **7th day**
- **B lymphocytes** do not appear to play a significant role in wound healing
- **Lymphocytes** exert their effects on **fibroblasts** by producing cytokines (Stimulatory: **IL-2 and fibroblast-activating factor**. Inhibitory: **TGF-β, TNF-α, and IFN-γ**
- The **macrophage** processes bacteria and serves as **an antigen-presenting cell to lymphocytes**.
- This stimulates **lymphocyte proliferation** and release of **cytokines**. T cells produce **IFN-γ**, which inhibits macrophages from leaving the site of injury

INFLAMMATORY PHASE

Lymphocytes

- **IFN-γ** is an important mediator of **chronic non-healing wounds**, and its presence suggests that **T lymphocytes are primarily involved in chronic wound healing**

- **Drugs that suppress T-lymphocyte** function and proliferation, such as **steroids and immunosuppressive agents** (e.g., cyclosporine, tacrolimus), have been found to result in **impaired wound healing** through decreased NO synthesis

PROLIFERATIVE PHASE

- As the acute phase begin to resolve, the **scaffolding is laid** for repair of the wound through **angiogenesis, fibroplasia, and epithelialization**

- **Granulation tissue** is formed, which consists of a capillary bed, fibroblasts, macrophages, collagen, fibronectin, and hyaluronic acid

Angiogenesis

- Angiogenesis is **stimulated** by **cytokines** produced by **macrophages and platelets**

- **VEGF** has potent **angiogenic activity**; it is produced by keratinocytes, macrophages, endothelial cells, platelets, and fibroblasts

PROLIFERATIVE PHASE

- **Quiescient fibroblasts** are chemoattracted to the inflammatory site, where they proliferate and **produce the ECM**

- **Fibroblasts** (normally arrested in G0 phase) stimulated by macrophage and platelet-derived growth factors and cytokines, undergo **replication and proliferation.** Platelet-derived **TGF-β** stimulates fibroblast proliferation

- They **synthesize collagen.** Mesenchymal cells need time to differentiate into fibroblasts. This accounts for the delay between injury and the appearance of collagen: generally **3 to 5 days** (**lag phase of wound healing**)

- Collagen synthesis declines after **4 weeks**, which is followed by phase of **collagen maturation**, which lasts for **months or years**

- Glycoprotein and mucopolysaccharide ↓, and new capillaries regress and disappear. These changes ↑ **wound's strenght**

Epithelialization

- **Re-Epithelialization** begins within **hours** after injury.

- **Keratinocytes** located at the basal layer of the residual epidermis migrate to resurface the wound

- Epithelialization involves 4 phases: **detachment, migration, proliferation, differentiation and stratification**

Extracellular matrix (ECM)

- ECM is a scaffold that stabilizes tissues, but it also plays a role by **regulating the behavior of cells**
- Cells within it produce
- 1) **glycosaminoglycans (GAGs)**, or polysaccharide chains usually linked to proteins (**proteoglycans**)
- (2) **fibrous proteins** (collagen, elastin, fibronectin, and laminin)

