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Wound Healing Subsequent to Injury

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Definition

The generally accepted definition of wound healing is: 'a reaction of any multicellular organism on tissue damage in order to restore the continuity and function of the tissue or organ'. This is a functional definition saying little about the process itself and which factors are influential.

Traumatic dental injuries usually imply wound healing processes in the periodontium, the pulp and sometimes associated soft tissue. The outcome of these determines the final healing result (Fig. 1.1). The general response of soft and mineralized tissues to surgical and traumatic injuries is a sensitive process, where even minor changes in the treatment procedure may have an impact upon the rate and quality of healing.

In order to design suitable treatment procedures for a traumatized dentition, it is necessary to consider the cellular and humoral elements in wound healing. In this respect considerable progress has been made in understanding the role of the different cells involved.

In this chapter the general response of soft tissues to injury is described, as well as the various factors influencing the wound healing processes. For progress to be made in the treatment of traumatic dental injuries it is necessary to begin with general wound healing principles. The aim of the present chapter is to give a general survey of wound healing as it appears from recent research. For more detailed information about the various topics the reader should consult textbooks and review articles devoted to wound healing (1–23, 607–612, 626, 631, 640–647).

Nature of a traumatic injury

Whenever injury disrupts tissue, a sequence of events is initiated whose ultimate goal is to heal the damaged tissue. The sequence of events after wounding is: control of bleeding; establishing a line of defense against infection; cleansing the wound site of necrotic tissue elements, bacteria or foreign bodies; closing the wound gap with newly formed connective tissue and epithelium; and finally modifying the primary wound tissue to a more functionally suitable tissue.

This healing process is basically the same in all tissues, but may vary clinically according to the tissues involved. Thus wound healing after dental trauma is complicated by the multiplicity of cellular systems involved (Fig. 1.2).

During the last decades, significant advances have been made in the understanding of the biology behind wound healing in general and new details concerning the regulating mechanisms have been discovered.

While a vast body of knowledge exists concerning the healing of cutaneous wounds, relatively sparse information exists concerning the healing of oral mucosa and odontogenic tissues. This chapter describes the general features of wound healing, and the present knowledge of the cellular systems involved. Wound healing as it applies to the specific odontogenic tissues will be described in Chapter 2.

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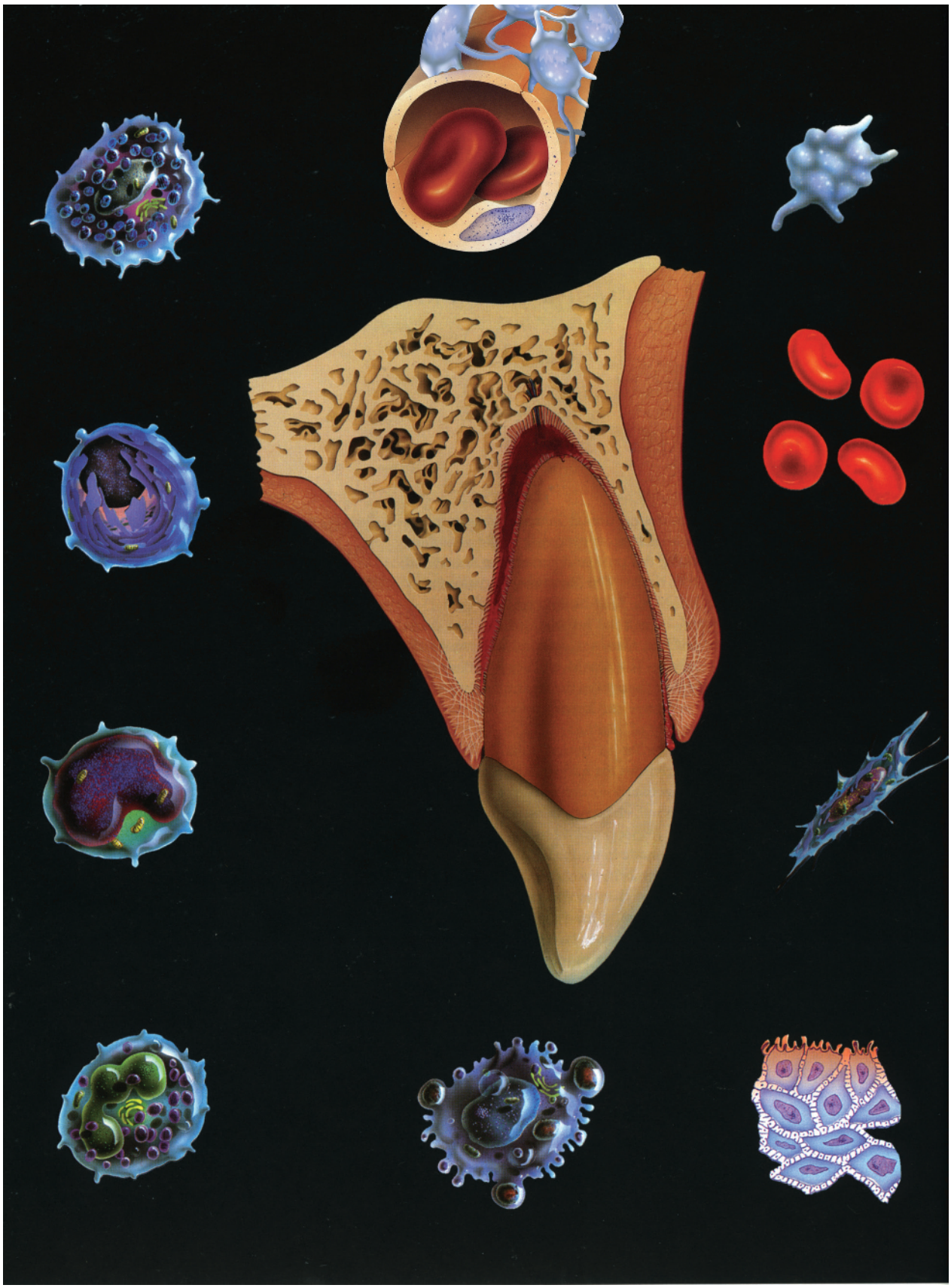

Fig. 1.1 Cells involved in the healing event after a tooth luxation. Clockwise from top: endothelial cell and pericytes; thrombocyte (platelet); erythrocyte; fibroblast; epithelial cell; macrophage; neutrophil; lymphocytes; mast cell.

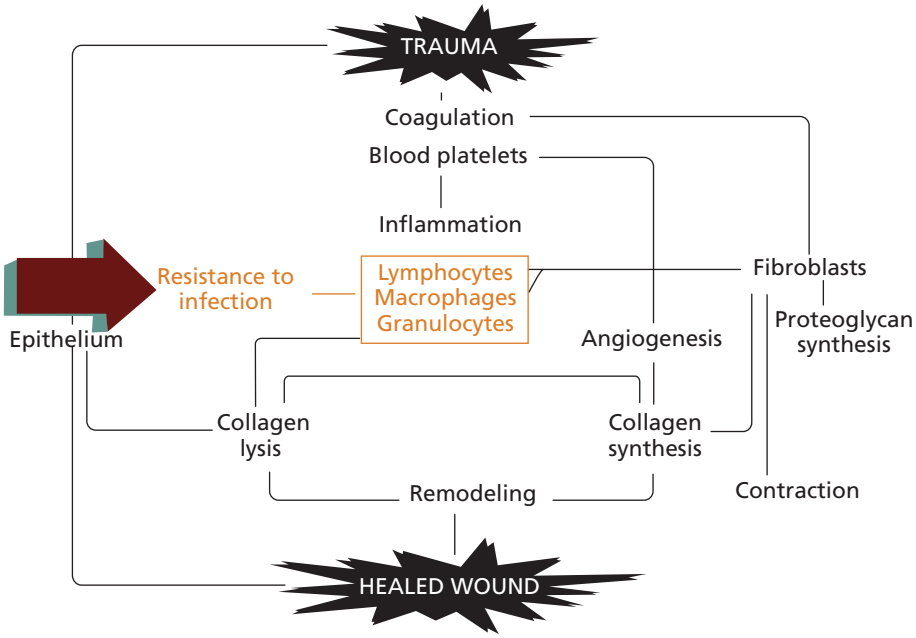

Fig. 1.2 Modified Hunt flow diagram for wound healing.

Wound healing biology

Wound healing is a dynamic, interactive process involving cells and extracelluar matrix and is dependent on internal as well as external factors. Different schemes have been used in order to summarize the wound healing process. With increasing knowledge of the involved processes, cell types, etc., a complete survey of all aspects will be hugely difficult to overview. The authors have for many years used a modification of the original Hunt flow diagram for wound healing (19) (Fig 1.2). This diagram illustrates the main events in superficial epithelialization and production of granulation tissue.

The wound healing process will be described in detail in the following section.

Repair versus regeneration

The goal of the wound healing process after injury is to restore the continuity between wound edges and to re-establish tissue function. In relation to wound healing, it is appropriate to define various terms, such as *repair* and *regeneration*. In this context, it has been suggested that the term *regeneration* should be used for a biologic process by which the structure and function of the disrupted or lost tissue is completely restored, whereas *repair* or scar formation is a biologic process whereby the continuity of the disrupted or lost tissue is regained by new tissue which does not restore structure and function (14). Throughout the text, these terms will be used according to the above definitions. The implication of repair and regeneration as they relate to oral tissues is discussed in Chapter 2.

Cell differentiation

Cell differentiation is a process whereby an embryonic non-functional cell matures and changes into a tissue-specific cell, performing one or more functions characteristic of that cell population. Examples of this are the *mesenchymal paravascular cells* in the periodontal ligament and the pulp, and the basal cells of the epithelium. A problem arises as to whether already functioning odontogenic cells can revert to a more primitive cell type. Although this is known to take place in cutaneous wounds, it is unsettled with respect to dental tissues (see Chapter 2). With regard to cell differentiation, it appears that extracellular matrix (ECM) compounds, such as proteoglycans, have a significant influence on cell differentiation in wound healing (25).

Progenitor cells (stem cells)

Among the various cell populations in oral and other tissues, a small fraction are *progenitor cells*. These cells are self-perpetuating, non-specialized cells, which are the source of new differentiating cells during normal tissue turnover and healing after injury (17–19, 24). The role of these in wound healing is further discussed in Chapter 4.

Cell cycle

Prior to mitosis, DNA must duplicate and RNA be synthesized. Since materials needed for cell division occupy more than half the cell, a cell that is performing functional synthesis (e.g. a fibroblast producing collagen, an odontoblast producing dentin or an epithelial cell producing keratin) does not have

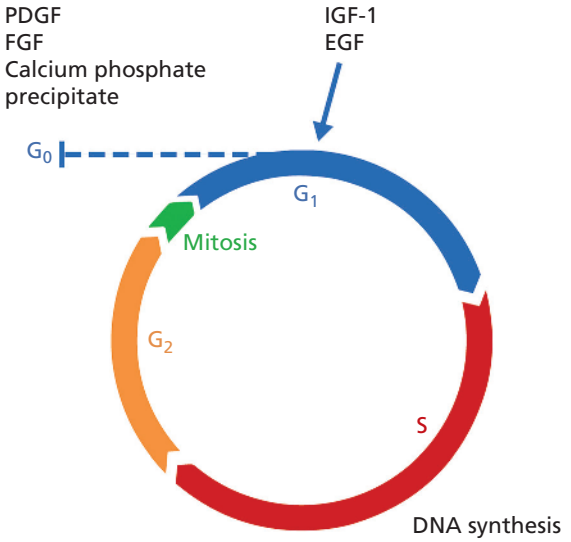

Fig. 1.3 Cell cycle: G_0, resting phase; G_1, time before onset of DNA synthesis; S, replication of DNA; G_2, time between DNA replication and mitosis.

the resources to undergo mitosis. Conversely, a cell preparing for or undergoing mitosis has insufficient resources to undertake its functions. This may explain why it is usually the least differentiated cells that undergo proliferation in a damaged tissue, and why differentiated cells do not often divide (15).

The interval between consecutive mitoses has been termed the *cell cycle*, which represents an ordered sequence of events that are necessary before the next mitosis (Fig. 1.3). The cell cycle has been subdivided into phases such as G_1, the time before the onset of DNA synthesis; in the S phase the DNA content is replicated, G_2 is the time between the S phase and mitosis, and M the time of mitosis (Fig. 1.3). The cumulative length of S, G_2 and M is relatively constant at 10–12 hours, whereas differences occur among cell types in the duration of G_1 (26).

Cells that have become growth arrested enter a resting phase, G_0, which lies outside the cell cycle. The G_0 state is reversible and cells can remain viable in G_0 for extended periods.

In vivo, cells can be classified as continuously dividing (e.g. epithelial cells, fibroblasts), non-dividing post-mitotic (e.g. ameloblasts) and cells reversibly growth arrested in G_0 that can be induced to re-enter the proliferative cycle.

Factors leading to fibroblast proliferation have been studied in the fibroblast system. Resting cells are made *competent* to proliferate (i.e. entry of G_0 cells into early G_1 stage) by so-called *competence factors* (i.e. platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and calcium phosphate precipitates). However, there is no progression beyond G_1 until the appearance of progression factors such as insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF) and other plasma factors (26) (Fig. 1.3).

Cell migration

Optimal wound repair is dependent upon an orderly influx of cells into the wound area. Directed cell motion requires polarization of cells and formation of both a leading edge that attaches to the matrix and a trailing edge that is pulled along. The stimulus for directional cell migration can be a soluble attractant (*chemotaxis*), a substratum-bound gradient of a particular matrix constituent (*haptotaxis*) or the three-dimensional array of ECM within the tissue (*contact guidance*). Finally there is a *free edge effect* which occurs in epithelial wound healing (27, 28).

Typical examples of cells responding to *chemotaxis* are circulating neutrophils and monocytes and macrophages. The chemoattractant is regulated by diffusion of the attractant from its source into an attraction-poor medium.

Cells migrating by *haptotaxis* extend lamellipodia more or less randomly and each of these protruding lamellipodia competes for a matrix component to adhere to, whereby a leading edge will be created on one side of the cell and a new membrane inserted into the leading edge. In that context, fibronectin and laminin seem to be important for adhesion (27).

Contact guidance occurs as the cell is forced along paths of least resistance through the ECM. Thus, migrating cells align themselves according to the matrix configuration, a phenomenon that can be seen in the extended fibrin strands in retracting blood clots, as well as in the orientation of fibroblasts in granulation tissue (29). In this context it should be mentioned that mechanisms also exist whereby spaces are opened within the extracellular area when cells migrate. Thus both fibroblasts and macrophages use enzymes such as plasmin, plasminogen and collagenases for this purpose (30).

During wound repair, a given parenchymal cell may migrate into the wound space by multiple mechanisms occurring concurrently or in succession. Factors related to cell migration in wound healing are described later for each particular cell type.

Dynamics of wound repair

Classically, the events taking place after wounding can be divided into three phases, namely the *inflammation*, the *proliferation* and the *remodeling phases* (5, 13, 20–23, 31). The inflammation phase may, however, be subdivided into a *hemostasis* phase and an *inflammatory* phase. But, it should be remembered that wound healing is a continuous process where the beginning and end of each phase cannot be clearly determined and phases do overlap.

Tissue injury causes disruption of blood vessels and extravasation of blood constituents. Vasoconstriction provides a rapid, but transient, decrease in bleeding. The extrinsic and intrinsic coagulation pathways are also immediately activated. The blood clots together with vasoconstriction re-establish hemostasis and provide a provisional ECM for cell migration. Adherent platelets undergo morphologic changes to facilitate formation of the hemostatic plug and secrete several mediators of wound healing such as PDGF, which attract and activate macrophages and fibroblasts.

Other growth factors and a great number of other mediators such as chemoattractants and vasoactive substances are also released. The released products soon initiate the inflammatory response.

Inflammation phase

Following the initial vasoconstriction, a vasodilation takes place in the wound area. This supports the migration of inflammatory cells into the wound area (Fig. 1.4).

These processes take place in the coagulated blood clot placed in the wound cavity. When prothrombin changes to thrombin, cleaving the fibrinogen molecule to fibrin, the clot turns into a fibrin clot, which later becomes the wound crust in open wounds. Fibronolytic activity is, however, also present in this early stage of healing. From plasminogen is produced plasmin which digests fibrin leading to the removal of thrombi. Fibrin has its main effect when angiogenesis starts and the restoration of vascular structure begins.

Neutrophils, lymphocytes and macrophages are the first cells to arrive at the site of injury. Their major role is to guard against the threat of infection, as well as to cleanse the wound site of cellular matrix debris and foreign bodies. The macrophages appear to direct the concerted action of the wound cell team (Fig. 1.4).

Proliferative phase (fibroplasia)

This is called the *fibroplasia phase* or *regeneration phase* and is a continuation of the inflammatory phase, characterized by fibroblast proliferation and migration and the production of connective tissue. Once fibroblasts have migrated into the granulation tissue, their primary role is to rapidly produce new connective tissue ECM to re-establish tissue strength and function. It appears that fibroblast migration into the wound provisional matrix continues along an increasing but relatively low concentration gradient of a given chemoattractant, e.g. EGF or transforming growth factor (TGF)-band PDGF. It starts about day 2 after the tissue trauma and continues for 2–3 weeks after the trauma in the case of a closed wound. This phase can be extended significantly in the case of an open wound with severe tissue damage, where complete closure will require production of a large amount of connective tissue.

In response to chemoattractants created in the inflammation phase, fibroblasts invade the wound area and this starts the proliferation phase. The invasion of fibroblasts starts at day 2 after injury and by day 4 they are the major cell type in normal healing. Fibroblasts are responsible for replacing the fibrin matrix (clot) with collagen-rich new stroma often called *granulation tissue*. In addition, fibroblasts also produce and release proteoglycans and glycosaminoglycan (GAG), which are important components of the ECM of the granulation tissue. Vascular restoration uses the new matrix as a scaffold and numerous new capillaries endow the new stroma with a granular appearance (angiogenesis). Macrophages provide a continuing source of growth factors necessary to stimulate fibroplasia and angiogenesis. The structural molecules of newly formed ECM, termed *provisional matrix*, produce a scaffold or conduit for cell migration. These molecules include fibrin, fibronectin and hyaluronic acid. Fibronectin and the appropriate integrin receptors bind fibronectin, fibrin or both on fibroblasts, appearing to limit the rate of formation of granulation tissue.

Stimulated by growth factors and other signals, fibroblasts and endothelial cells divide, and cause a capillary network to move into the wound site which is characterized by ischemic-damaged tissue or a coagulum.

The increasing numbers of cells in the wound area induce hypoxia, hypercapnia and lactacidosis, due to the increased need for oxygen in an area with decreased oxygen delivery because of the tissue injury (32, 33).

At cellular level oxygen is an essential nutrient for cell metabolism, especially energy production. This energy is supplied by the coenzyme adenosine triphosphate (ATP), which is the most important store for chemical energy on the molecular/enzymatic level and is synthesized in mitochondria by oxidative phosphorylation. This reaction is oxygen dependent.

NADPH-linked oxygenase is the responsible enzyme for the respiratory burst that occurs in leukocytes. During the inflammatory phase of the healing process NADPH-linked oxygenase produces high amounts of oxidants by consuming high amounts of oxygen (34). The first event in wound healing is activation of an NADPH-linked oxidase (630). Successful wound healing can only take place in the presence of the enzyme, because oxidants are required for the prevention of wound infection.

Not only phagocytes, but almost every cell in the wound environment is fitted with a specialized enzyme to convert O_2 to *reactive oxygen species* (ROS), including oxidizing species such as free radicals and hydrogen peroxide (H_2O_2) (35, 36). These ROS act as cellular messengers to promote several important processes that support wound healing. Thus O_2 has a role in healing beyond its function as nutrient and antibiotic. Given the growth factors, such as PDGF, require ROS for their action on cells (35, 37), it is clear that O_2 therapy may act as an effective adjunct. Clinically this has been found in chronic granulomatous disease (CGD) where there are defects in genes that encode NADPH oxidase. The manifestations of this defect are increased susceptibility to infection and impaired wound healing (625).

Simultaneously, the basal cells in the epithelium divide and move into the injury site, thereby closing the defect. Along with revascularization, new collagen is formed which, after 3–5 days, adds strength to the wound. The high rate of collagen production continues for 10–12 days, resulting in strengthening of the wound. At this time healing tissue is dominated by capillaries and immature collagen.

The fibroblasts are responsible for the synthesis, deposition and remodeling of the ECM, which conversely can have an influence on the fibroblast activities. Cell movements at this stage into the fibrin clot or tightly woven ECM seem to require an active proteolytic system that can cleave a path for cell migration. Fibroblast-derived enzymes (collagenase,

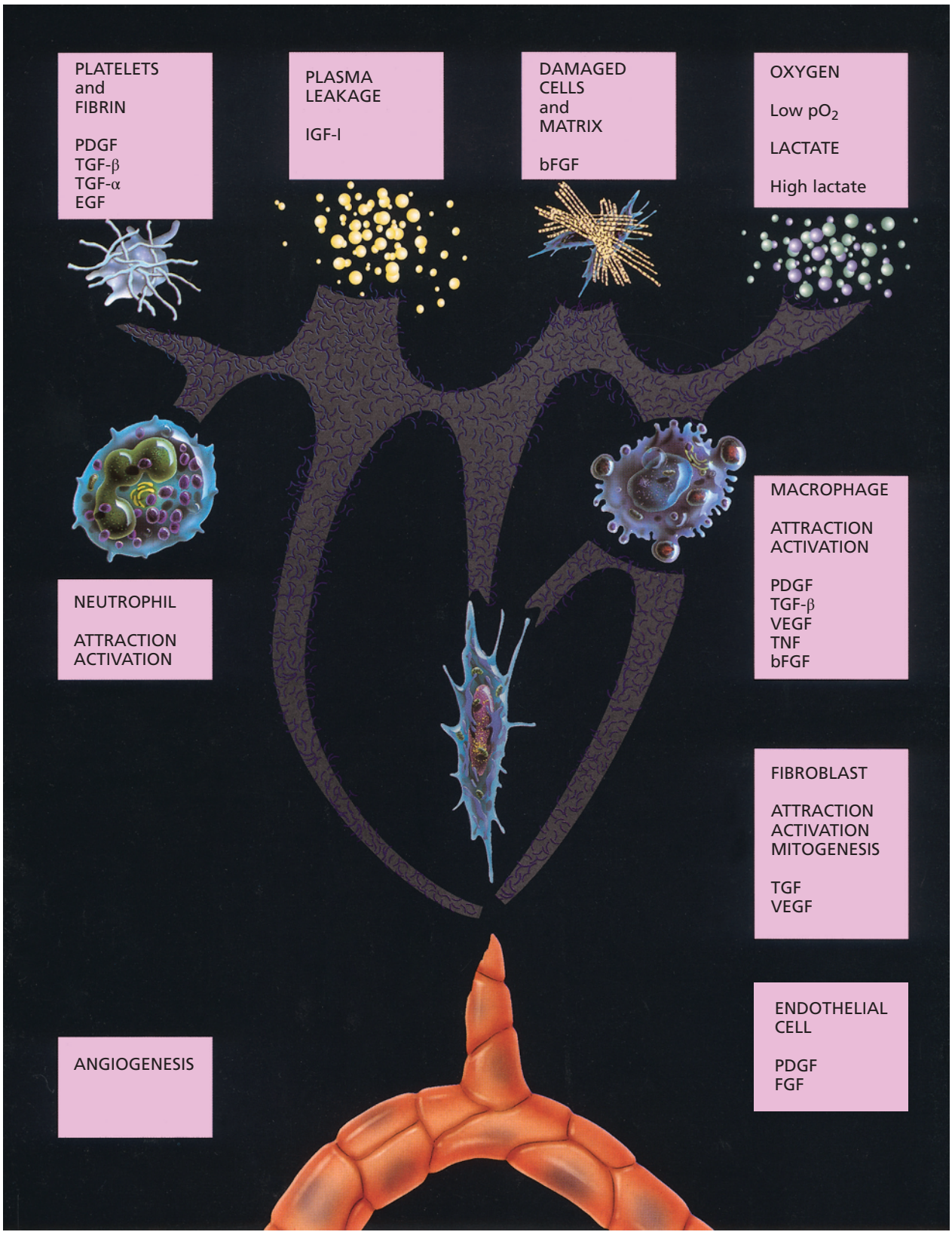

Fig. 1.4 Cellular components and mediators in the wound healing module. Signals for wound healing are released by platelets, fibrin, plasma leakage, damaged cells and matrix. Furthermore low oxygen tension and a high lactate concentration in the injury site contribute an important stimulus for healing.

gelatinase A, etc.) and serum plasmin are potential candidates for this task (23).

After fibroblast migration into the wound cavity, the provisional ECM is gradually replaced with collagenous matrix. It appears that fibroblast migration into the wound provisional matrix continues along an increasing, but relatively low, concentration gradient of a given chemoattractant, e.g. EGF or TGF-band PDGF. New connective tissue begins to form approximately 2–4 days after wounding, and it is called granulation tissue due to its granular appearance when examined visually. Once an abundant collagen matrix has been deposited in the wound, the fibroblasts stop producing collagen, and the fibroblast-rich granulation tissue is replaced by a relatively acellular scar. Cells in the wound undergo apoptosis (cell death) triggered by unknown signals, but doing so the fibroblast dies without raising an inflammatory response. Deregulation of these processes occurs in fibrotic disorders such as keloid formation, morphea and scleroderma. Collagen synthesis and secretion requires hydroxylation of proline and lysine residues. Sufficient blood flow delivering adequate molecular oxygen is pivotal for this process.

Collagen production/deposition and development of strength of the wound is directly correlated to the partial pressure pO_2 of the tissue (p_tO_2) (38–40). Synthesis of collagen, crosslinking and the resulting wound strength relies on the normal function of specific enzymes (41, 42). The function of these enzymes is directly related to the amount of oxygen present, e.g. hydrolyzation of proline and lysine by hydroxylase enzymes (43).

Recently it has been shown that oxygen also may trigger the differentiation of fibroblasts to myofibroblasts, cells responsible for wound contraction (44).

Neovascularization/angiogenesis

Early in the healing process there is no vascular supply to the injured area, but the stimulus for angiogenesis is present: growth factors released by especially macrophages, low oxygen and elevated lactate. The angiogenesis starts the day after the lesion. Angiogenesis is complicated, involving endothelial cells and activated epidermal cells. Proteolytic enzymes degrade the endothelial basement membrane allowing endothelial cells from the surroundings of the wound area to proliferate, migrate and form new vessels. The establishment of new blood vessels occurs by the budding or sprouting of intact venoles and the sprouts meet in loops (see p. 32) (259, 377). The presence of capillary loops within the provisional matrix provides the tissue with a red granular appearance. Once the wound is filled with new granulation tissue, angiogenesis ceases and many of the blood vessels disintegrate as a result of apoptosis. Angiogenesis is dependent upon the ECM (623, 624).

