Textbook and Color Atlas of

Traumatic Injuries to the Teeth 5th edition

Edited by
Jens O. Andreasen
Frances M. Andreasen
Lars Andersson

WILEY Blackwell

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Fifth Edition

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Preface

It is now more than 50 years since the first edition of *Traumatic Injuries of the Teeth* was published. It has served clinicians in providing evidence-based treatment and also provided a complete overview of the scientific literature on traumatic dental injuries. The fourth edition came out in 2007 and this new, fifth, edition contains new information on the role of stem cells. This is presented together with a chapter updating us on research behind the creation of bio-roots attached by a functional periodontal ligament.

Traumatic dental injuries are very frequent and there are now data from almost every country in the world to support this. To reach a higher level of evidence we must, in the future, standardize data collection to enable new meta-analyses. For this reason we have expanded the chapter on classification and epidemiology, with standardized registration of important outcome variables according to the core outcome set as defined by the International Association of Dental Traumatology.

New data on root fractures and alveolar bone fractures are also included. The chapters on endodontics have been expanded and now also include procedures aimed at regenerative endodontics. In line with this, much emphasis has been placed on pulpal healing responses in the chapters dealing with tooth luxation and horizontal root fractures.

New chapters include those on reinforcing endodontically treated teeth and restoration using porcelain veneers. A special chapter is now devoted to the management of dentoalveolar ankylosis where emphasis is placed on preserving the alveolar bone rather than saving the tooth, which is especially important in the young patient who is still growing. The new interactive program Dental Trauma Guide is based on a database from Copenhagen. It illustrates the principles of diagnosis and treatment of traumatic dental injuries, and is presented in a special chapter. Finally, there is a new chapter presenting the International Association of Dental Traumatology.

We hope this new edition will continue to serve clinicians and researchers in this field in their efforts to provide optimal treatment for the benefit of victims of dental trauma.

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Wound Healing Subsequent to Injury

F. Gottrup & J. O. Andreasen

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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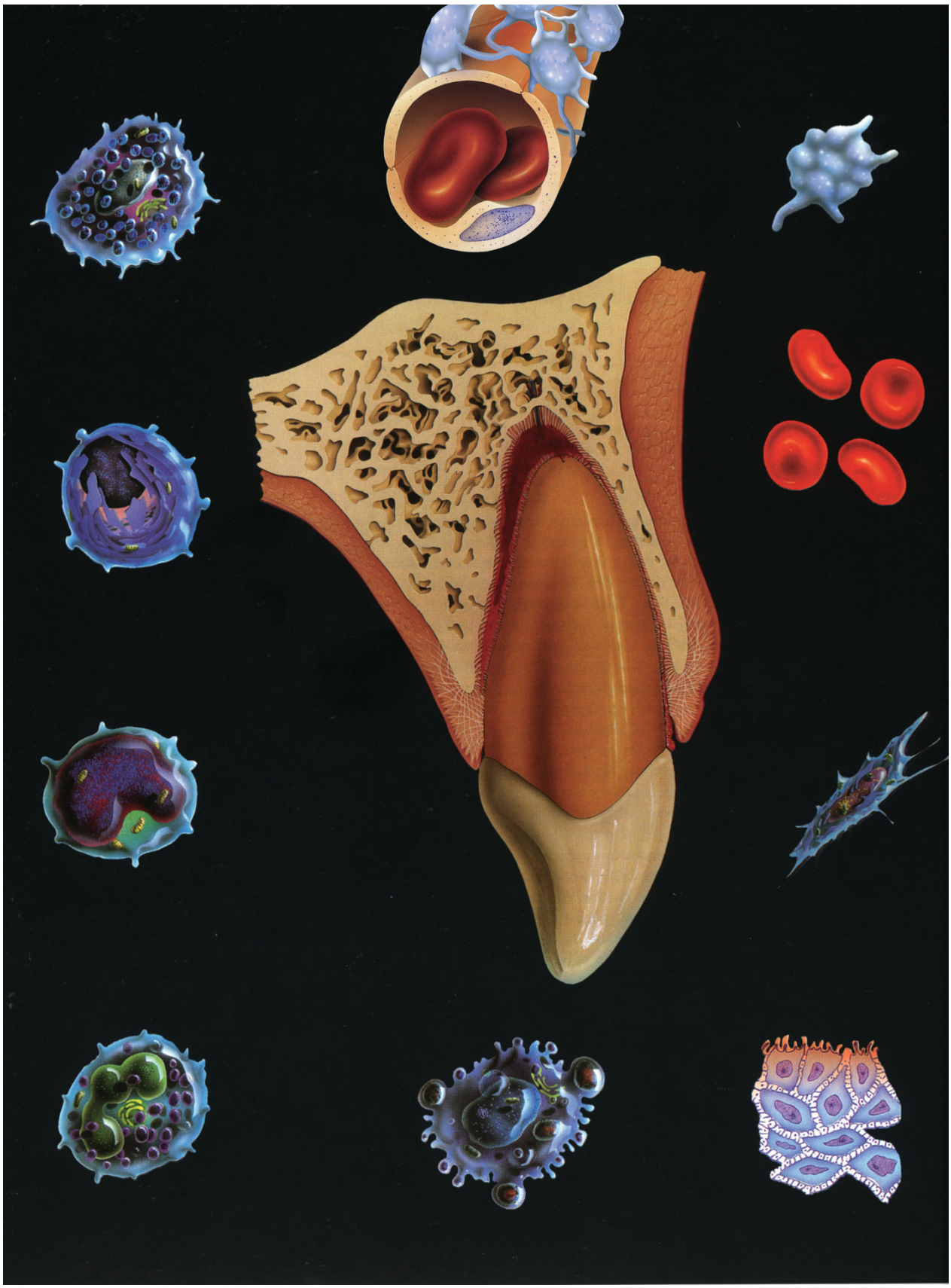

