Cutaneous Response to Injury And Wound Healing

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Wound healing

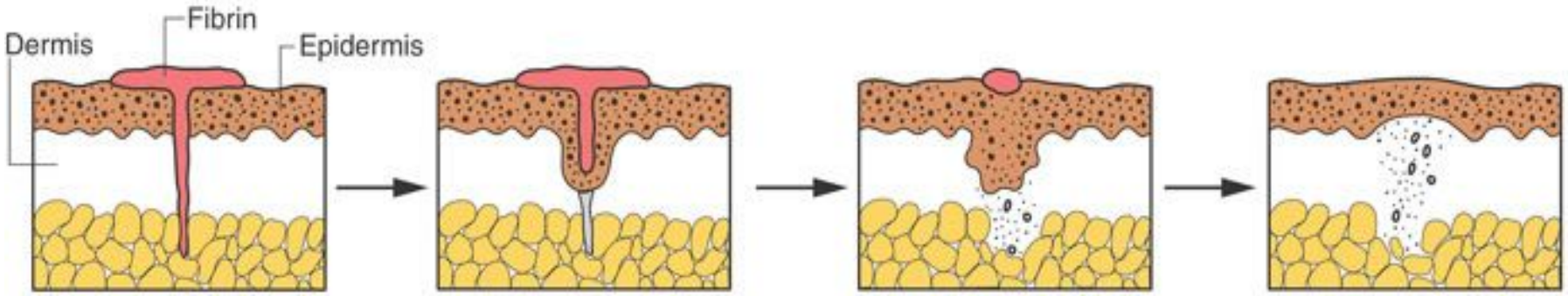
- It is a natural restorative response to tissue injury.
- Healing is the interaction of a complex cascade of cellular events that generates resurfacing, reconstitution, and restoration of tensile strength of injured skin.

Types of Wound Healing

- **Primary Healing (first intention)**
  - Clean incised wound or surgical wound.
  - Wound with opposed edges
  - More epithelial regeneration than fibrosis
  - Wound heals rapidly with complete closure

- **Secondary Healing (second intention)**
  - Occurs in a wound with extensive soft tissue loss.
  - Wound with separated edges.
  - Heals slowly with fibrosis.
  - It may leads into a wide scar, often hypertrophied and contracted.