While hypoxia can initiate neovascularization, it cannot sustain it. Supplementary oxygen administration accelerates vessels' growth (35, 45). Vascular endothelial growth factor (VEGF) has been established as a major long-term angiogenetic stimulus at the wound site. Recently, the cell response to hypoxia has been further elucidated. Hypoxia inducible factor 1 (HIF-1) has been identified as a transcription factor that is induced by hypoxia (46, 48).

In the presence of normal oxygen tensions HIF-1 transcriptional activity is ubiquinated and degraded (47). HIF-1 seems to upregulate genes involved in glucose metabolism and angiogenesis under hypoxia and in a model of myocardial and cerebral ischemia the factor seems to protect cells from damage. The exact molecular mechanisms of how hypoxia is sensed by the cells are still unknown.

The arrangement of cells in the proliferative phase has been examined in rabbits using ear chambers where wounds heal between closely approximated, optically clear membranes (33, 49). It appears from these experiments that macrophages infiltrate the tissue in the dead space, followed by immature fibroblasts. New vessels are formed next to these fibroblasts that synthesize collagen. This arrangement of cells, which has been termed the *wound healing module*, continues to migrate until the tissue defect is obliterated. The factors controlling the growth of the wound healing module are described on p. 32.

Epithelialization

Re-epithelialization of wounds begins within hours after injury. Within 24 hours after wounding, epithelial cells at the margin of the wound dissolve their hemidesmosomal adhesions and show the first signs of migration. In 48 hours, proliferation starts behind the leading edge, seeding more cells into the wound site. Epithelial cells migrate through the fibrin–fibronectin provisional matrix until they contact the front of leading cells coming from the other side of the wound (626).

If parts of the dermis layers are intact, epidermal cells from skin appendages such as hair follicles quickly remove clotted blood and damaged stroma and cover the wound space. This results in fast epithelialization. If the dermis is totally destroyed, the epithelialization only takes place from the wound edges and epithelialization can continue for a considerable time dependent on wound area.

The trauma of being wounded causes an activation of epithelial keratinocytes by exposure to the pro-migratory matrix molecules, growth factors and cytokines that are released, and wound-generated electrical fields. During epithelialization the cells undergo considerable phenotypic alteration including retraction of intracellular tonofilaments, dissolution of most intercellular desmosomes and formation of peripheral cytoplasmatic actin filaments, which allow cell movement. Furthermore, the cells no longer adhere to one another and the basement membrane. This allows migration of the cells dissecting the wound and separating scar from viable tissue. Integrin expression of the migrating epidermal cells appears to determine the path of dissection (23). Epidermal cell migration between collagenous dermis and the fibrin scar requires degradation of ECM. This is achieved by production of proteinases (collagenases, e.g. matrix metalloproteinase-1) and activation of plasmin by activators produced by epidermal cells. In well-adapted, non-complicated

surgical incisional wounds the first layers of epidermal cells move over the incisional line 1–2 days after suturing. At the same time, epidermal cells at the wound margin in open wounds begin to proliferate behind the actively migrating cells. The stimulus for migration and proliferation of epidermal cells is unknown, but the absence of neighbor cells at the margin of the wound (free edge effect), local release of growth factors and increased expression of growth factor receptors may be a suggestion.

During dermal migration from the wound margin, a basement membrane reappears in a zip-like fashion and hemidesmosomes and type VII collagen anchoring fibrils form. Epidermal cells firmly attached to the basement membrane and underlying dermis reverts to normal phenotype.

The production of epithelial tissue is primarily dependent on the degree of hydration and oxygen. While a moist wound environment increases the rate of epithelialization by a factor of two or three (50, 51), the optimal growth of epidermal cells is found at an oxygen concentration of 10–50% (52–54).

Wound contraction

Wound contraction is a complex process, and beneficial because a portion of the lesion is covered by skin despite scar tissue and thus it decreases complications by decreasing the open skin wound area. In human skin, contraction can account for about 50% of wound closure, but in rodents it is more extensive and makes up to 90% of the wound closure (631). During the second week of healing, fibroblasts assume a myofibroblast phenotype characterized by large bundles of actin-containing microfilaments (55). The stimulus for contraction probably is a combination of growth factors, integrin attachment of the myofibroblasts to collagen matrix and crosslinks between collagen bundles (23). Wound contraction seems to be related to the early wound healing period and the effect decreases in time; in chronic unclosed wounds, no wound contraction exists.

Scar contracture

As opposed to the process of wound contraction of skin edges, this is a late pathologic process in wound healing. It consists of a contraction of large amounts of scar tissue followed by immobilization of the affected area (e.g. a joint). In scar contracture, the wound area as well as adjacent tissue shrinks, as opposed to contraction where only the wound area is involved. The morbidity of scar contracture is a major problem in the rehabilitation of severely injured patients.

Remodeling phase

The *remodeling phase* is also called the *moderation phase* or the *scar phase*.

The wound remodeling phase turns the abundant and poorly organized granulation tissue ECM into a mature connective tissue. Remodeling starts when wound contraction has assembled the collagen fibrils into thicker bundles and aligned them perpendicularly to the wound edges. During the remodeling stage, collagen crosslinking also gradually increases, improving the stability of the tissue, and there is a gradual maturation of the tissue so that the aligned collagen fiber bundles are reorganized to the typical and more resilient basketweave organization found in normal connective tissue (628). Remodeling continues slowly and can last for months or in some cases for years.

In closed wounds this phase starts 2–3 weeks after closure, while it does not start in open wounds before the wound has healed. Granulation tissue covered by epidermis is known to undergo remodeling earlier than uncovered granulation tissue. The length of this phase is unknown; some have argued 1 year but others have claimed the rest of the patient's life.

During this phase the granulation tissue is remodeled and matured to a scar formation. When granulation tissue is covered by epithelium it undergoes remodeling. Similarly, a wound covered by a graft will continue the remodeling phase. This results in a decrease in cell density, numbers of capillaries and metabolic activity (55). The collagen fibrils will be united into thicker fiber bundles. There is a major difference between dermis and scar tissue in the arrangement of collagen fiber bundles. In scar tissue, as in granulation tissue, they are organized in arrays parallel to the surface, while in dermis they are more in a basketweave pattern (21). This difference results in a more rigid scar tissue. The collagen composition change from granulation tissue to scar tissue, where there is collagen type III, decreases from 30% to 10%. In the remodeling phase the biomechanical strength of a scar increases slightly, despite no extra collagen being produced. This increase relates primarily to a better architectural organization of the collagen fiber bundles.

The epidermis of a scar differs from normal skin by lacking the rete pegs, which are anchored within the underlying connective tissue matrix (21). Furthermore there is no regeneration of lost subepidermal appendages such as hair follicles or sweat glands in a scar.

Types of wound after injury

Wounds can be divided into different types, according to healing and associated wound closure methods (56–58) (Fig. 1.5). This distinction is based on practical treatment regimens while the basic biological wound healing sequences are similar for all wound types.

Primary healing, or healing by *first intention*, occurs when wound edges are anatomically accurately opposed and healing proceeds without complication. This type of wound heals with a good cosmetic and functional result and with a minimal amount of scar tissue. These wounds, however, are sensitive to complications, such as infection.

Secondary healing, or healing by *second intention*, occurs in wounds associated with tissue loss or when wound edges are not accurately opposed. This type of healing is usually

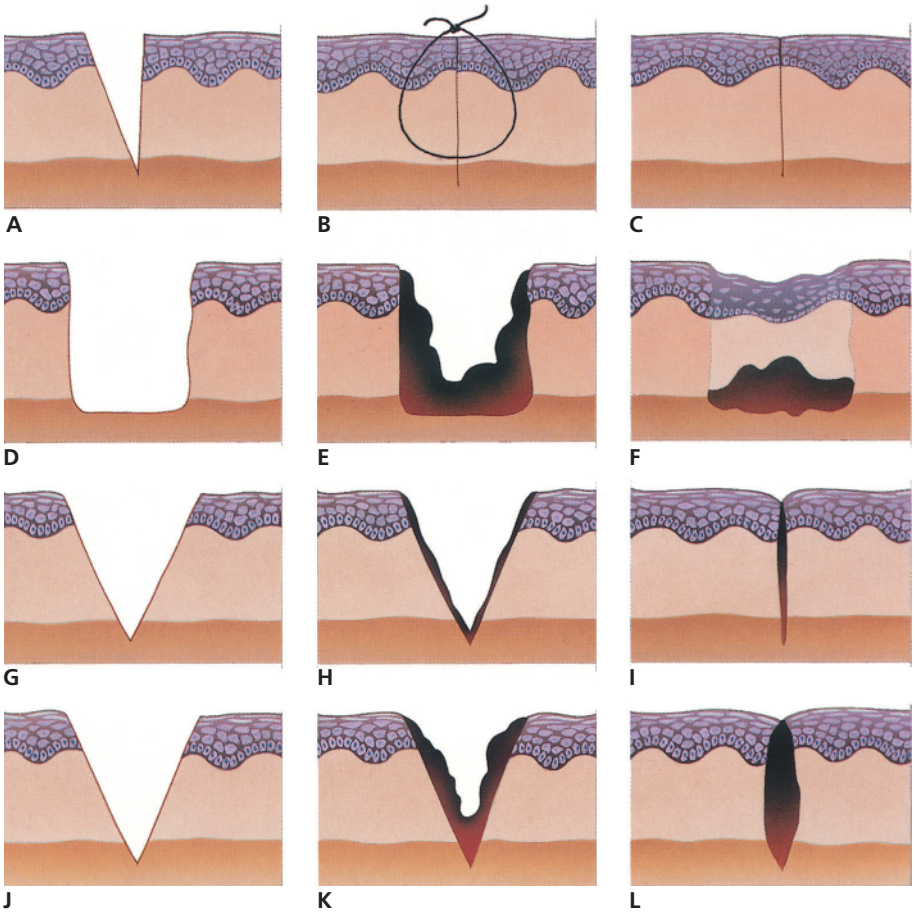

Fig. 1.5 Wound healing events related to the type of wound and subsequent treatment. A–C. Incisional wound with primary closure. D–F. Open and non-sutured wound. G–I. Delayed primary closure. J–L. Secondary closure. From (56).

the natural biologic process that occurs in the absence of surgical intervention. The defect is gradually filled by granulation tissue and a considerable amount of scar tissue will be formed despite an active contraction process. The resulting scar is less functional and often sensitive to thermal and mechanical injury. Furthermore, this form of healing requires considerable time for epithelial coverage and scar formation, but is rather resistant to infection, at least when granulation tissue has developed.

Surgical closure procedures have combined the advantages of the two types of healing. This has led to a technique of *delayed primary closure*, where the wound is left open for a few days but closure is completed before granulation tissue becomes visible (usually a week after wounding) and the wound is then healed by a process similar to primary healing (59, 60, 435, 614). The resulting wound is more resistant to healing complications (primarily infection) and is functionally and cosmetically improved. If visible granulation tissue has developed before either wound closure or wound contraction has spontaneously approximated the defect, it is called *secondary closure*. This wound is healed by a process similar to secondary healing and scar formation is more pronounced than after delayed primary closure. The different closure techniques are shown in Fig. 1.5. The following section describes the sequential changes in tissue components and their interactions seen during the wound healing process.

Tissues and compounds in wound healing

Hemostasis phase and coagulation cascade

An injury that severs the vasculature leads to extravasion of plasma, platelets, erythrocytes and leukocytes. This initiates the coagulation cascade that produces a blood clot usually after a few minutes and which, together with the already induced vascular contraction, limits further blood loss (Fig. 1.6). The tissue injury disrupts the endothelial integrity of the vessels, and exposes the subendothelial structures and various connective tissue components. Exposure of type IV and V collagen in the subendothelium promotes binding and aggregation of platelets and their structural proteins (61, 62). Exposure of collagen and other activating agents provokes endothelial cells and platelets to secrete several substances, such as fibronectin, serotonin, PDGF, adenosine diphosphate (ADP), thromboxane A and others. Following this activation, platelets aggregate and platelet clot formation begins within a few minutes. The clot formed is impermeable to plasma and serves as a seal for the ruptured vasculature as well as to prevent bacterial invasion (62). In addition to platelet aggregation and activation, the coagulation cascade is initiated (Fig. 1.6).

The crucial step in coagulation is the conversion of fibrinogen to fibrin, which will create a thread-like network to entrap plasma fractions and formed elements. This fibrin

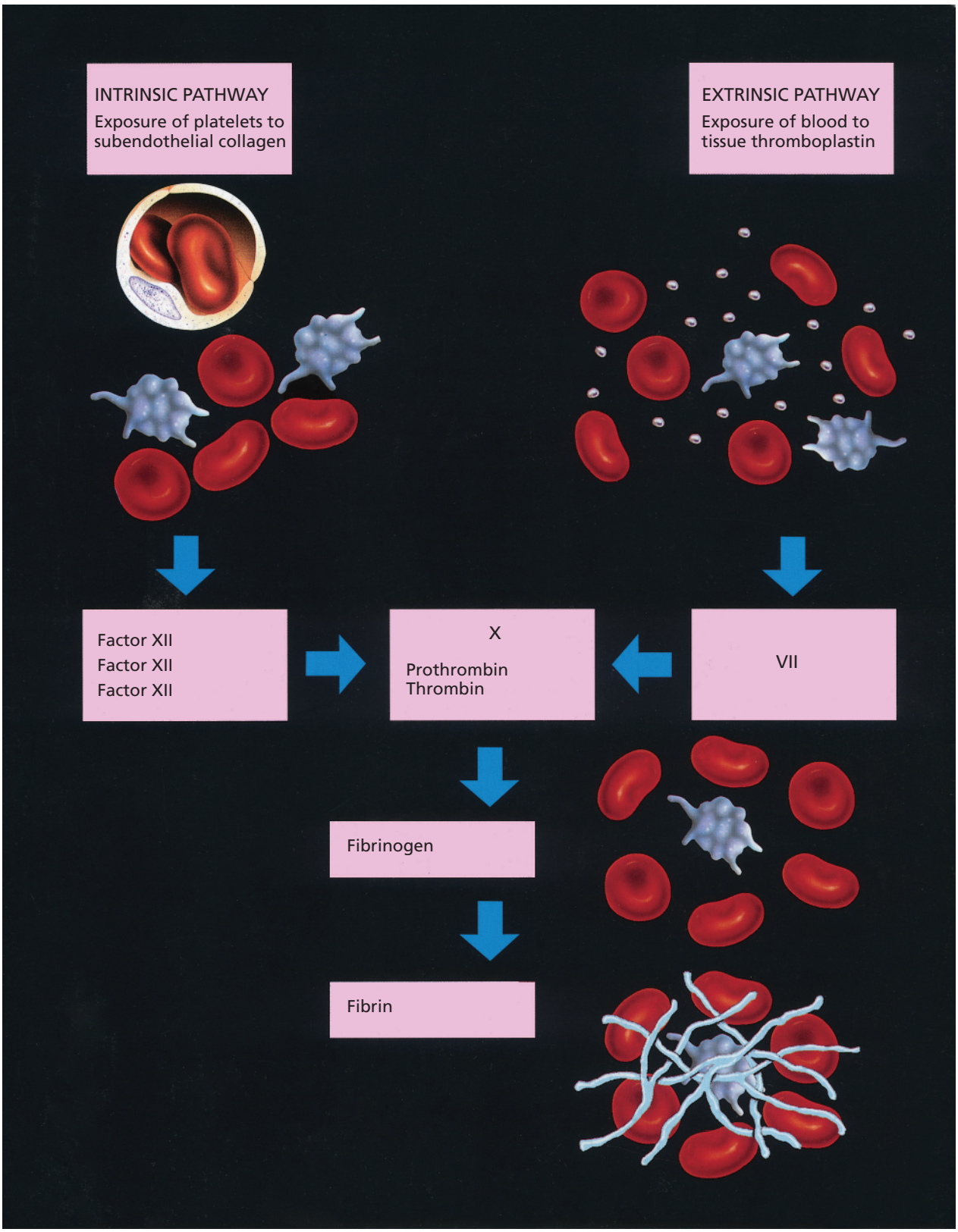

Fig. 1.6 Extrinsic and intrinsic coagulation cascade.

blood clot is formed both intravascularly and extravascularly and supports the initial platelet clot (Fig. 1.6). Extrinsic and intrinsic clotting mechanisms are activated, each giving rise to cascades that will convert prothrombin to thrombin, and in turn cleave fibrinogen to fibrin which then polymerizes to form a clot (63).

The *extrinsic* coagulation pathway is initiated by tissue thromboplastin and coagulation factor VII, whereas the

initiator of the *intrinsic* coagulation cascade consists of Hageman factor (factor XII), prekallikrein and high molecular weight kinogen. The extrinsic coagulation pathway is the primary source of clotting, while the intrinsic coagulation pathway is probably most important in producing bradykinin, a vasoactive mediator that increases vascular permeability (64).

Products of the coagulation cascade regulate the cells in the wound area. Thus *intact thrombin* serves as a potent growth stimulator for fibroblasts and endothelial cells (65, 66) whereas *degraded thrombin* fragments stimulate monocytes and platelets (67–69). Through its chemotactic and mitogenic activities towards macrophages, fibroblasts and endothelial cells thrombin directly supports wound healing (632). Likewise, *plasmin* acts as a growth factor for parenchymal cells (69). *Fibrin* acts as a chemoattractant for monocytes (70) and induces angiogenesis (64). Other mediators created by blood coagulation for wound healing include *kallikrein*, *bradykinin*, and *C3a* and *C5a* through a spillover activation of the complement cascade, and most of these factors act as chemoattractants for circulating leukocytes. Thus apart from ensuring hemostasis, the clot also initiates healing (Fig. 1.4).

If the blood clot is exposed to air it will dry and form a scab which serves as a temporary wound dressing. A vast network of fibrin strands extends throughout the clot in all directions (Fig. 1.6). These strands subsequently undergo contraction and become reoriented in a plane parallel to the wound edges (71, 72). As the fibrin strands contract, they exert tensional forces on the wound edges whereby serum is extruded from the clot and the distance between wound edges is decreased. Contraction and reorientation of the fibrin strands later serve as pathways for migrating cells (see p. 24).

If proper adaptation of the wound edges has occurred, the extravascular clot forms a thin gel filling the narrow space between the wound edges and gluing the wound edges together with fibrin.

If hemostasis is not achieved, blood will continue to leak into the tissue, leading to a hematoma and a coagulum which consists of serum plasma fraction, formed elements and fibrin fragments. The presence of such a hematoma will delay the wound healing and increase the risk of infection (77).

Coagulation

More extensive blood clot formation is undesirable in most wounds as the clots present barriers between tissue surfaces and force wounds that might have healed without a clot to heal by secondary intention. In oral wounds such as extraction sockets, blood clots are exposed to heavy bacterial colonization from the saliva (74). In this location neutrophil leukocytes form a dense layer on the exposed blood clot and the most superficial neutrophils contain many phagocytosed bacteria (75).

The breakdown of coagulated blood in the wound releases ferric ions into the tissue, which have been shown to decrease the non-specific host response to infection (76). Furthermore, the presence of a hematoma in the tissue may increase the chance of infection (77).

Clot adhesion to the root surface appears to be important for periodontal ligament healing. Thus an experiment has shown that heparin-impregnated root surfaces, which prevented clot formation, resulted in significantly less connective tissue repair and an increase in downgrowth of pocket epithelium after gingival flap surgery (78).

Coagulation and sustained thrombin production seem also to be essential for wound healing in genral. Mice deficient in FXIII or FIX have delayed wound healing because of continued bleeding into the granulation tissue and reduced thrombin production (633).

Fibrin

Fibrin in the wound provisional matrix provides adhesion for leukocytes, fibroblasts and endothelial cells (693). In addition, fibrin provides a reservoir for many growth factors such as FGF-2 and VEGF that stimulate wound healing. During coagulation, fibrin is crosslinked to plasma fibronectin to create a plug for hemostasis. Fibrinogen is converted to fibrin which, via a fishnet arrangement with entrapped erythrocytes, stabilizes the blood clot (Figs 1.6 and 1.7).

In the early acute inflammatory period extravasation of a serous fluid from the leaking vasculature accumulates as an edema in the tissue spaces. This transudate contains fibrinogen which forms fibrin when acted upon by thrombin (Fig. 1.7). Fibrin plugs then seal the damaged lymphatics and thereby confine the inflammatory reaction to an area immediately surrounding the wound.

Formation of a fibrin clot is also essential for the initiation of wound healing. Fibrin has been found to play a significant role in wound healing by its capacity to bind to fibronectin (63). Thus fibronectin present in the clot will link to both fibrin and to itself (79, 80).

Fibrin clots and fibrinopeptides are weak stimulators of fibroblasts (81), an effect which is prevented by depletion of fibronectin (82). It has also been proposed that an interaction may take place between hyaluronic acid and fibrin which creates an initial scaffold on which cells may migrate into the wound (83).

The extravascular fibrin forms a hygroscopic gel that facilitates migration of neutrophils and macrophages, an effect which possibly reflects a positive interaction between the macrophage surface and the fibrin matrix. Fibrin has also been shown to elicit fibroblast migration and angiogenesis, both of which initiate an early cellular invasion of the clot (63, 64, 84–86).

Fibrin clots are continuously degraded over a 1–3 week period (73, 87, 88). This occurs during the fibrinolysis cascade, which is activated by the plasminogen present in damaged endothelial cells and activated granulocytes and macrophages (87–89) (Fig. 1.7).

In experimental replantation of teeth in monkeys it has been found that collagen fiber attachment to the root surface was preceded by fibrin leakage, and that this leakage was an initial event in the wound healing response (90).

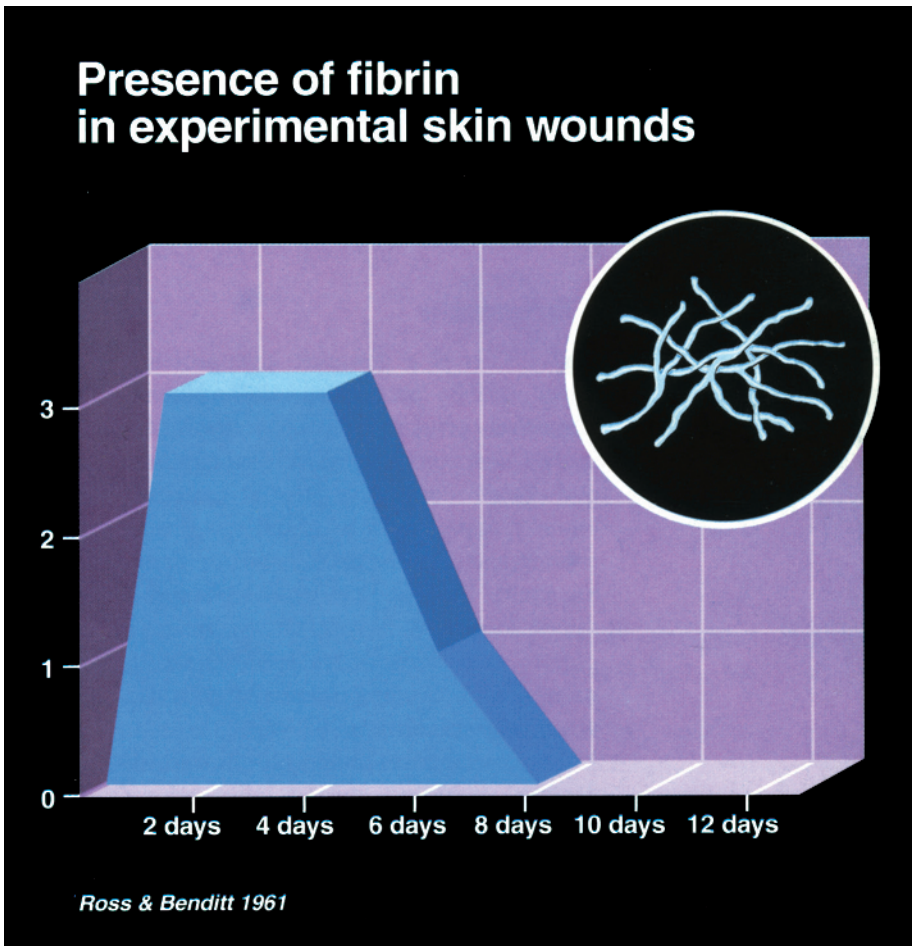

Fig. 1.7 Schematic illustration of the presence of fibrin in experimental skin wounds in guinea pigs. The scale is semiquantitative, graded from 0 to 3. Adapted from (73).

In summary, the blood clot, apart from being responsible for hemostasis, also serves the purpose of initiating wound healing including functioning as a matrix for migrating connective cells.

Fibronectin

Fibronectin is a complex glycoprotein, which can be present as soluble plasma fibronectin, produced by hepatocytes, or stromal fibronectin, found in basal laminae and loose connective tissue matrices where it is produced by fibroblasts, macrophages and epithelial cells (91, 92). During wound healing, fibronectin is also produced locally by fibroblasts (93), macrophages in regions where epidermal cell migration occurs (92), endothelial cells (94, 95), and by epidermal cells (96).

In normal resting adult epithelium, keratinocytes do not interact with fibronectin and thus do not typically express fibronectin receptors (629). However, in wound healing fibronectin plays many roles, including platelet aggregation, promotion of re-epithelialization, cell migration, matrix deposition and wound contraction (92, 97).

In wound healing, fibronectin is the first protein to be deposited in the wound (98) and therefore, together with fibrin, serves as a preliminary scaffold and matrix for migrating cells (99). Thus plasma fibronectin is linked to fibrin that has been spilled from damaged vessels or from highly permeable undamaged vessels (97, 100). The fibrin–fibronectin complex forms an extensive meshwork throughout the wound bed which facilitates fibroblast attachment and migration into the clot (80, 101–103). Furthermore, soluble fibronectin fragments are chemotactic for fibroblasts and monocytes (104).

Fibronectin appears also to guide the orderly deposition of collagen within the granulation tissue. Thus fibronectin serves as the scaffold for deposition of types III and I collagen (105–109) as well as collagen type VI (109). As dermal wounds age, bundles of type I collagen become more prominent at the expense of type III collagen fibronectin (106). Finally, fibronectin seems to represent a necessary link between collagen and fibroblasts, which makes it possible to generate the forces in wound contraction (92, 110).

Although plasma fibronectin does not appear to be essential for hemostasis (635), it can provide a substratum for epithelial and fibroblast migration towards the clot.

