NORMAL WOUND HEALING

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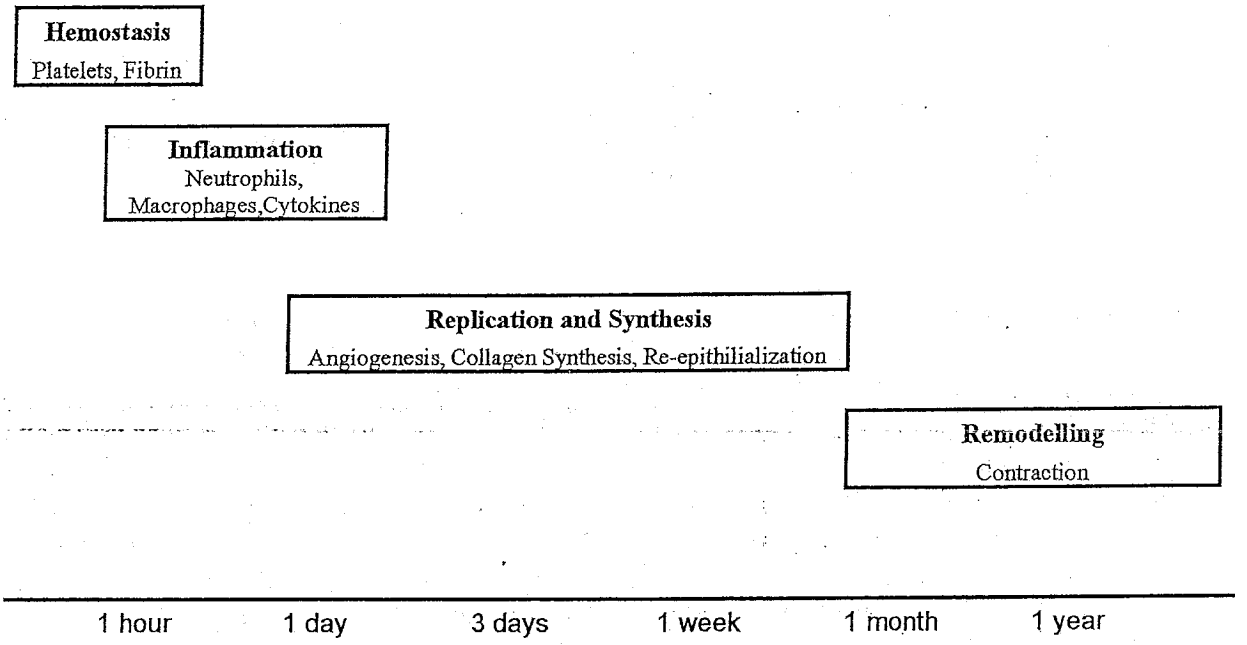
Wounds heal through a series of dynamic, overlapping processes that begin in response to tissue injury. Cellular and extracellular matrix components interact to restore tissue integrity. Multiple growth factors are known to modulate tissue repair by influencing cell proliferation, migration, and other cellular metabolic activity. Advancement in understanding of the complex mechanisms of wound repair and the function of growth factors promises to enable the implementation of new therapeutic interventions.

Hemostasis

The initial response to injury is to achieve hemostasis through vasoconstriction and activation of the clotting mechanism[1]. The formation of a blood clot serves the dual role of preserving vascular integrity and providing a temporary scaffold for the wound healing process to begin[2]. The conversion of fibrinogen to fibrin and formation of the fibrin clot is the first step in the assembly of a provisional extracellular matrix (ECM). In addition to contributing to hemostasis, thrombin and fibrin contribute to other aspects of wound healing. Thrombin contributes to the increased vascular permeability seen after injury and also facilitates the migration of inflammatory mediators. It may also have a role in both epithelialization and angiogenesis. Fibrin provides a scaffold for the migration of the inflammatory and mesenchymal cells[3].