Healing by primary intention



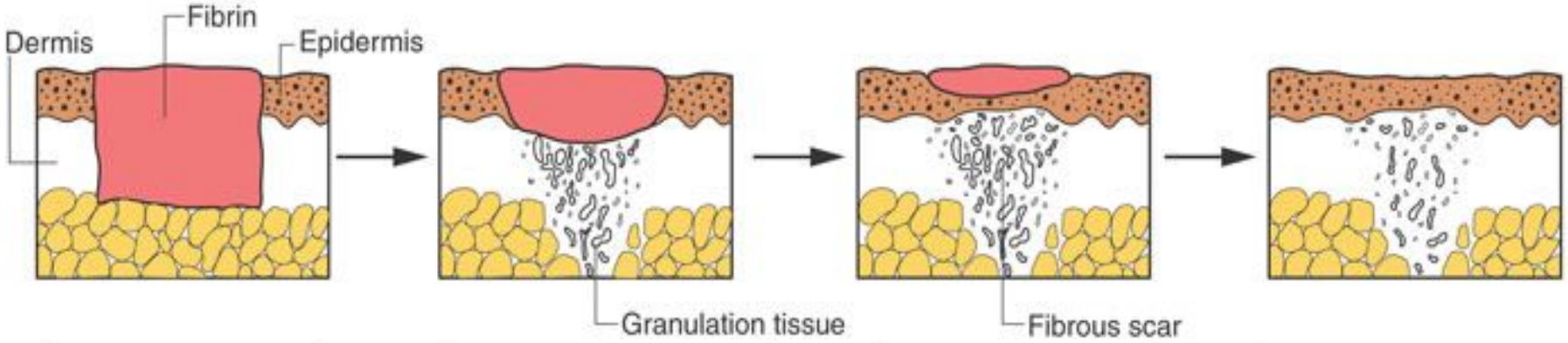
Wound edges joined by fibrin plug

Regrowth of basel layer of epidermis

Lysis of fibrin and re-epithelialisation

Restoration to intact skin

Healing by secondary intention



Large defect filled by fibrin clot

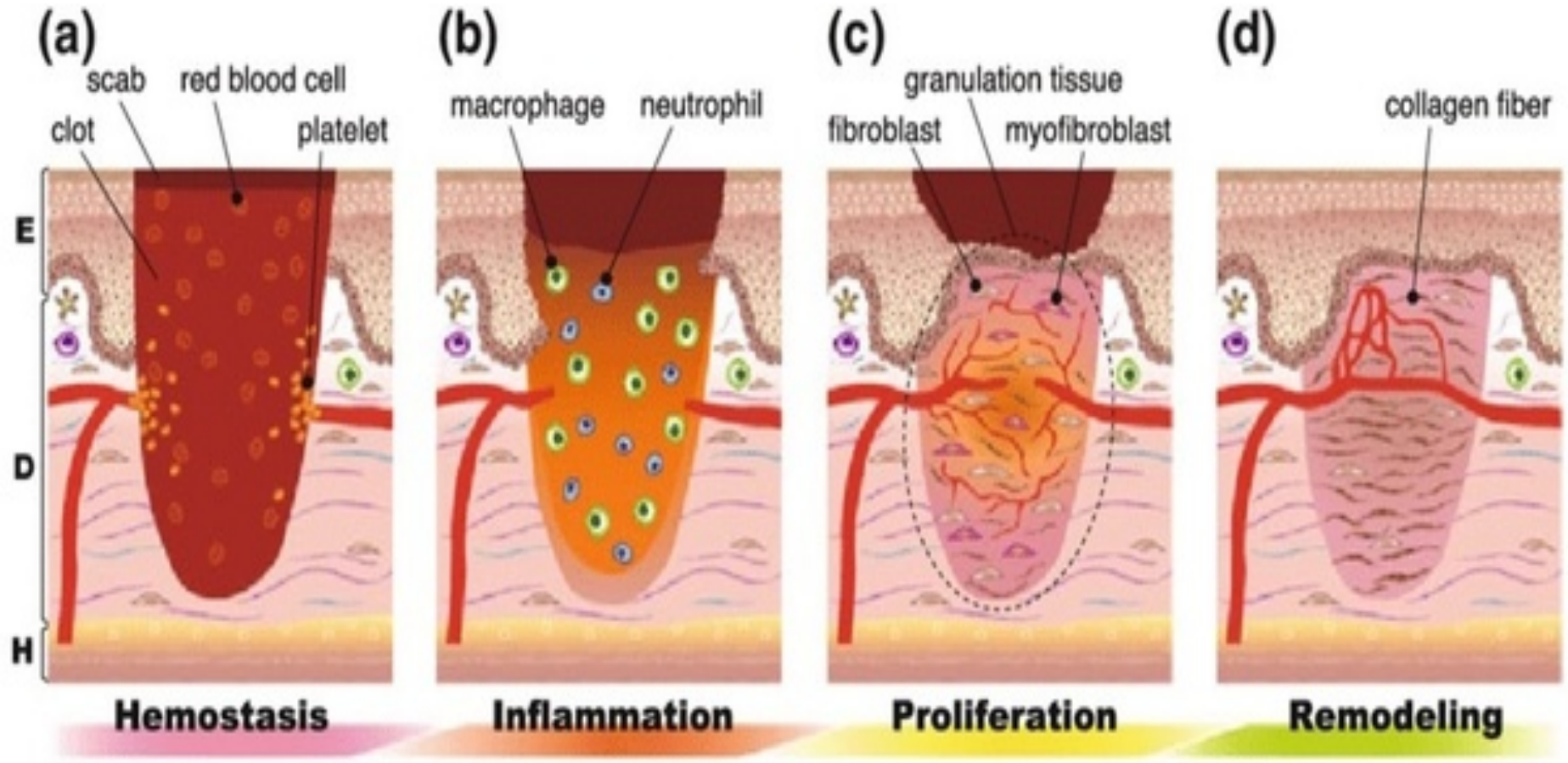
New blood vessels and fibroblasts (granulation tissue) grow from the demis into fibrin

Collagen laid down by granulation tissue fibroblasts to restore integrity

Maturation of collagen achieves structural integrity and allows regrowth of epidermis

- **Tertiary Healing**
  - It refers to wound that are closed to allow primary intention healing but wound dehiscence occur and wound heals by secondary intention.

- **Healing is a systematic process, explained in terms of 4 overlapping classic phases:**
  - Hemostasis
  - Inflammatory phase
  - Proliferative phase
  - Maturation phase


(a)
scab
red blood cell
clot
platelet
E
D
H
Hemostasis
(b)
macrophage
neutrophil
Inflammation
(c)
granulation tissue
fibroblast
myofibroblast
Proliferation
(d)
collagen fiber
Remodeling

Hemostasis

- Hemostasis starts within very first movements of the injury, where platelets play a role by aggregating, as well as by releasing variety of factors, including thromboxanes, prostaglandins, 12- lipoxygenase products, serotonin, adhesive glycoproteins and growth factors.

- These factors contribute to the initial fibrin plug that closes the wound.

- Chemokines released by platelet activation attract inflammatory cells to the area, leading to next phase in healing process

Inflammatory phase

- The cellular aspect of the inflammatory phase occurs within hours of injury, and it includes neutrophils, macrophages, and lymphocytes.

Neutrophils:

- These are predominant cell type for the first 48 hours after injury.
- They releases cytokine and growth factors that amplify the inflammatory response, resulting in influx of monocytes and lymphocytes, proliferation of monocytes precursors within the wound and their differentiation into mature macrophages
- Neutrophils release proteolytic enzymes that degrade cell debris and infective bacteria .

Macrophages:

- Macrophages are essential to wound healing and perhaps are the most important cells in the early phase of wound healing.
- Produces a range of cytokines in response to pro-inflammatory signals including TGF-b and TGF-a, basic fibroblast growth factor, VEGF, PDGF.
- In addition to cytokines, wound macrophages also produce a range of extracellular matrix molecules which together with fibrinogen and fibrin from blood clot, matrix molecules from degranulating platelets, recruited fibroblasts and endothelial cells form granulation tissue or provisional matrix.

Lymphocytes:

- Lymphocytes migrates into wound healing during inflammatory phase, approximately 72 hours following injury.
- T lymphocytes are attracted to the wound by cellular release of interleukin 1.
- Lymphocytes secrete lymphokines such as heparin-binding EGF and basic FGF.
- Lymphocytes also play a role in cellular immunity and antibody production.