In the endothelium during wound healing, fibronectin is found in the basement membrane and reaches a maximum at approximately the same time as the peak in endothelial cell mitosis occurs, indicating a possible role of fibronectin in endothelial cell migration (111).

In epithelialization, it has been found that fibronectin is implicated in epidermal cell adhesion, migration and differ-

entiation (96, 111–116). Thus migrating epithelial cells are supported by an irregular band of fibrin–fibronectin matrix which provides attachment and a matrix for prompt migration (87, 108).

Clinically, fibronectin has been used to promote attachment of connective tissue to the exposed root and surfaces, thereby limiting epithelial downgrowth (117–123). Furthermore, fibronectin has been shown to accelerate healing of periodontal ligament fibers after tooth replantation (120). This effect has also been shown to occur in experimental marginal periodontal defects in animals (121, 122) as well as in humans (123).

Complement system

The complement system consists of a group of proteins that play a central role in the inflammatory response. One of the activated factors, C5a, has the ability to cleave its C-terminal arginine residue by a serum carboxypeptidase to form C5a-des-arg which is a potent chemotactic factor for attracting neutrophils to the site of injury (124, 125).

Necrotic cells

Dead and dying cells release a variety of substances that may be important for wound healing such as tissue factor, lactic acid, lactate dehydrogenase, calcium lysosomal enzymes and FGF (126).

Matrix

Proteoglycans and hyaluronic acid

All connective tissues contain proteoglycans. In some tissues, such as cartilage, proteoglycans are the major constituent and add typical physical characteristics to the matrix (127).

Chondroitin sulfate proteoglycans

Chondrocytes, fibroblasts and smooth muscle cells are all able to produce these proteoglycans. Chondroitin sulfate impairs the adhesion of cells to fibronectin and collagen and thereby promotes cell mobility. Skin contains proteoglycans, termed dermatan sulfates, which are involved in collagen formation.

Heparin and heparan sulfate proteoglycans

Heparins are a subtype with an anticoagulant activity. Heparan sulfates are produced by mast cells and adhere to cell surfaces and basement membranes.

Keratan sulfates are limited to the cornea, sclera and cartilage. Their role in wound healing is unknown.

Hyaluronic acid is a ubiquitous connective tissue component and plays a major role in the structure and organization of the ECM. Hyaluronic acid has been implicated in the detachment process of cells that allows cells to move. Furthermore, hyaluronic acid inhibits cell differentiation. Because of its highly charged nature, hyaluronic acid can absorb a large volume of water (128).

The role of proteoglycans during wound healing is not fully understood (129). Heparin may play a role in the control of clotting at the site of tissue damage. Proteoglycans are also suspected of playing an important role in the early stages of healing when cell migration occurs. Thus *hyaluronic acid* may be involved in detachment of cells so that they can move (130). Furthermore, proteoglycans may provide an open hydrated environment that promotes cell migration (129, 131, 133, 135).

The proliferative phase of healing involves cell duplication, differentiation and synthesis of ECM components. Thus hyaluronidate has been found to keep cells in an undifferentiated state which is compatible with proliferation and migration (127). At this stage chondroitin and heparan sulfates are apparently important in collagen fibrillogenesis (127) and mast cell heparin promotes capillary endothelial proliferation and migration (132). Furthermore, when endothelium is damaged, a depletion of growth-suppressing heparan sulfate may allow PDGF or other stimuli to stimulate angiogenesis (133).

The combined action of substances released from platelets, blood coagulation and tissue degradation results in hemostasis, initiation of the vasculatory response and release of signals for cell activation, proliferation and migration.

The role of the anticoagulant heparin is to temporarily prevent coagulation of the excess tissue fluid and blood components during the early phase of the inflammatory response.

Inflammatory phase mediators

The sequence of the inflammatory process is directed by different types of chemical mediators which are responsible for vascular changes and migration of cells into the wound area (Fig 1.6).

Mediators responsible for vascular changes

Inflammatory mediators such as histamine, kinins and serotonin cause vasodilation unless autonomic stimulation overrules them.

The effect of these mediators is constriction of smooth muscles. This influences endothelial and periendothelial cells, providing reversible opening of junctions between cells and permitting a passage of plasma solutes across the vascular barrier. These mediators are released primarily during the process of platelet aggregation and clotting. The best known mediators related to the vascular response are shown in Table 1.1.

Histamine

The main sources of histamine in the wound appear to be platelets, mast cells and basophil leukocytes. The histamine release causes a short-lived dilation of the microvasculature (137) and increased permeability of the small venules. The endothelial cells swell and separations occur between the individual cells. This is followed by plasma leaking through the venules and the emigration of polymorphonuclear leukocytes (137–141).

Table 1.1 Mediators of vascular response in inflammation. Adapted from (136).

	Mediator	Originating cells
Humoral	Complement Kallikrein–kinin system Fibrin	
Cellular	Histamine	Thrombocytes Mast cells Basophils
	Serotonin	Thrombocytes Mast cells
	Prostaglandins	Inflammatory cells
	Thromboxane A_2	Thrombocytes Neutrophils
	Leukotrienes	Mast cells Basophils Eosinophils Macrophages
	Cationic peptides	Neutrophils
	Oxygen radicals	Neutrophils Eosinophils Macrophages

Serotonin

Serotonin (5-hydroxytryptamine) is generated in the wound by platelets and mast cells. Serotonin appears to increase the permeability of blood vessels, similarly to histamine, but appears to be more potent (139, 140). Apart from causing contraction of arterial and venous smooth muscles and dilation of arterioles, the net hemodynamic effect of serotonin is determined by the balance between dilation and contraction (137, 142).

Prostaglandins

Other mediators involved in the vascular response are prostaglandins (PGs). These substances are metabolites of arachidonic acid and are part of a major group called eicosanoids, which are also considered primary mediators in wound healing (143). Prostaglandins are the best known substances in this group and are released by cells via arachidonic acid following injury to the cell membrane. These include PGD_2, PGE_2, PGF_2, thromboxane A_2 and prostacycline (PGI_2). These components have an important influence on vascular changes and platelet aggregation in the inflammatory response and some of the effects are antagonistic. Under normal circumstances, a balance of effects is necessary. In tissue injury, the balance will shift towards excess thromboxane A_2, leading to a shutdown of the microvasculature (143).

New research suggests that prostaglandins, and especially PGF_2, could be endogenous agents that are able to initiate repair or reconstitute the damaged tissue (144). Thus biosynthesis of PGF_2 has been shown to have an important effect on fibroblast reparative processes (145), for which reason this prostaglandin may also have an important influence on later phases of the wound healing process. The effect of prostaglandins on the associated inflammatory response elicited subsequent to infection is further discussed in Chapter 2.

Bradykinin

Bradykinin released via the coagulation cascade relaxes vascular smooth muscles and increases capillary permeability leading to plasma leakage and swelling of the injured area.

Neurotransmitters (norepinephrine, epinephrine and acetylcholine)

The walls of arteries and arterioles contain adrenergic and cholinergic nerve fibers. In some tissues the sympathetic adrenergic nerve fibers may extend down to the capillary level. Tissue injury will stimulate the release of neurotransmitters which results in vasoconstriction.

Mediators with chemotactic effects

These mediators promote migration of cells to the area of injury and are thus responsible for the recruitment of the various cells that are involved in the different phases of wound healing (Fig. 1.4).

The first cells to arrive in the area are the leukocytes. The chemotactic effects are mediated through specific receptors on the surface of these cells. Complement-activated products like C5a, C5a-des-arg and others cause the leukocytes to migrate between the endothelial cells into the inflammatory area. This migration is facilitated by the increased capillary permeability that follows the release of the earlier mentioned mediators. Further leukocyte chemoattractants include kallikrein and plasminogen activator, PDGF and platelet factor 4.

Other types of chemotactic receptors are involved when leukocytes recognize immunoglobulin (Ig) and complement proteins such as C3b and C3bi. The mechanism appears to be that B lymphocytes, when activated, secrete immunoglobulin, which again triggers the activation of the complement system resulting in production of chemoattractants such as C5a-des-arg (146).

Other mediators involved in chemoattraction will be mentioned in relation to the cell types involved in the wound healing process.

Growth factors

Growth factors are a group of polypeptides involved in cellular chemotaxis, differentiation, proliferation and synthesis of ECM during embryogenesis, postnatal growth and adulthood.

All wound healing events in both hard and soft tissues are influenced by polypeptide growth factors, which can be released from the traumatized tissue itself, can be harbored

in the quickly formed blood clot or brought to the area by neutrophils or macrophages.

Growth factors are local signaling molecules. They can act in a paracrine manner where they bind to receptors on the cell surface of neighboring target cells, leading to initiation of specific intracellular transduction pathways; or they can act in an autocrine manner, whereby the function is elicited on the secreting cell itself. Additionally, elevated serum levels have been demonstrated for a few growth factors which may indicate an endocrine effect. Complex feedback loops regulate the production of the individual growth. The effect of each growth factor is highly dependent on the concentration and on the presence of other growth factors. A growth factor can have a stimulatory effect on a specific cell type, whereas an increased concentration may inhibit the exact same cell type. Two different growth factors with a known stimulatory effect on a cell type can in combination result in both an *agonistic*, *synergistic* and even *antagonistic* effect.

Growth factors may have the potential to improve healing of traumatized tissues in several ways. First, some growth factors have the ability to recruit specific predetermined cell types and pluripotent stem cells to the wounded area by chemotaxis. Second, they may induce differentiation of mesenchymal precursor cells to mature secreting cells. Third, they often stimulate mitosis of relevant cells, and thereby increase proliferation. Fourth, several growth factors have the ability to increase angiogenesis, the ingrowth of new blood vessels. Finally, they can have a profound effect on both secretion and breakdown of ECM components.

The most important growth factors are listed in Table 1.2 and a brief summary of their characteristics, including their presumed role in wound repair and regeneration is given below.

Dentoalveolar traumas may involve a multitude of tissues like oral mucosa, periodontal ligament, root cementum, dentin, dental pulp, bone, skin, blood vessels and nerves. Only a few clinical studies have evaluated the use of growth factors specifically for oral and maxillofacial traumas (see Chapter 2).

Platelet-derived growth factor (PDGF)

PDGF consists of two amino acid chains and comes in homo- and heterodimeric isoforms (AA, AB, BB, CC and DD, where AA, AB and BB are the best documented) (147). PDGF binds to two specific receptors: α and β. Differential binding of the different isoforms to the receptors contributes to the varying effects of PDGF. As the name implies, PDGF is released from platelets, where it is present in large amounts in α-granules. Platelets are activated by thrombin or fibrillar collagen. Other sources of PDGF are macrophages, endothelial cells and fibroblasts. PDGF was the first growth factor shown to be chemotactic for cells migrating into the wound area, such as neutrophils, monocytes and fibroblasts. Additionally, PDGF stimulates proliferation and ECM production of fibroblasts (148) and activates macrophages to debride the wound area (149).

Platelet-rich plasma (PRP)

PRP has been advocated for periodontal regeneration as well as pulp regenerative therapy. PRP is prepared in the office from patients' own blood using centrifugation and platelets are then activated by thrombin (658). PRP contains several growth factors released mainly by thrombocytes such as PDGF-AB, PDGF-BB, TGF-β, IGF-1 and VEGF (657).

The use of PRP in regenerative periodontics has shown both positive and negative results (658, 659). PRP has also been used as a scaffold with growth factors in regenerative endodontic treatment. In case reports it seemed to have a positive effect (688–691); however, a larger clinical study could not support such a finding (692). The effect of PRP has been examined in furcation defects and sinus graft procedures, but it does not seem to optimize healing (670).

Transforming growth factors (TGFs)

TGFs comprise a large family of cytokines with a widespread impact on the formation and development of many tissues (among those, the bone morphogenetic proteins, which are described separately). This factor has been divided into α and β subtypes, where the latter is the most important for the wound healing process (150). TGF-β is mainly released from platelets and macrophages as a latent homodimer that must be cleaved to be activated. This latent form is present in both wound matrix and saliva. TGF-β is known to be a strong promoter of ECM production of many cell types (e.g. collagen and mucopolysaccharide) including periodontal ligament fibroblasts. TGF-β encourages ECM reorganization and increased stability by collagen crosslinking (628).

Proliferation of fibroblasts is also induced by TGF-β, whereas mitogenesis of most other cell types is inhibited like keratinocytes, lymphocytes and most epithelial cells. Additionally, TGF-β plays a role in immune and inflammatory regulation. TGF-β is also deposited in bone matrix where it is released during bone remodeling or in relation to trauma and acts as a chemotactic on osteoblasts. The effects of TGF-β are extremely complex and strongly dependent on the concentration of the growth factor itself, the concentration of other growth factors and the differentiation state of the target cells.

Epidermal growth factor (EGF)

EGF was one of the first growth factors to be isolated (151). It is produced by platelets, salivary glands and duodenal glands. TGF-α is today considered to be a member of the EGF family. The receptors for EGF have been found in oral epithelium, enamel organ, periodontal ligament fibroblasts and preosteoblasts (152, 153). Stimulation of the EGF receptor causes the cells to become less differentiated and to divide and grow rapidly. In wounds, EGF has been found to encourage cells to continue through the cell cycle. Such a cell proliferative effect has been demonstrated in epithelial cells (154), endothelial cells and periosteal fibroblasts (155). EGF has also been shown to be chemotactic for epithelial cells (156) and to stimulate fibroblast collagenase production

Table 1.2 Characteristics of growth factors involved in healing after dental trauma.

Growth factor	Originating cells	Target cells	Main effect	Tissue response
PDGF	Platelets Macrophages Endothelial cells Osteoblasts	Neutrophils Monocytes Fibroblasts Osteoblasts	Chemotaxis Proliferation	Angiogenesis Macrophage activation
TGF	Platelets Macrophages Fibroblasts Lymphocytes Osteoblasts	Fibroblasts Monocytes Neutrophils Macrophages Osteoblastic precursor cells	Chemotaxis ECM production Proliferation	Collagen production (scarring) Downregulation of other cell types but fibroblasts Immunoregulation
IGF	Hepatocytes Osteoblasts	Fibroblasts Osteoblasts Epithelial cells	Proliferation ECM production Chemotaxis Cell survival	Stimulated DNA synthesis Growth promotion of committed cells
EGF	Platelets Salivary glands	Epithelial cells Enamel organ Periodontal ligament fibroblasts Preosteoblasts	Proliferation Chemotaxis ECM production	Epithelialization Tooth eruption
FGF	Endothelial cells Macrophages Keratinocytes Osteoblasts	Endothelial cells Fibroblasts Keratinocytes	Proliferation Migration ECM formation	Angiogenesis Epithelialization
VEGF	Keratinocytes Macrophages Fibroblasts	Endothelial cells	Proliferation	Angiogenesis
BMP	Osteoblasts	Undifferentiated mesenchymal cells Osteoblastic precursor cells Osteoblasts	Differentiation Proliferation ECM production	Bone formation Cementum formation Dentin formation PDL formation
GDF-5	Osteoblasts	Fibroblasts Osteoblasts Chondroblasts	Proliferation	Recruitment of stem cells for ligament repair Cartilage formation Bone formation Cementum formation Periodontal ligament Fibroblast proliferation
EMP	Ameloblasts	Angiogenesis Macrophage activation Periodontal ligament fibroblasts Osteoblasts Cementoblasts	Proliferation	Angiogenesis PDL formation Cementum formation Bone formation
P-15	Collagen cell binding region	Osteoblast precursors Osteoblasts Fibroblasts	Adhesion and proliferation of fibroblasts Osteogenic differentiation	Bone formation

ECM, extracellular matrix; PDL, periodontal ligament.

(157). In oral tissues it has been shown that EGF controls the proliferation of odontogenic cells (158) and accelerates tooth eruption (159).

Insulin-like growth factor (IGF)

IGF is a single chain polypeptide which structurally is very similar to proinsulin. Two isoforms, IGF-1 and IGF-2 are mainly produced in the liver and exert their effects in autocrine, paracrine and endocrine manners. The endocrine effect is mainly controlled by growth hormone. Osteoblasts also produce IGF that is stored in the bone matrix and acts as paracrine and autocrine (160, 161). IGF alone has hardly any major effect on wound healing (162). Combinations with other growth factors, such as PDGF and FGF, have, however, been shown to have a pronounced stimulatory effect on fibroblast proliferation, collagen synthesis, bone formation and epithelialization (162).

Fibroblast growth factors (FGFs)

FGFs comprise a growing family of polypeptides, currently consisting of more than 20 members. They are mainly produced by endothelial cells and macrophages. FGFs are mitogenic for several cell types involved in wound healing and support cell survival under stress conditions. FGFs are involved in angiogenesis and epithelialization. FGF-1 and FGF-2 (earlier known as acidic FGF and basic FGF) are potent stimulators of angiogenesis in the early formation of granulation tissue (days 1–3) by recruiting endothelial cells and inducing proliferation. Neither has a transmembrane sequence and can therefore not be secreted. Instead they are probably released from disrupted cells by tissue damage (163). After release, FGFs interact with heparin and heparan sulfate, with which they can be stored in the ECM. Here FGF can be activated when injury causes platelets to degranulate and among many other substances release heparin degrading enzymes.

Vascular endothelial growth factor (VEGF)

VEGF is, as far as we know today, the only endothelial-specific growth factor enhancing cell proliferation, and its activity is therefore probably essential for angiogenesis in all tissues during both development and repair. VEGF is produced in large quantities by keratinocytes, macrophages and, to a lesser extent, fibroblasts in the epidermis during wound healing, where it seems to be critical for angiogenesis in the granulation tissue formation from days 4 to 7. Hypoxia, a hallmark of tissue injury, induces VEGF production. Reduced expression and accelerated degradation of VEGF has been shown to cause skin wound defects (163) and the addition of VEGF has promoted angiogenesis in skin wounds in diabetic mice (164).

In vivo, VEGF has resulted in increased capillary density and bone formation in standardized bone defects in rabbits (165).

No clinical studies have evaluated the effect of VEGF in relation to oral and maxillofacial trauma.

Bone morphogenetic proteins (BMPs)

BMPs are members of the TGF-β superfamily. More than 20 different BMPs have been identified. BMPs are found in bone matrix and in periosteal cells and mesenchymal cells of the bone marrow (166). BMP-2, -4, and -7 (also called osteogenic protein-1 (OP-1)) are the most involved in bone healing, whereas increased BMP-6 has been described in skin wounds. The main task of BMP is to commit undifferentiated pluripotential cells to become bone or cartilage forming cells. BMPs are the only known factors that are capable of forming bone in extraskeletal sites, a phenomenon referred to as osteoinduction (167).

Growth and differentiation factor 5 (GDF-5)

This growth factor is a member of the large BMP family. This factor has been shown to enhance bone and cartilage formation in a series of animal studies (662). Furthermore, in animal models GDF-5 used with a carrier (βTCP or PLGA) has been shown to augment cementum, periodontal ligament (PDL) and bone formation (663–667).

Enamel matrix proteins (EMPs)

EMPs, commercially sold under the name Emdogain®, have been used for decades in periodontics to promote periodontal regeneration in relation to attachment loss, and many reviews have described their effects in relation to bone grafting or guided tissue regeneration (668–670). Several studies have shown that EMPs can stimulate the expression of TGF-β1 and IGF-1 in PDL fibroblasts (671). Furthermore, EMPs have been shown to stimulate phagacytic activity of macrophages in relation to tissue repair (672, 673) and angiogenesis (674).

Experimental data indicating clinical implications of growth factors

Angiogenesis

During healing after trauma, *de novo* formation of the disrupted vascular supply is a prerequisite for most of the healing events. This is supported by the finding that hyperbaric oxygen (HBO) is a potent stimulator of healing of both hard and soft tissue healing (168) in sites with a compromised healing potential such as diabetic ulcers and irradiated bone (169, 170). The primary long-term effect of HBO is increased angiogenesis. VEGF, FGF, TGF-β and PDGF are known to be involved in angiogenesis during wound healing (259). Exactly how these growth factors interact with the ECM environment in the blood clot and in granulation tissue, are, however, not known in detail. Revascularization of the dental pulp is necessary after both tooth fractures and luxation injuries. VEGF, PDGF and FGF have been identified in the soluble and insoluble part of human dentin matrix (171). These may be released during injury and contribute to pulpal wound healing.

Wounds in skin and oral mucosa

In most instances, healing proceeds rapidly in healthy individuals. Research has therefore mainly been focused on situations where the healing potential is seriously compromised such as diabetes, malnutrition and infection. In skin wounds, PDGF is known to be chemotactic to neutrophils, monocytes and fibroblasts. In addition, PDGF is a mitogen for fibroblasts, which has led to US Food and Drug Administration (FDA) approval for the treatment of non-healing ulcers (172, 173). In addition, PDGF stimulates new vascularization of an injured area (174). Exogenously applied TGF-β has been demonstrated to induce fibroblast infiltration in the wound and increased collagen deposition (175), as well as angiogenesis and mucopolysaccharide synthesis (175, 176). This results in an accelerated healing of incisional wounds (177, 178). Due to the same mechanisms, however, TGF-β is also intimately related to scar formation. Thus the elimination of TGF-β from incisional wounds in rats (by neutralizing antibody) is able to prevent scar tissue

formation (179, 620–622). Furthermore, it has been shown that the effect of TGF-β can be potentiated by the presence of PDGF and EGF (180). In experimental skin wounds in *animals*, an acceleration of both connective tissue and epithelial healing was found after topical application of EGF (181, 182). However, results after topical application of EGF to experimental wounds in *humans* have shown contradictory results on re-epithelialization (178, 183–186). In the oral mucosa, salivary EGF has been shown to stimulate migration of oral epithelial cells (187).

An interesting observation in mice has been that saliva rinsing of skin wounds (by communal licking) both enhances coagulation and leads to acceleration of wound healing (182, 188–190). Due to the high concentration of EGF found in saliva (191) this effect has been suggested to be caused by EGF. Later experiments with induced tongue wounds in mice have shown that EGF (and possibly also TGF-β) is involved in healing of wounds of the oral mucosa (192, 193). Salivary EGF is suggested not only to accelerate wound healing in the oral cavity, but also to contribute to preserving integrity of the oral mucosa (194). Administration of IGF-1 in skin wounds has no influence upon fibroblast proliferation or activity, or upon epithelialization (195–197). However, if IGF-1 is administered together with PDGF or FGF, a marked fibroblast proliferation and collagen production can be observed as well as enhanced epithelialization (197).

Periodontal healing

Experimental studies have suggested that PDGF-BB alone could have a regenerative effect on the formation of root cementum, periodontal ligament and alveolar bone (151, 198–200, 675, 676). PDGF has clinically, however, mainly been evaluated in combination with IGF-1 where an increased bone fill could be observed both around periodontally compromised teeth and in peri-implant defects (199, 201, 202, 677). IGF used alone, TGF-β used alone, and the combination IGF-2/FGF-2/TGF-β has not been able to generate noteworthy periodontal regeneration in experimental studies (197, 203, 204).

FGF-2 has resulted in increased periodontal regeneration compared to control sites in experimentally created defects (206). Its biologic action has recently been described in a review article (678). Clinically, FGF-2 has in a randomized multicenter study been shown to stimulate periodontal regeneration (679).

Experimental studies have reported regeneration of a periodontal ligament with Sharpey's fibers, inserted in the newly formed cementum and alveolar bone by using recombinant BMP-2, BMP-7 (OP-1) and recently also BMP-12. The treated periodontal defects have been either surgically created (207–209) or experimentally induced (210). This pronounced periodontal regeneration could not be obtained when BMP-12 was applied to extracted dog teeth before replantation. In contrast, ankylosis developed whether BMP-12 was applied or not (211). In cats ankylosis was also created when BMP-2 was applied to furcation defects (680). EMPs in the form of Endogain® have been tested for their capacity to promote healing in relation to periodontal healing in the replantation of extracted dog teeth. In one study a significantly better healing was found (681), whereas a similar designed study could not demonstrate any effect (682).

ABM/P-15 has been used in periodontal regeneration both in animals and in humans with good results in regard to bone healing and gain in attachment (683–687).

Bone healing

Information of the role of growth factors in bone healing mainly comes from preclinical studies of periodontal lesions and bone augmentation procedures before or in relation to implant placement. Numerous growth factors are deposited in bone matrix during bone formation (e.g. PDGF, TGF-β, FGF-2 and IGF-1). These are released during bone remodeling and in relation to trauma (256).

Contradictory results have been reported regarding the bone regenerative potential of PDGF. Both inhibition and stimulation of bone formation has been observed in rat calvarial defects (212, 213). PDGF alone has little impact on bone healing *in vivo*. However, a couple of studies have reported significant bone regeneration in periodontal and peri-implant defects, when PDGF is combined with IGF (102, 201, 202, 214, 215). Likewise, IGF must be combined with other growth factors to promote bone healing. TGF-β has a strong impact on the healing of long bone fractures (216). Only a few clinical data from the use of BMP in humans exist (217, 218). Experimental data, however, suggest enhanced bone formation using BMP-2 and -7 for bone regeneration procedures (219–221).

Pulp-dentin complex

Attempts to regenerate the pulp-dentin complex have mainly focused on the possibility of generating a hard tissue (dentin) closure to an exposed pulp in relation to pulp capping. The key question is how to induce uncommitted pulpal cells to differentiate into odontoblast-like cells secreting reparative dentin. TGF-βs, BMPs, FGFs and IGFs are harbored in dentin and are known to influence dentinogenesis during embryogenesis (222). BMP-2, BMP-4 and BMP-7 (OP-1) have all been shown to induce widespread dentin formation in the pulp, even leading to total occlusion of the pulp cavity when applied in high doses (223). Numerous studies have evaluated the revascularization of avulsed replanted teeth, but none have specifically studied the role of growth factors in this process.

Platelet concentrate/platelet-rich plasma

In the past few years, utilization of platelet concentrate (PC), also called platelet-rich plasma (PRP), has been increasingly recommended in patients undergoing osseous reconstruction and periodontal regeneration. PRP has gained much attention since the presentation of very promising data for the resulting bone density by adding PC to iliac cancellous cellular bone marrow grafts in the reconstruction of mandibular continuity defects (224). An

accelerated graft maturation rate and a denser trabecular bone configuration were observed in defects where PRP had been added. It was speculated that the stimulating effect of PRP was due to the accumulation of autogenous platelets, providing a high concentration of platelet growth factors with a well-documented impact on bone regeneration (225, 226). The concept of using autogenous growth factors is attractive since there is no risk of disease transmission, and as it is relatively inexpensive compared to growth factors produced by recombinant techniques.