Fig. 1.1 Cells involved in the healing event after a tooth luxation. Clockwise from top: endothelial cell and pericytes; thrombocyte (platelet); erythrocyte; fibroblast; epithelial cell; macrophage; neutrophil; lymphocytes; mast cell.

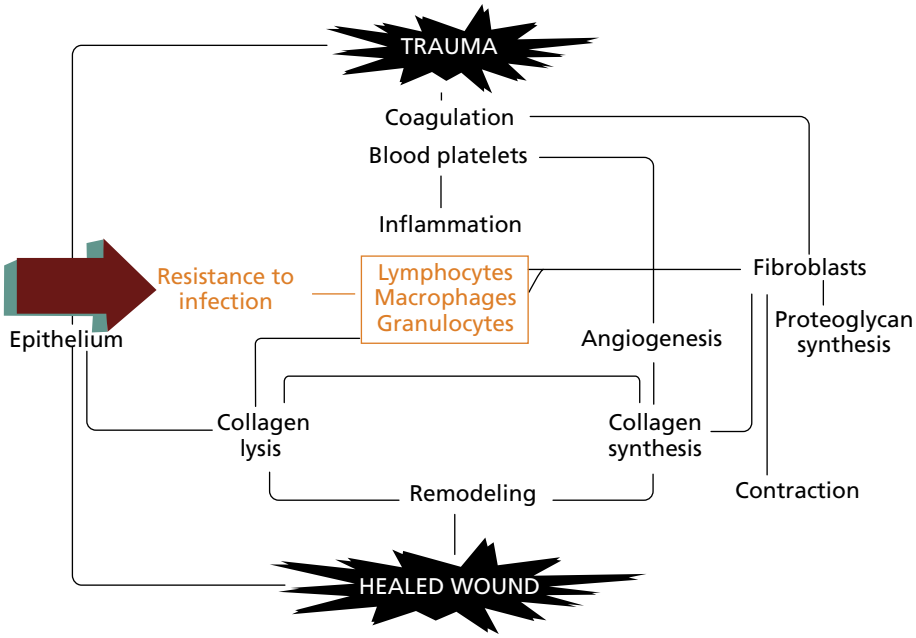

Fig. 1.2 Modified Hunt flow diagram for wound healing.