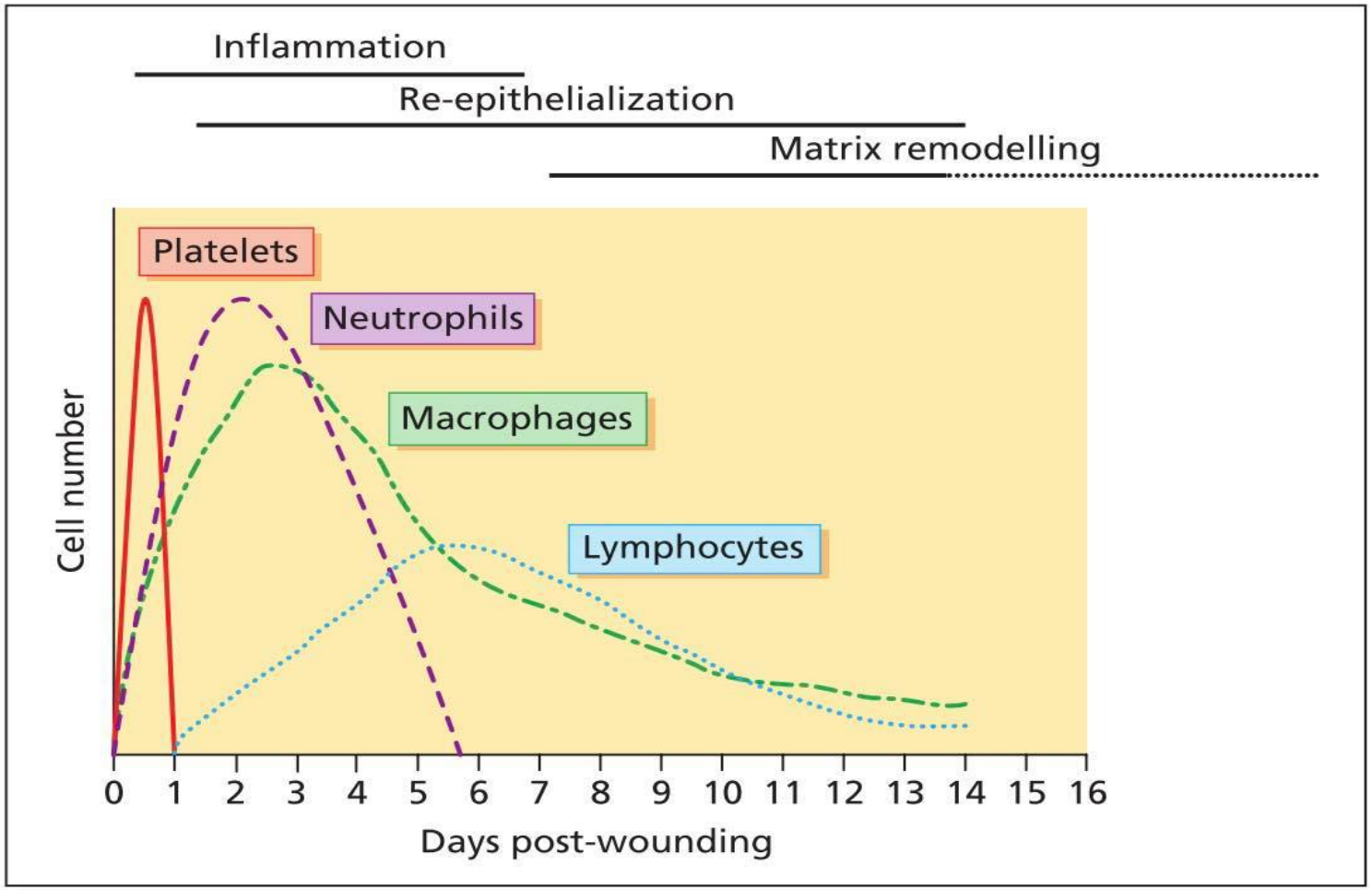


Figure 10.1 Wound inflammatory cells during the first 2 weeks of wound healing showing the stages of acute wound healing and the inflammatory infiltrate present in the wound bed.

Proliferative phase

- Formation of granulation tissue is central event during during proliferative phase. Its formation occurs 3-5 days following injury and overlaps with the preceding inflammatory phase.
- **Main event in this phase are**
    - Re-epithelialization
    - Fibroplasia
    - Angiogenesis
    - Contraction

Re-epithelialization

- The process of re-epithelialization begins about 24h after wounding where keratinocytes migrate from wound edges across the provisional wound matrix to invade the wound bed, where they proliferate to form new epidermis.
- Migrating cells dissect the wound and separate the overlying eschar from underlying viable tissue.