Additionally, one clinical study (227) and a series of clinical case reports and case series have presented the use of PRP in different applications (228–236), leading to divergent recommendations. Data from experimental studies, evaluating the addition of PRP to bone graft materials have also been conflicting (237–247). In these studies a wide range of different animal models and PRP preparation techniques have been used. Therefore, they are difficult to compare. Moreover, none of the studies have analyzed the growth factor content in the applied PRP. Just one study analyzed the influence of PRP platelet concentration in an *in vivo* model. The authors demonstrated a certain platelet concentration interval with the most positive biologic effect on bone regeneration, corresponding to a 3–5-fold increased concentration compared to whole blood. There was no effect using low concentrations (0–2-fold increased concentration), and there seemed to be an inhibitory effect on bone regeneration when higher concentrations were used (6–11-fold increased concentration compared to whole blood) (248).

The use of PRP in regenerative periodontics has shown both positive and negative results (658, 659). PRP has also been used as a scaffold with growth factors in regenerative endodontic treatment. In case reports it seemed to have a positive effect (688–691). However, a larger clinical study could not support such a finding (62).

In conclusion, no methods are currently available to produce standardized PRP in which a certain whole blood platelet count will result in PRP with a predictable amount of platelets and a predictable combination of growth factors. Use of autologous growth factors is simple and safe as compared with allogenic and xenogenic preparation methods. Consistent results, however, cannot be expected until the ideal concentration of platelet growth factors has been identified and reliable PC preparation methods have been developed.

Carriers/delivery systems for growth factors

Growth factors are in general volatile and need carriers to ensure continuance of the growth factor at the relevant site, and to provide sustained release of the growth factor in therapeutic doses. A carrier must be biocompatible. In addition, the carrier should be substituted concurrently with healing of the traumatized tissue, without causing an inflammatory reaction. Collagen can bind and release bioactive substances with some predictability (249, 250). Like other natural polymers, however, collagen has limitations in clinical use due to difficulties in engineering its properties, handling problems, immunogenicity and lack of resorption resistance (251, 252). Synthetic carriers for tissue promotive agents have therefore been extensively investigated. Traditionally copolymers such as lactic and glycolic acid have been utilized as vehicles for bioactive molecules due to their handling properties and biodegradability. They may, however, be associated with protein denaturation and inflammatory reactions along with the degradation process. More hydrophilic materials with controlled network properties thus offer an attractive alternative, but problems with loading the bioactive protein into the material is a common limitation related to these materials. A new polyethylene glycol (PEG) hydrogel may meet these demands. This hydrogel polymer network is synthesized around the bioactive molecules without modifying its action; it is highly water soluble, non-toxic and non-immunogenic (253).

In bone regeneration, a certain mechanical stability of the carrier is often required in order to avoid collapse of soft tissue into the defect and to protect against pressure from the overlying periosteum. *In vitro* investigations have shown that both adsorption of the bioactive substance and release kinetics exhibit pronounced variation when different carriers and growth factors are combined (254). In addition, the growth factor may be inactivated in relation to the release (254). PDGF-BB has, compared to IGF-1, been shown to adsorb better, be released more completely and keep its bioactivity in combination with an anorganic bovine bone substitute material (255).

In dental traumatology a carrier will probably be needed in case of pulp and PDL regeneration.

Cells in wound healing

Platelets

Platelets (thrombocytes) are anucleate discoid fragments with a diameter of 2 µm (Fig. 1.8). They are formed in the bone marrow as fragments of cytoplasmatic buddings of

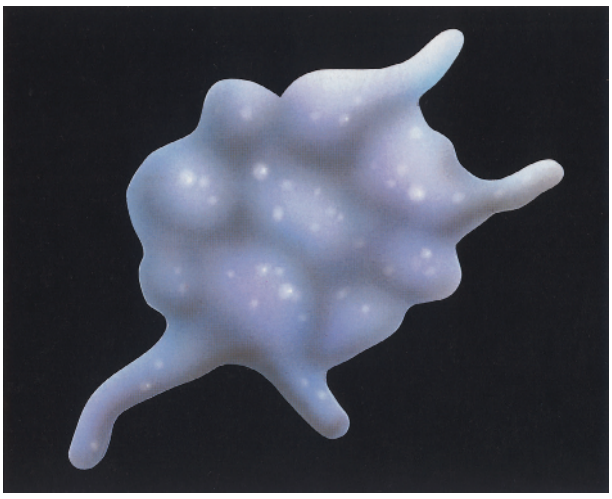

Fig. 1.8 Activated platelet (thrombocyte).

megakaryocytes and have a life span of 7–10 days in the blood (260). Platelets contain various types of granules which, after release, have a number of effects upon hemostasis and initiation of wound healing processes (261–263) (Fig 1.4). Under physiologic conditions, platelets are limited to the intravascular space where they circulate in the blood without adhesion to each other or to the vessel walls.

The capacity of the platelets to adhere to exposed tissue surfaces as well as to each other after vessel injury is decisive for their hemostatic capacity (256–259). Adhesion and activation of platelets occurs when they contact collagen and microfibrils of the subendothelial matrix and locally generated factors such as thrombin, ADP, fibrinogen, fibronectin, thrombospondin and von Willebrand factor VIII. Platelet adhesion and aggregation are also influenced by the particular matrix proteins exposed during injury and by the local hydrodynamic conditions including shear stress and shear rate (632–635).

Platelet activation results in degranulation and release of ADP, serotonin, thromboxane, prostaglandins and fibrinogen. The release of these substances initiates binding of other platelets to the first adherent platelets whereby blood loss is limited during formation of a hemostatic platelet plug (264). The blood loss is further reduced by the vasoconstrictor effect of thromboxane and serotonin. Platelet activation during primary hemostasis also initiates the wound healing response via release of cytokines such as PDGF and TGF-β1 that serve as a chemotactic and stimulatory signal for many cell types essential for wound healing (627).

The inflammatory response is initiated by activation of platelets due to liberation of serotonin, kinins and prostaglandins which leads to increased vessel permeability.

The platelet release of cytokines such as PDGF, platelet-derived angiogenesis factor (PDAF), TGF-α, TGF-β and platelet factor 4 leads to an initiation of the wound healing process (Fig. 1.9). Thus PDGF has been shown to have a chemotactic and activating effect upon neutrophils, monocytes and fibroblasts as well as a mitogenic effect upon fibroblasts and smooth muscle cells (263, 265). The release of TGF-β has been found to induce angiogenesis and collagen deposition (266, 267). PDAF has been shown to cause new capillary formation from the existing microvasculature (268–270). Finally, platelet factor 4 has been found to be a chemoattractant for neutrophils (271).

In summary, the platelets are the first cells brought to the site of injury. Apart from their role in hemostasis, they exert an effect upon the initiation of the vascular response and attraction and activation of neutrophils, macrophages, fibroblasts and endothelial cells. As wound healing progresses, the latter tasks are gradually assumed by macrophages.

Erythrocytes

The influence of erythrocytes upon wound healing is not adequately documented except for the effect of carrying oxygen to healing tissue (40, 42, 48, 618). In one study it was found that neovascularization was stimulated in areas with erythrocyte debris (272). Another effect of the breakdown of erythrocytes is the liberation of hemoglobin, which has been found to enhance infection (273–276). In addition, the heme part of hemoglobin may contribute to the production of oxygen free radicals that can produce direct cell damage (277).

In summary, the role of erythrocytes in wound healing is doubtful, apart from being oxygen carriers.

Mast cells

Masts cells, distinguished by their large cytoplasmatic granules, are located in a perivenular position at portals of entry of noxious substances and are especially prominent within the body surfaces that are subject to traumatic injury, such as the mucosa and skin (278, 279) (Fig. 1.10).

The mast cell participates in the initial inflammatory response after injury via a series of chemical mediators such as histamine, heparin, serotonin, hyaluronic acid, prostaglandins and chemotactic mediators for neutrophils.

Mast cells, which reside in tissues in a resting state, may release mast cell mediators through direct trauma inflicted on the cell. Another means of activation after trauma appears to be when coagulation generates the mast cell activator bradykinin. An alternative means of mast cell activation appears to be the release of endotoxin during infection and the generation of C3a, C5a and cationic neutrophil protein during the inflammatory response (278).

The release of the mast cell mediators such as histamine, heparin, serotonin and slow reacting substance of anaphylaxis (SRS-A) results in active vasodilation of the small venules, which allows for the entrance of water, electrolyte and plasma proteins into the microenvironment. The maintenance of an open channel for this influx is promoted by the anticoagulant activity of heparin and by the proteolytic enzymes such as chymase. Histamine and heparin may also potentiate the angiogenesis when other angiogenic factors are present (280).

The liberation of a neutrophil chemotactic factor and a lipid chemotactic factor from activated mast cells both result in the attraction of neutrophils and the release of a platelet-activating factor which results in degranulation and aggregation of platelets. Finally, hyaluronic acid promotes cell movement and may be crucial for cell division, which is essential in this phase of wound healing (9).

In summary, the mast cell plays a role, together with platelets, in being the initiator of the inflammatory response. However, experiments with corneal wounds have shown that healing can proceed in the absence of mast cells (281) and in mice mast cells do not seem to play a role in the healing of full thickness excisional cutaneous wounds (639).

Neutrophils

The first wave of cells entering the wound site are neutrophil leukocytes which migrate from the microvasculature (Figs 1.11 and 1.12). The primary function of neutrophils is to phagocytize and kill microorganisms present within the wound (282, 283). They then degrade tissue macromolecules

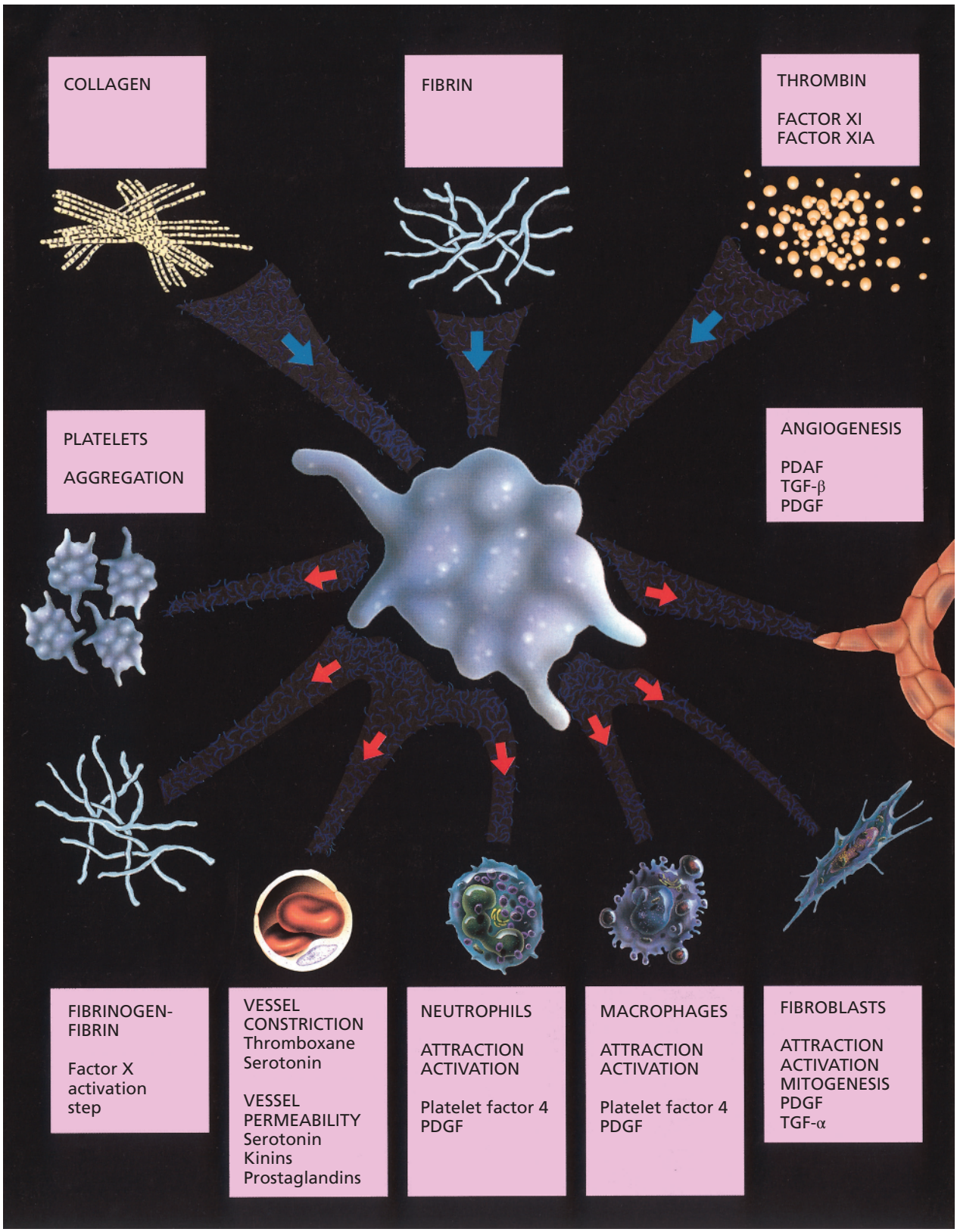

Fig. 1.9 Role of platelets in wound healing. Exposure of platelets to collagen, fibrin, thrombin, factor XI and factor XI-A results in activation and degranulation. This then results in the release of a series of mediators influencing coagulation, vessel tone and permeability. Furthermore the initial cellular response of neutrophils, macrophages, fibroblasts and endothelial cells is established.

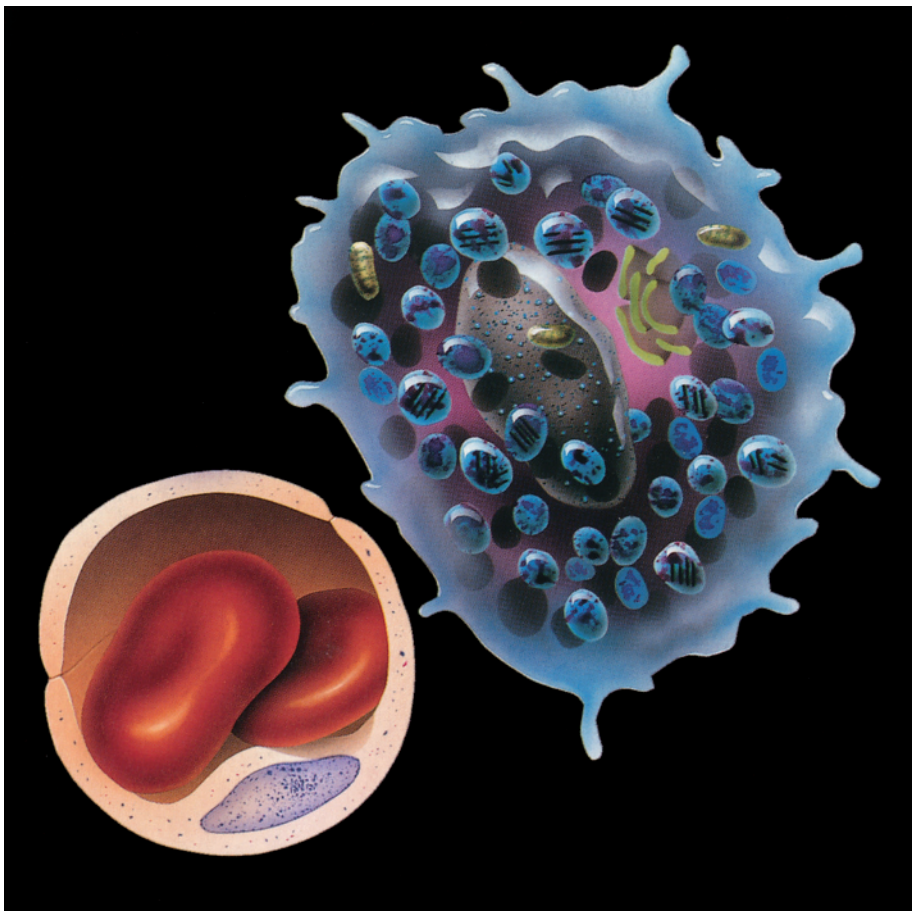

Fig. 1.10 Mast cell in perivenular position.

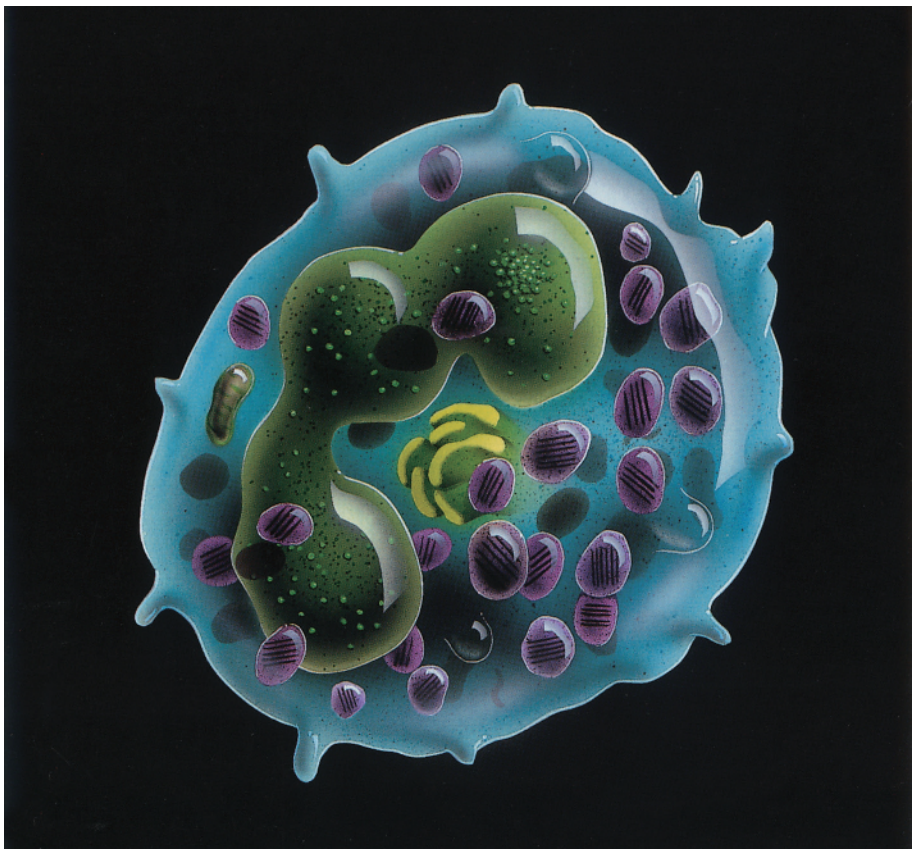

Fig. 1.11 Neutrophil leukocyte.

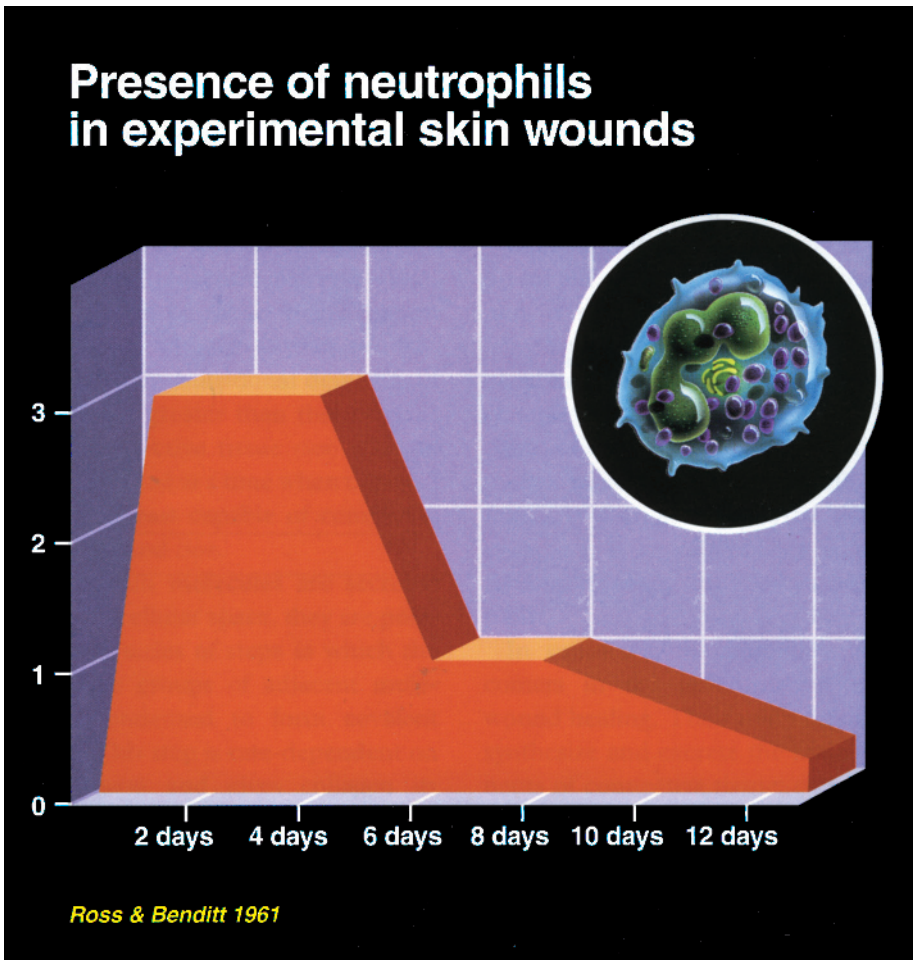

Fig. 1.12 Schematic illustration of the presence of neutrophils in experimental skin wounds in guinea pigs. The scale is semiquantitative, graded from 0 to 3. Adapted from (73).

Table 1.3 Neutrophil-produced degrading products.

Primary granules (unspecific, azurophilic)
Cathepsin A, G Elastase Collagenase (unspecific) Myeloperoxidase Lyzozyme
Secondary granules (specific)
Lactoferrin Collagenase (specific) B_{12} binding protein Lyzozyme
Other products
Gelatinase Kininogenase Oxygen radicals

such as collagen, elastin, fibrin and fibronectin by liberation of digestive enzymes (Table 1.3). Finally, neutrophils release a series of inflammatory mediators that serve as chemotactic or chemokinetic agents (Table 1.4, Fig. 1.4).

Table 1.4 Some chemotactic and chemokinetic agents. Modified from (136).

Humoral	
Chemotactic	C3 and C5 fragments Fibrin degradation products Kallikrein Plasminogen activator Fibronectin Casein
Chemokinetic	C3 and C5 fragments Acute phase proteins (Orosomucoid, α_1-antitrypsin, α_2-macroglobulin) Hyaluronic acid
Cellular	
Chemotactic	Leukotriene B_4 (precursors and derivatives) Platelet-activating factor (PAF) Transforming growth factor-β (TGF-β) Lymphokines NCFs (neutrophil chemotactic factors) ECFs (eosinophil chemotactic factors) MCFs (monocyte chemotactic factors) Formylated tripeptides (e.g. FMLP)
Chemokinetic	Cathepsin G

Upon exposure to chemoattractants from the clot, such as platelet factor 4, PDGF kallekrein, C5a, leukotriene B4 (284, 285) and bacterial endotoxins (286–288), the granulocytes start to adhere locally to the endothelium of the venular part of the microvasculature next to the injury zone (289). Neutrophils begin to penetrate the endothelium between endothelial cells, possibly by active participation of the endothelium 2–3 hours after injury (290–293), and migrate into the wound area (291–293). Once a neutrophil has passed between endothelial cells, other leukocytes and erythrocytes follow the path (293, 294).

Once the neutrophil has passed between the endothelial cells it traverses the basement membrane by a degradation process and then moves into the interstitial tissue in the direction of the chemoattractant. This movement may be facilitated by proteolytic activity and enhanced by contact guidance. Thus neutrophils move preferentially along fiber alignments, suggesting that tissue architecture may be a significant determinant of the efficacy of cellular mobilization.

At this point the wound contains a network of fibrin, leukocytes and a few fibroblasts. By the end of the second day most of the neutrophils have lost their ameboid properties and have released their granula into the surrounding tissue. This event apparently triggers a second migration where plasma, erythrocytes and neutrophils again leave the venules (295). In the case of uncomplicated non-infected healing, the numbers of neutrophils decrease after 3–5 days (Fig. 1.12).

When the neutrophils have reached the site of injury they form a primary line of defense against infection by phagocytosis and intracellular killing of microorganisms (288). In this process each phagocyte may harbor as many as 30 or more bacteria (296).

Phagocytosis of bacteria by neutrophils induces a respiratory burst that produces toxic oxygen metabolites. These products include hypohalides, superoxide anion and hydroxyl radicals (288). Furthermore, they can generate chloramine formed by the reaction of hypochlorite with ammonia or amines (297). As a result of stimulation, phagocytosis or lysis, the neutrophils may release the content of their granules into the extracellular space. These granules contain oxygen radicals and neutral proteases, such as cathepsin G, elastase, collagenases, gelatinase and cationic proteins (288). All these products result in tissue damage and breakdown at an acid pH (298). A decrease or elimination of these products provided by experimental neutropenia has prevented the normally found decrease in wound strength in early intestinal anastomosis (299).

Despite these effects upon the wound, the presence of neutrophils is not essential for the wound healing process itself. Thus wound healing has been found to proceed normally as scheduled in uncontaminated wounds in the absence of neutrophils (283, 300). A recent study, however, demonstrating neutrophil expression of cytokines and TGF-β may indicate a positive influence on wound healing (301).

A role of neutrophils in healing of chronic wounds has been elucidated (302–308). A consistent feature of chronic wounds is chronic inflammation associated with increased neutrophil infiltration (302). Once initiated, the inflammatory response is perpetuated and gradually converted into a chronic inflammatory state. Morphologically, the chronic inflammatory infiltrate is predominantly composed of macrophages and lymphocytes (304, 305). Mast cells may also contribute to the fibrotic response (304). It is likely that different polypeptidic cytokines and growth factors mediate some of these processes. One candidate is the profibrotic and proinflammatory TGF-β1, which is also increased in the mRNA and protein levels in the lower leg skin of class 4 patients (305, 306).

It is thought that excessive local proteolytic activity results in the breakdown of the matrix components of the skin with the end result of an ulcer.

Proteinases in skin homeostasis have multiple biologic functions. Proteinases not only remodel ECM proteins but they also modulate the bioactivity of cytokines and growth factors by several different mechanisms (307, 308). In chronic wounds neutrophils are strong protease producers, delaying the wound healing process.

In summary, the main role of neutrophils in wound healing appears to be limited to the elimination of bacteria within the wound area.

Macrophages

Following the initial trauma and neutrophil accumulation, monocytes become evident in the wound area (Fig. 1.13). These cells arise from the bone marrow and circulate in the blood (309, 310). In response to release of chemoattractants, monocytes leave the bloodstream in the same way as neutrophils. These cells are a heterogeneous group of cells which can express an almost infinite variability of phenotypes in response to changes (311–313).