PROLIFERATIVE PHASE

Collagen

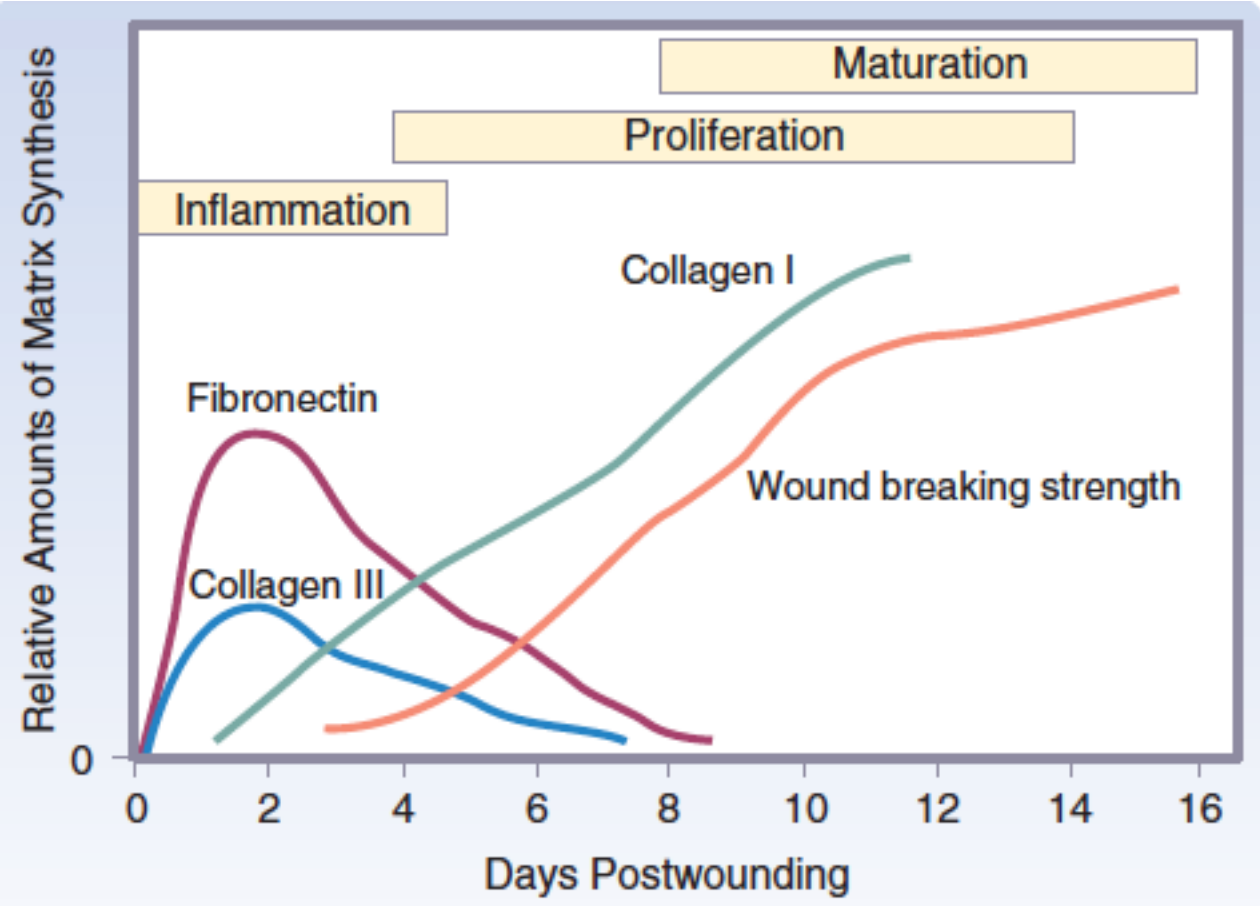
- Collagen fibers **give strength to ECM**, whereas **elastin give resilience**
- There are **20 types of collagen**, the main constituents of the connective tissue being types **I, II, III, V, XI**.
- **Type I**: found in skin and bone – it is the most common

- ECM changes in composition as healing progresses (new deposition and degradation processes). Matrix modulation is also seen in tumor metastasis (role of MMPs)

- The **provisional matrix** is composed of **fibrin, fibrinogen, fibronectin, and vitronectin**. **GAGs and proteoglycans** are synthesized next. **Collagens**, which are the predominant scar proteins, are the end result.

- **Fibrin and fibronectin** bind to cell surface **integrin receptors**

PROLIFERATIVE PHASE



Wound matrix deposition over time. **Fibronectin** and type **III collagen** constitute the early matrix. **Type I collagen** accumulates later and **↑ wound-breaking strength**

Degradation of the extracellular matrix

- Regulated turnover of the ECM occurs during **metastasis** when neoplastic cells migrate from their site of origin to distant organs
- In injury or infection, **localized degradation of the ECM** occurs so that cells can migrate to reach the site of injury
- **Cellular proteases (MMP)** degrade the ECM components. Matrix proteolysis helps the cell to migrate
- **Proteolysis** is tightly regulated. Many **proteases are secreted as inactive** precursors that are activated when required.