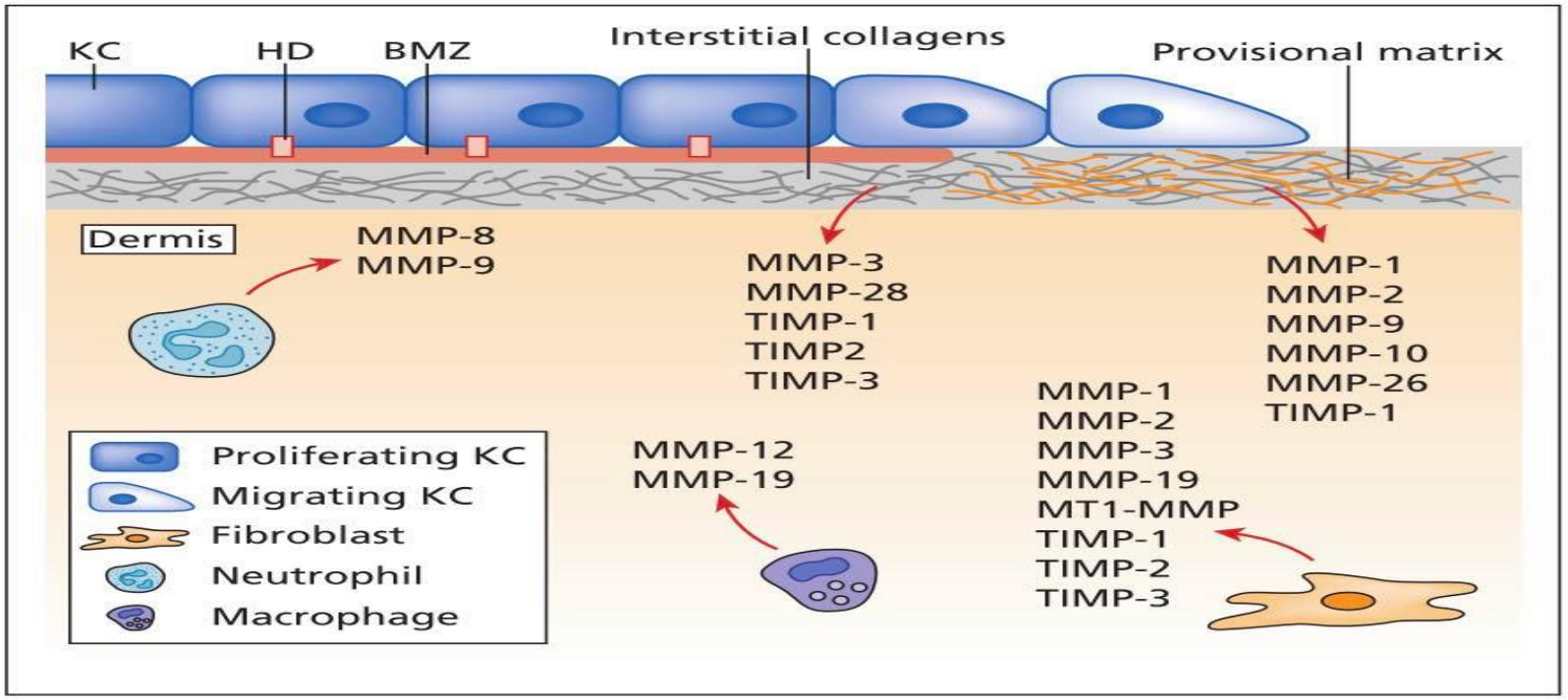


Figure 10.2 Matrix metalloproteinases (MMPs) and tissue inhibitor of matrix metalloproteinases (TIMPs) in wound healing showing the expression and cellular source of MMPs and TIMPs in cutaneous wound healing. Keratinocytes (KC) change their phenotype as they lose their hemidesmosomes (HD), which attach them to the underlying basement membrane zone (BMZ), thus coming in contact with a provisional wound matrix made of fibronectin and fibrin (in orange). Below the basement membrane lies the interstitial matrix composed mainly of type I collagen (in grey). A differential pattern of expression of MMPs and their inhibitors seem to be critical for wound healing, with some MMPs being expressed by migrating keratinocytes at the leading edge of the wound, while others are only expressed by the proliferating population of keratinocytes adjacent to the wound edge. MMPs expressed by fibroblasts and inflammatory cells such as neutrophils and macrophages also contribute to regulating the wound healing process. (Adapted from Martins *et al.* 2013 [35]. Reproduced with permission of Springer.)

Angiogenesis

- Formation of new blood vessels within provisional matrix is stimulated by day 2 of the inflammatory stage of wound healing.
- Immediately following injury, angiogenic factors including VEGF, PDGF, FGFs, TGF-b and complement 1q are secreted by platelets, fibroblasts, wound keratinocytes, and macrophages.
- Adequate revascularization of the wound is essential for healing.

