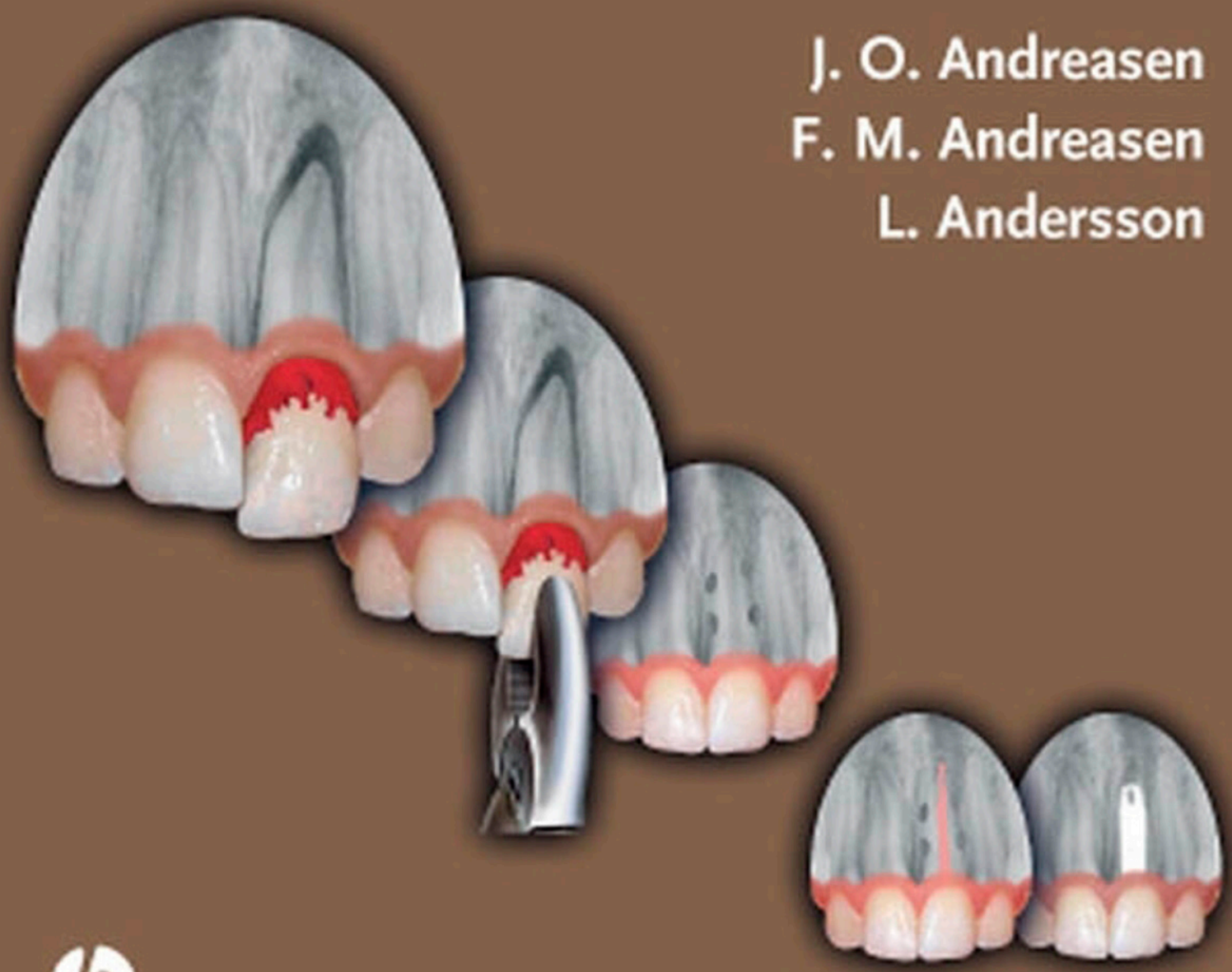
Textbook and Color Atlas of

Traumatic Injuries to the Teeth

4th edition

J. O. Andreasen
F. M. Andreasen
L. Andersson



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Fourth Edition

Edited by
J. O. Andreasen
F. M. Andreasen
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Preface

More than thirty years has elapsed since the publication of the first edition of this textbook in 1972. At that time, table clinics were being held, where the theme of treatment was to extract traumatized teeth at the time of injury, and then the problem was solved. Since then, the biology of acute dental trauma has been elucidated through clinical and experimental research and in subsequent editions of this book used as the guiding light in defining treatment strategy.