Fibronectin is a major constituent of the provisional ECM that is deposited during the first 24 hours after tissue injury. It promotes the adhesion and migration of inflammatory and epithelial cells[4]. Fibronectin exists in a soluble, nonreactive form in the blood. Fibroblasts, endothelial cells and vascular smooth muscle cells can secrete, bind , and assemble fibronectin into fibrils in the ECM. Both plasma fibronectin and cellular fibronectin, synthesized locally, have the potential to be deposited into the ECM. Once polymerized into its insoluble form within the ECM, fibronectin becomes a highly adhesive protein[5]. Polymerized fibronectin interacts with cells through integrins, heterodimeric transmembrane receptors linking the ECM to the intracellular cytoskeleton and signaling pathways. Cross-linking of fibronectin to the fibrin clot promotes fibroblast adhesion and migration into the provisional ECM. Fibronectin also acts as a ligand for platelet integrins contributing to platelet adhesion and aggregation[6].

Platelets are early modulators of the wound healing process. The exposed collagen in the injured blood vessels and dermis stimulates platelet aggregation and degranulation. Platelets adhere to extravascular collagen and release adenosine diphosphate (ADP), which in turn stimulates further platelet aggregation[7]. Platelet aggregation leads to the release of cytokines and growth factors that reside in the alpha granules. These include polypepdtide growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor I (IGF-I), and transforming growth factor beta (TGF-β). These growth factors are released into the wound and the surrounding tissues and exert their effect on neutrophils, macrophages, smooth muscle cells, fibroblasts. They control cellular growth, migration, differentiation, proliferation as well as protein production throughout the wound healing process[1,8]



Phases of Wound Healing
TABLE 1

Inflammation

The early inflammatory phase is marked by vasodilatation and increased vascular permeability. These changes are mediated by histamine, kinins, prostaglandins, and possibly additional factors such as leukotrienes, proteases, and acid hydrolases. Mast cells are the primary source of histamine in connective tissue[9]. Histamine directly increases vascular permeability and indirectly causes vasodilatation through stimulation of prostaglandin synthesis. The kinins are a family of small peptides that act

predominantly as short duration vasodilators. They are released from protein-binding molecules after activation of kallikrein, another byproduct of the clotting cascade[10]. PGE1 and PGE2 are prostaglandins that increase capillary permeability. They result from phospholipase activation on injured cell membranes that leads to arachidonic acid release and subsequent prostaglandin synthesis and accumulation. In addition to stimulating vasodilatation, histamine and prostaglandins contribute to gap formation between endothelial cells, thus promoting plasma leak from the intravascular space to the ECM[11].

Leukocytes migrate into the injured tissue through an active phenomenon known as diapedesis. This process involves adherence to endothelial cells lining the capillaries and migration between the endothelial cells into the ECM. Neutrophils and monocytes migrate into the wound in numbers directly proportional to the respective serum concentrations[12,13]. They are attracted to the site of injury by chemoattractants such as bacterial products, complement factors (C5a) , histamine, PGE2, leukotrienes and PDGF. Neutrophils are the first of the leukocytes to be found in the wound. They control local bacterial contamination by engulfing bacteria and foreign material through the process of phagocytosis[14]. Neutrophils also contribute to the acute inflammation by the release of cytokines such as TNF-α and interleukin-1 (IL-1). Furthermore, they release proteases such as collagenase and elastase that help remove damaged tissue. After these acute inflammatory activities, neutrophils die. They do not appear to have a role in the ensuing normal wound healing process[15]. Neutropenic animals exhibit similar hydroxyproline content as well as similar wound tensile strength as their normal counterparts. Moreover, clinical studies reveal that although neutropenic patients have a higher incidence of wound infection and secondary wound healing problems, they seldom exhibit primary deficiency of wound healing[12].

Monocytes migrate from the capillaries into the ECM and transform into macrophages in a process that is mediated by inflammatory mediators such as TGF-β and breakdown products of fibronectin from the provisional ECM[16]. Chemotactic factors stimulate the migration of macrophages throughout the injured tissue. Similar to neutophils, macrophages begin their phagocytic activity and aid in the debridement of devitalized tissues through the secretion of collagenases and other proteases[17,18]. Moreover, macrophages are a primary source of cytokines and growth factors that stimulate fibroblast proliferation and collagen production. For instance, TGF-β activates fibroblasts and stimulates collagen deposition by enhancing collagen synthesis and inhibiting collagen degradation. Macrophages are the most abundant source of TGF-β in the healing wound. They also secrete additional growth factors such as PDGF, TGF-α, basic fibroblast growth factor (bFGF), and heparin-binding epidermal growth factor (HB-EGF) in addition to IL-1 and TNF-α. These factors not only control the ongoing inflammatory response, but also modulate epithelialization, collagen deposition and angiogenesis in the healing wound[19,20].