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

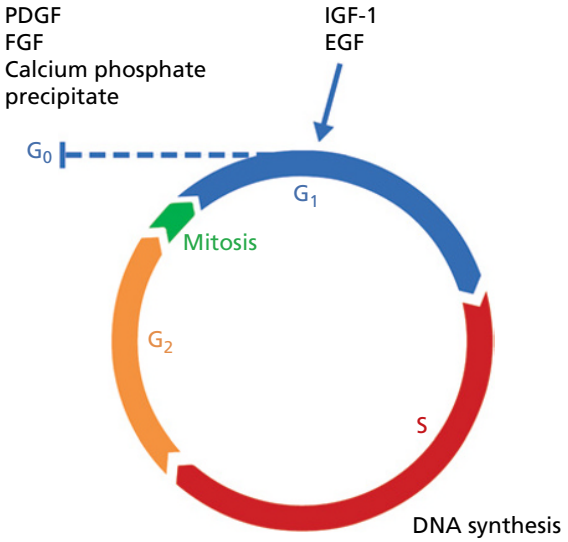

Fig. 1.3 Cell cycle: G_0, resting phase; G_1, time before onset of DNA synthesis; S, replication of DNA; G_2, time between DNA replication and mitosis.

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,

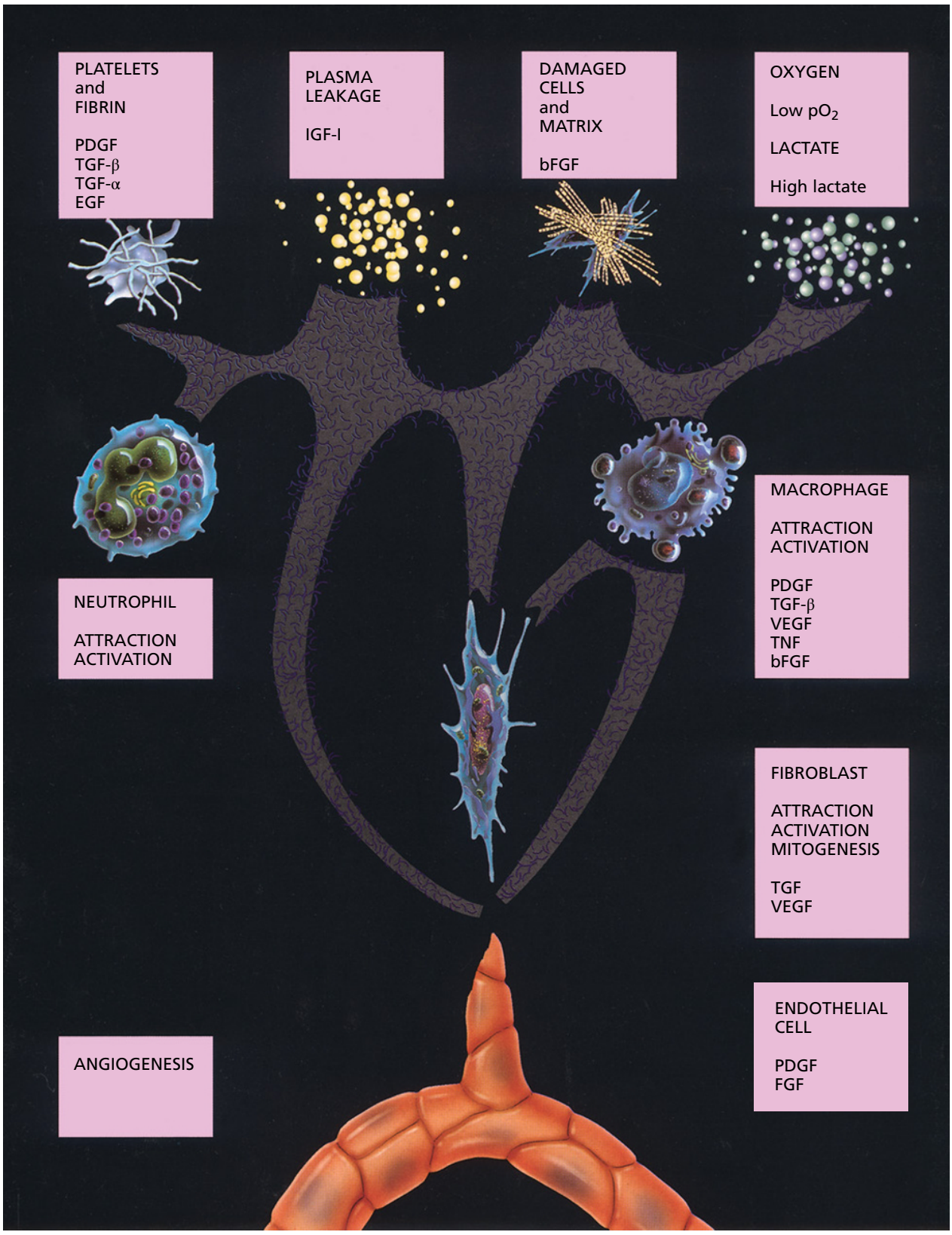

Fig. 1.4 Cellular components and mediators in the wound healing module. Signals for wound healing are released by platelets, fibrin, plasma