A disturbing finding from several recent studies is that acute treatment of dental injuries can sometimes lead to inferior healing. This naturally leads to a rethinking of strategy for treatment of the injured patient. Until now, accepted treatment has been to reposition traumatically displaced teeth or bone fragments into an anatomically correct position. However, this procedure itself may further damage already traumatized tissues. This might explain the negative effect of many forceful reductions, as after luxation injuries, particularly upon periodontal healing. Likewise, the idea that an exposed pulp is a diseased and infected pulp which requires immediate or delayed extirpation has not been substantiated in real life. On the contrary, given the right healing conditions, exposed pulp is a survivor. Similarly, root fractured incisors are often removed due to a lack of understanding of the healing capacity of the pulp and periodontium and their respective roles in the healing process.

Previously, acute dental trauma was considered an event encompassing certain treatment problems that could be adequately resolved by proper endodontic, surgical or orthodontic intervention. However, recent new research has altered this view. Now we know that most healing complications following trauma are related to pre-injury or injury factors, and that treatment should be very specific and restricted in order to optimize healing. This edition is devoted to a biologic approach in understanding the nature of trauma and subsequent healing events and how these events can be assisted by treatment interventions.

The study and understanding of healing in hard and soft tissues after trauma is probably one of the most serious challenges facing the dental profession. That this task presently rests with only a handful of researchers is out of proportion with the fact that perhaps half of the world's population today has suffered oral or dental trauma – a paradox that dental trauma is dentistry's stepchild.

In the decade that has elapsed since the third edition of this textbook, the impact of dental implants has been felt. In the wake of esthetically and functionally successful implant therapy, there is a growing tendency towards the approach of: 'If in doubt, take it out' and replace with a dental implant. This mind-set has seriously colored many professionals' perception of conservative therapy, be it active observation or interceptive endodontic therapy. Moreover, it has led to an explosion in the cost of treatment following dental trauma. For the sake of completeness, the chapter on dental implants has therefore been expanded with respect to primary biologic principles, with particular emphasis on problems related to the use of implants following dental trauma. In this regard, it should be borne in mind that trauma patients are most often young patients in whom the placement of an implant is contraindicated because it interferes with growth and development of the jaw. Furthermore, the fact that many families in the world today may live on a few dollars a day brings the cost–benefit aspect of treatment into focus. In such a world, sophisticated treatment modalities that are now available may only be realistic for very few. And then what? This reality is also described.

The enormous impact of a traumatic event on the mental health of the patient has long been ignored. In some situations, the loss of a tooth or parts of teeth may result in a difficult psychological situation, which so far has been completely underplayed and neglected in dental traumatology. This void is addressed in this new edition by a chapter on the psychological impact of dental trauma on the patient. Moreover, a traumatic event, whether crown fracture or tooth loss, usually results in severe esthetic problems. A further chapter is now devoted specifically to esthetic rehabilitation of the traumatized patient.

In this edition, an evidence-based approach to treatment has been chosen. This implies that any treatment procedure

must be carefully screened for its effect upon healing processes. Due to the fact that treatment approaches, by their very nature, are usually traumatogenic, treatment principles for traumatized teeth become critical. Randomized clinical studies would be desirable, but inevitably there are practical and ethical problems with this: it would be difficult to ask for signed patient approval to place the patient in group A or B to test the difference between acute treatment procedures.

Acute dental trauma implies severe pain and psychological impact for many of us. There may also be severe economic consequences for trauma victims, especially in less privileged social groups. The dental profession must cope with these problems. One approach could be prevention of dental injuries, but previous efforts in this direction have not always been cost-effective. In this fourth edition, accident-prone sports activities have been identified where mouthguards could be of value.

Statistics from most countries show that one third of all preschool children have suffered a dental trauma involving the primary dentition and 20–25% have suffered a trauma to the permanent dentition. With such statistics it is likely that more than 3 billion of the world's population are trauma victims and, considering the general lack of continuing and serious research into dental trauma, the importance of this area of dentistry cannot be overstated.