T-lymphocytes migrate into the ECM along with leukocytes. They exert both stimulatory and inhibitory effects on fibroblast proliferation and collagen synthesis through the

release of lymphokines. T cell-derived lymphokines interact with other inflammatory mediators and modulate fibroblast and endothelial cell activity[21,22]. Fibroblast activating factor (FAF) is a stimulatory lymphokine that is produced by activated T-lymphocytes. It has been demonstrated that the continued presence of antigenic stimulus at a healing site leads to excessive fibrosis, presumably due to continued activation of T lymphocytes and continued production of FAF[23]. Interferon gamma (IFN-γ) is a potent inhibitor of fibroblast proliferation and collagen synthesis. TNF-α stimulates fibroblast activity in low doses[8,24]. However, in higher doses, TNF-α has been shown to inhibit collagen and fibronectin synthesis by fibroblasts, and to diminish endothelial cell proliferation. Thus, T lymphocytes possess the capacity to modify the tissue repair mechanisms[25]. An intact cellular immune response appears to play an important role in the wound healing process. The role of B lymphocytes and the humoral immune system in normal wound healing is not fully understood[26,27].

Foreign material or bacteria can alter the acute inflammatory process and lead to chronic inflammation. The persistence of inflammation complicates the normal wound healing process. Neutrophils release proteases and generate free oxygen radicals that cause damage to the ECM[28]. Complement products form cytotoxic membrane attack complexes and contribute to tissue destruction. Encapsulation of foreign body or chronic bacterial infections can lead to granuloma formation that interferes with successful wound healing[29].

Replication/Synthesis

Following hemostasis and inflammation, processes which normally last 2-3 days, the wound healing intensifies the process of tissue restoration. The cellularity of the wound increases as various cell types migrate and proliferate in the ECM. Fibroblasts secrete IGF-I, bFGF, TGF-β, PDGF and epidermal growth factor (EGF). Endothelial cells synthesize vascular endothelial growth factor (VEGF), bFGF, and PDGF. Keratinocytes produce TGF-β, TGF-α, and keratinocyte-derived autocrine factor (KDAF). These factors stimulate and modulate extracellular matrix deposition, epithelialization and angiogenesis[30].

---Matrix Synthesis

Fibroblasts are the primary mesenchymal cells involved in wound healing[31]. Undifferentiated mesenchymal cells in the dermis turn into fibroblasts under the stimulation of cytokines and growth factors released by platelets, neutrophils and macrophages. Fibroblasts and smooth muscle cells are attracted to the site of tissue injury by growth factors such as PDGF, EGF, IGF-I, and TGF-β[32,33,34]. Fibronectin facilitates fibroblast adhesion and migration in the ECM[35]. The ECM cellularity is further augmented by proliferation of fibroblasts and smooth muscle cells stimulated by PDGF, TGF-β, TNF-α, IL-1, lymphokines, and IGF-I[36,37].

Proteoglycans are hybrid molecules composed of a central core protein to which one or more glycosaminoglycans (GAGs) are covalently attached. GAGs are known as hyaluronic acid, chondroitin sulfate A, B, and C, keratan sulfate, and heparan sulfate. Proteoglycans influence a variety of events during tissue repair. A GAG chain has the capacity for encoding a unique structural pattern to transmit information. Functional information is encoded in the sequence of amino acids of the core protein in the sequence of sugars in the GAG chain, and on the final enzymatic processing of the proteoglycan or GAG in the tissue. The core protein of the proteoglycan adds further information the complex by locating GAG to specific intercellular and extracellular locations[38,39].

Proteoglycans and GAGs are present in all tissues but have a complex expression pattern in the skin. Hyaluronic acid is synthesized in the epidermis and hyaluronan is abundant there[40]. Syndecan-1 and syndecan-4 are also abundant in the epidermis where they are expressed at the cell surface as heparin sulfate proteoglycans. In the basement membrane, the extracellular matrix heparin sulfate proteoglycan perlecan is most abundant. In the dermis, fibroblasts produce proteoglycans containing dermatan sulfate such as decorin and versican. Other cell types within the skin add to the complex environment of proteoglycans and GAGs. Mast cells synthesize large amounts of heparin sulfate. Neural cells express syndecan-3. Endothelial cells primarily produce heparin sulfate syndecan-4. Specificity of the skin proteoglycans and GAG environment effects cell behavior and function during the wound healing process[38,41].