Monocytes appear in the wound after 24 hours, reach a peak after 2–4 days, and remain in the wound until healing is complete (73) (Fig. 1.14). When monocytes invade the wound area, they undergo a phenotypic metamorphosis to macrophages (314, 315). It should be mentioned that tissue macrophages can proliferate locally (314–317) and possibly play a significant role in the initial inflammatory response.

The arrival of macrophages in the wound area is a response to various chemoattractants released from injured tissue, platelets, neutrophils, lymphocytes and bacteria (Fig. 1.4). In Table 1.4 a series of chemotactic factors are listed which have been shown to be chemotactic for macrophages. In this context, it should be mentioned that monocytes express multiple receptors for different chemotactic factors (317). As shown in Table 1.4, connective tissue fragments appear to be *chemotactic* for macrophages (i.e. forming gradients enabling directional movements) and *chemokinetic* (i.e. alter the rate of cell movements). These tissue fragments are possibly generated by neutrophils which precede the appearance of monocytes. It has been shown that neutrophils contain enzymes such as elastase, collagenase and cathepsin, which may

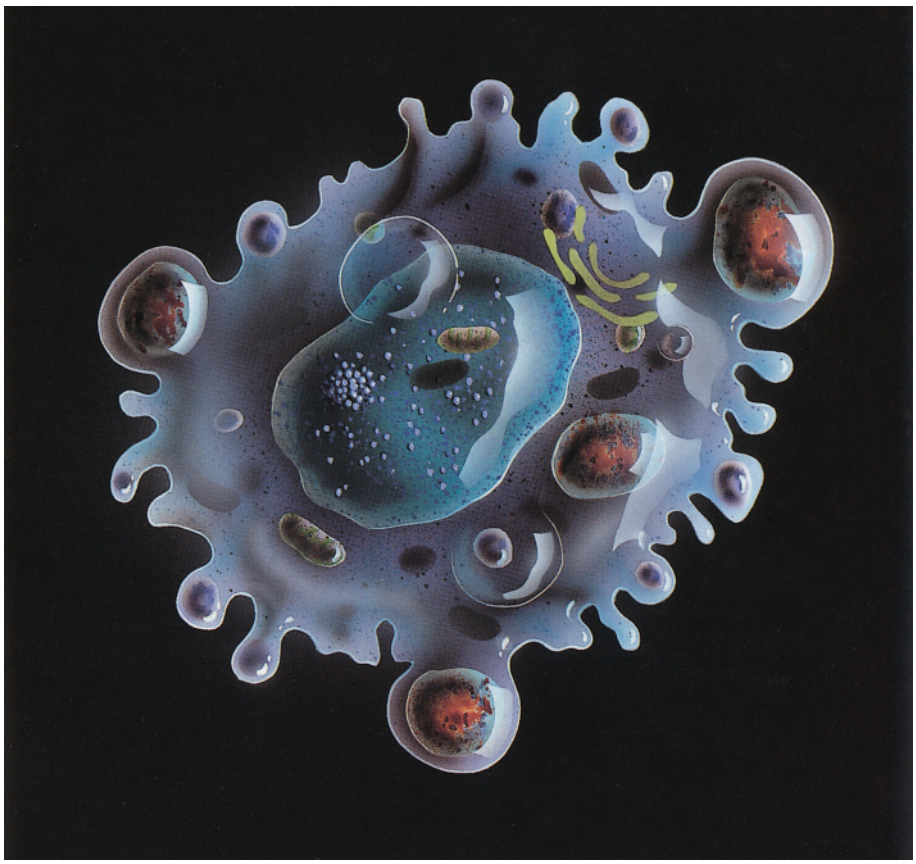

Fig. 1.13 Macrophage.

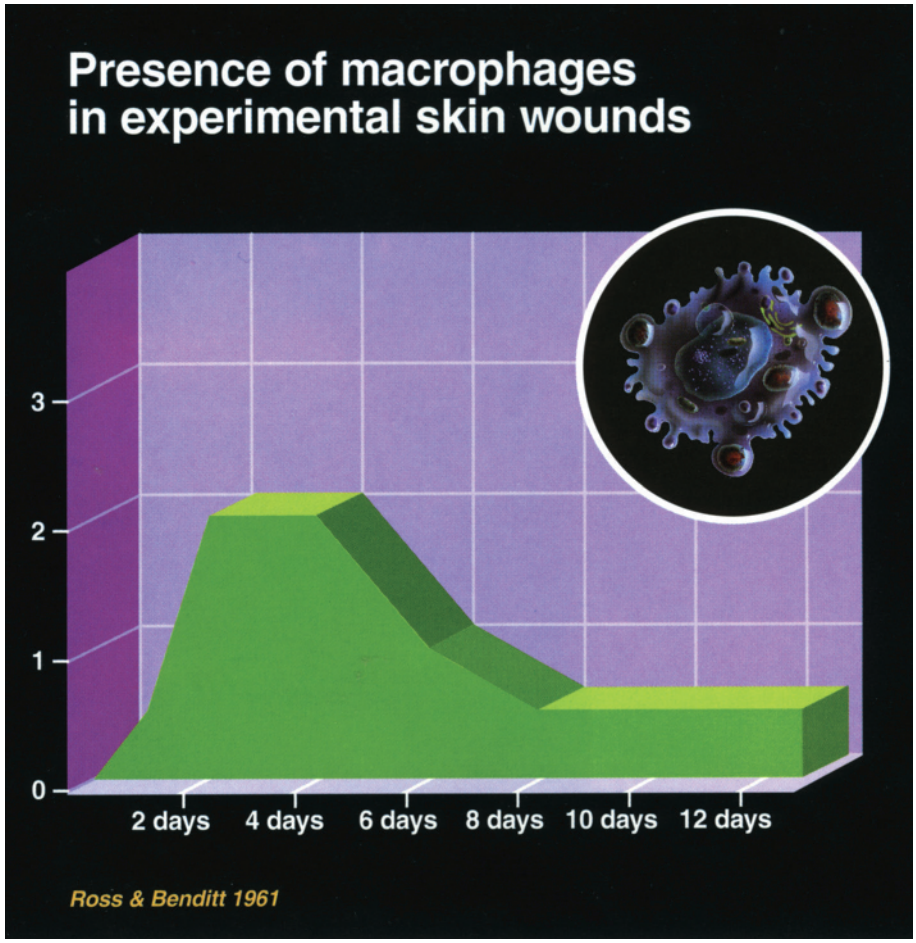

Fig. 1.14 Schematic illustration of the presence of macrophages in experimental skin wounds in guinea pigs. The scale is semiquantitative, graded from 0 to 3. Adapted from (73).

degrade collagen and elastin, and fibronectin (Table 1.3) and thereby attract monocytes (317).

In contrast to neutrophils, depletion of circulating blood monocytes and tissue macrophages results in a severe retardation of tissue debridement and a marked delay in fibroblast proliferation and subsequent wound fibrosis (309). Macrophages therefore seem to have an important regulatory role in the repair process.

After migration from the vasculature into the tissue, the monocyte rapidly differentiates to an inflammatory macrophage, the mechanism of which is largely unknown. However, the binding of monocytes to connective tissue fibronectin has been found to drive the differentiation of monocytes into inflammatory macrophages (318, 319).

The regulatory and secretory properties of macrophages seem to vary depending on the state of activity: at rest, intermediate or in an activated state. Macrophage activation can be achieved by the already mentioned chemoattractants in higher concentrations. Further activation can be achieved through the products released from the phagocytotic processes described. These various stimuli induce macrophages to release a number of biologically active molecules with potential as chemical messengers for inflammation and wound repair (320) (Tables 1.1 and 1.2).

At the inflammatory site macrophages undertake functions similar to neutrophils, i.e. bacterial phagocytosis and killing and secretion of lysozomal enzymes and oxygen radicals O_2, H_2O_2 and OH (62). Activated inflammatory macrophages have been found to be responsible for the degradation and removal of damaged tissue structures such as elastin, collagen, proteoglycans and glycoproteins by the use of secreted enzymes such as elastase, collagenase, plasminogen activator and cathepsin B and D. Both extra- and intracellular tissue debridement can occur (317).

Macrophages have been shown to release growth factors such as PDGF, tumor necrosis factor (TNF) and TGF-β which stimulate cell proliferation in wound healing (321–323) (Fig. 1.4). These growth factors are collectively known as macrophage-derived growth factors (MDGFs). The level of MDGF can be significantly increased following stimulation of macrophages with agents such as fibronectin and bacterial endotoxin (324, 325).

Activation of macrophages has been found to lead to fibroblast proliferation (321, 326), increased collagen synthesis (322) and neovascularization (327–336).

Macrophages release their angiogenic mediator only in the presence of low oxygen tension in the injured tissue (i.e. 2–30 mmHg) (268, 269). However, as macrophages have been found to release lactate even while they are well oxygenated, the stimulus to collagen synthesis remains even during hyperoxygenation (331, 332), a finding which is of importance in the use of hyperbaric oxygen therapy.

Finally, macrophages can release a polypeptide, interleukin-1, that can function as a messenger for lymphocytes.

In summary, the macrophage seems to be the key cell in the inflammatory and proliferative phase of wound healing by secreting factors that stimulate the proliferation of fibroblasts and secretion of collagen, as well as stimulation of neovascularization (Fig. 1.4). The macrophages also act as scavengers in the wound area and remove traumatized tissue and bacteria and neutralize foreign bodies by forming giant cells that engulf or surround the foreign matter. Moreover, signals released by traumatized bone or tooth substances cause some monocytes to fuse and form osteoclasts which will subsequently resorb damaged hard tissue (see Chapters 2 and 7). Macrophages play an important role in the immune response to infection (see Chapter 2).

Lymphocytes

Lymphocytes emigrating from the bloodstream into the injury site become apparent after 1 day and reach a maximum after 6 days (Figs 1.15 and 1.16). The role of these lymphocytes in the wound healing process has for many years been questioned as earlier investigations have pointed out that lymphocytes, like neutrophils, were not necessary for normal progression of healing in non-infected wounds (300). However, recent research has demonstrated that lymphocytes together with macrophages may modulate the wound healing process (333–335) (Fig. 1.17).

Lymphocytes can be divided into *T lymphocytes* (thymus-derived lymphocytes) and *B lymphocytes* (bone marrow-derived lymphocytes). Both types are attracted to the wound area, probably by activated complement on the surface of macrophages and neutrophils.

Lymphocyte infiltration in wounds is a dynamic process where both T-helper/effector and T-suppressor/cytotoxic lymphocytes are present in the wound after 1 week (336). These activated lymphocytes produce a variety of lymphokines of which interferon (IFN-α) and TGF-β have been shown to have a significant effect on endothelial cells and thereby may have an effect on angiogenesis. This effect may be secondary to other effects such as macrophage stimulation and activation (337). TGF-β is also a potent fibroblast chemotactic molecule and, in addition, induces monocyte chemotaxis and secretion of fibroblast growth factor and activating factors (338).

Recent studies have indicated that there are at least two populations of T cells involved in wound healing. One population bearing the T-cell marker appears to be required for successful healing, as shown by the impairment in healing caused by their depletion. The T-suppressor/cytotoxic subset appears to have a counterregulatory effect on wound healing, as their depletion enhances wound rupture strength and collagen synthesis (333–335, 337).

Based on present evidence, Barbul has postulated the following theory: macrophages exert a direct stimulatory effect on endothelial cells and fibroblasts (Fig. 1.17). A T-cell marker positive subset (T^+), which is not yet fully characterized, has a direct action on endothelial cells and fibroblasts and acts indirectly by stimulating macrophages. T-suppresser/cytotoxic cells (Ts/c) downregulate wound

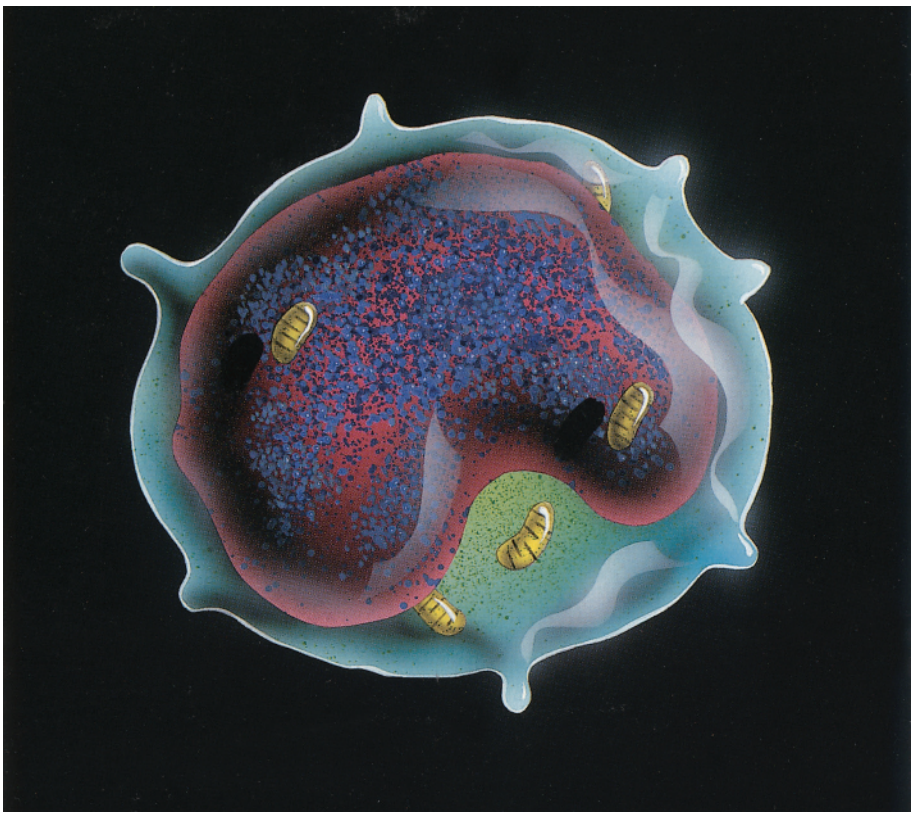

Fig. 1.15 Lymphocyte.

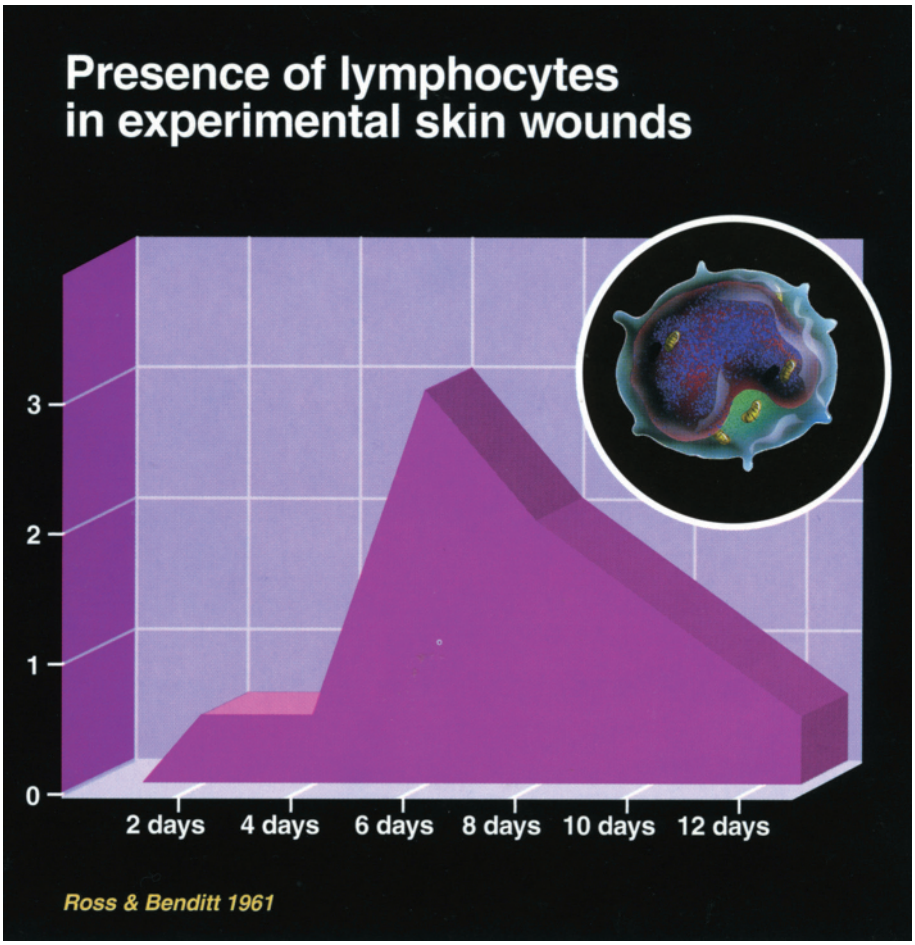

Fig. 1.16 Schematic illustration of the presence of lymphocytes in experimental skin wounds in guinea pigs. The scale is semiquantitative, graded from 0 to 3. Adapted from (73).

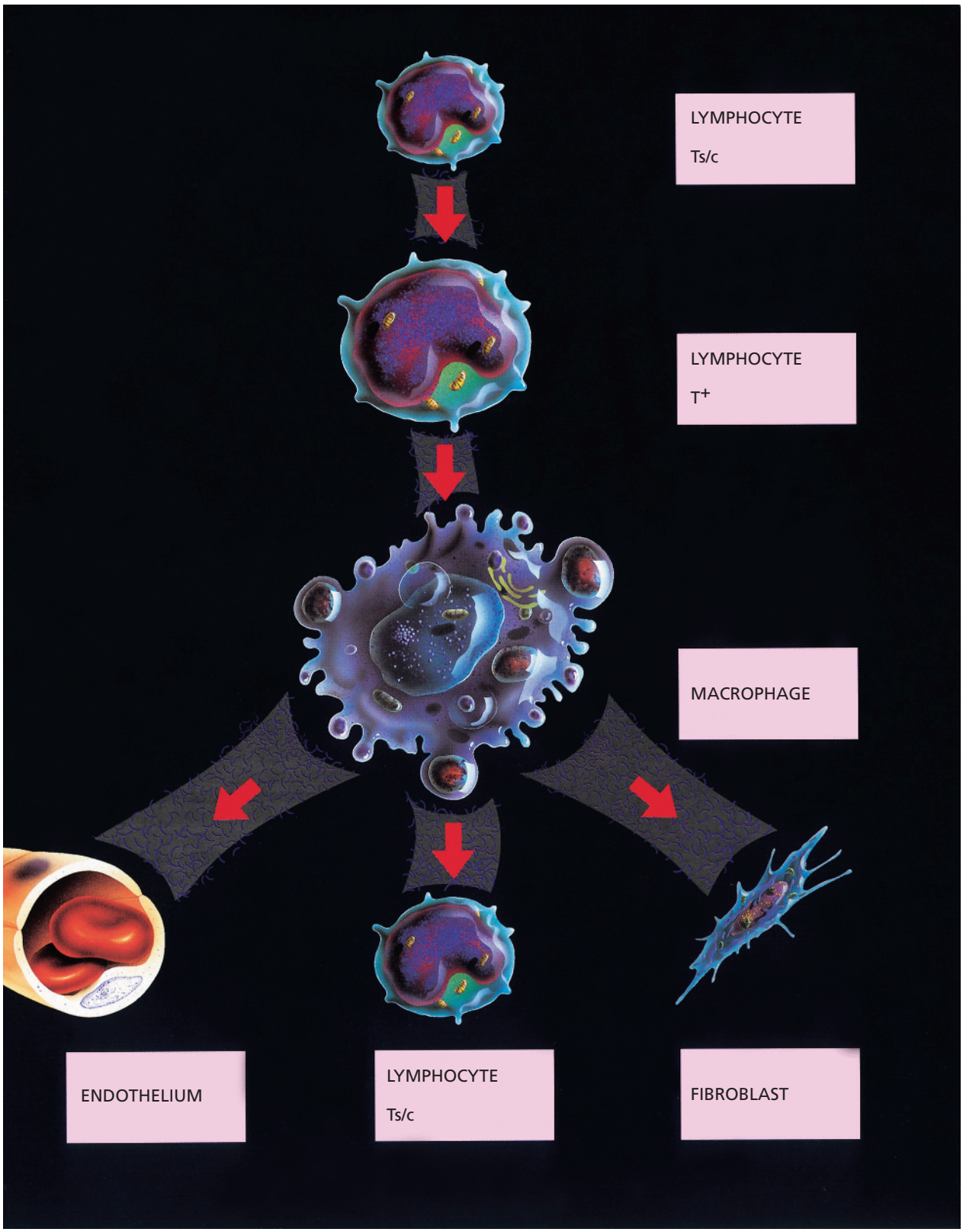

Fig. 1.17 Role of lymphocytes in wound healing. Macrophages exert a direct stimulatory effect on endothelial cells and fibroblasts. A T-cell marker positive subset (T^+), which is not fully characterized, has direct action on endothelial cells and fibroblasts and acts indirectly by stimulating macrophages. T-suppressor/cytotoxic cells (Ts/c) downregulate wound healing by direct action on macrophages and T cells. Reproduced from (337) with permission.

healing by direct action on macrophages and T cells (337, 338) (Fig. 1.17).

There is presently no evidence to suggest that the humoral immune system (B lymphocytes) participates in the wound healing process. The influence of lymphocytes therefore seems primarily to be through T lymphocytes.

In summary, lymphocytes appear indirectly to influence the balance between the stimulatory and inhibitory signals to fibroblasts and endothelial cells via the macrophages.

Fibroblasts

The fibroblast is a pleomorphic cell. In the resting, nonfunctional state it is called a *fibrocyte*. The cytoplasm is scanty and often difficult to identify in ordinary histologic sections. In the activated, mature form the cell becomes stellate or spindle shaped and is now termed a *fibroblast*. The most characteristic feature is now the extensive development of a dilated endoplasmic reticulum, the site of protein synthesis (339) (Fig. 1.18). In normal connective tissue, fibroblasts are attached to a fibrillar ECM mesh that contains an abundance of structural proteins such as crosslinked type I collagen and elastin produced by fibroblasts themselves (628). The ECM proteins present in the fibroblast microenvironment serve as a conduit for cell migration into the wound. In order to migrate, fibroblasts therefore need to modify their interaction with the existing ECM and be able to recognize and interact with the novel proteins present in the wound provisional matrix. To facilitate cell migration, fibroblasts use focalized proteolysis to release their adhesion to the pericellular ECM and to remodel the ECM (632, 633). The mode of fibroblast migration is different from that of leukocytes, which appear to use a more dynamic ameboid migration that does not require extensive ECM remodeling (634).

In the wound the fibroblast will produce collagen, elastin and proteoglycans (340). After injury, fibroblasts start to invade the area after 3 days, stimulated by platelets and macrophage products, and they become the dominating cell 6–7 days after injury, being present in considerable concentration until the maturation phase of the healing process (73) (Fig. 1.19).

Several studies have suggested that new fibroblasts arise from the connective tissue adjacent to the wound, principally from the perivascular undifferentiated mesenchymal cells (stem cells) (341–346) and not from hematogenous precursors (346).

Once fibroblast precursors receive the proper signal they begin to reproduce and a mitotic burst is seen between the 2nd and 5th day after injury (347). Proliferating fibroblasts develop through cell divisions every 18–20 hours and remain in the mitotic phase for 30 minutes to 1 hour. The primary function of the activated fibroblast in the wound area is to produce collagen, elastin and proteoglycans. However,

Fig. 1.18 Active fibroblast.

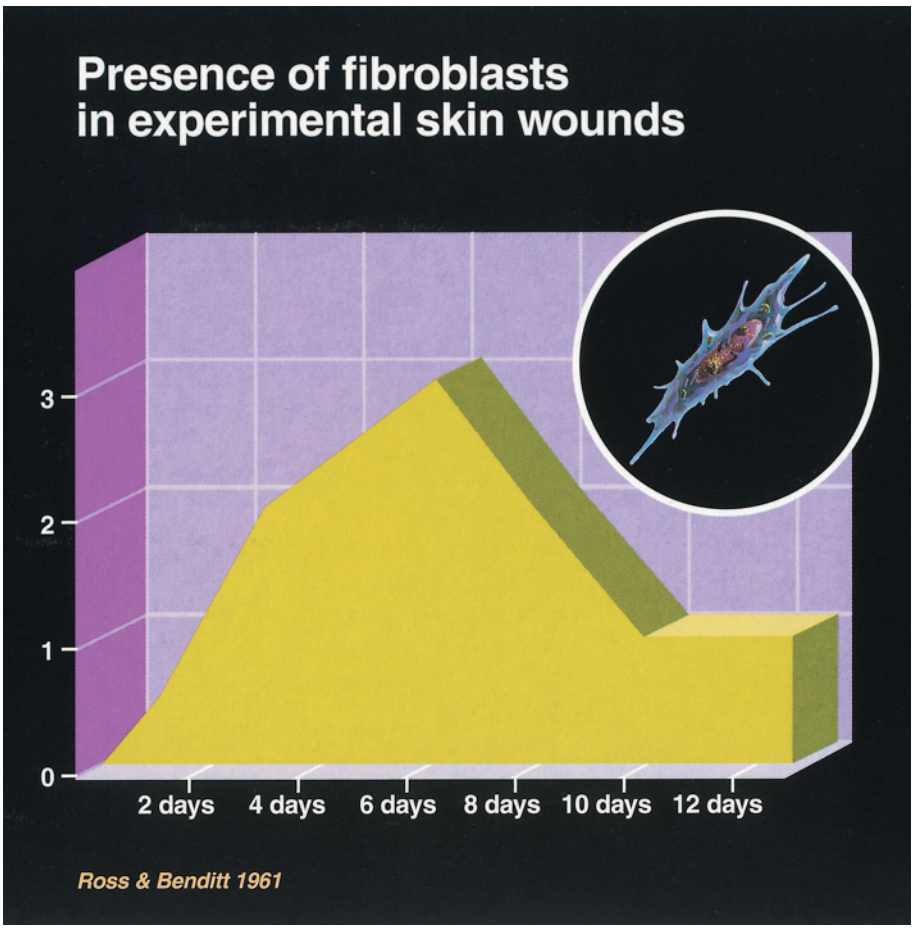

Fig. 1.19 Schematic illustration of the presence of fibroblasts in experimental skin wounds in guinea pigs. The scale is semiquantitative, graded from 0 to 3. Adapted from (73).

during the mitotic phase, the fibroblast does not synthesize or excrete external components. Progression factors are necessary to stimulate the fibroblast to undergo replication. Before this can happen the fibroblast must be made competent. Factors that induce this competence are PDGF, FGF and calcium phosphate precipitates (26). PDGF-induced competence requires only transient exposure of cells to the factor. When competent, the fibroblast can replicate after stimulation by progression factors such as IGF-1, EGF and other plasma factors (26). This dual control of fibroblast proliferation explains why fibroblasts can remain in a reversible quiescent state in the presence of progression factor. The transition to a proliferative stage then awaits the release of competence factors by activated cells, such as platelets, macrophages and lymphocytes.