It is the authors' hope that a better knowledge of the biology of dental trauma and wound healing will lead to a more intelligent treatment strategy, where the slogan 'Hands off where you can!' to save teeth could mean that the traumatic episode might be a short-lived one and not a protracted story of treatment and re-treatment – not only for the victim of dental trauma, but also for the dental practitioner.

The aim of this textbook is to ignite interest in this stepchild of dentistry, a discipline where all the skills of dentistry are needed to help victims of dental trauma. Please be involved!

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1

Wound Healing Subsequent to Injury

F. Gottrup, S. Storgård Jensen & J. O. Andreasen

F. Gottrup

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 of the role of 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).

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 two 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 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.

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.

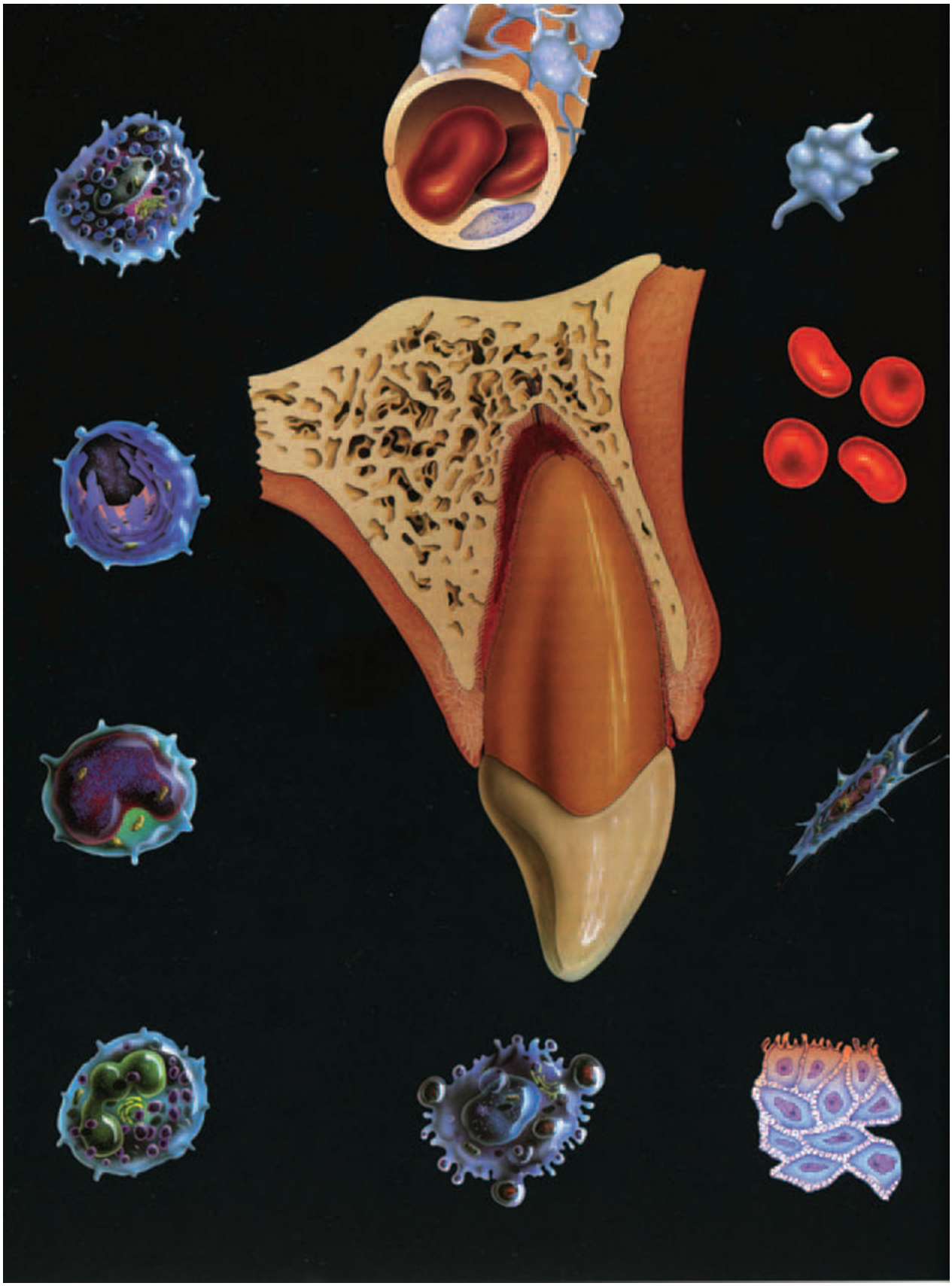

Fig. 1.1 Cells involved in the healing event after a tooth luxation. Clockwise from top: endothelial cell and pericytes; thrombocyte (platelet); erythrocyte; fibroblast; epithelial cell; macrophage; neutrophil; lymphocytes; mast cell.