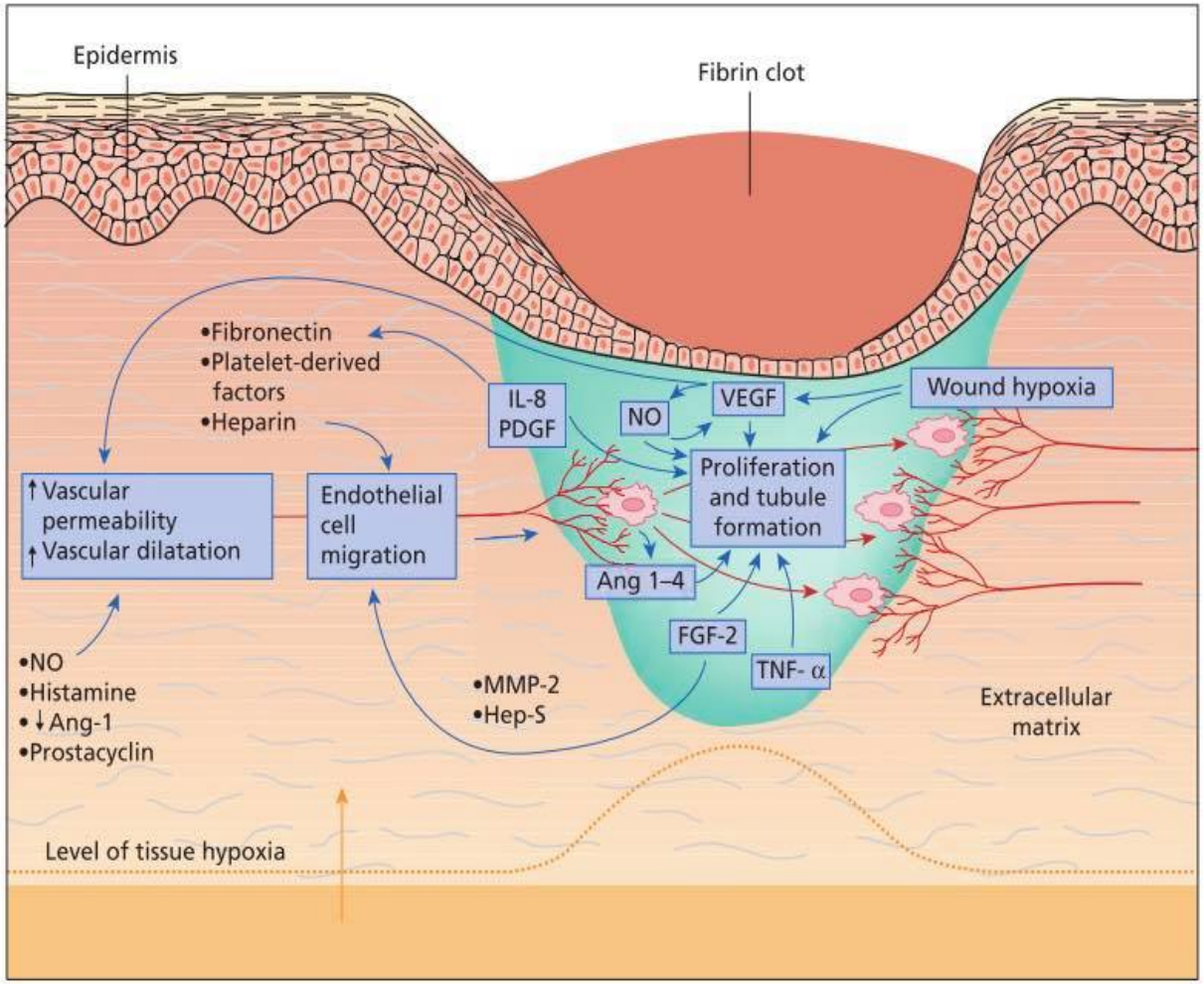


Figure 10.4 Molecular and cellular factors required for angiogenesis in order to promote endothelial cell migration, proliferation and tubule formation. After wounding, angiogenesis functions to re-establish normoxia and nutrient delivery to damaged and regenerating tissue. Existing capillaries in the wound margin become dilated with increased permeability secondary to the influence of inflammatory mediators including nitric oxide (NO), histamine, angiopoietin 1 (Ang-1), prostacyclin and vascular endothelial growth factor (VEGF). This facilitates endothelial cell extravasation and migration into the perivascular space. The process is further encouraged by platelet-derived factors, extracellular matrix components (heparan sulphate (HEP-S) and fibronectin), platelet-derived growth factor (PDGF), interleukin 8 (IL-8) and fibroblast growth factor 2 (FGF-2), which may be fibrin or fibrinogen bound. FGF-2-induced integrin expression facilitates matrix metalloproteinase 2 (MMP-2) localization, resulting in collagen degradation and thereby facilitating endothelial cell migration. Once present in the wound bed, endothelial cells proliferate and form new capillary tubules contributing to granulation tissue formation and restoration of circulatory integrity. VEGF replaces FGF-2 as the predominant stimulant of this process after 7 days and is secreted by a range of cellular mediators including macrophages, neutrophils, endothelial cells, keratinocytes and fibroblasts. Other important proliferative factors include tissue hypoxia, NO, tumour necrosis factor α (TNF-α) and Ang-1–4. (Adapted from Greaves *et al.* 2013 [**3**].)

Fibroplasia

- Most wound fibroblast are derived from proliferation of fibroblast progenitor cells in the lower dermis and the septae of underlying fat.
- Fibroblast migrate and proliferate in response to fibronectin, PDGF, fibroblast growth factors, TGF and C5a.
- Produce collagen and other component of ECM.
- Early wound being characterize by elevated levels of type III collagen, whereas later in wound healing type I collagen predominant.

Contraction

- Wound contraction, defined as the centripetal movement of wound edges that facilitates closure of a wound defect, essentially begins concurrent with collagen synthesis.
- Wound contraction depend on the myofibroblast located at the periphery of the wound, its connection to component of the ECM, and myofibroblast proliferation.