leakage, damaged cells and matrix. Furthermore low oxygen tension and a high lactate concentration in the injury site contribute an important stimulus for healing.

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

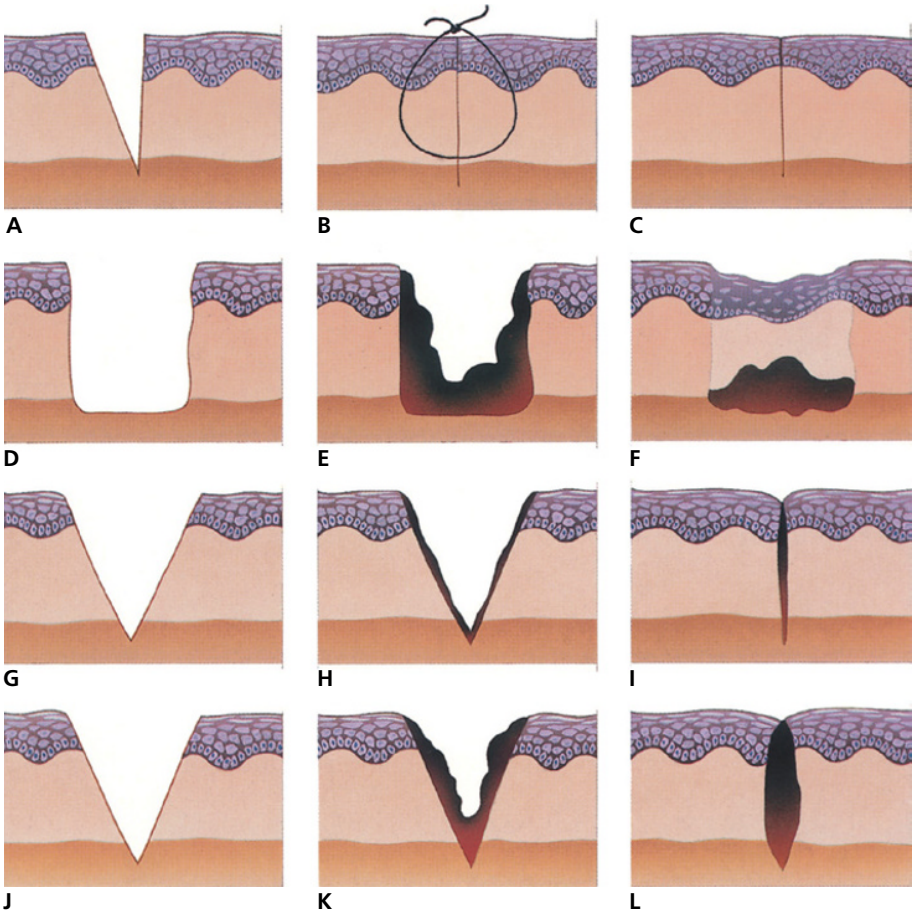

Fig. 1.5 Wound healing events related to the type of wound and subsequent treatment. A–C. Incisional wound with primary closure. D–F. Open and non-sutured wound. G–I. Delayed primary closure. J–L. Secondary closure. From (56).