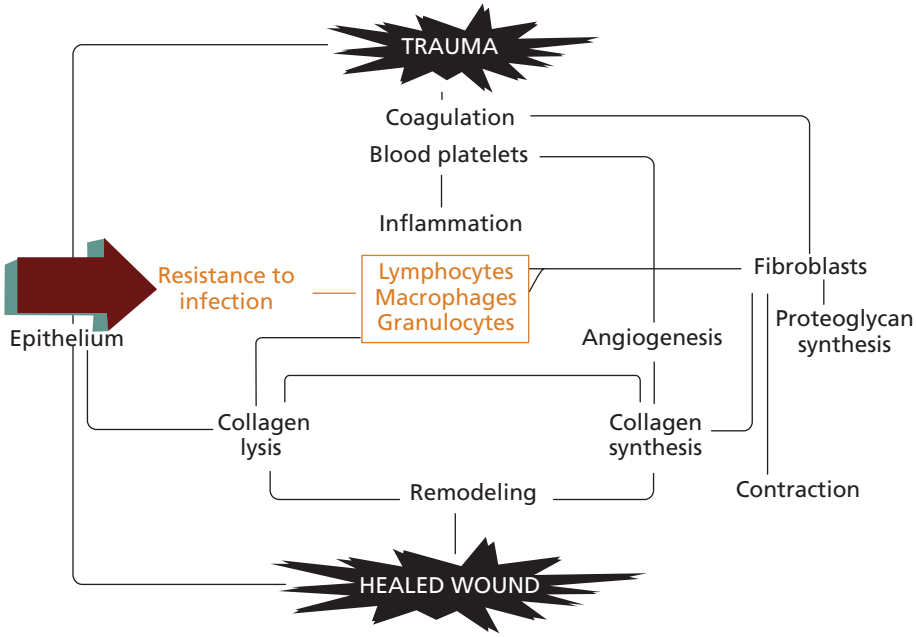

Fig. 1.2 Modified Hunt flow diagram for wound healing.

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 nonfunctional 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 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, nonspecialized cells, which are the source of new differentiating cells during normal tissue turnover and healing after injury (17–19). The role of these in wound healing is further discussed in Chapter 3.

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 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.

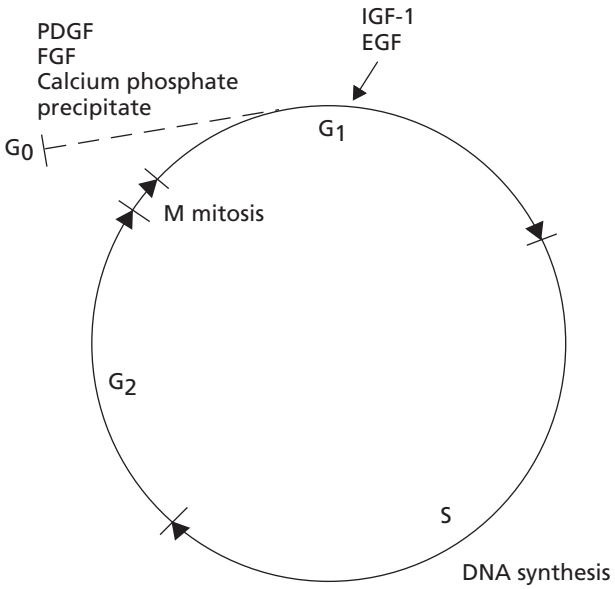

Fig. 1.3 Cell cycle. G_0 = resting phase; G_1 = time before onset of DNA synthesis; S = replication of DNA; G_2 = time between DNA replication and mitosis; M = mitosis.

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 extracellular matrix within the tissue (*contact guidance*). Finally there is a *free edge effect* which occurs in epithelial wound healing (see p. 36) (27, 28).