The adhesive properties of GAGs are the main mechanism by which they control cell function. Binding to GAGs is a necessary element in the activation of many growth factors and cytokine[42]s. One example of this process is the activation of FGF-2 by heparan sulfate that is mediated by conformational changes that occur when GAG and FGF-2 bind[43]. GAG association can serve to either activate or deactivate the function of the protein factor. Regulation of the location of proteoglycans and/or the sequence of the GAG creates an environment that may be permissive or resistant to a complex mixture of soluble signaling molecules in the wound[41].

Engagement of proteoglycans with cell surface receptors or extracellular matrix proteins occurs through binding to the core protein or GAG. For example decorin core protein stabilizes dermal collagen structure and deletion of this core protein results in increased skin fragility and disorganized collagen[44,45]. Another example is the interaction between cell surface heparan sulfate proteoglycan such as syndecan with fibronectin through integrin receptors. Syndecan-4 becomes inserted into the focal adhesions of a number of cell types such as fibroblasts, smooth muscle cells and endothelial cells[46].

Fibroblasts, smooth muscle cells as well as endothelial cells synthesize collagen. Fibroblasts under the stimulation of TGF-β are the primary source of collagen fibrils[47]. Collagenous matrix replaces the provisional extracellular matrix and leads to the formation of a relatively acellular scar. Collagen makes up approximately 25 % of all body proteins and more than 50% of the protein found in the scar tissue. Collagen levels rise continuously for 3 weeks. As the amount of collagen increases in the wound, the

number of fibroblasts decreases. Collagen homeostasis is achieved when the rates of collagen synthesis and degradation are equivalent[48].

The collagen superfamily includes 19 proteins formally defined as collagens[49]. All collagen molecules consist of three polypeptide chains, called alpha chains that are wrapped around each other into a triple helix. In some collagen types, all three alpha chains of the molecule are identical, while in other types the molecule contains two or three different alpha chains. In each of the polypeptide chains every third amino acid is glycine. The sequence of an alpha chain in a protein can be expressed as $(GLY\text{-}X\text{-}Y)_n$, where X and Y represent amino acids other than glycine and n varies based on the collagen type. Proline is frequently found in the X position and 4-hydroxyproline in the Y position. The triple helix is further stabilized by hydrogen bonds and water bridges, many of which require the presence of 4-hydroxyproline residues[50,51].

The superfamily of collagens can be further divided into various families based on their supramolecular assemblies. Collagen types I, II, III, V and XI are a family of collagens that form fibrils[50]. Type I collagen makes up 80-90% of collagen in skin. The remaining 10-20% is comprised of type III collagen. Thus, normal skin contains type I and III collagen in a 4:1 ratio. Increased levels of type III collagen are seen embryonically and in the early phases of wound healing. In hypertrophic and immature scars the type I to type III ratio may be 2:1. Type IV collagen is found in the basement membranes whereas types II and XI are predominantly seen in cartilage[48,49].

The fibril-forming collagens are first synthesized as procollagen molecules that have pro-peptide extensions at both their N and C terminal ends. The main intracellular steps in the assembly of procollagen from pro-alpha chains include the cleavage of the signal peptides, hydroxylation certain proline and lysine residues, glycosylation of some of the hydroxylysine residues, and association of the C propeptides which initiates the formation of the triple helix. The procollagen molecules are then transported from the endoplasmic reticulum across the Golgi apparatus and are secreted in the form of granules. The extracellular steps in biosynthesis include cleavage of the N and C propeptides, self-assembly of the collagen molecules into fibrils by the process of nucleation and propagation, and formation of covalent cross-links[50,52].

Other components of the extracellular matrix include fibronectin and elastin. As mentioned previously, fibronectin is a component of the provisional matrix and aids in the migration of various cell types into the wound and it facilitates binding of epithelial cells to matrix[4]. Elastin is a component of normal connective tissue. It is not synthesized in response to tissue injury. This may contribute to diminished elastic properties of the scars relative to normal skin[53].