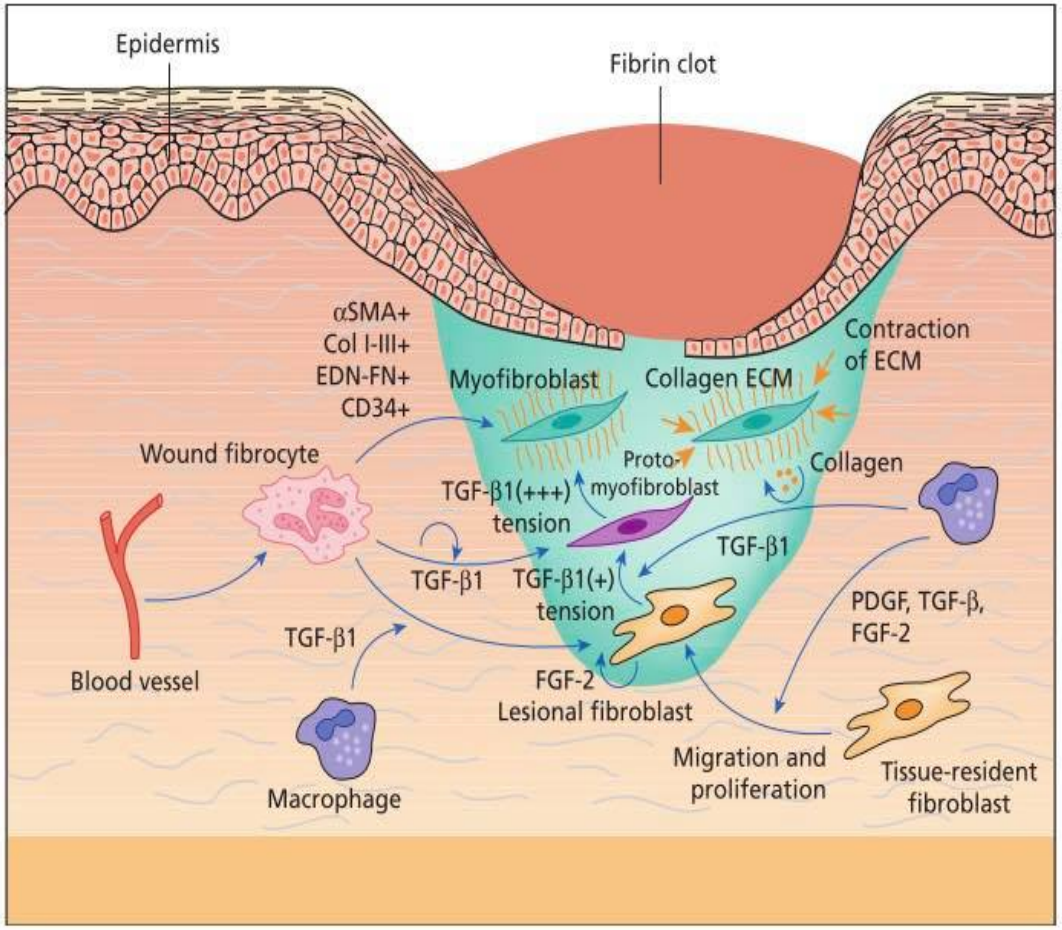


Figure 10.5 Wound contraction to initiate scarring. Macrophage-derived platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and fibroblast growth factor 2 (FGF-2) result in the migration of fibroblasts from surrounding healthy tissue to the wound site where they begin the process of fibroplasia. TGF-β1 can also induce bone-marrow-derived fibrocyte differentiation into fibroblasts, proto-myofibroblasts and myofibroblasts. Later in the proliferative phase fibroblasts convert to proto-myofibroblasts in response to increased tissue tension and TGF-β. A positive feedback loop is created where tension facilitates further TGF-β release and final maturation into myofibroblasts, which generate the majority of contractile forces in the wound. Myofibroblasts are recognized by increased expression of α-smooth muscle actin (αSMA), collagen I and III, extra domain A fibronectin (EDA-FN) and CD34+ cells. ECM, extracellular matrix. (Adapted from Greaves *et al*. 2013 [31].)

Maturation phase

- As healing proceeds, the number of fibroblasts and endothelial and inflammatory cells decrease, and the predominantly collagenous matrix become organized into thicker, more heavily cross-linked bundles.
- This marks the establishment of the mature scar.
- Scar continue to remodel for a long time after wounding, and cannot be considered to be in a steady state condition until atleast 2 years post wounding.

Growth Factors and Cytokines Affecting Various Steps in Wound Healing	
Monocyte chemotaxis	PDGF, FGF, TGF-ß
Fibroblast migration	PDGF, EGF, FGF, TGF-ß, TNF, IL-1
Fibroblast proliferation	PDGF, EGF, FGF, TNF
Angiogenesis	VEGF, Ang, FGF
Collagen synthesis	TGF-ß, PDGF
Collagenase secretion	PDGF, EGF, FGF, TNF, TGF-ß inhibits

PDGF- platelet-derived growth factor
FGF- fibroblast growth factor
TGF- transforming growth factor
EGF- epidermal growth factor
IL- interleukin
TNF- tumor necrosis factor
VEGF- vascular endothelial growth factor

Abnormal wound healing

- **Chronic wounds**
  - Sometimes, the wound healing does not proceed normally and chronic wound results include:
    - Venous ulcer
    - Diabetic ulcers
    - Pressure sores
  - Chronic wound environment may be deficient in the stimulating growth factors, growth factors receptors or proteolytic enzymes required for growth factor activation, or may be overproducing any of these factors.