Typical examples of cells responding to *chemotaxis* are circulating neutrophils and monocytes and macrophages (see p. 23). 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 extracellular matrix. 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 (see p. 9), 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 extracellular matrix for cell migration. Adherent platelets undergo morphological changes to facilitate formation of the hemostatic plug and secrete several mediators of wound healing such as platelet derived growth factor, 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 vasodilatation 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

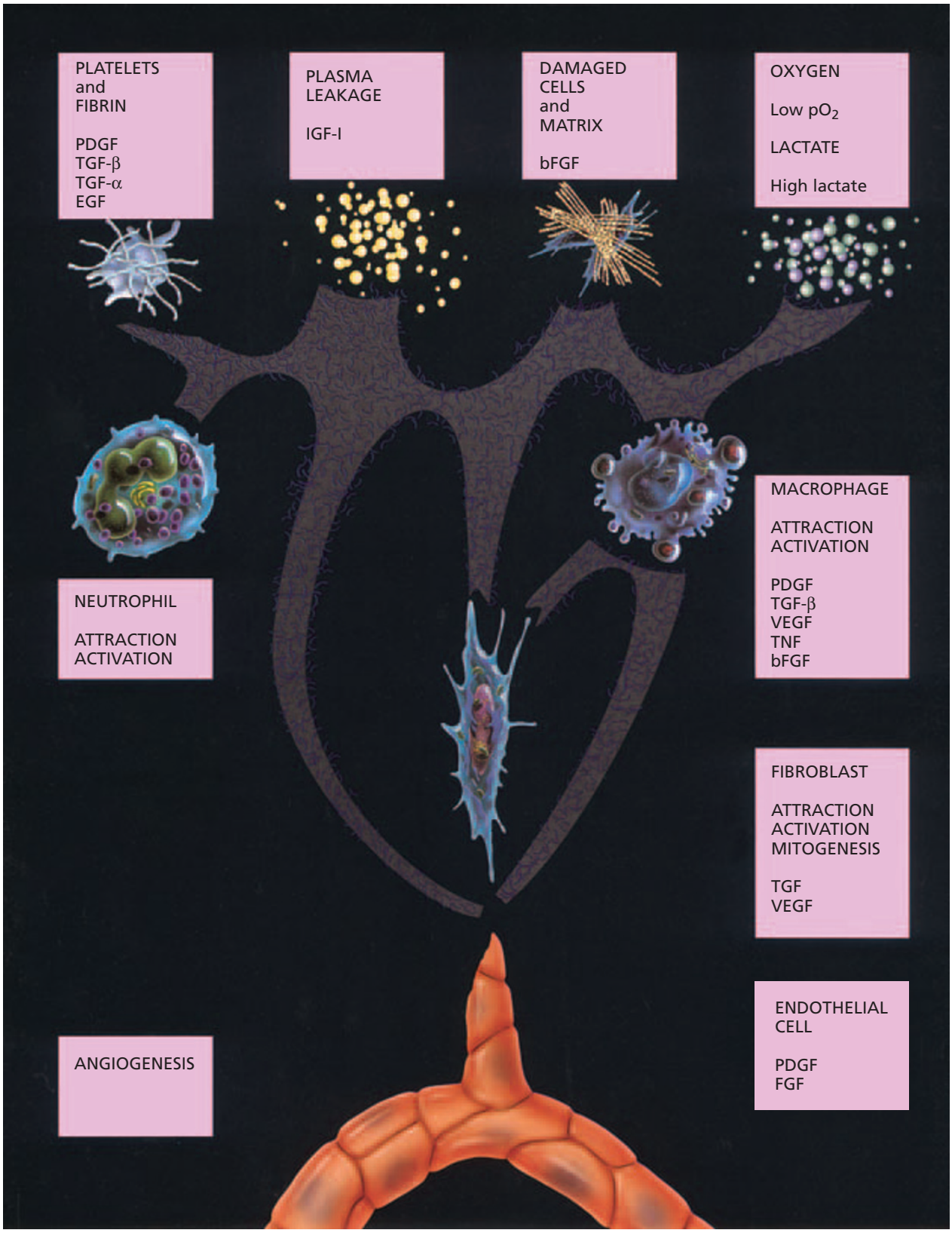

Fig. 1.4 Cellular components and mediators in the wound healing module. Signals for wound healing are released by platelets, fibrin, plasma leakage, damaged cells and matrix. Furthermore low oxygen tension and a high lactate concentration in the injury site contribute an important stimulus for healing.