--Epithelialization

The epidermis consists of multiple layers of epithelial cells. Immediately above the dermis is a layer of cuboidal basal cells that are attached to the basement membrane thru hemidesmosomes. Above is, first, a squamous cell layer, then a granular layer and,

finally, a most superficial layer that is called the stratum corneum. The stratum corneum consists primarily of dead cell fragments and keratin. Epithelialization refers to the process of epithelial renewal in response to injury. Epithelial cells involved in the closure of wounds derive from both the wound edges and the epithelial appendages, hair follicles, sweat glands, and sebaceous glands in the de-epithelialized wound itself. These appendages extend into the underlying dermis and subcutaneous tissues and are present in partial thickness injuries[1,54].

The sequence of events that comprise epithelialization include cellular detachment, migration, proliferation, and differentiation. The basal cell layer begins to enlarge, elongate and detach from the underlying basement membrane during the first 24 hours after tissue injury[55]. This process of keratinocyte activation is thought to be mediated by cytokines, IL-1, IL-8 and TNF-α. Keratinocyte growth factor KGF, EGF, bFGF, PDGF, TGF-α and TGF-β are also stimulators of keratinocyte activation, although TGF-β is inhibits keratinocyte proliferation[30,56,57].

In recent years, there has been growing evidence that matrix metalloproteinases have a role in the remodeling of connective tissue during wound healing. Two such proteinases, MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) are thought to mediate the separation of basal keratinocytes from the basement membrane in preparation for migration across the wound bed[58,59,60]. The basal cells migrate as a monolayer by aligning themselves with the collagen fibers[61]. Fibronectin in the provisional matrix promotes keratinocyte adhesion and migration. Laminin and type IV collagen are usually not present during this migratory phase. The basal keratinocytes undergo cell division to provide more cells to the advancing epithelial layer. The leading cells acquire phagocytic properties and help create a smooth path for the moving cells. Migration ceases once the cells reach other migrating cell from another direction. Once the initial epithelialization is complete, keratinocytes continue proliferation in order to restore a thick epithelial layer. The process of differentiation then reestablishes the multiple layers of normal epithelium, from basal cells to the stratum corneum. Laminin and type IV collagen are produced and wound fibronectin is replaced by keratinocyte-derived fibronectin. The newly formed epithelial layer has fewer basal keratinocytes compared with uninjured skin. Moreover, the epidermal-dermal interface lacks the normally present rete pegs[54,56,62].

--Angiogenesis

The new cells and tissue which are created as part of wound healing require the formation of new blood vessels to provide the nutrients needed for homeostasis. This angiogenic response is a critical component of the wound healing process. The endothelial cells from the underlying tissue invade the ECM of the provisional matrix, form tubular structures that branch and form a network. This process is mediated by spatial and temporal interactions between the endothelial cells, angiogenic mediators, matrix metalloproteases (MMPs), and integrins that recognize fibrin, fibronectin and matrix GAG's[63,64].

Endothelial cells present in the wound bed become activated in response to tissue destruction and hypoxia. Angiogenic factors such as αFGF (FGF-1), βFGF (FGF-2), vascular endothelial growth factor (VEGF), EGF, and angiopoietins are released by activated inflammatory and epithelial cells or become available following cellular disruption[65,66,67]. These factors act as direct chemotactic and mitogenic stimuli for endothelial cells. In addition, many growth factors indirectly contribute to angiogenesis through the stimulation of fibroblasts and macrophages. Thrombospondin and angiostatin are known negative angiogenic factors. Angiogenesis is modulated through a balance between these positive and negative regulators of homeostasis[64].

The ECM microenvironment plays an important role in directing angiogenesis. Specific GAG's promote the adhesion and migration of the endothelial cells. Endothelial cells express members of the integrin superfamily of cell surface receptors[68]. One such receptor, αvβ3, recognizes most ECM proteins including fibrin, fibronectin and GAG's. During the early phases of angiogenesis, αvβ3 receptor is heavily expressed on the advancing capillary sprouts. Recent evidence suggests that ECM proteins can upregulate angiogenesis by the modulation of αvβ3 receptor expression[69,70].