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

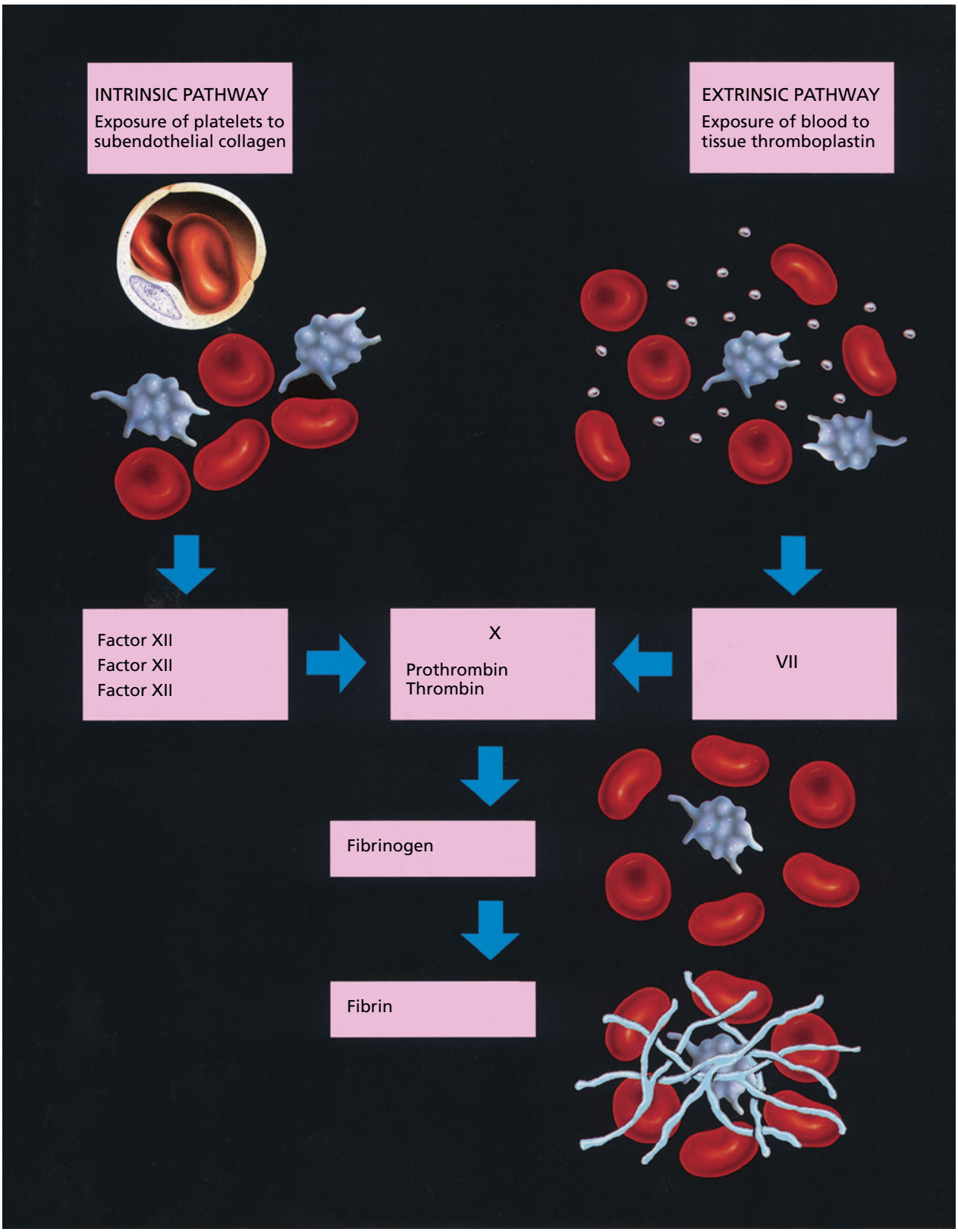

Fig. 1.6 Extrinsic and intrinsic coagulation cascade.

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).