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. It starts about day 2 after the tissue trauma and continues for two to three 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 extracellular matrix 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 extracellular matrix, 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). Successful wound healing can only take place in the presence of the enzyme, because oxidants are required for 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 platelet-derived growth factor (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 extracellular matrix, which conversely can have an influence on the fibroblast activities. Cell movements at this stage into the fibrin clot or tightly woven extracellular matrix seem to require an active proteolytic system that can cleave a path for cell migration. Fibroblast derived enzymes (collagenase, gelatinase A, etc.) and serum plasmin are potential candidates for this task (23).

After fibroblast migration into the wound cavity the provisional extracellular matrix is gradually replaced with collagenous matrix. 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, cross-linking 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 (259, 377; see p. 30). 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 extracellular matrix (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 which 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. 38.

Epithelialization

Re-epithelialization of wounds begins within hours after injury. If parts of 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 dermis is totally destroyed the epithelialization only takes place from the wound edges and epithelialization can continues for a considerable time dependent on wound area.

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 extracellular matrix. This is achieved by production of proteinases (collagenases, e.g. MMP-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 ziplike fashion and hemidesmosomes and type VII collagen anchoring fibrils form. Epidermal cells firmly attached to the basement membrane and underlying dermis revert 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 2–3 (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, 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. 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 cross-links 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 pathological 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 shrink 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.

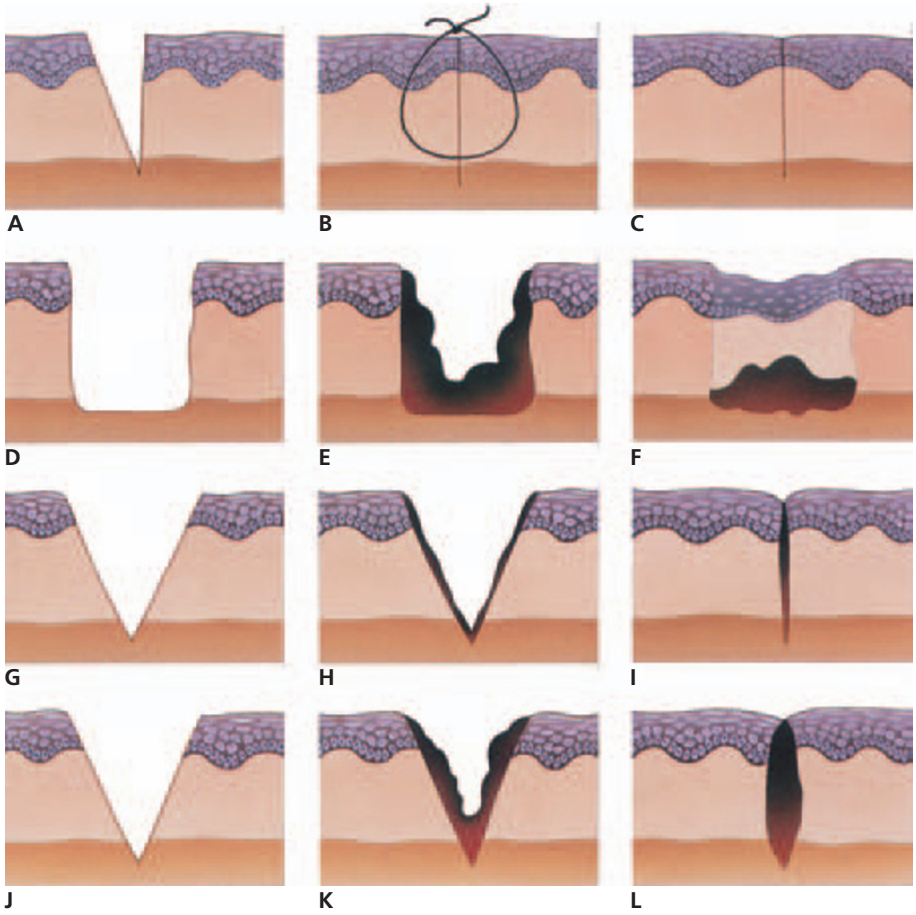

Fig. 1.5 Wound healing events related to the type of wound and subsequent treatment. A–C: incisional wound with primary closure; D–F: open and non-sutured wound; G–I: delayed primary closure; J–L: secondary closure. From GOTTRUP (56) 1991.