PDGF is released in response to injury by platelets and production is continued by activated macrophages, which can induce migration into the wound and proliferation of fibroblasts over an extended period of time (Fig. 1.4).

TGF-β is a growth stimulator for mesenchymal cells and has been found to accelerate wound healing in rats (348, 349) by direct stimulation of connective tissue synthesis by fibroblasts and indirect stimulation of fibroblast proliferation by PDGF (350, 351). Other factors that may be involved in fibroblast proliferation include TNF-α and TNF-β (26).

The best characterized inhibitor of fibroblast proliferation is β-fibroblast interferon (IFN-β). It has been suggested that IFN-β inhibits events involved in fibroblast competence and induction of competence of fibroblasts and by PDGF inhibition (352, 353).

Regulatory systems of fibroblast activity may operate through activation of macrophages, which generate an endogenous stimulus to fibroblast proliferation. Alternatively, fibroblast proliferation might be slowed by either inhibition of the release and activation of PDGF and TGF-β or by stimulation of inhibitory substances such as IFN-β. Clinically, this may suggest a specific stimulation of fibroblast proliferation by treatment with exogenous growth factors such as PDGF and TGF-β or by their activation (26).

Once fibroblasts have migrated into the wound, they produce and deposit large quantities of fibronectin, types I and III collagen and hyaluronidate. TGF-β is considered to be the most important stimulator of ECM production (354, 355).

Multiplication and differentiation of fibroblasts and synthesis of collagen fibers require oxygen as well as amino acids, carbohydrates, lipids, minerals and water. Collagen cannot be made in the mature fibroblast layer without oxygen (356–359). Consequently, the nutritional demands of the wound are greater than that of non-wounded connective tissue (315) and the demand is greatest at a time when the

local circulation is least capable of complying with that demand (316).

After collagen molecules are secreted into the extracellular space, they are polymerized in a series of steps in which the hydroxylysine groups of adjacent molecules are condensed to form covalent crosslinks. This step is rate dependent on oxygen tension and gives collagen its strength (356, 361).

Fibroblasts have been shown to have chemotactic attraction to types I, II, and III collagen as well as collagen-derived peptides, with binding of these peptides directly to fibroblasts (362, 363).

Fibroblasts have been known for many years to be involved in wound contraction. In relation to this phenomenon, a specific type of fibroblast has been identified which has the characteristics of both fibroblasts and smooth muscle cells for which reason they have been termed myofibroblasts. These cells are richly supplied with microfilament bundles that are arranged along the long axis of the cells and are associated with dense bodies for attachment to the surrounding ECM (364, 365). Besides numerous cytoplasmic microfilaments, large amounts of endoplasmic reticulum are also seen. In this respect these cells have characteristics of fibroblasts.

The myofibroblast has contractive properties and has been demonstrated in many tissues that form contracted and/or nodular scars (366). Myofibroblasts are present throughout granulation tissue and along wound edges at the time when active contraction occurs (367). For this reason the most generally accepted theory of wound contraction has involved a contribution by myofibroblasts (366).

Ehrlich and co-workers have presented a new theory for the phenomenon of wound contraction (368). In this fibroblast theory it is suggested that fibroblast locomotion is the mechanism that generates the contractive forces in wound contraction and that the connective tissue matrix is important in controlling these forces. It is suggested that the histologic existence of myofibroblasts is a transitional state of the fibroblast in granulation tissue.

In summary, the fibroblasts are latecomers in the inflammation phase of wound healing. Their main function is to synthesize and excrete the major components of connective tissue: collagen, elastin and proteoglycans. The fibroblast is also involved in wound contraction through a specific cell called the myofibroblast which has characteristics of both fibroblasts and smooth muscle cells.

Endothelial cells and pericytes

The capillaries play an essential role in wound healing. The caliper of capillaries averages 9–12 µm, which is just enough to permit unimpeded passage of cellular elements. In cross-section the capillary wall consists of 1–3 endothelial cells (Fig. 1.20). Capillaries converge to form post-capillary venules of slightly larger size (15–20 µm). The endothelial cells are surrounded by a network of pericytes (Fig. 1.20). These cells appear to represent a pool of undifferentiated mesenchymal cells which have been found to participate in wound healing, and this applies also to the pulp and periodontium (see Chapter 5).

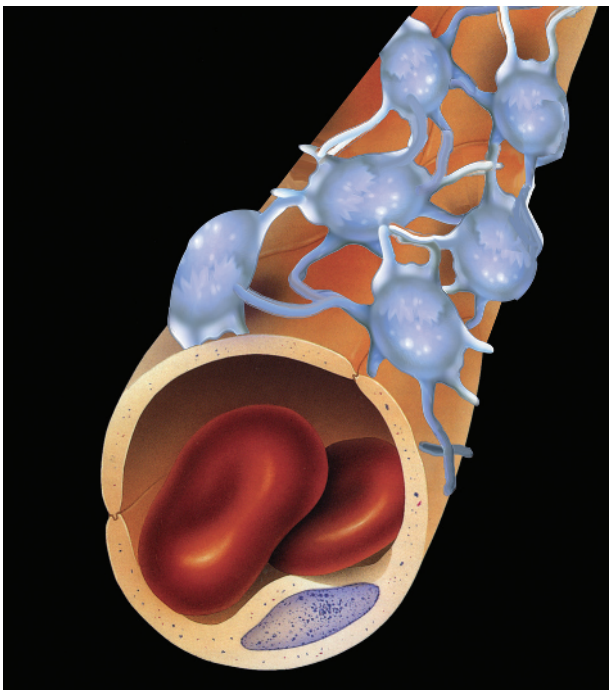

Fig. 1.20 Endothelial cells and pericytes.

Angiogenesis

Between 2 and 4 days after wounding, proliferation of capillaries and fibroblasts begins at the border of the lesion. However, studies have shown that blood cells and plasma perfuse the wound tissue several hours before the space is invaded by sprouting capillaries (295, 369). At first the blood cells move around randomly in the meshes of the fibrinous network but gradually preferential channels are formed in the wound through which cells pass more or less regularly. This phenomenon has been termed open circulation and it is suggested that the blood cells at this time are transported in a simple tube system which has not yet acquired an endothelial lining (295, 369, 370).

Angiogenesis is the process of formation of new blood vessels by directed endothelial migration, proliferation and lumen formation (262, 371–376, 628) (Fig. 1.21). In wound healing, angiogenesis is crucial for oxygen delivery to ischemic or newly formed tissue. New vessels arise in most cases as capillaries from existing vessels and only from venules (376, 377) (Fig. 1.21). In early granulation tissue, after the wound healing module is assembled, capillary sprouts move just behind the advancing front of macrophages. Collagen-secreting fibroblasts are placed between these sprouts and are nourished by the new capillaries (see Fig. 1.26).

Variants in healing of the vascular network are found according to the type of tissue involved and the extent of the injury. In skin or mucosal lacerations, primarily closed, existing vessels may anastomose spontaneously and thereby re-establish circulation. In wounds with tissue defects or in non-closed wounds, a new vascular network has to be created via granulation tissue. The third variant in vascular healing is

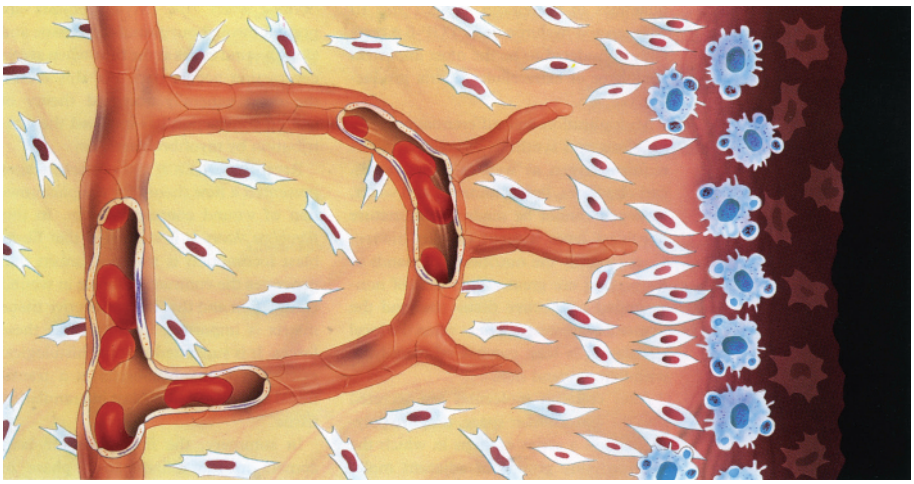

Fig. 1.21 Neovascularization. New capillaries start as outgrowths of endothelial cells lining existing venules. Subsequent arcading sprouts unite and tubulization allows circulation to be established.

the revascularization of ischemic tissue as seen after skin grafting, tooth luxation, tooth replantation and transplantation. In these situations angiogenesis takes place in existing ischemic or necrotic tissue. The healing in these cases usually occurs as a mixture of gradual ingrowth of new vasculature combined with occasional end-to-end anastomosis between existing and ingrowing vessels (see Chapter 2).

Angiogenesis in wounds has been examined in different *in vivo* assays such as the rabbit ear chamber (269, 378), the Algire chamber where a transparent plastic window is placed in the dorsal subcutaneous tissue of a mouse (379) or the hamster cheek pouch (380, 381). Furthermore, angiogenesis has been tested in corneal pockets (379, 382, 383), and chicken chorioallantoic membrane (384). These assays have been used to describe the dynamic process of angiogenesis together with the influence of different types of external factors on vascular proliferation.

Our current knowledge of the biochemical nature of the signals that induce angiogenesis has been derived primarily from *in vitro* observations using cultured vascular endothelial cells. *In vitro* assays have been used extensively in the identification and purification of angiogenetic factors. In this context, as angiogenesis is considered to be a process of capillary growth, cultured capillary endothelial cells seem to be optimal for testing angiogenesis (385).

Cellular events in angiogenesis

New capillaries usually start as outgrowths of endothelial cells lining existing venules. After exposure to an angiogenic stimulus, endothelial cells of the venules begin to produce enzymes that degrade the vascular basement membrane on the side facing the stimulus (636). After 24 hours the endothelial cells migrate through the degraded membrane in the direction of the angiogenic stimulus (Fig. 1.21). Behind the tip of the migrating wound edges, trailing endothelial cells divide and differentiate to form a lumen. The sprouts or buds can either connect with other sprouts to form vascular loops or can continue migrating. Capillary bud formation is found after 48 hours and these buds arise solely from venules (377).

When endothelial migration tips join to form capillary loops or join across a wound edge, blood flow begins within the formed lumen. As vessels mature, ECM components are laid down to form a new basement membrane (371, 376). Recent studies have shown that angiogenesis is closely related to fibroblast activity. Thus it appears that new vessels cannot grow beyond their collagenous support (356, 386).

The speed of neovascularization has been investigated in ear chambers and been found to range from 0.12 to 0.24 mm per day (272, 295, 387, 388). In dental pulps which become revascularized after replantation or transplantation, the speed is approximately twice this rate (see Chapter 2).

In raised and repositioned skin flaps, angiogenesis along the cut margins is rapid and capillary sprouts advance across the wound space from the host bed. In rats, new vascular channels across the wound margin can be demonstrated within 3 days; and in pigs, normal blood flow has been observed within 2–3 days (389–391). After tissue grafting, specific vascular healing processes take place. Thus it has been shown in skin transplants that after an initial contraction of the vessels, a so-called plasmatic circulation takes place in the zone next to the graft bed (392–400). This supply of fluids serves to prevent drying of the graft before blood supply has been restored (398). The role of the plasmatic circulation as a source of nourishment is, however, debatable (398).

Vascularization of skin transplants, although initially sluggish, takes place after 3–5 days (401–410). The role of already existing graft vessels is unsettled. Thus, in some studies, it has been shown that the original vessels act only as non-viable conduits for the ingrowth of new vessels (388), and that revascularization takes place primarily from invading new vessels (408, 411). In other experiments, however, it has been shown that, depending upon the degree of damage to the grafted tissues and on local hemodynamic factors, the original graft vessels may be incorporated in the established new vascular network (409–413).

Teeth have a vascular system which in some situations is dissimilar to skin. In replantation and transplantation procedures of immature incisors, the severed periodontal ligament and the pulp can be expected to become revascularized.

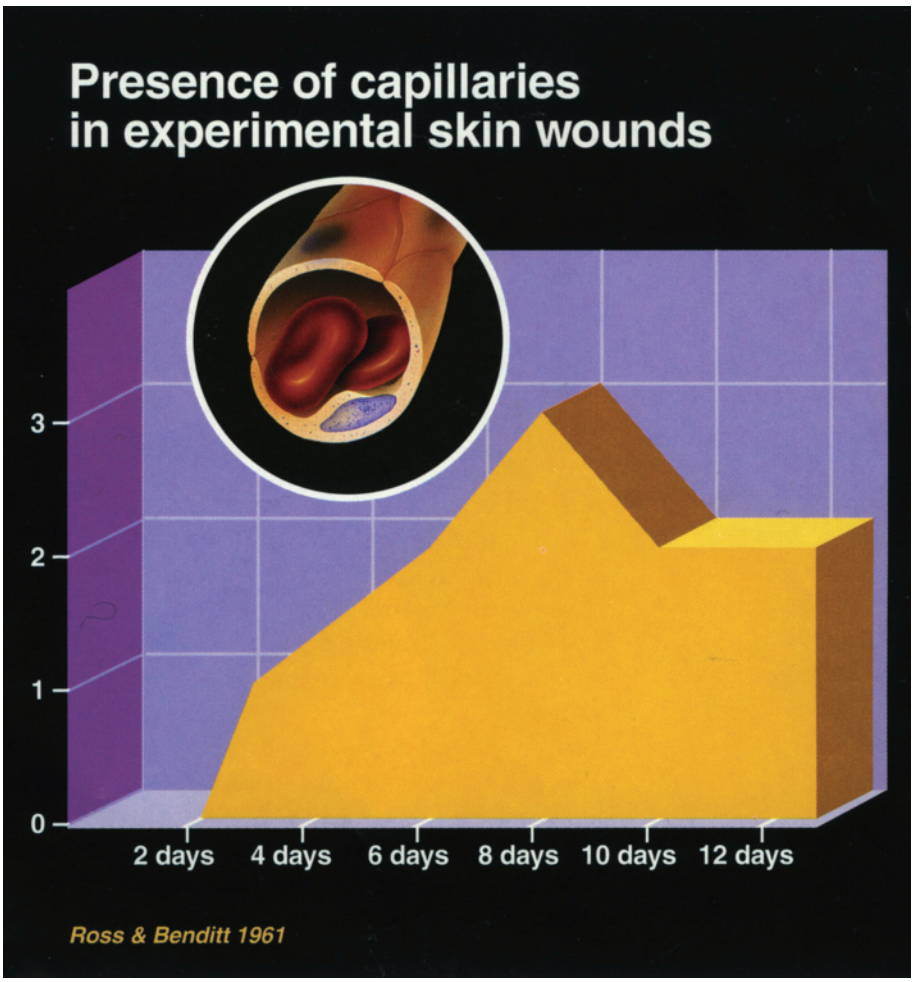

Fig. 1.22 Schematic illustration of the presence of new capillaries in experimental skin wounds in guinea pigs. The scale is semiquantitative graded from 0 to 3. Adapted from (73).

The process of revascularization of the periodontal ligament seems to follow the pattern of skin grafts (see Chapter 2). There is limited diffusion of nutrients from the graft bed to the dental pulp due to its hard tissue confinement and extended length, which again leads to specific vascular healing events (see Chapter 2).

In a closed skin wound, circulation bridging the wound edges can be established as early as the 2nd or 3rd day and appears to be at a maximum after 8 days (73) (Fig. 1.22). The new vascular network is then remodeled. Some vessels differentiate into arteries and veins while others recede. The mechanism regulating this process is largely unknown. Active blood flow within the lumen may be a factor, as capillaries with decreased blood flow typically recede while those with active flow are usually maintained or expand into larger vessels (378, 414).

Factors determining angiogenesis represent a series of cellular and humoral events that lead to the initiation, progression and termination of angiogenesis (Figs 1.4 and 1.23). Initiation of angiogenesis appears to be related to signals released from activated platelets and fibrin at the site of vascular rupture (86, 285, 415, 416). During platelet activation, enzymes are released that degrade heparin and heparan sulfate components from the vascular basement membranes, whereby stored basic FGF (bFGF) is liberated (417–421). This liberation of FGF has been shown to induce angiogenetic activity (422). Other bFGF signals are released from injured cells and matrix (376). This growth factor is partly responsible for angiogenesis through initiating a cascade of events (423). Thus bFGF stimulates endothelial cells to secrete procollagenase, plasmin and plasminogen. Plasmin, as well as plasminogen, activates procollagenase to collagenase. Together, these enzymes can digest the blood vessel basement membrane. Subsequently, endothelial chemoattractants, such as fibronectin fragments generated from ECM degradation and heparin released from mast cells, draw endothelial cells through the disrupted basement membrane to form a nascent capillary bud (132).

A variety of growth factors, cytokines and lipid mediators produced in response to injury can stimulate angiogenesis. One of the most important proangiogenic mediators is vascular endothelial growth factor (VEGF or VEGF-A), and sufficient VEGF levels are believed to be essential for proper wound healing. VEGF is one of the most potent proangiogenic growth factors in the skin, and the amount of VEGF present in a wound can significantly impact healing. The activity of VEGF was once considered to be specific for endothelial cells lining the inside of blood vessels, partly because VEGF receptor (VEGFR) expression was believed to be restricted to endothelial cells. It is now known, however, that VEGFRs can be expressed by a variety of other cell types involved in wound repair. For example, keratinocytes and

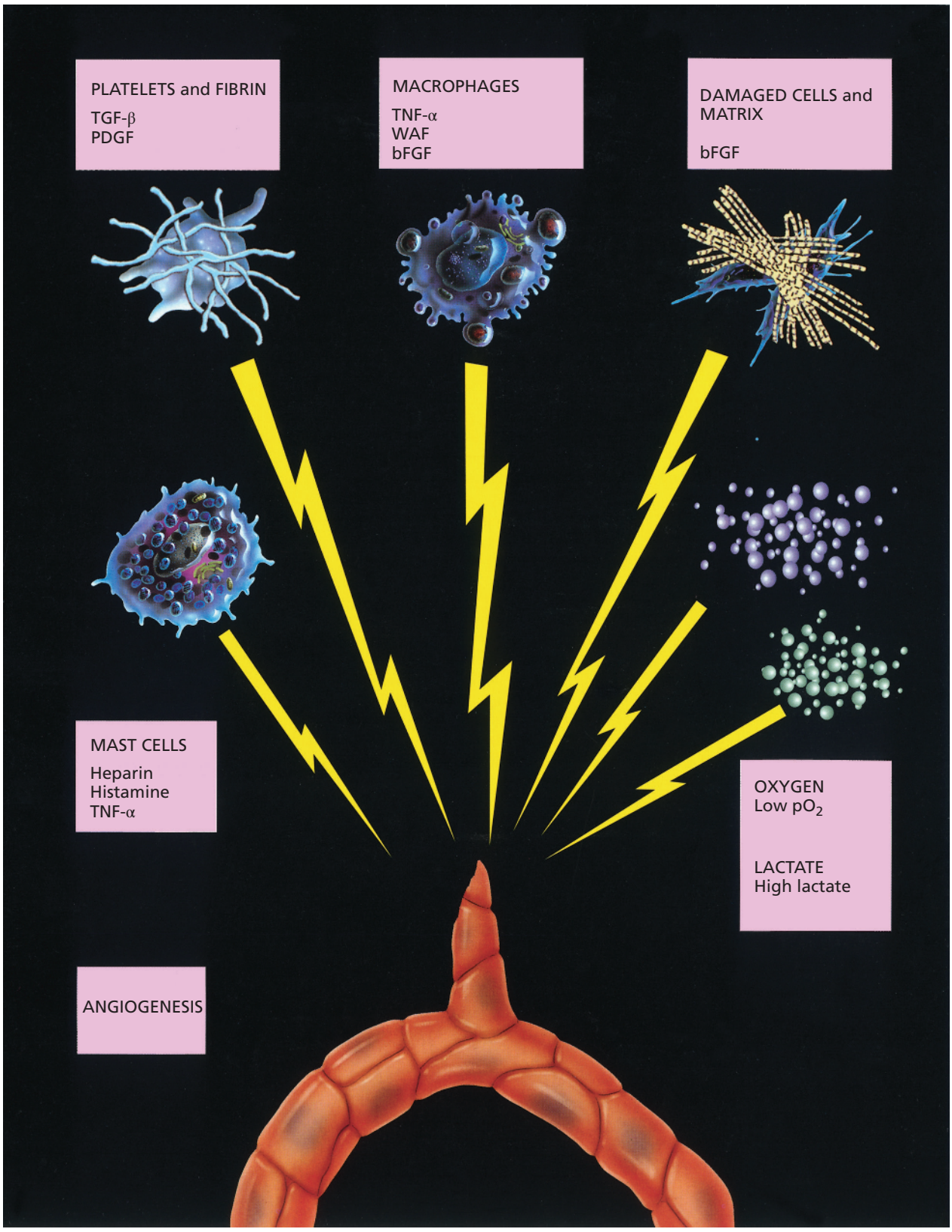

Fig. 1.23 Cellular and humoral events leading to angiogenesis. Platelets, fibrin, mast cells, macrophages, injured cells and matrix all release angiogenic signals. Low oxygen tension and a high lactate concentration in the wound space represent also an important stimulation to angiogenesis.

macrophages, which both carry out important functions during wound healing, express VEGFRs and are capable of responding directly to VEGF (635).

Recruited and activated macrophages soon also promote angiogenesis (424) by liberating potent direct acting angiogenic factors such as TNF-α, wound angiogenesis factor (WAF) and FGF. The macrophage signal seems to diminish as angiogenesis proceeds (268, 269, 356, 361, 425–428). Recent studies indicate that the effect of hypoxia within the wound upon angiogenesis is possibly mediated via stimulated macrophages (see p. 42) (268, 429, 430).

Other factors controlling angiogenesis

A number of angiogenetic factors have been isolated which either have a direct effect on endothelial cell migration/proliferation or have an indirect effect via other cells. The exact mechanisms behind indirect angiogenetic activity are not yet known, but it is possible that they cause accumulation of other types of cells, e.g. platelets or macrophages, that release direct acting factors (126, 376, 431).

Once new blood vessels form, they acquire a layer of pericytes and the composition of their basement membrane changes. Pericytes inhibit the growth of adjacent endothelial cells and thereby direct growth toward the site of attraction (432).

Finally, it should be mentioned that angiogenesis is dependent upon the composition of the ECM (433, 623, 624). Thus, fibrin appears to promote angiogenesis (64) and the fibrin–fibronectin extravascular clot serves as a provisional stroma providing a matrix for macrophages, fibroblasts and new capillary migration. In this way, the fibrin–fibronectin gel is transformed to granulation tissue.

Wound strength development

Wound strength, from a functional point of view, is the most important property for a healing wound. The time interval from injury to a healed wound that is strong enough to resist mechanical stress in the tissue is important for the surgeon to consider. It is essential that an early return to normal life is facilitated with the development of significant wound strength. For ethical reasons this has to be based on investigations of the tensile strength of experimental wounds.

In early wounds the tensile strength is low and insufficient to keep the tissue edges together without sutures. The strength of the wound is in this stage mainly based on fibrin in the wound cavity. Later, in the proliferation phase, the strength increases rapidly as granulation tissue is formed. The strength of the wound lies in the collagen fibers, and is directly related to the collagen content of the tissue (434, 435) (Fig. 1.24). Some collagenous elements can be seen already after 2–3 days of injury, but the maximum period of collagen synthesis most often starts during the 5th to 6th days of healing. The collagen fibers are laid down in a random pattern and in the beginning possess little mechanical strength. Gradually, a more systematic pattern of collagen fibrils develops, leading to stabilization by crosslinking and assembly of fibers into a more correct anatomic pattern. Experimental studies have shown that the 'biochemical active zone' encompasses tissue 5–7 mm from the incisional line. Wound contraction also brings collagen fibrils together and organizes them perpendicular to the wound edges, increasing the mechanical strength of the tissue (644–646).

The resulting tensile strength of a wound is the combined strength of the old collagen (present in the wound area before injury), which diminishs through lyses of collagenases, and the increasing strength of the new-build collagen. The lowest tensile strength of a healing wound therefore occurs after some days of healing (20) (Fig. 1.25).

Wound strength can in a functional way be described as the relative tensile strength of the wound. This is the actual tensile strength of the wound in relation to the strength of intact tissue and is expressed as a percentage. Figure 1.25 shows the relative tensile strength of healing incisional wounds in different types of tissues. In tissues with a low collagen content in intact tissue (gastrointestinal tract, muscle) (436, 437), the relative tensile strength increases rapidly and reaches an intact level after 10–20 days. In tissues with a high collagen content (fascia, skin, tendon), the relative strength increases slowly and in skin and tendon the strength has after 60 days only reached 60% of the intact level (435) (Fig. 1.25).

Collagen constitutes the principal structural protein of the body and is the main constituent of ECM in all species. At least 13 types of collagen have been identified. Despite their differences, all collagen molecules consist of a triple helix matrix protein which gives the tissues their strength (15, 437–442). Literature on wound healing and collagen contains only sparse information about the different types of collagens (437).

Type I collagen is the major structural component of skin, mucosa, tendons and bone (440).

Type II collagen is located almost exclusively in hyaline cartilage.

Type III collagen, also called reticular fibers (443), is found in association with type I collagen although the ratio varies in different tissues (440). In a rat model, type III collagen could be demonstrated 10 hours after the start of wound healing in skin (444), and after 3 days in healing (PDL wounds (445, 446). The early appearance of type III collagen has been found associated with the deposition of fibronectin, indicating that type III collagen together with fibronectin may provide the initial scaffolding for subsequent healing events (447, 448).

In children, type III collagen can be detected between 24 and 48 hours in skin wounds, whereas no type I collagen is found in this type of wound (449). From 72 hours and onwards a substantial increase in type I collagen is found, together with the appearance of mature fibroblasts (449).

Type IV collagen, together with other components, including heparan sulfate, proteoglycans and laminin, makes up the basement membranes in both the epidermis and endothelium.