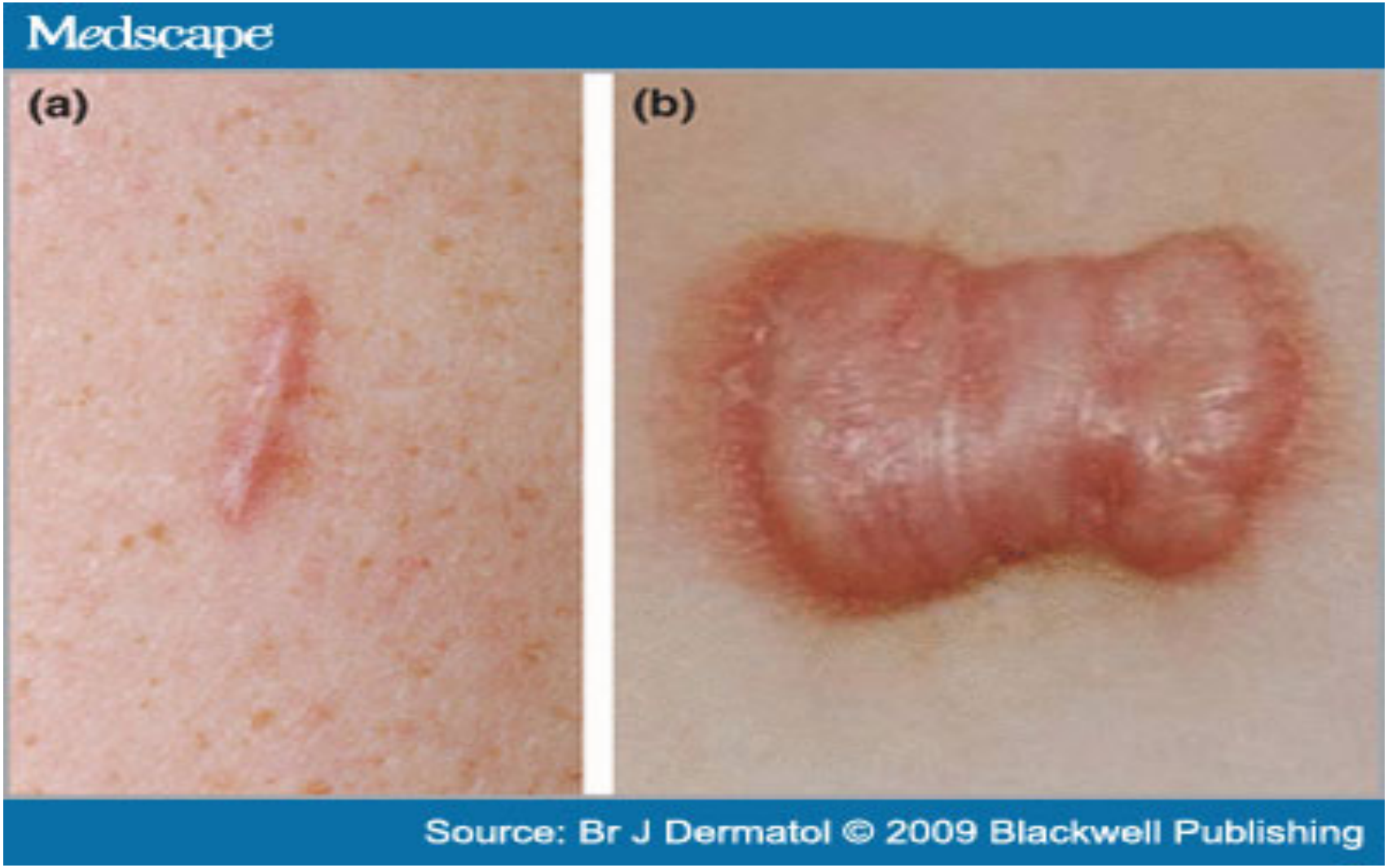
- **Hypertrophic and keloid scarring:**

  These are abnormal fibrous reactions to trauma, inflammation, surgery or burns in predisposed individuals, particularly in Afro-caribbean skin, and most occur between the ages of 10 and 30 years.

Difference between Keloid and Hypertrophic scars

KELOIDS	HYPERTROPHIC SCARS
Grow beyond borders of the original wound	Remain within the boundaries of the original wound
Size varies between a pea and a football; growth may be widespread, vertical or both	Rarely more than a centimeter in thickness or width
Itchy and painful	Less itchy and painful
Appear within several months after initial scar	Generally arise within 4 weeks and grow intensely for several months
Commonly occur on the chest, shoulders, upper back, back of the neck and earlobes, rarely on the palms or soles.	No predominant site on the body
Do not go away on their own	Will spontaneously get smaller often within a year
Larger, thicker and more wavy collagen fibers than normal skin, random collagen fiber orientation, increased ratio of type I to type III collagen	Fine collagen fibers running parallel to the epidermis

Source: Archives of Dermatological Research. May 2009, Volume 301, Issue 4, pp 259-272