Matrix metalloproteases (MMP's) also play an important role in angiogenesis. The matrix metalloproteases are a family of secreted and membrane-associated extracellular endopeptidases that selectively degrade components of the extracellular matrix. These enzymes may be produced by epithelial cells, endothelial cells,, fibroblasts and inflammatory cells. MMP's are released as zymogens that need to be activated in the extracellular space. MT-1-MMP which is a membrane-bound MMP is an exception as it is present in its activated form on the cell surface. Tissue inhibitors of MMP's are identified as TIMP. This family consists of four members, TIMP-1 through 4. Endothelial cells produce MMP-1, MMP-2, MMP-3, MMP-9, MMP-14, and MT-1-MMP as well as TIMP-1 and TIMP-2[71]. As a group, these proteins are involved in the breakdown of the connective tissue barriers that is a necessary step for new vessel formation. Proteases help with degradation of the basement membrane and create a physical pathway to accommodate endothelial capillary sprouts within the ECM. They also function to promote angiogenesis by regulating endothelial cell attachment, proliferation, migration and growth. Finally, they may stimulate angiogenesis indirectly by causing the release of growth factors sequestered in the ECM[72,73].

After surgical trauma or burn, which represents the normal healing, the MMP-9 is transiently expressed, and then diminishes (Salo et al(72); Tarlton et al(75); Young and Grinnell(59)). There is significant clinical evidence shows that such proteinases are persistently presented in chronic wounds (Wysocki et al,1993(76); Wysocki et al,1999(77); Yager et al(78)). Because MMP-9 can digest type IV collagen (Seltzer et al(79) and other components in basement membrane zone (BMZ), non-healing could derive from the over destruction of BMZ or other ECM by the elevated active MMP-9. Obviously reestablishing BMZ is essential for re-epithialization.

Growth Factors

Growth factors are a group of proteins that have been identified to play a role in tissue repair[80]. The terminology may be misleading as the action of these mediators is not restricted to the modulation of growth. Moreover, the labels attribute specific origin or action to these factors that only reflect the circumstances that were present at the time of the discovery of the factor. For instance, platelet derived growth factor was originally identified in the α-granule of the platelets, but since then has also been found in macrophages, endothelial cells, and smooth muscle cells. Many of these proteins exert both chemotactic and mitogenic effects in the healing tissue. As such, they are important in both determining the sequence of cells that appear throughout the wound healing process as well as in modulating their activities[81,82].

Growth Factor	Source	Function
EGF	Macrophages Platelets, Endothelial Cells	Epithelial Cell Proliferation and Migration, Fibroblast and Endothelial Cell Activity, Fibronectin Synthesis
FGF	Macrophages, Endothelial Cells, Fibroblasts	Endothelial Cell, Fibroblast and Smooth Muscle Cell Proliferation
IGF-I	Fibroblasts, Hepatocytes	Fibroblast and Epithelial Cell Proliferation and Chemotaxis
PDGF	Macrophages, Platelets, Endothelial Cells, Fibroblasts	Endothelial Cell, Fibroblast and smooth Muscle Cell Proliferation and Chemotaxis Fibronectin and Proteoglycan Synthesis
TGF-β	Macrophages, Platelets, Endothelial Cells, Lymphocytes	Fibroblast, Endothelial Cells Proliferation and Chemotaxis Fibronectin and Proteoglycan Synthesis
TNF-α	Macrophages, Lymphocytes	Inflammatory Process and Fibroblast Activity
VEGF	Endothelial Cells	Endothelial Cell and SmoothMuscle Cell Proliferation

TABLE 2

Growth factors exert their effects through specific receptors on the target cells. Receptors are composed of three parts: a cytoplasmic region, a hydrophobic transmembrane region, and an extracellular ligand binding domain[83]. The exact mechanism of receptor-ligand binding and subsequent cell stimulation in not fully known. Receptor activation does lead to the activation of intracellular enzyme tyrosine kinase with resultant

phosphorylation of intracellular serine, threonine, or tyrosine residues[84]. Another pathway of cell activation is mediated through the activation of G-protein coupled receptors. G-proteins are GTP-binding proteins that affect a variety of intracellular activities, including regulation of enzymes and ion channels within the cell membrane[85]. Proto-oncogenes such as c-fos and c-myc represent another receptor-effector mechanism which are thought to modulate DNA synthesis and division[86]. Growth factors can also modulate cell-matrix interactions through interactions with integrins[87].