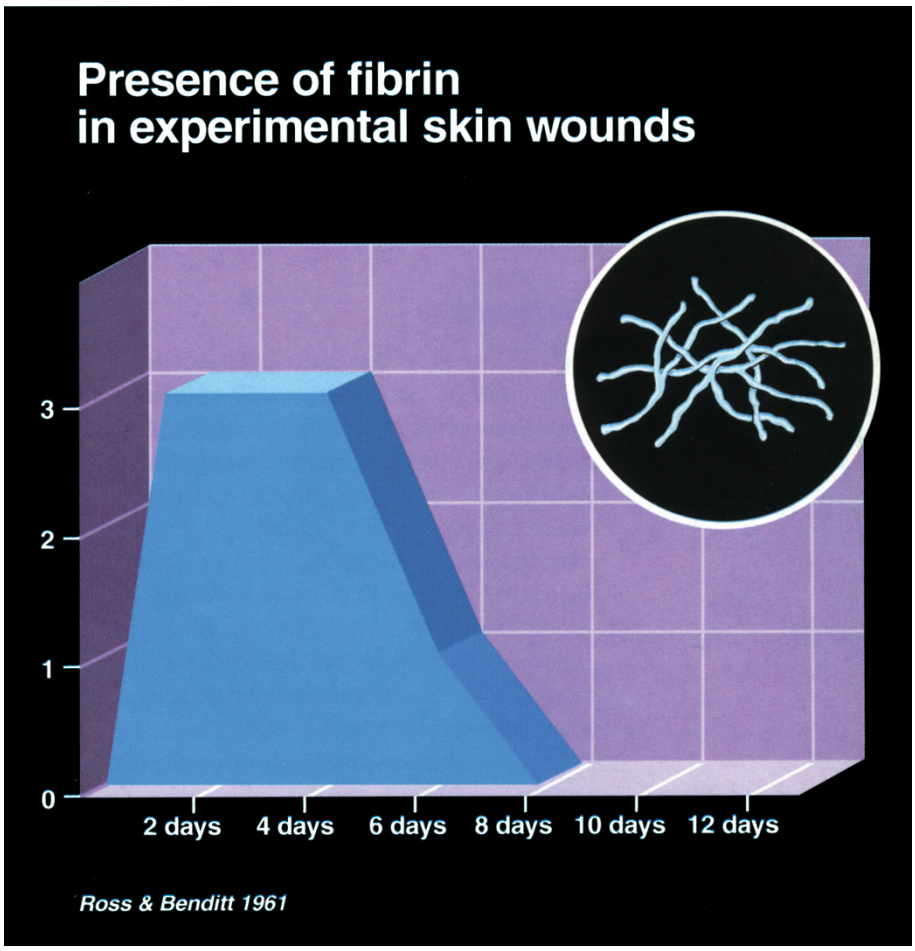

Fig. 1.7 Schematic illustration of the presence of fibrin in experimental skin wounds in guinea pigs. The scale is semiquantitative, graded from 0 to 3. Adapted from (73).

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

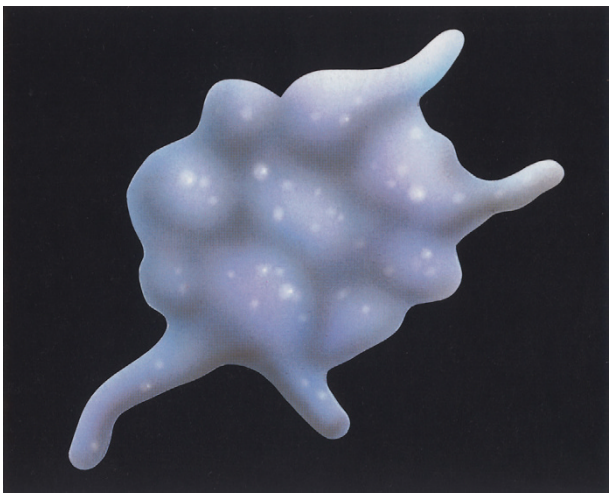

Fig. 1.8 Activated platelet (thrombocyte).