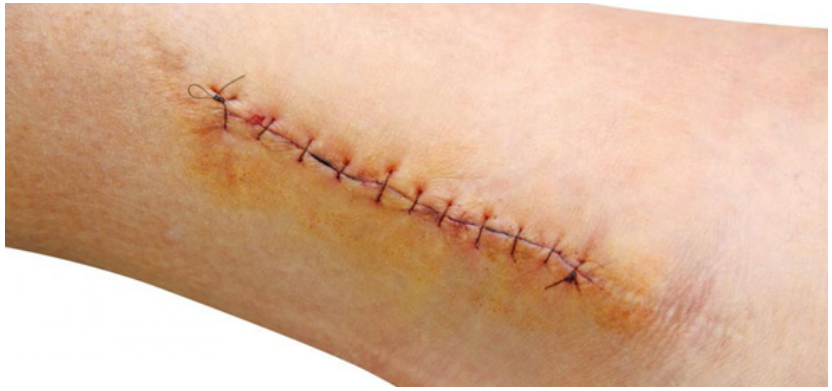
MATURATIONAL PHASE

- **Wound contraction** reduces the amount of disorganized scar
- Contractures occur when excessive scar exceeds normal wound contraction
- Stimulated fibroblast develops contractile ability related to the formation of cytoplasmic actin-myosin complexes (**myofibroblast**)
- Myofibroblasts, are found in abundance in diseases involving excessive fibrosis, (hepatic cirrhosis, renal and pulmonary fibrosis, Dupuytren's contracture, and desmoplastic reactions)
- **MMPs** may be necessary to allow cleavage of the attachment between the fibroblast and the collagen so that the lattice can be made to contract

Remodeling

- The fibroblast population decreases, and the dense capillary network regresses. Wound strength increases rapidly within 1 to 6 weeks and then appears to plateau up to 1 year after the injury

- Factors that impede wound healing can be divided into local and systemic.



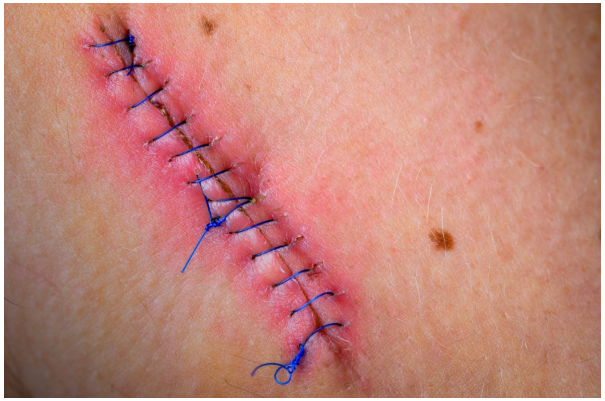


Table 10.3 Factors that impede wound healing, divided into local (factors related to the wound itself) and systemic (factors that affect the entire body)

Local	Systemic
Inadequate blood supply	Advancing physiological age
Increased skin tension	Obesity
Poor surgical wound apposition	Smoking
Wound dehiscence	Diabetes mellitus
Poor venous drainage	Malnutrition
Presence of foreign bodies	Vitamin/trace elements deficiency
Haematoma Infection Excess local mobility (e.g. over a joint) Topical medicine	Systemic malignancy Shock Chemotherapy or radiotherapy Immunosuppressants Corticosteroids Anticoagulants Chronic renal/hepatic failure

Excessive wound healing: occurs when the normal **balance of collagen deposition and degradation** during remodelling is disturbed. There are two main types:

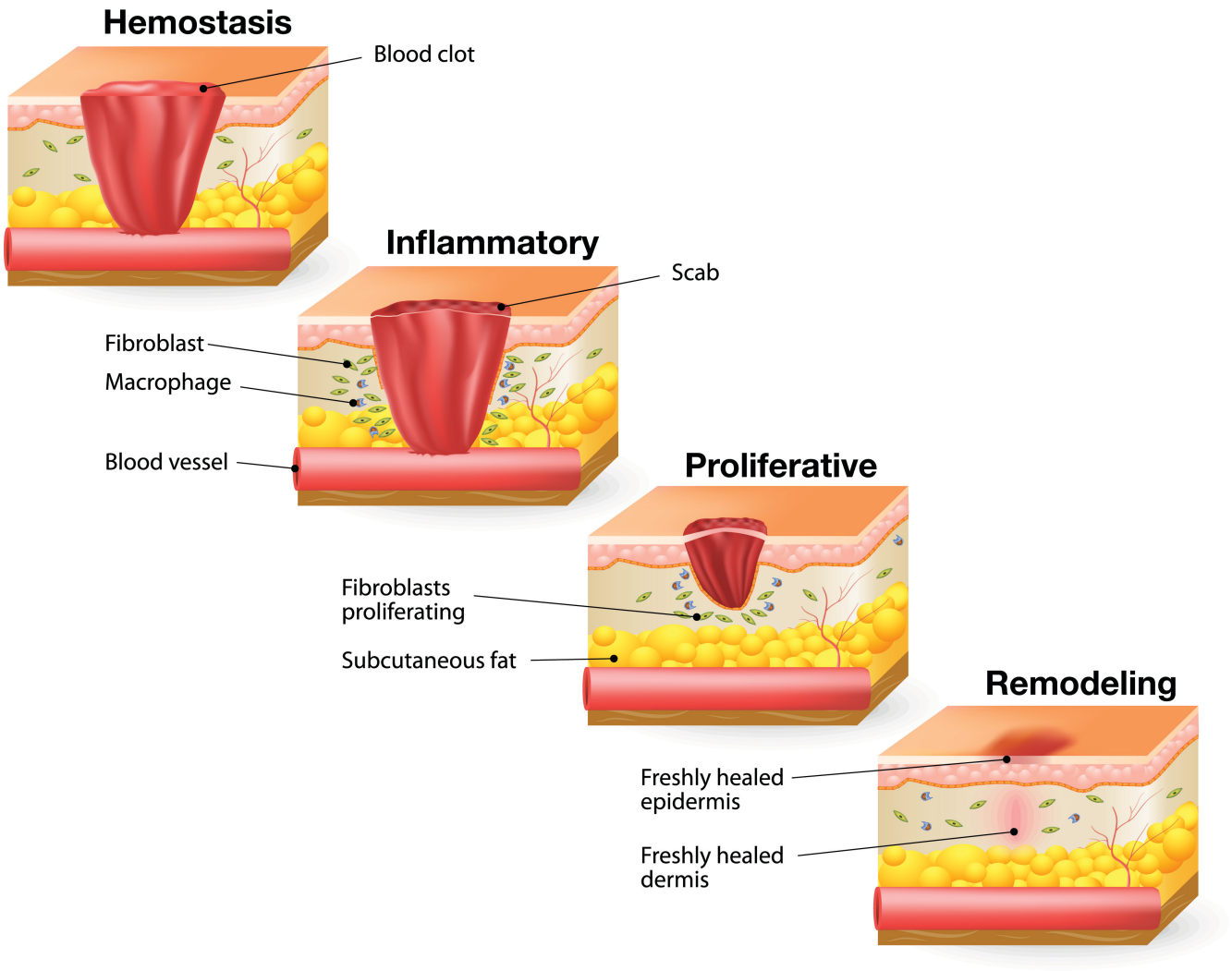
- **Keloids :** the **raised area extends beyond the wound margins** and there is no wound contracture. They are treated by excision, steroids and cryotherapy, but typically recur. These are uncommon in children