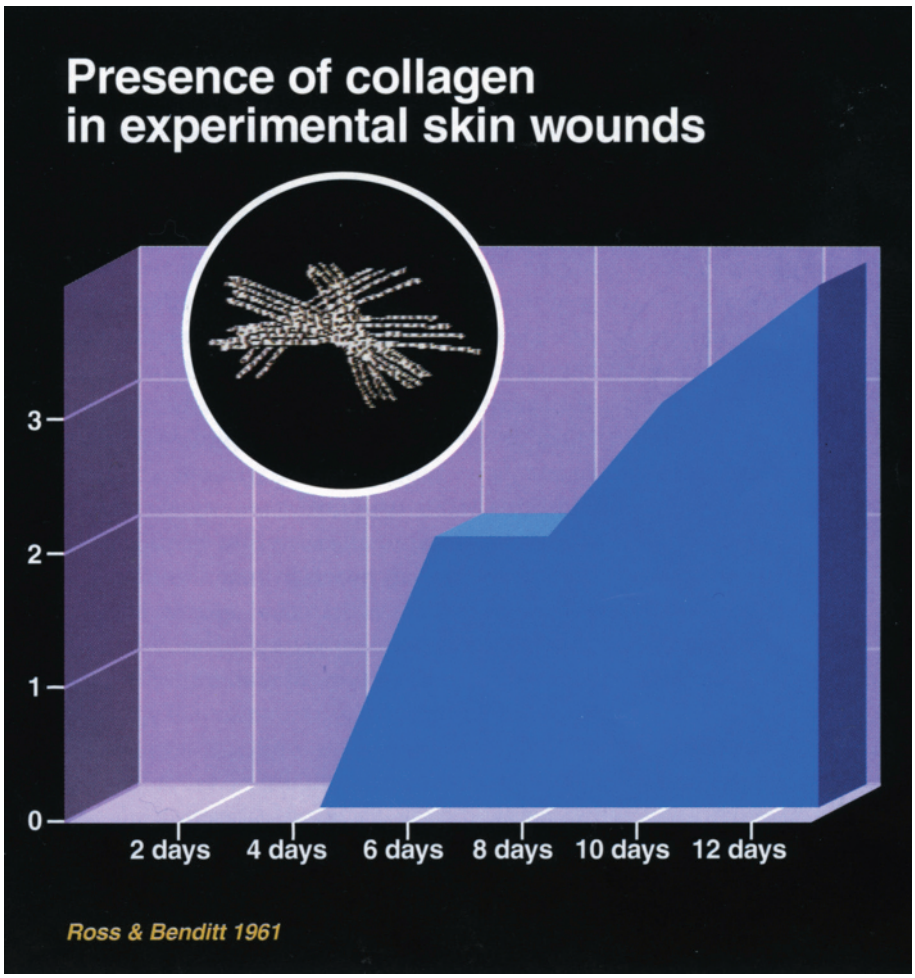

Fig. 1.24 Schematic illustration of the presence of collagen in experimental skin wounds in guinea pigs. The scale is semiquantitative graded from 0 to 3. Adapted from (73).

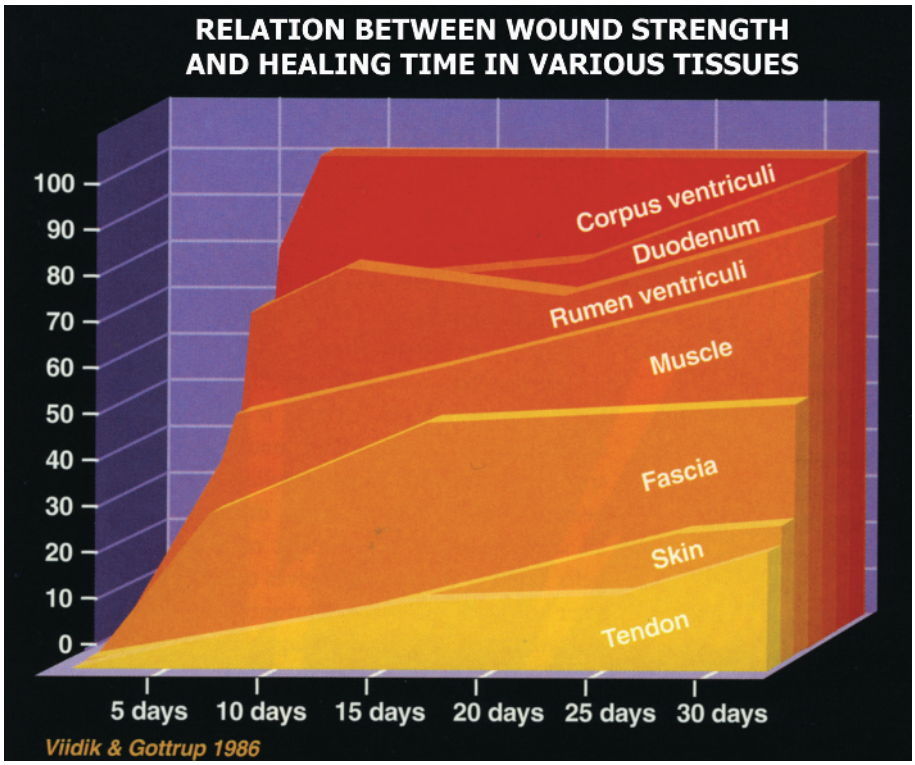

Fig. 1.25 Relative healing rates for linear incisional wounds in different tissues in rats and rabbits; the tensile strength is calculated as a percent of that of the respective intact tissues (taken as 100%). Reproduced from (457) with permission.

In dermal wound healing, type IV collagen synthesis by epidermis is connected with the reformation of the basement membrane and is a relatively late event in the wound healing process (450–452).

Type V collagen is found in almost all types of tissue and has been proposed to be involved in the migration of capillary endothelial cells during angiogenesis (441). Type V collagen is synthesized while epidermal cells migrate; however, the regeneration of the basement membrane is delayed until the wound defect is covered and the epidermal cells are no longer in a migrating phase (451).

Type VII collagen has been found to be an anchoring fibril that attaches the basement membrane to the underlying connective tissue (454–456).

The remaining types of collagen are less known in relation to the wound healing process and are therefore not discussed.

Collagen represents a key component in wound healing. Thus, immediately after injury the exposure of collagen fibers to blood results in platelet aggregation and activation with resultant coagulation and the release of chemotactic factors from platelets that initiate healing (e.g. PDGF, platelet factor IV, IGF-1, TGF-β and an unidentified chemoattractant to endothelial cells). Collagen fragments are then degraded by the attracted neutrophils and leukocytes, which leads to attraction of fibroblasts.

Synthesis of new collagen in a wound starts with fibroblast proliferation and invasion and the deposition of a collagen-based ECM. This event takes place after 3–5 days and persists for 10–12 days. During this period of time, there is a rapid synthesis primarily of type III and later of type I collagen, resulting in an increase in the tensile strength of the wound which is primarily dependent on the build up of type I collagen (449, 458–460).

Remodeling phase

Remodeling of the ECM is a continuous process that starts early in the wound healing process and turns the abundant and poorly organized granulation tissue ECM into a mature connective tissue. Thus most fibronectin is eliminated within 1 or 2 weeks after granulation tissue is established. Hyaluronidate is replaced or supplemented with heparan sulfate proteoglycans in basement membrane regions and with dermatan chondroitin sulfate proteoglycans in the interstitium (61).

Type III collagen fibers are gradually replaced by type I collagen, which becomes arranged in large, partly irregular, collagen bundles. These fiber bundles become oriented according to lines of stress and provide a slower increase in the tensile strength of the healing wound than that found during the proliferation phase (460). In most tissues, this remodeling phase ultimately leads to the formation of a scar.

The functional properties of the scar tissue vary considerably depending on the content of collagen in the intact original tissue. The healing rate, measured as the mechanical strength of the wound compared to adjacent intact tissue, therefore varies from one tissue type to another. In tissue with a low collagen content before injury (e.g. gastrointestinal tract (461) and other intra-abdominal organ systems), the primarily closed wound shows a rapid increase in relative strength (strength of wounded tissue compared to intact tissue) (457). As shown in Fig. 1.25, tensile strength close to that of intact tissue levels is reached after 10–20 days of healing in tissues with a low collagen content before injury. In tissues with a high collagen content (e.g. tendon and skin) the increase in relative strength is much slower; and more than 100 days of healing are needed to achieve half the strength of intact tissue. In the wounded PDL, a very rapid increase in tensile strength has been found after severance of Sharpey's fibers (see Chapter 2) Investigations of wounds closed 3–6 days after injury (delayed primary closure) has shown that this type of wound was significantly stronger than primarily closed wounds after 20 days of healing. After 60 days of healing, delayed primarily closed wounds were almost twice as strong as primarily closed wounds; and this difference persisted after 120 days (59, 60, 462). The mechanisms behind the different wound strength in primarily and delayed primarily closed wounds are probably related to an increase in tissue perfusion and oxygenation due to increased angiogenesis and oxygen delivery to the tissues (463).

Remodeling starts when wound contraction has assembled the collagen fibrils into thicker bundles and aligned them perpendicularly to the wound edges. The timing of the start of this stage depends on the size of the wound, and whether the wound healing occurs by primary or secondary intention. During the remodeling stage, collagen crosslinking also gradually increases, improving the stability of the tissue, and there is a gradual maturation of the tissue so that the aligned collagen fiber bundles are reorganized to the typical and more resilient basketweave organization found in normal connective tissue. Remodeling is a slow process and continues slowly and can last for months or in some cases for years (639, 640).

Hypertrophic scar and keloid

As mentioned above, if remodeling does not lead to complete structural regeneration of the tissue, scar formation may occur. In the mildest form, scars may contain some excess of collagen-rich ECM where collagen is organized in thick parallel bundles instead of the mechanically stronger basketweave that is present in normal tissue. Therefore, scars can gain at best only about 70–80% of the breaking strength of normal skin. However, in more severe cases, ECM deposition and degradation is not balanced, leading to progressive ECM accumulation and formation of pathologic scars, namely keloids or hyperthrophic scars in the skin. Therefore, biologic processes that regulate the ECM abundance and organization are critical for appropriate wound healing outcome. At least four different mechanisms likely contribute to these processes: First, the number of cells secreting ECM is reduced. Second, cells degrade and

remodel ECM components to reduce their abundance. Third, ECM is reorganized and its stability is increased by collagen crosslinking. Fourth, secretion of ECM by the cells is downregulated (628).

Excessive deposition of scar tissue is a clinical problem that has been difficult to resolve because of the lack of reliable animal models. Hypertrophic scar and keloid are both characterized by excessive accumulation of ECM, especially collagen. The etiology is not known but abnormalities in cell migration and proliferation, inflammation, syntheses and secretion of ECM proteins and cytokines, and remodeling of wound matrix, have been described. Also, increased activity of fibrogenic cytokines (e.g. TGF-β1, interleukin-1), abnormal epidermal–mesenchymal interaction and mutations in regulatory genes have been proposed (464). In healed burns the development of hypertrophic scars seems not to be the result of a continued proliferation phase, rather an alteration of the remodeling phase (465).

The hypertrophic scar results from a full thickness injury and is characterized by a thick, raised scar that stays within the boundaries of the original injury. Keloids can develop from superficial injuries and exceed the boundaries of the initial injury. Histologically hypertrophic scars contain nodules and keloid does not. The collagen bundles on the surfaces of the nodule are arranged in parallel sheets, while randomly arranged fibrils occur within the centre (466). The collagen bundles of keloid are arranged in braided sheets running parallel to each other.

Hypertrophic scars most often regress over time and can be corrected by surgical intervention (620). Keloid scars as a rule do not regress over time and frequently recur after removal. Treatment of keloid scars is difficult, but pressure dressings and local application of glucocorticoids have been used with limited success. Silicone dressings and local use of calcium channel blockers have recently shown promising results.

Epithelial cells

Different epithelial cells have various features of interest:

- Keratinocytes become exposed to novel ECM molecules in wounds.
- Integrins are signaling molecules that mediate cell adhesion to ECM and cell migration.
- Epithelial keratinocytes need to readjust their cell adhesion receptors to interact with the wound matrix and to modulate cellular signaling.
- Fibronectin receptor integrins regulate multiple aspects of re-epithelialization.
- Several types of cell adhesion molecules in different wound cells jointly contribute to wound healing.
- The EGF family of growth factors promote keratinocyte migration and proliferation.
- TGF-β1 promotes keratinocyte migration but inhibits proliferation.
- Wound-induced electrical fields direct re-epithelialization, but may fail to re-epithelialize chronic wounds (629).

Epithelium covers all surfaces of the body, including the internal surfaces of the gastrointestinal, respiratory and genitourinary tracts. The major function of epithelium is to provide a selective barrier between the body and the environment. The epithelial barrier is the primary defense against threats from the environment and is also a major factor in maintaining internal homeostasis. Physical and chemical injury of the epithelial layer must therefore be repaired quickly by cell proliferation.

After injury to the epidermis, wound protection is provided in two steps: within minutes there is a temporary coverage of the wound by coagulated blood which serves as a barrier to arrest the loss of body fluids. The second step is the movement of adjacent epithelium beneath the clot and over the underlying dermis to complete wound closure. Re-epithelialization of an injured surface is achieved either by movement or growth of epithelial cells over the wound area (467–469). In early phases of wound healing the most important process is cell migration, which is independent of cell division (470–472).

In deep wounds the new epithelial cover arises from the wound periphery, whereas shallow wounds usually heal from residual pilosebaceous or eccrine structures (405, 473–475).

The cellular response of epithelial cells to an injury can be divided into four basic steps: *mobilization* (freeing of cells from their attachment); *migration* (movement of cells); *proliferation* (replacement of cells by mitosis of pre-existing cells); and *differentiation* (restoration of cellular function, e.g. keratinization).

The first response of the epithelium after injury is *mobilization*, which starts after 12–24 hours. This involves detachment of the individual cells in preparation for migration. Epithelial cells lose hemidesmosomal junctions; the tonofilaments withdraw from the cell periphery; and the basal membrane becomes less well defined (15, 467, 476, 477). In addition, the cells of the leading edge become phagocytic, engulfing tissue debris and erythrocytes. Epithelialization occurs most rapidly in superficial wounds where the basal membrane is intact. Short tongues of epithelial cells grow out from the residual epithelial structures. By the 2nd or 3rd day, most of the wound base is covered with a thin epithelial layer; and, by the 4th day, by layered keratinocytes (453, 478, 479).

Migration of epithelial cells occurs as the movement of clusters or sheets of cells. This movement of epithelial cells has been proposed to take place in a 'leapfrog' fashion of epidermal sheet movement (480). Fibronectin in connection with fibrin seems to make a provisional matrix for cellular anchorage and self-propelling traction of the epithelial cell for migration. A speculative mechanism has been that fibronectin is produced in front of the wound edge by epidermal cells, and these then slide over the deposited fibronectin matrix and finally break down this matrix at a distance of some cell diameters behind the wound edge (481, 482). It seems that the motile cells use secreted fibronectin as a temporary basal membrane and use collagenase and plasminogen activators to facilitate passage through reparative connective tissue (483).

The specific signals or stimuli for epithelialization are unknown. However, it is known that the trauma of wounding causes an activation of epithelial keratinocytes by the exposure to pro-migratory matrix molecules, growth factors and cytokines that are released, and leads to wound-generated electrical fields (629).

Migration of epithelial cells takes place in a random fashion; however, orientation of the substrate on which the cells move, as well as the presence of other cells of the same type, are determinants of the extent and direction of cell movement. Furthermore, cell migration appears to be at least in part initiated by a negative feedback mechanism from other epithelial cells in the free edge of the wound (5). Substances in the substrate which are important for direction of the migration seem to be collagen fibers, fibrin and fibronectin, as earlier described. Fibronectin appears to be a substrate for cell movement and to have a binding capacity for epithelial cells as well as monocytes, fibroblasts and endothelial cells (101, 482–484).

Proliferation of epidermal cells starts after 1–2 days in the cells immediately behind the migrating edge, thereby generating a new pool of cells to cover the wound (472, 485). Mitosis in epidermal epithelium has a diurnal rhythm, being greatest during rest and inactivity. In normal epidermis, very few basal cells are in mitosis at any given time. Epidermal wounds, however, result in a change of the diurnal mitotic rhythm in cells adjacent to the wound, resulting in an absolute increase in mitotic activity and an increase in the size of epidermal cells (453). The maximal mitotic activity is found on the 3rd day and continues until epithelialization is complete and epithelial cells have reverted to their normal phenotype and reassumed their intercellular and basement membrane contacts by differentiation (486).

A number of stimuli for epidermal cell growth and thereby wound closure have been indentified, such as calcium in low concentration, interleukin-1, bFGF, EGF, PDGF and TGF-α (467, 482). The only factor known to block epithelial growth is TFG-β (478, 487). Most of these molecules are released from cells within the wound environment such as platelets, inflammatory cells, endothelial cells and smooth muscle cells (Fig. 1.4).

Another factor that influences epithelialization is oxygen tension. Thus a high pO_2 has been found to increase epithelialization (488, 489).

Epithelial repair differs temporally in different types of wound. In incisional wounds, mobilization and migration of epithelial cells is a rapid response compared to other events of the wound healing process. Already after 24–48 hours the epithelial cells have bridged the gap in clean incisional and sutured wounds (142). In small excised and non-sutured wounds which heal by secondary intention the surface is initially covered by a blood clot. Migrating epithelium does not move through the clot, but rather beneath it in direct contact with the original wound bed. Epithelial cells appear to secrete a proteolytic enzyme that dissolves the base of the clot and permits unimpeded cell migration.

In large excised wounds, all stages of epithelial repair may be seen simultaneously. In such wounds epithelialization will not be complete before granulation tissue has developed. Epithelial cells will use this bed for subsequent migration. Depending upon the size of wound, the surface will subsequently be covered by a scar epithelium which is thin, and lacks strong attachment to the underlying dermis as well as lacking Langerhans' cells and melanocytes.

One factor that has a strong influence upon epithelial healing is the depth of the wound. In superficial wounds, the regeneration from hair follicles coincides with epithelialization from the wound edges. In deeper wounds, all epidermal regrowth occurs from the wound edge.

Finally, it should be mentioned that re-epithelialization is significantly enhanced if the wound is kept moist (481).

In the oral cavity the morphological changes seen during epithelialization of the rat molar extraction socket appear to be similar to wounds that involve oral mucosa (74, 490–494). Thus the epithelium migrates down into and across the wound with either fibrin–fibronectin or granulation tissue–fibronectin below it and the superficial wound contents (i.e. neutrophil leukocytes, tissue debris, food elements and bacteria) above it. This layer is subsequently lost in the form of a scab following re-epithelialization (492).

Microenvironment in wounds

Microenvironments in wounds are the sum of the single processes mentioned earlier. Of particular interest for the wound healing process is the influence of the wound microenvironment as an initiator, supporter and terminator of the wound healing processes (495).

Cellular activity in the wound has already been discussed in detail, but can be described as three waves of cells invading the wound area. Apart from their role in hemostasis, platelets serve as the initiators of wound healing by their release of substances such as growth factors (e.g. PDGF, platelet factor IV, IGF-1 and TGF-β) and an uncharacterized chemoattractant of endothelial cells at the moment of injury (Fig. 1.4). As the access of platelets to the wound area is limited by the coagulation process, the supply of these factors is limited.

The second set of cells, polymorphonuclear leukocytes, migrate into the wound after a few hours largely under the direction of complement factors. Their role in the wound healing process appears mainly to be the control of infection.

The third type of cell invading the wound area are the monocytes, which are attracted to the injury site by platelet factors, complement and fibrinopeptides. After entering the wound, these cells are transformed to macrophages and take over the control of healing processes. It would appear that macrophages have the capacity to detect and interpret changes in the wound environment and thereby initiate appropriate healing responses.

In the early wound, cells float around in the tissue fluid of the wound and their function and movement are directed by different growth factors (e.g. TGF-β, IL-6, IGF-1 and insulin produced by platelets and/or macrophages).

The amino acid content of wound fluids reflects to some extent the metabolic events. Amino acid concentrations are initially close to those of serum. Later, they approach those of inflammatory cells. After some time, levels of glutamine and glutamate in particular, rise well over those of serum, whereas arginine concentration falls to low levels due to conversion to ornithin and citrullin (495). Arginine has been shown to be active in influencing the wound healing process and seems to activate macrophages (496, 497).

It would seem that the wound fluid, with its mixture of growth factors, amino acids and other components, is conducive to cell proliferation. Thus, it has been found experimentally that cell growth was optimized in the presence of wound fluid compared to cell growth in serum (327).

The concentration of oxygen in the wound plays a very significant role in activation of the wound healing process and control of wound infection (648–653).

Wound microenvironments have been studied in rabbit ear chambers in which healing tissues can be visualized between closely approximated, optically clear membranes mounted under a microscope (Fig. 1.26). This narrow space, as thin as 50 µm, forces healing cells to travel in coherent order, so that one or two cells pass at a time (49). In this model, the influence of oxygen tension and lactate concentration could be measured by microprobes and has been characterized (49). An oxygen gradient from the central wound space in the chamber to the peripheral normal tissue has been described. While oxygen tension along the edges of the wound is very low, with hypoxia close to 0 mmHg, the oxygen tension at the periphery of the wound is up to 100 mmHg. This oxygen tension gradient seems to be important in the initiation of the wound healing process. Concurrently, lactate concentration in the wound space is 10–20 times that of venous blood, resulting in a fall in pH to 7.25 (498).

Wound healing module

After the first phase of the inflammatory process occurs, a characteristic cell build-up is found after 3–4 days (Fig. 1.26). At the edge of a wound is a vanguard of scavenging cells, the majority of which in the non-infected wound are macrophages. If the wound is infected, these macrophages are accompanied by large numbers of neutrophilic leukocytes.

Beneath the phagocytes is a layer of immature fibroblasts, floating in a gelatinous, non-fibrillar matrix, which are unable to divide because of local hypoxia. Beneath this layer of fibroblasts is a group of dividing fibroblasts which are associated with the most distal perfused arcaded capillaries, and behind the first perfused capillary loops are more dividing fibroblasts which provide cells to form the new tissue.

Distal to this zone, blood vessels increase in size and become less dense, probably as a result of enlargement or coalescence of a few vessels, as the other channels recede from the pattern of vascular flow. Between these vessels lie mature fibroblasts and new fibrillar collagen. This arrangement of

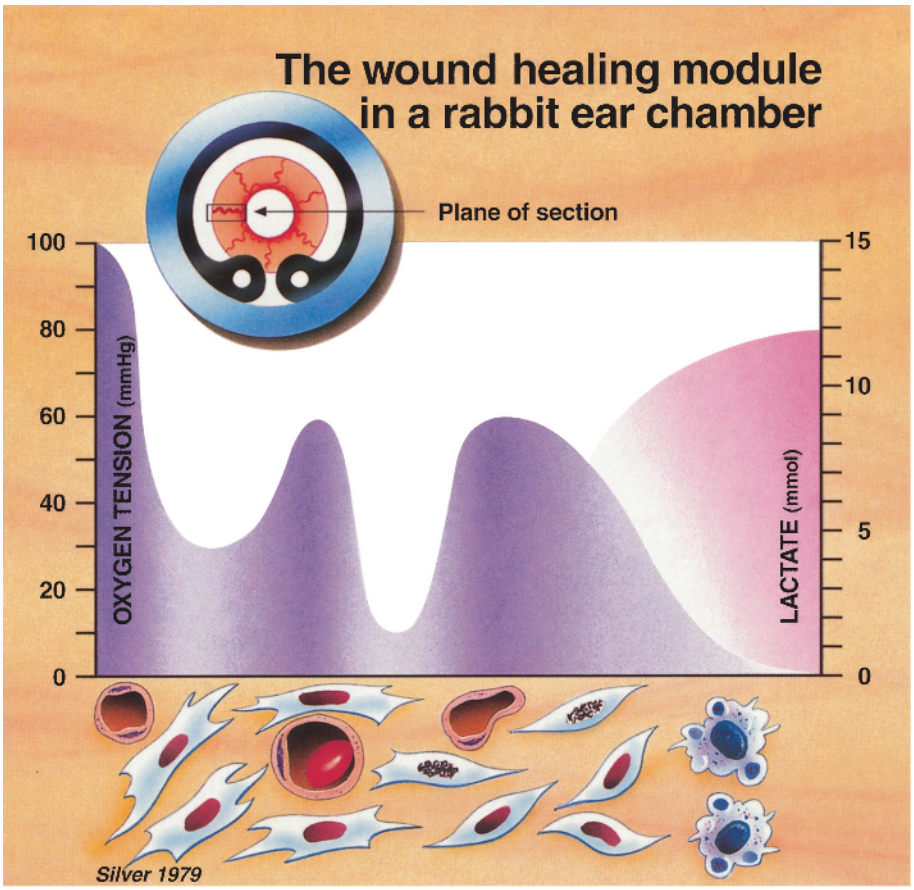

Fig. 1.26 Cellular build up, oxygen tension and lactate concentration in a rabbit ear chamber The center of the wound is to the right on the drawing. At this location where macrophages operate there is very low oxygen tension and a high lactate concentration. It appears that oxygen tension is closely related to the location of the vasculature. Replication of fibroblasts takes place ahead of the regenerating vessels, whereas fibroblasts begin collagen production when the neovascular front reaches the proliferating fibroblasts. From (499).

cells creates an environment that is favorable to angiogenesis and collagen deposition and has been termed the *wound healing module* (Fig. 1.4).

Fibroblasts in mitosis are always found just ahead of the regenerating vessels, where the tissue oxygen tension is optimal for replication (i.e. about 40 mmHg). It is assumed that these fibroblasts behave as growth centers and that new fibroblasts remain at this location until reached by the neovascular zone, whereafter they initiate collagen production (499). The hyperemic neovascular front has a higher pO_2 (Fig. 1.26), which is optimal for collagen synthesis. Thus the fibroblasts replicate and produce collagen in different wound environments.

It has been found that hypoxia or exposure to a high lactate concentration increases the capacity of fibroblasts to synthesize collagen when they are subsequently placed in an oxygenated environment (500, 501). It has been suggested that lactate stimulates collagen synthesis (495). When lactate increases, the construction phase of healing starts by stimulating the transcription and post-translational modification of wound-related genes such as collagen genes, matrix metalloproteins and others (647). This carries healing on to its proliferative phase and thus collagen deposition. The lactate also enhances TNF production by lipopolysaccharide, collagen lysis (remodelling), hif production and angiogenesis (648).

Thus lactate has been shown to induce an increase in procollagen mRNA. Lactate concentration in test wounds is greatest in the central space and persists well into the zone of collagen synthesis (Fig. 1.26). It is therefore suggested that new vessels overtake the immature fibroblasts and change their environment from high lactate and low oxygen tension to high lactate and high oxygen tension and thereby increase both their collagen synthesis and their deposition. When the wound cavity is totally filled with granulation tissue, the hypoxia and high lactate concentration gradually diminish as does macrophage stimulation, whereupon the wound healing process will stop.