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

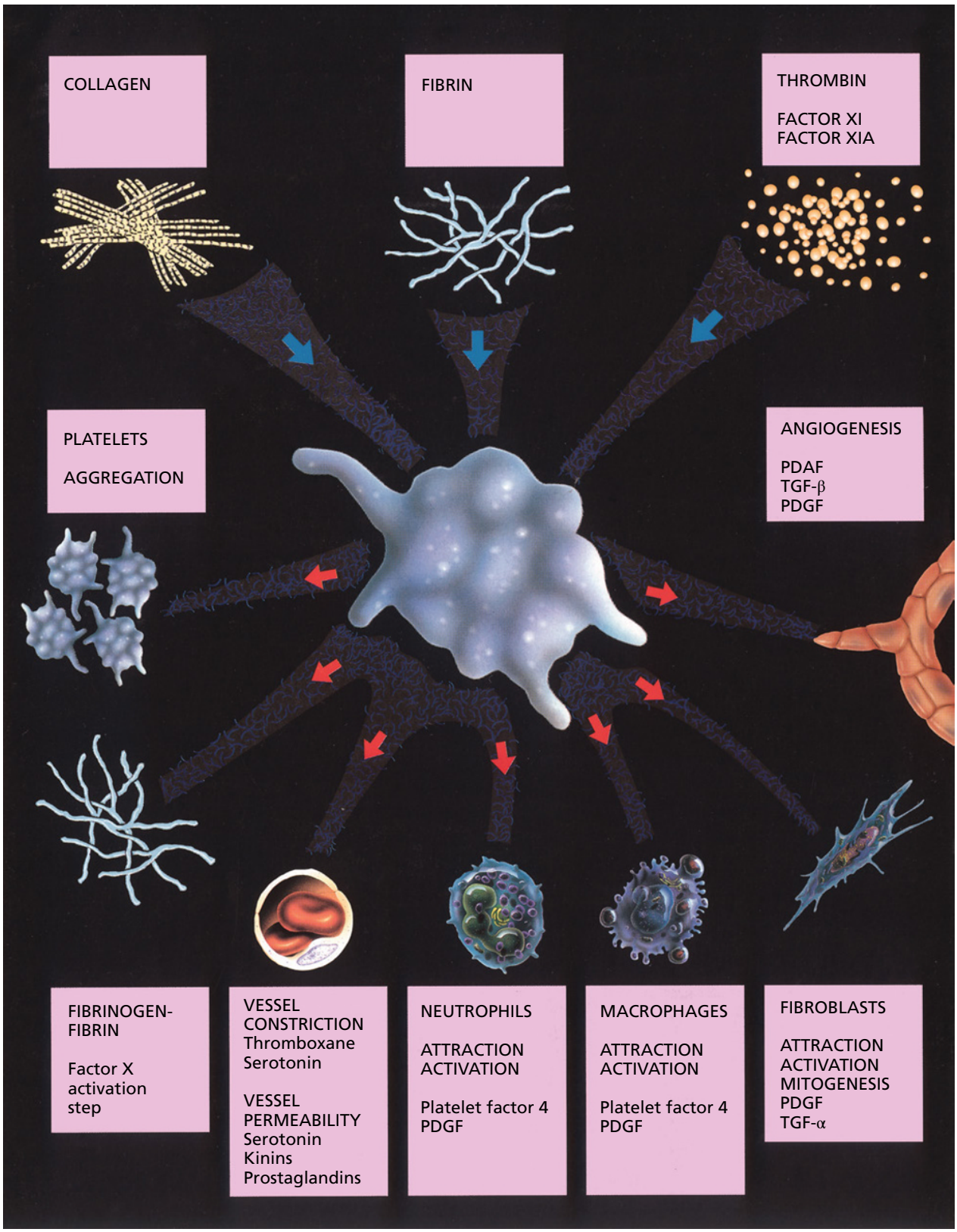

Fig. 1.9 Role of platelets in wound healing. Exposure of platelets to collagen, fibrin, thrombin, factor XI and factor XI-A results in activation and degranulation. This then results in the release of a series of mediators influencing coagulation, vessel tone and permeability. Furthermore the initial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