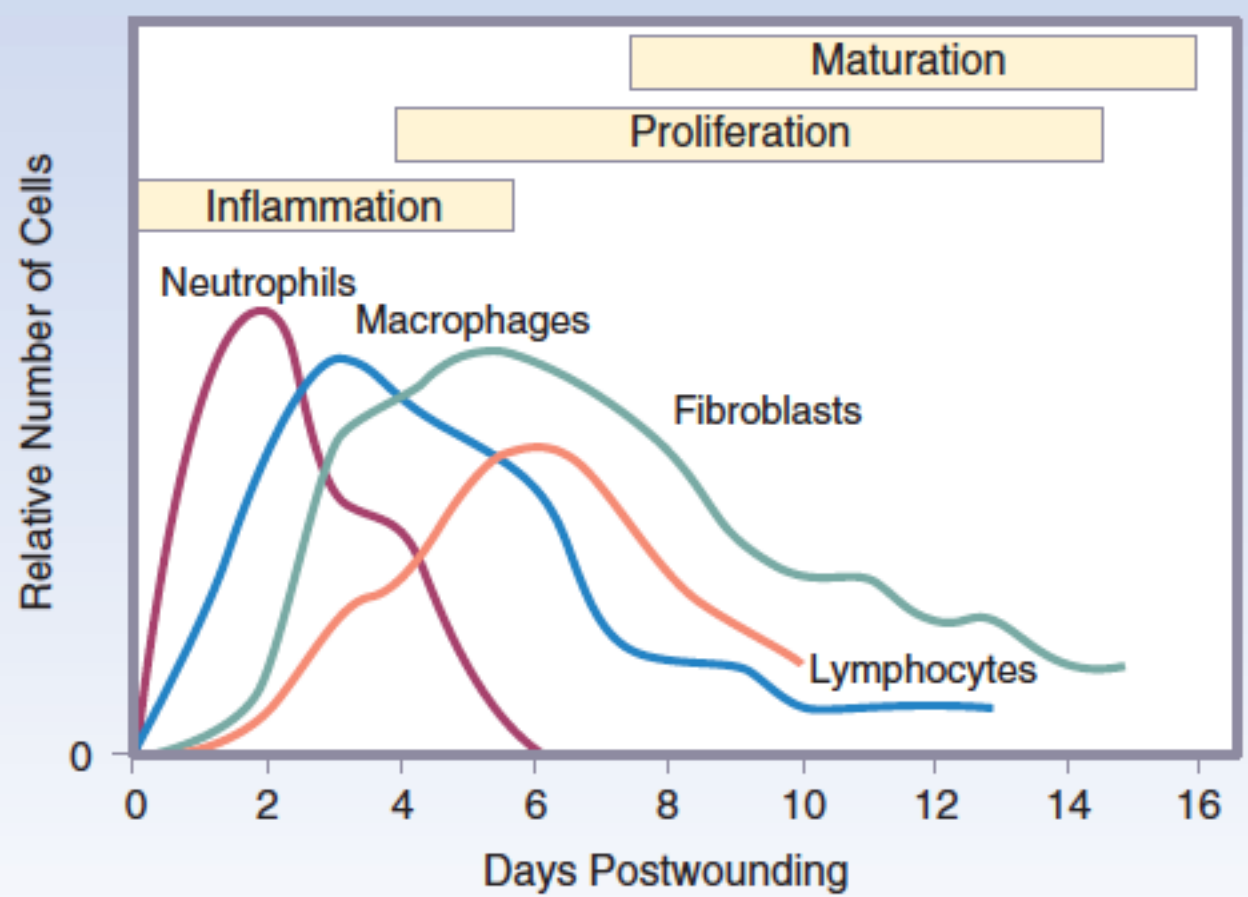
- **Hypertrophic scars : the raised area is confined to the wound margins** and there is wound contracture. They are treated by excision, steroids and cryotherapy, and typically do not recur. These can occur at any age.

Wound Dressing: there are many types of wound dressing. The most appropriate is linked to the properties of the wound.

- **Semipermeable film dressings** : made of a polyurethane film that adheres to intact skin. Used in dry, superficial wounds.
- **Semipermeable pad dressing**: an interface between the wound and the dressing allows exudate to pass. Indicated with wounds with low exudate.
- **Hydrocolloid dressing**: when contact with exudate is made, a gel from cellulose or gelatin is formed. Indicated in wounds with low or moderate exudate.
- **Alginate dressing**: derived from seaweed. When contact with exudate is made, a gel is formed. Indicated in wounds with low or moderate exudate.
- **Hydrofibre dressing**: a textile fibre dressing formed of carboxymethyl cellulose. Reduces risk of skin maceration. Indicated in wounds with moderate to heavy exudate.
- **Foam dressing**: formed from silicon or polyurethane. Indicated in wounds with heavy exudate.
- **Hydrogel dressing**: formed from an insoluble polymer. Aids in wound debridement and slough. Indicated in dry, necrotic wounds with minimal exudate.