Oxygen tension in the wound has been shown to be important for collagen deposition and the development of the tensile strength of the wound (488, 502), for regulation of angiogenesis (369) and for the epithelialization of wounds (472). Oxygen in the wound's extracellular environment also has an important role in the intracellular killing of bacteria by granulocytes (503).

Neutrophil migration, attachment and ingestion of bacteria apparently are independent of oxygen; however, the killing of the most important wound pathogens is achieved by mechanisms that require molecular oxygen (504). These mechanisms are introduced through reducing extracellular molecular oxygen to superoxide which is then inserted into phagosomes. Here the superoxide is converted to high-energy bactericidal oxygen radicals for optimal bacterial killing (505).

Several studies in normal volemic animals have shown that they clear bacteria from wounds in proportion to the fraction of oxygen in the inspired gas. Infection was less invasive or failed to develop in hyperoxygenated guinea pigs when bacteria were injected into skin (505). Furthermore, infected skin lesions in dogs became invasive in tissues with an extracellular fluid oxygen tension under 60 mmHg; however, they remained localized if the tissue was better oxygenated, as in the case of higher inspiratory oxygen concentration (506). Experience in human subjects tends to support these findings, but no trial has yet been performed supporting these observations.

Factors affecting the wound healing process

External factors have an influence on the wound healing process. The categorization is generally divided into local or systematic. In acute surgical wounds a classification based on patient- and surgical-related factors is most convenient. Patient-related factors are associated with the single patient and can alone, or together with other factors, inhibit or prevent wound healing. Surgical-related factors can be separated in pre-, peri- and postoperative influencing factors.

Blood circulation and oxygenation of the wound

Continuous supply of oxygen to the tissue through a sufficient tissue perfusion is vital for the healing process as well as resistance to infection (360, 488, 502, 507–509, 648, 649). Collagen production and development of strength of the wound is directly correlated to the partial pressure pO_2 of the tissue (p_tO_2). Epithelialization is also dependent on oxygen, but the humidity of the wound healing environment seems of more importance. Moist wound healing increases the epithelialization by a factor of 2–3, while the optimal growth of epidermal cells is found at an oxygen concentration of between 10% and 50%.

Anemia with hematocrite values of 15–20 has, in experimental animal studies, in cases of normal function of the heart and normal tissue perfusion, been of minor importance for the pO_2 in the wound area and consequently for the healing. Evaluation of tissue perfusion and oxygenation is important in all types of wound. Monitoring systems should measure the hemodynamic situation and the ability of the cardiovascular system to deliver an adequate volume of oxygen to meet the metabolic demands of the peripheral tissue.

Tissue perfusion is determined by a variety of general and local factors. Peripheral tissue perfusion is influenced by multiple cardiovascular regulatory mechanisms. In response to hemorrhage, these mechanisms maintain blood flow to vital organs, such as the heart and brain, while blood flow to other tissues is decreased (510, 511). Circulatory adjustments are effected by local as well as systemic mechanisms that change the caliber of the arterioles and alter hydrostatic pressure in the capillaries. Detection of poor tissue perfusion, and especially tissue oxygenation, is crucial in the post-injury and care periods.

If wound edges show signs of ischemia, there is a risk of impaired wound healing with the development of wound leakage and infection. From the knowledge of healing wounds

of the oral cavity and the anus, it is obvious that perfusion is the major factor in resistance to infection. Despite contamination of both types of wound with bacteria, they almost always heal without infection if patients have a normally functioning immune system. The difference between these and other wounds (e.g. in the extremities) is not related to local immunity, but to differences in perfusion and oxygenation.

Increased oxygen tension improves resistance to infection through local leukocyte function (512). Thus, experimental data have shown that the killing capacity of granulocytes is normal only to the extent to which molecular oxygen is available (503, 513–519). Bacteria killing involves two major components. The first is degranulation of neutrophils in which bacteria located within the phagosome are exposed to various antimicrobial compounds from the granules. The effect of this system is unrelated to the environment of the leukocytes. The second system is the so-called oxidative killing and this depends upon molecular oxygen absorbed by the leukocytes and converted to high energy radicals such as superoxide, hydroxyl radical, peroxides, aldehydes, hypochlorite and hypoiodite – all substances which to varying degrees are toxic to bacteria. In this regard it is important to consider that the efficacy of oxidative bacteria killing is directly proportional to local oxygen tension (539).

The clinical relevance of blood flow and oxygen supply to healing and infection has been shown experimentally in skin flaps in dogs (32, 392, 395). In flaps with a high perfusion and tissue oxygen tension, no infection was found after injection of bacteria; whereas invasive, necrotizing infections were found in flap areas in which oxygen tension was less than 40 mmHg. When oxygen tension in inspired gas and arterial blood was raised or lowered, the infection rate corresponded to tissue oxygen tension, but not to oxygen-carrying capacity.

With respect to the healing of dental tissues, the oxygen tension may have a significant effect. Thus, several *in vivo* tissue culture studies have shown that low oxygen tension (e.g. a 5% oxygen atmosphere) results in reduced collagen and bone formation. A concentration of 35% O_2 was found to be optimal for collagen and bone formation, while a high oxygen concentration (95%) resulted in depression of collagen and bone formation as well as osteoclastic resorption of bone and cartilage (520–522). This *in vitro* relation between high oxygen concentration and osteoclastic activity may have an *in vivo* counterpart in vanishing bone disease (Gorham's disease or vanishing facial bone) (523, 524) as well as in the internal surface resorption phenomenon seen in revascularization of the pulps of luxated or root-fractured teeth (525). In both instances active local hyperemia and increased oxygen supply may be related to osteoclastic activity.

Among local factors which can control blood supply and tissue perfusion to the injured tissue, tension caused by splints or sutures may seriously jeopardize local circulation. Thus, splinting types exerting pressure on the periodontium may disturb or prevent uneventful PDL or pulp healing and lead to disturbances, such as root resorption, ankylosis and pulp necrosis (see Chapter 2). Furthermore, tension of sutured soft tissue wounds may lead to ischemia with subsequent risk of wound infection (see Chapter 21).

Topical oxygenation

Locally, oxygen has been applied to the wound surface in order to increase regeneration of epithelium, but topical oxygen therapy approaches are not yet widely used in wound management anywhere in the world. While the effect of this treatment has been well documented, there has been scepticism largely because of the absence of randomized controlled trials. However, growing evidence of its effectiveness suggests it has the potential to form a regular part of adjunctive therapies in treatment regimens to speed up healing of acute and chronic wounds (650–652, 694).

Hyperbaric oxygenation

Hyperbaric oxygen (HBO) has been introduced in the treatment of various oral conditions such as problem wounds subsequent to irradiation as well as in cases of grafting procedures where vascularization appears compromised, as well as in other types of chronic wounds (526, 617, 618, 694).

HBO is administered in pressurized tanks where the patients inhale 100% oxygen at a pressure of 2 atmospheres. The interaction of HBO with hypoxic tissue has a range of effects (7). The most significant effect of HBO is that it augments the oxygen gradient within the wound healing site, thus leading to increased fibroblast and endothelial activity (269, 527–530) as well as increased epithelialization (489). HBO may also suppress the growth of certain bacteria (512).

In experimental gingival wounds in rats, it has been found that HBO augments gingival connective tissue healing during the first 2 weeks, whereafter no difference was seen in comparison to wounds healed at normal atmosphere (531). In more extensive wounds in rats, where mandibular ramus osteotomy wounds severed the neurovascular supply in the mandibular canal, it was found that HBO reduced or prevented ischemic damage to pulp cells, ameloblastema and adjacent bone on a short-term basis (i.e. after 10 days). With an observation period of 30 days, HBO was found to stimulate osteodentin and bone formation in the zones of injury (532).

This beneficial effect of HBO on bone healing after injury is supported by a human study where acceleration of bone healing after osteotomy could be demonstrated after the use of hyperbaric oxygen (533, 619). Osteoradionecrosis of the jaws is another condition treated with HBO and the timing of surgical intervention is an important variable with respect to success (647). A standard protocol shown to be effective is 20 preoperative HBO treatments before oral surgery. When HBO is used without surgical intervention it has no lasting benefit (648). The effect of HBO in the treatment of diabetic foot lesions is, however, still debated. In a longitudinal observational cohort study Margolis et al. (654) it was concluded that the usefulness of HBO in the treatment of

diabetic foot ulcers needs to be better clarified, preferably using well-designed randomized controlled trials and perhaps using other healing-based end points other than a healed wound.

Smoking and alcohol

Smoking influences the healing process by different mechanisms. Nicotine is quickly absorbed and starts a release of catecolamines resulting in a peripheral vascular constriction followed by a decrease in perfusion rate of 42% (534). Furthermore the CO in the cigarette smoke will reduce the oxygen content of the blood. These combined effects have been shown to decrease the tissue perfusion by more than 30% in more than 45 minutes in specific areas of the body (535). In such areas the production of collagen is 1.8 times higher in non-smokers compared to smokers (536). Leukocytes in smokers have also shown a decreased ability to kill bacteria resulting in a higher risk of wound infections in smokers. In surgical patients an increased risk of necrosis of the wound edge, diminished cosmetic result, increased risk of anastomic leakage after bowel surgery, and increased recurrence rate after hernia surgery have been described (49).

A study found that healthy smokers have a higher incidence of wound infections and wound ruptures than never-smokers, and 4 weeks of abstinence from smoking reduces wound infections to a level similar to that found in never-smokers (537). Smoking has to be considered within a pathophysiologic model as delaying healing and increaseing wound infection rate. Biologic markers of acute wound healing affected by smoking may appear to be valuable predictors for adverse wound events after surgery (650, 651, 655).

Alcohol has also been shown to increase the risk of postoperative infection, bleeding, exudation and wound/anastomoses rupture. The specific influence on the wound healing process is not known, but alcohol consumption decreases total protein but not collagen in artificial wounds. These changes are reversible after stopping alcohol intake (538).

Infection

Infection is the most common complication that can disturb wound healing (342). Development of infection is determined by the number and type of contaminating organisms, host defense capability and local environment (540, 614).

When bacteria invade a wound, the final outcome of this event is related to the success of the initial phase of the inflammatory response in establishing an antibacterial defense (Fig. 1.27). Timing is critical and the fate of the contaminating bacteria appears to be determined within the first 3–4 hours after injury. During this period, the early inflammatory process is established and will usually result in the elimination of bacteria (539, 541, 542).

The risk of infection appears to be directly related to the number of bacteria initially present in the wound (540). If the bacteria are not eliminated during these first critical hours, a series of events will occur that affect wound healing. Thus the formation of fibroblasts will be disturbed in several ways and fibroblast proliferation is generally inhibited; but stimulation may occur in certain circumstances (541–547). Liberation of toxins, enzymes and waste products from bacteria decrease or inhibit collagen synthesis (548) and increase the synthesis of collagenase, resulting in lysis of collagen (549). Furthermore, some bacteria decrease the amount of oxygen available in the infected tissue (550, 551) whereby healing processes suffer. Collagen formation is reduced, cell migration is delayed or arrested, and cellular necrosis and microvascular thrombosis may result (552). Wounds have been classified as:

- *Clean wounds*, which are uninfected operative wounds in which no inflammation is encountered.
- *Potentially contaminated wounds*, in which the respiratory, alimentary or genitourinary tracts are entered under controlled conditions during surgery.
- *Contaminated wounds* where acute inflammation (without pus) is encountered or where there is a gross spillage from a hollow viscus during surgery.
- *Dirty wounds and infected wounds*, which are old traumatic wounds and operating wounds in the presence of pus or those involving clinical infection of perforated viscera (552).

The level of aerobic and anaerobic contamination expressed as bacteria in the wound (i.e. colony forming units per unit area) is for clean wounds 2.2, for potentially contaminated wounds 2.4×10^1, for contaminated wounds 1.1×10^3, and for dirty wounds 3.7×10^3 (553).

Oral wounds are associated with a high risk of contamination as saliva contains 10^{8-9} bacteria per milliliter (554).

The infective dose of bacteria that results in a microscopic infection has been found to be 10^5 bacteria per gram of tissue (555). A correlation has been found in different types of wounds between preclosure bacterial density of aerobic and anaerobic bacteria and postsurgical wound infections (553). It is recognized that both aerobic and anaerobic organisms are implicated in most wound infections.

The use of antibiotics to supplement the natural host resistance (i.e. the early inflammatory response) has been found to be additive and sometimes even synergistic in bacteria killing. However, if antibiotics are given with more than a 3-hour delay, animal experiments have shown that the effect of antibiotics such as penicillin, erythromycin, chloramphenicol and tetracyclin was eliminated (556). The timing of antibiotics therefore seems to be of utmost importance.

Many factors are described as contributing to impaired wound healing as well as increased risk of infection. Some of these factors may have a direct influence on the healing process while other factors have an indirect influence by changing circulation and thereby oxygenation.

Foreign bodies

The presence of foreign bodies can contribute to delayed healing, but is normally not by itself sufficient to prevent healing. Foreign bodies provide a focus for bacterial growth, and consequently a smaller amount of bacteria is needed to

cause infection in the wound area. More than 50 years ago it was observed that just a single silk suture present in the wound area increased the susceptibility to bacteria (*Staphylococcus*) by a factor of × 10 000 (557, 558). Other types of foreign materials, such as soil, clothing and drains have also been shown to increase the risk of postoperative infection and impaired wound healing (559–561). Recently, it has been found that bacteria may be camouflaged on artificial surfaces by producing an extracellular carbohydrate film (562). This film seriously affects the host response by inhibiting chemotaxis, bacterial engulfment and the oxidation response of the phagocytes.

Foreign bodies in oral and other soft tissue wounds consist mainly of soil and its contaminants (563), but tooth fragments can also be found. Soil has four major components: inorganic minerals, organic matter, water and air. The coarser components of soil are stone, gravel and sand. The smallest inorganic particle found in soil is clay. Not only does soil carry bacterial contamination into the wound, but the mere presence of inorganic and organic particles has been shown to lead to impairment of leukocyte ability to ingest and kill bacteria (559). Therefore very few bacteria are able to elicit purulent infections in the presence of foreign bodies. As there is no way of neutralizing the effect of soil, therapeutic efforts should be directed towards removing it from the wound area (see Chapter 24).

In traumatic wounds, foreign bodies can usually be removed, improving wound healing and decreasing the risk of infection. In surgical wounds, however, this may not always be the case. The most common foreign bodies in surgical wounds are sutures, drains and biological materials such as hematomas. A article emphasized that the knowledge of the foreign body reaction in tissue continuously needs to be re-established in the health care system especially in areas where implantation of foreign material is used (657).

Sutures

The ideal suture can be described as free of infection, non-irritating to tissues, achieving its purpose and disappearing when the work is finished (435, 563). Such ideal sutures are still not available; but by choosing the best material, the complication rate provided by the suture material itself can be decreased. Bulky and braided suture materials are generally more likely to cause trouble than fine monofilament sutures (564, 565) (see Chapter 24).

In non-infected multifilamentous sutures, fibroblasts and giant cells appear early and the suture strands remain tightly bound in comparison to infected sutures where bacteria are entrapped within the braids, leading to pus formation (566).

The reaction around a monofilament suture is minimal and a fibrous capsule appears after 10 days, even in the presence of infection. Apart from the knots, there is no space for bacteria to lodge (559). The ideal suture is therefore a monofilament type of suture with sufficient strength to hold the wound edges together until significant healing has occurred, even in delayed healing. The use of absorbable and non-absorbable sutures in relation to wound healing and infection is still controversial (564–569). The use of sutures in soft tissue wounds is further discussed in Chapter 21.

Distant wound response

For decades it has been known that a wound preceded by a previous injury heals faster than a primary wound. Thus from a mechanical point of view (wound strength), a second wound heals faster than the first (570). The explanation for this phenomenon is still uncertain. Another distant wound response is found when two wounds occur simultaneously in distant parts of the body. In these cases impaired blood circulation may be found in the wounds leading to impaired healing (571).

Age

Fetal wound healing

Healing of experimental oral wounds in a mammalian fetus differs greatly from the healing of similar wounds in adults (572–576). Thus accelerated healing without scarring is found in fetal wounds, even in defect wounds. The wound response appears to be without acute inflammation and with minimal fibroblast and endothelial cell proliferation (577). Furthermore the ECM appears collagen poor, and rich in hyaluronic acid.

Hyaluronic acid is laid down early in the matrix of both fetal and adult wounds, but sustained deposition of hyaluronic acid is unique to fetal wound healing (578). Hyaluronic acid is presently thought to play a decisive role in the regenerative process, as it provides a permissive environment for cell proliferation and mobility (572–579, 658, 659) and suppresses macrophage-effected postnatal repair (579). It has been shown that the elimination of TGF-β from healing wounds in adult rats reversed the typical fibrous scar formation to a stage of fetal wound healing without scar tissue (580). This implies that the control of selected cytokines may be a future approach to control scarring.

It is known that unique properties of fetal cells, ECM, cytokine profile and gene expression contribute to this scarless repair. However, the precise mechanism of fetal regeneration remains unclear with a number of differences identified between fetal and adult wound healing (651, 653). A number of potential antiscarring therapeutics have evolved from understanding fetal regeneration though to date none have completely prevented scar formation.

Adult wound healing

The relationship between age and healing of skin wounds in respect to speed of healing has been studied experimentally in rats (581–588), rabbits (589, 590) and in humans (591, 592). Experiments examining the role of the age factor upon wounding of oral mucosa and gingiva have been performed in rats (593–597), mice (598) and humans (599–601). In the most comprehensive studies using experimental skin wounds in rats and gingival wounds in humans, it was found that healing of gingival defects is slower and regeneration is more incomplete in old than in young individuals. Furthermore,

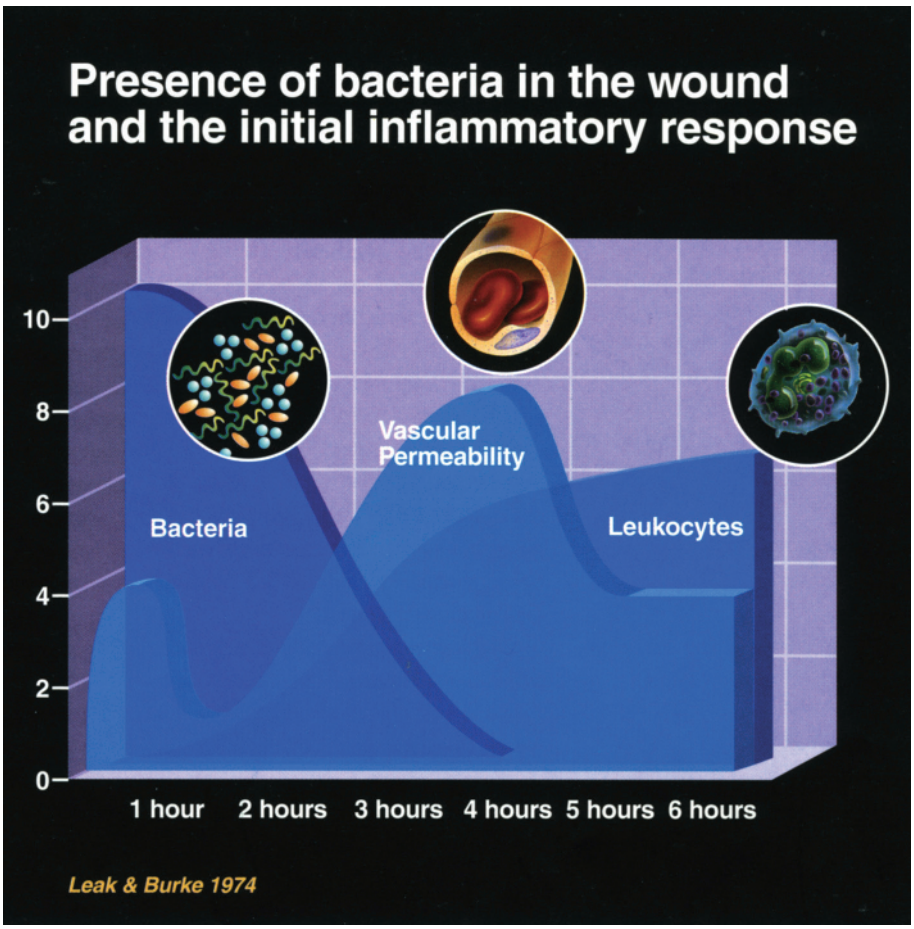

Fig. 1.27 Bacteria and the initial inflammatory response. The period of active tissue antibacterial activity (the decisive period) relates well to the establishing of the inflammatory response, as reflected by increased vascular permeability and leukocyte migration into the wound site. From (539).

wound strength develops more slowly in old rats than in young rats, a finding which was related to the better functional arrangement of collagen fibers in the young animals (602). In the gastrointestinal tract, however, aging seems not to have an adverse influence on wound healing (603). Surveys of the current literature conclude that wound healing in the skin of healthy elderly people is delayed, but scar maturation is improved in comparison with young individuals. Impaired wound healing leading to scar formation is primarily associated with comorbidities, which are more prevalent in old age (615, 616). Nevertheless, age (>60 years) is an independent risk factor for less frequent closure of wounds (661).

Wound infection is also strongly related to age (604, 605). Thus, in a prospective study on wound infection subsequent to surgical wounds, a wound infection rate of 0.6% was found in children aged 1–14 years and this rate rose to a maximum of 3.8% in patients over 66 years of age (604).

Optimizing oral wound healing

An important principle to consider in this context is that the mode of action of both normal wound healing and the response to infection seems to follow a general pattern which is sometimes in conflict with the regeneration of injured organs. This is apparent in skin wounds where the need for rapid wound closure (in order to prevent infection from invading microorganisms) usually results in the formation of a scar (613). In the dental organ, an effective response against bacteria takes priority with activation of the neutrophils, lymphocytes, macrophages and osteoclasts leading to frequent bone and tooth loss due to hard tissue resorption (see Chapter 2).

Presently, the most likely avenue whereby healing problems can be avoided appears to be careful tissue handling whereby tissue perfusion is re-established or stabilized and wound contaminants (e.g. foreign bodies and microbes) are reduced or eliminated.

To achieve this goal, various steps are necessary in the diffent phases of wound healing. In the coagulation phase, assistance in achieving hemostasis may be necessary. In performing this, it is important not to use excessive cautery, which results in tissue necrosis, or topical hemostatic agents (e.g. Surgicel®, Oxycel®, Gelfoam®) that may have a potentiating effect for infection (559). Instead, firm pressure exerted with a gauze sponge for several minutes usually results in hemostasis.

In handling oral wounds, a local anesthetic is usually necessary. In this regard it should be borne in mind that the vasoconstriction of the anesthetic solution increases the risk of infection of the wound due to interference with the inflammatory response in the critical first hours after injury (559, 606). Regional block anesthesia rather than local infiltration of the anesthetic solution is therefore to be recommended.

Wound debridement should be limited to the removal of foreign bodies and obviously damaged tissue that is not expected to survive or become revascularized (see Chapter 24).

The elimination and/or reduction in size of the blood clot should be attempted in order to facilitate wound healing, including revascularization. This applies to soft tissues as well as tooth and bone repositioning.

The value of complete immobilization of the wound edges is presently under debate so only a few treatment principles can be suggested.

In soft tissue wounds any sutures used to immobilize the wound edges must be regarded as foreign bodies which increases the risk of infection (276, 559, 652). Thus a minimal number of sutures should be used, and a suture type should be chosen which elicits minimal side effects (see Chapter 24).

In regard to hard tissue healing, splinting should generally be performed. These splints should not augment the risk of infection, whereby the application and the design of the splints becomes crucial (see Chapter 39).

The value of antibiotics in oral wound healing is presently unsettled (see Chapters 3 and 24). If indicated, antibiotics should be administered as early as possible and preferably not later than the first 3–4 hours after trauma and should only be maintained for a short period of time (540) (see Chapters 3, 21 and 24). Acceleration of oral wound healing by the use of growth factors is in its initial stages but has the potential to be an essential part of trauma treatment (see Chapter 2). A fascinating perspective of the use of growth factors is the achievement of an orchestrated healing response whereby certain parts of the cellular response are promoted (e.g. angiogenesis, fibrillogenesis, dentinogenesis, osteogenesis and epithelialization). The initial attempts at such an approach to oral wound healing appear very promising (see Chapter 24).

Essentials

Regeneration is a process whereby the original architecture and function of disrupted or lost tissue is completely restored.

Repair is a process whereby the continuity of disrupted or lost tissue is restored by new tissue, which does not reproduce the original structure and function.

General steps in wound healing

- Control of bleeding by the combined action of vasoconstriction and coagulation
- Inflammatory response, whereby leukocytes migrate into the wound in order to protect the area against infection and perform cleansing of the wound site
- Connective and epithelial tissue migration and proliferation, which obturate the wound defect and add mechanical strength to the wound
- Reorganization of the tissue by a remodeling process that results in more functionally oriented collagen fibers which increase the strength of the wound

Main roles of the individual tissue cells

- *Platelets*, apart from their role in initial hemostasis and their activation of the coagulation cascade, serve as initiators of the wound healing process
- *Polymorphonuclear leukocytes* prevent bacterial infection within the wound site
- *Macrophages* are scavengers of tissue remnants and foreign bodies including bacteria and are the key cells in coordinating the cellular events in wound healing
- *Fibroblasts* produce collagen and ground substance which fills out the wound defect and adds mechanical strength to the wound
- *Endothelial cells* in the venules are the key cells in angiogenesis. By coordinated endothelial cell proliferation and migration, a new vascular network is formed at the wound site
- *Pericytes* represent a pool of undifferentiated mesenchymal cells
- *Epithelial cells* close the gap against the external environment by cell migration and proliferation

The coordinated action of the cells mentioned here is found in the *wound healing module* created a few days after injury where leading macrophages clear damaged tissue, foreign bodies and bacteria with trailing fibroblasts and newly formed capillaries.

Significant stimuli for the invasive growth of newly formed connective tissue and also termination of the wound healing module appear to be *growth factors* as well as *oxygen tension* and *lactate concentration* in the injury zone ahead of the wound healing module.

Of the many factors known to disturb wound healing, the following are the most likely candidates affecting mucosa and skin, as well as the periodontium and the pulp of traumatized teeth:

- *Low oxygen* delivery to the wound site due to the initial trauma and/or improper tissue handling technique (e.g. suturing, splinting or lack of repositioning of tissues)
- *Infection* due to contamination of the injury site
- *Foreign bodies* including inappropriate use of sutures and drains

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