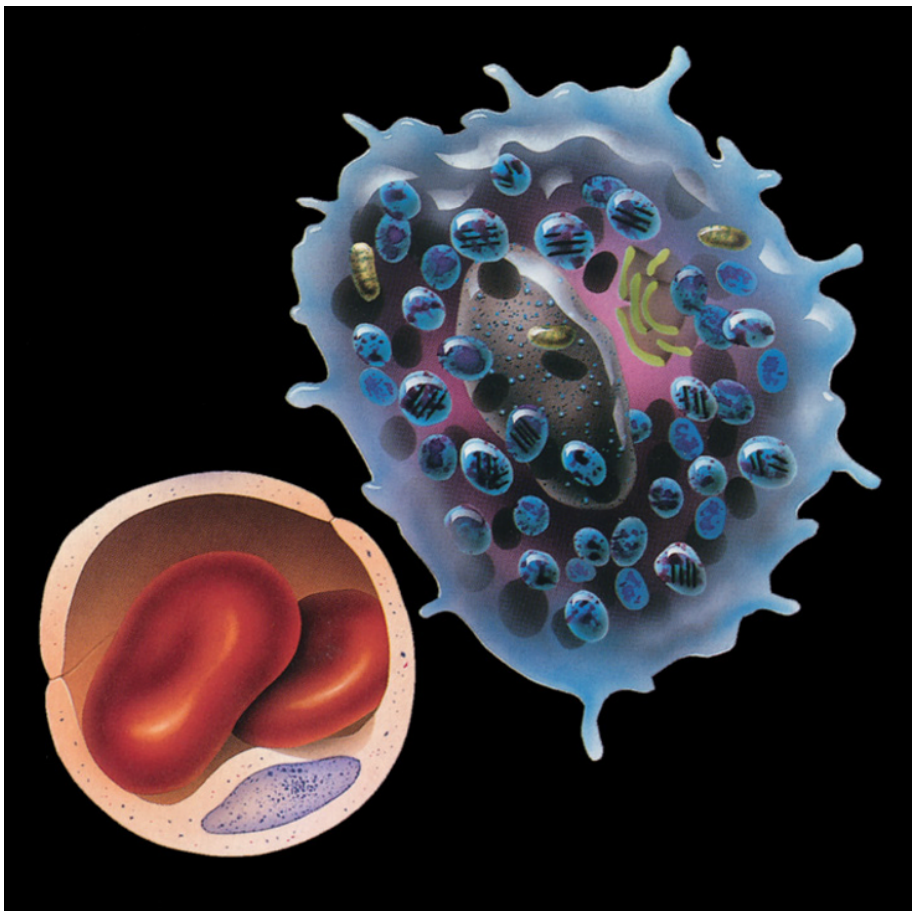

Fig. 1.10 Mast cell in perivenular position.

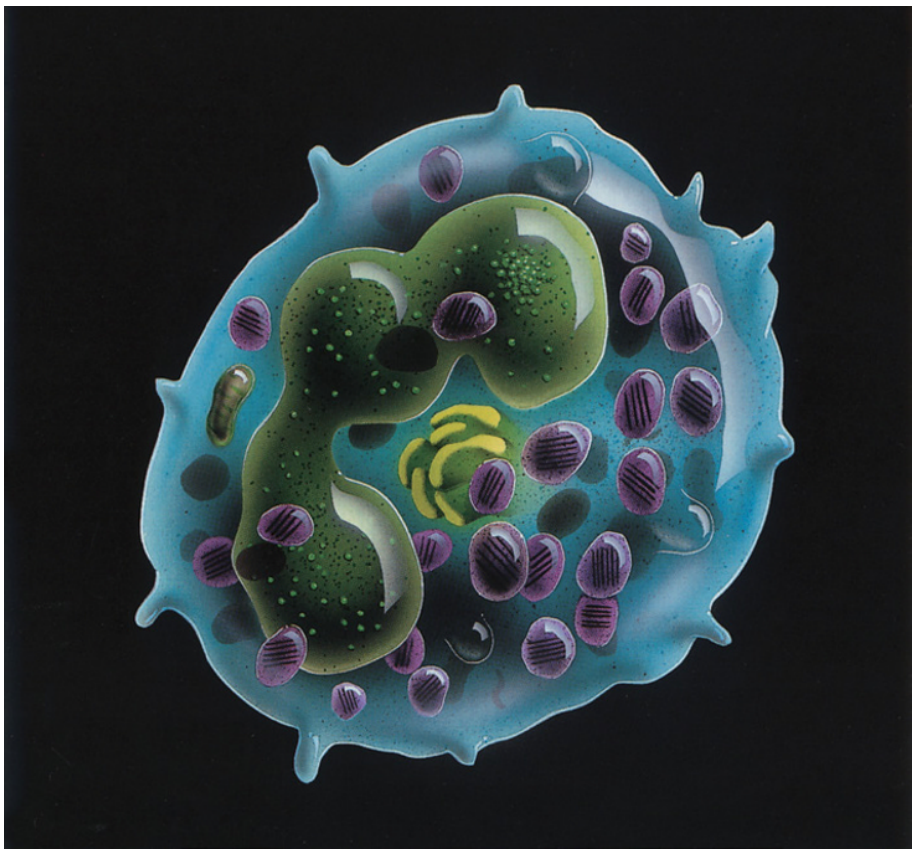

Fig. 1.11 Neutrophil leukocyte.