Remodeling phase

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

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 is unknown; some have argued one 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 more in basket weave 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

the natural biological 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, platelet derived growth factor (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 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 threadlike network to entrap plasma fractions and formed elements. This fibrin 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 HMW-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). 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. 29).

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

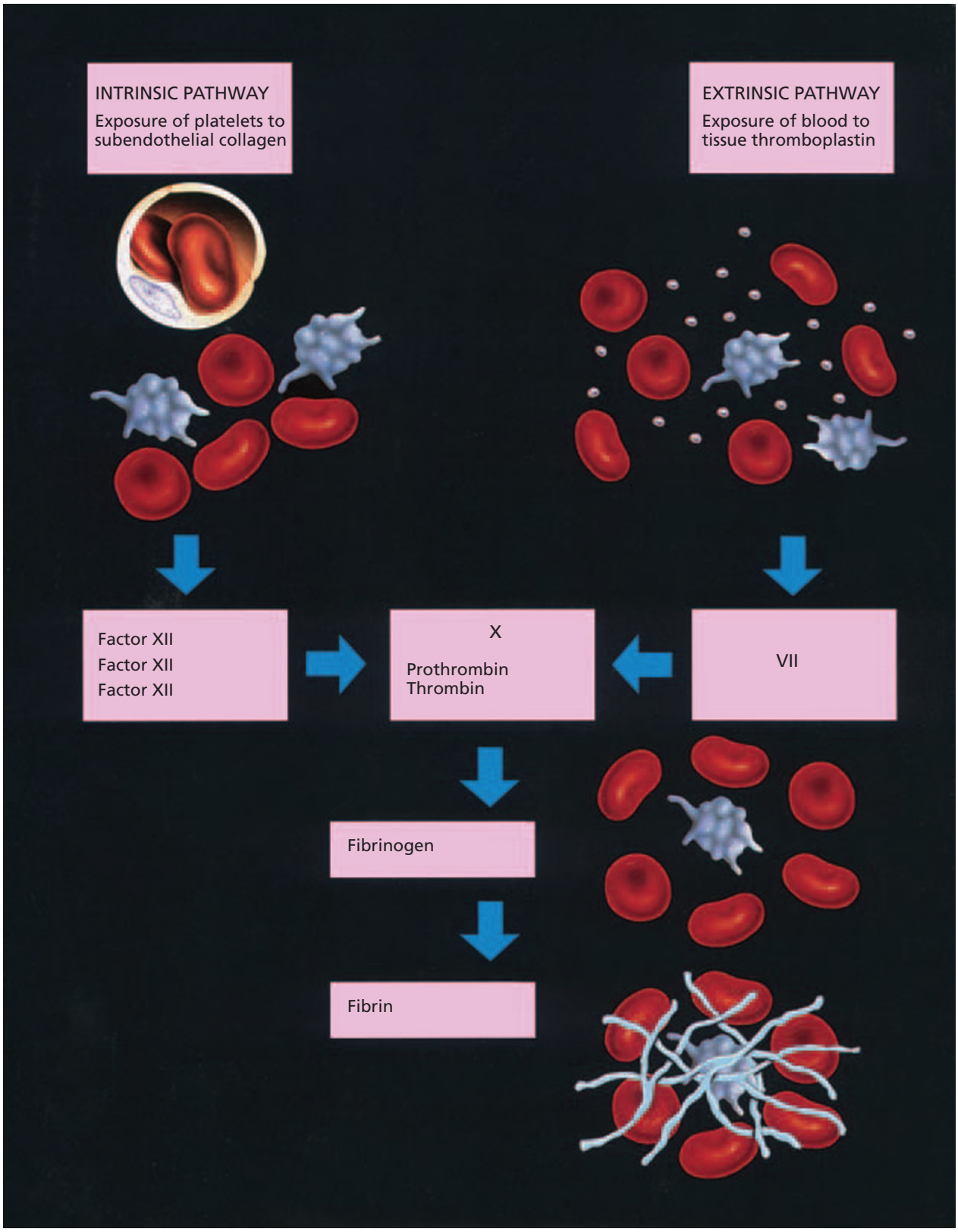

Fig. 1.6 Extrinsic and intrinsic coagulation cascade.