Medscape
(a)
(b)
Source: Br J Dermatol © 2009 Blackwell Publishing

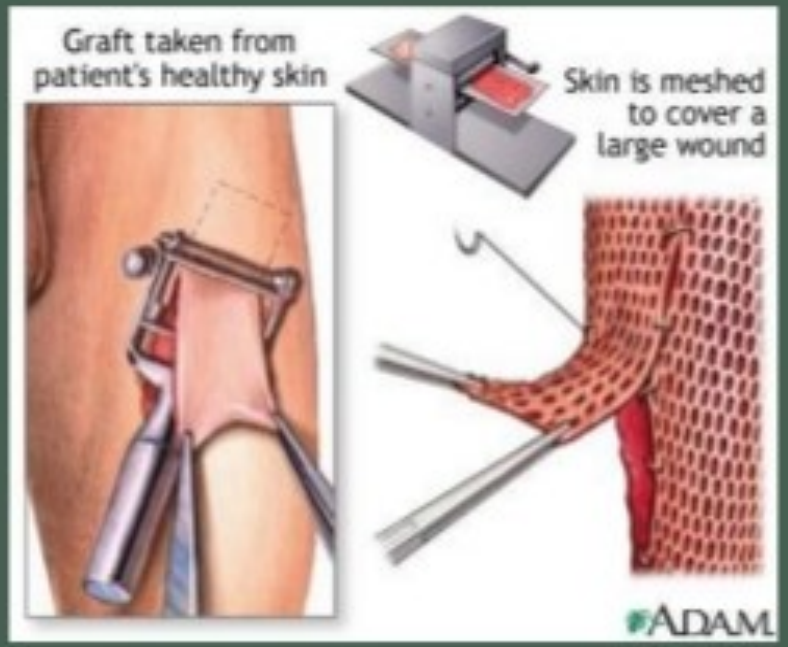
Novel therapies for wound healing

- **Growth factors to augment wound healing**
  - PDGF-BB (becaplermin gel) is currently the only licensed recombinant growth factor for treating recalcitrant wounds.

- **Stem cell therapy**
  - Several types of stems cells have been studied like:
    - Bone marrow derived stem cells
    - Adipose tissue-derived stem cells
    - Endothelial progenitor cells
    - Keratinocytes and fibroblast stem cells

• Skin grafting and biomaterials

A skin graft is a tissue of epidermis and varying amounts of dermis that is detached from its own blood supply and placed in a new area with a new blood supply.



Graft

Does not maintain original blood supply.

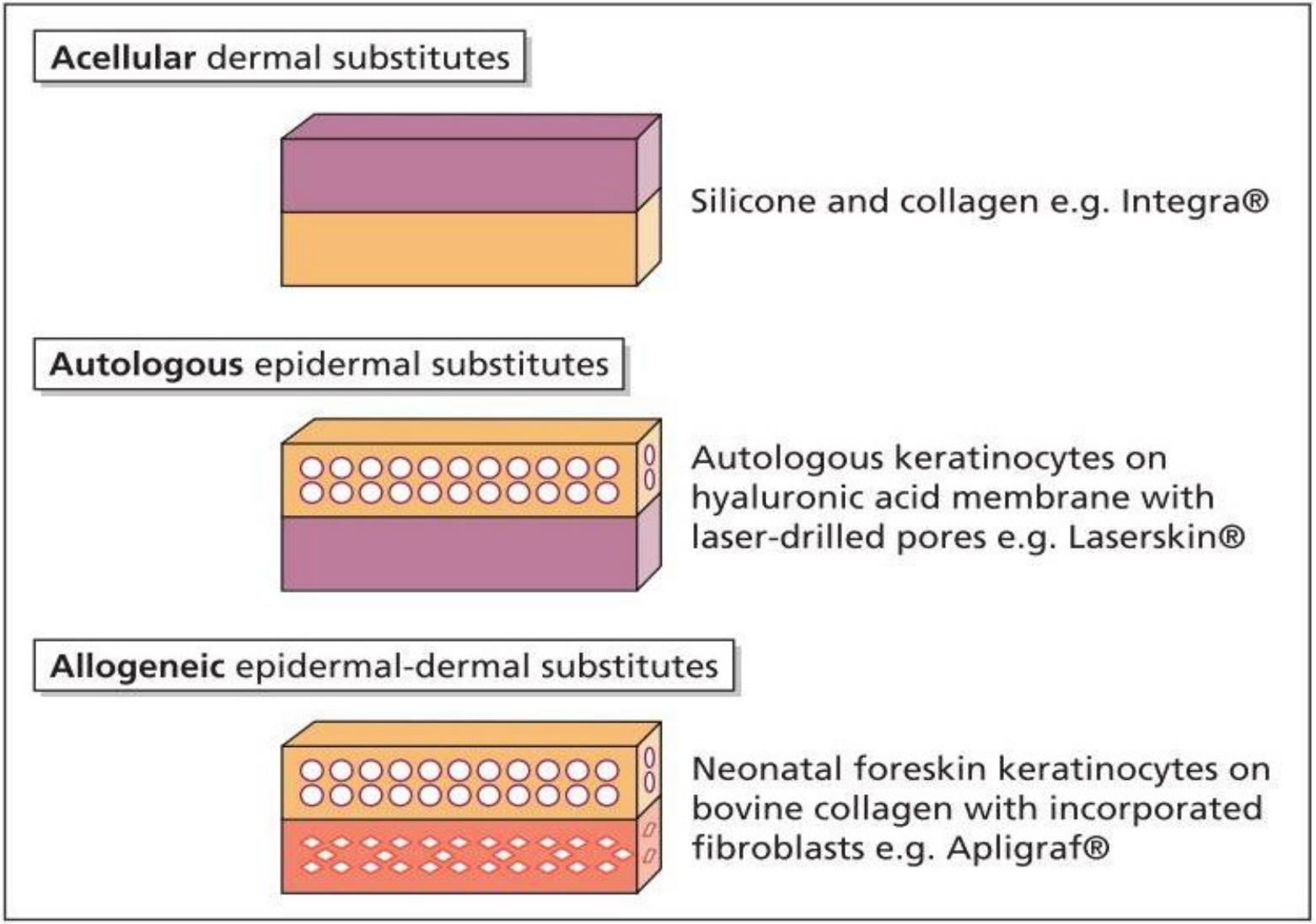


Figure 10.9 Examples of biological skin substitutes.

THANKYOU