WOUND HEALING




Maturation
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Fibroblasts
Lymphocytes
Relative Number of Cells
0
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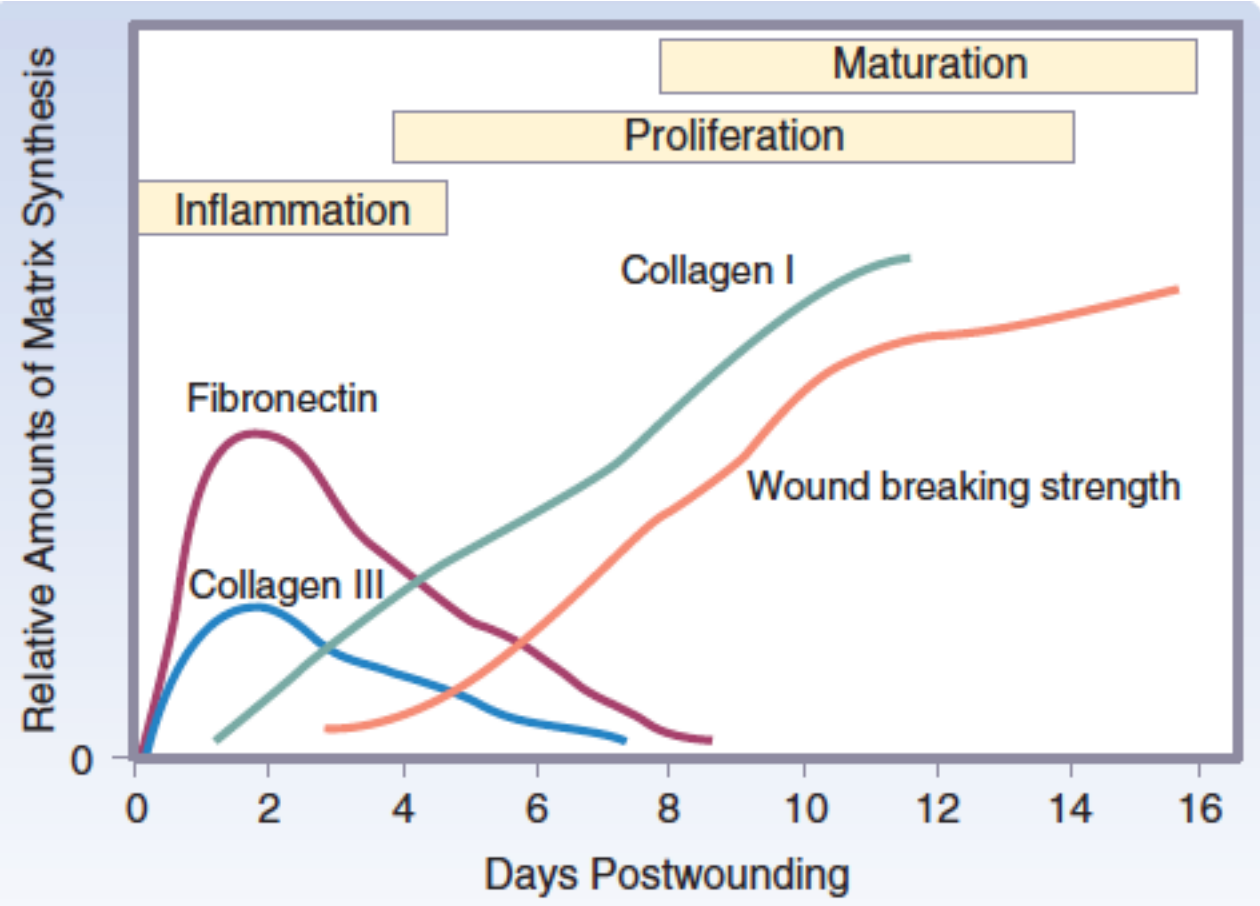
Days Postwounding

PROLIFERATIVE PHASE



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