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 nonspecific 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

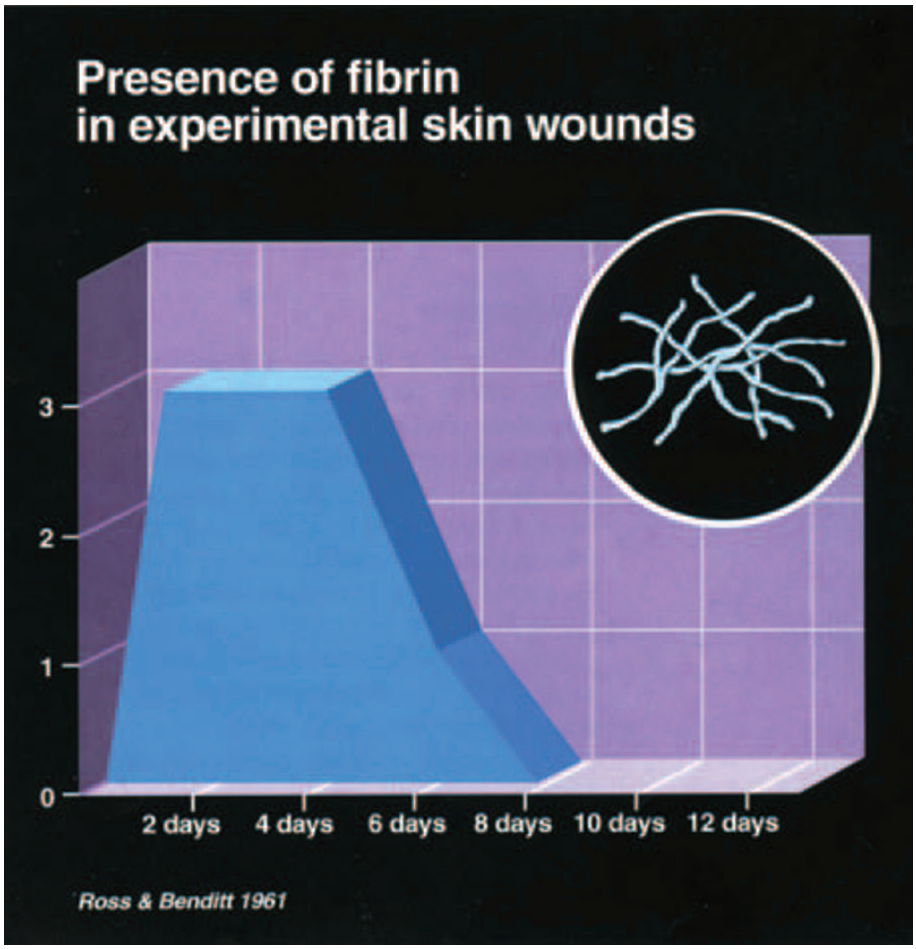

Fig. 1.7 Schematic illustration of the presence of fibrin in experimental skin wounds in guinea pigs. The scale is semiquantitative, graded from 0 to 3. From ROSS & BENDITT (73) 1961.

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).

Fibrin

During the coagulation process, 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 damaged lymphatics and thereby confine the inflammatory reaction to an area immediately surrounding the wound.

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).

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).

Fibronectin plays many roles in wound healing, 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) (see p. 29). Thus plasma fibronectin is linked to fibrin which has been spilled from damaged vessels or from highly permeable undamaged vessels (see p. 29) (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).

In 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 differentiation (96, 111–114). 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, and 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 fibroblast growth factor (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 extracellular matrix. 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).

The proliferative phase of healing involves cell duplication, differentiation and synthesis of extracellular matrix 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). Further-

more, 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 vasodilatation 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.

Table 1.1 Mediators of vascular response in inflammation. Modified from VENGE (136) 1985.

	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

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).

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 (PG). 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 A2 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 tissue 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 which 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.

S. Storgård Jensen

Growth factors

Growth factors are a group of polypeptides involved in cellular chemotaxis, differentiation, proliferation, and synthesis of extracellular matrix 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 an *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 extracellular matrix (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 (p. 16).

Platelet derived growth factor (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).

Transforming growth factors (TGFs) comprise a large family of cytokines with a widespread impact on the formation and development of many tissues (among those, the BMPs, which are described separately). This factor has earlier 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.