The transmission of growth factor from source to target can be accomplished in several ways. Factors such as insulin-like growth factor I and II can be secreted into the blood and transported to distant sites in an endocrine mode of delivery[88]. A paracrine mechanism in which the secretory products of one cell acts directly on another represent the mode of delivery for factors such as PDGF, TGF-α, and TGF-β[30]. Autocrine route serves an autoregulatory function and allows factors such as TGF-β to affect both their source and target activity[19]

Depending on their period of activity with respect to mitogenesis, growth factors can be further categorized[89]. Competence factors activate quiescent cells to enter the G_1 phase of the cell cycle. Progression factors act as a second signal to allow competent cells begin DNA synthesis. Competence factors such as PDGF and FGF need to be present only transiently as their resulting signal will be transferred from one cell to another. Progression factors such as EGF must be present throughout G_1 phase to convey their signal[90].

-- Epidermal Growth Factor (EGF)

Epidermal growth factor was first isolated from the salivary glands of mice. EGF is released by platelets as a component of the α–granule. EGF receptors are present on epithelial cells, endothelial cells, fibroblasts and smooth muscle cells. EGF promotes migration and division of epithelial cells and stimulates synthesis of fibronectin during the early phases of the wound healing process. It also acts as a potent mitogen and chemoattractant for fibroblasts and endothelial cells promoting matrix synthesis and angiogenesis[91,92]. Transforming growth factor α (TGF-α) is a peptide with structural and functional homology to EGF. It binds to EGF receptor and mirrors the stimulatory effects of EGF on epithelial cell proliferation, angiogenesis and matrix synthesis[93].

-- Fibroblast Growth Factor (FGF)

Fibroblast growth factor family includes the acidic FGF (αFGF), basic FGF (βFGF) and keratinocyte growth factor (KGF). FGFs are produced by fibroblasts, endothelial cells, smooth muscle cells and chondrocytes. FGFs stimulate the proliferation of endothelial cells, fibroblasts and smooth muscle cells. An important characteristic of FGF is their ability to bind heparin and heparan sulfate. Specifically, the binding of βFGF to extracellular matrix components may regulate its activity by acting as a potential storage and release site and by potentiating its effect on target cells[30,94,95]. KGF is thought to

stimulate keratinocyte proliferation and differentiation. It is also thought to promote keratinocyte migration by increasing the expression of MMP-2[56].

--Platelet-Derived Growth Factor (PDGF)

PDGF is stored in the α-granule of the platelets and released after activation of the platelets during the early phases of wound healing. This potent competence factor can also be secreted by macrophages, fibroblasts, endothelial cells and smooth muscle cells. PDGF acts through autocrine and paracrine mechanisms to stimulate proliferation and chemotaxis of fibroblasts, endothelial cells, and smooth muscle cells. It promotes the synthesis of the provisional extracellular matrix by stimulating fibronectin and proteoglycan synthesis[96,97]. PDGF may influence the expression of other growth factors such as TGF-β during the wound healing process and plays a role in epithelialization and matrix remodeling processesSignal transduction pathways for the action of PDGF include tyrosine kinase activation and proto-oncogenes c-fos and C-myc transcription[98]. Clinical applications of recombinant PDGF suggest a promising role for PDGF to stimulate wound healing by augmenting the inflammatory response, and promoting matrix deposition, angiogenesis, and epithelialization[90].

--Transforming Growth Factor β (TGF-β)

TGF-β was first isolated from the α-granule of the platelets and shown to stimulate normal cells to grow in soft agar as though they had been virally transformed. TGF-β regulates its own production by the macrophages in an autocrine fashion. It can function as both an agonist or an antagonist of the inflammatory process. TGF-β regulates PDGF, FGF, TNF-α, and IL-1 by inhibiting or stimulating their production or modulating their actions to synchronize and control tissue repair[99,100]. TGF-β stimulates fibronectin and proteoglycan synthesis, epithelial cell proliferation, collagen synthesis, angiogenesis and wound contraction. In different concentrations, TGF-β can either stimulate or inhibit collagen deposition and protease activity, thus playing an important role in collagen homeostasis[101].

Transduction of TGF-β activity is mediated by several kinase-dependent membrane receptors. Moreover, TGF-β has been shown to regulate the expression of integrin receptor proteins and change the balance of integrins in different cells. This property allows TGF-β to not only alter cell –matrix interactions, but also modulate the cellular response to TGF-β based on receptor configuration[47,102].

--Vascular Endothelial Growth Factor (VEGF)

VEGF is a heparin-binding growth factor with glycoprotein similarity to PDGF. It has potent vasopermeability activity which led to its initial designation as vasopermeability factor (VPF). VEGF is released in response to tissue hypoxia and acts as a mitogen for

endothelial cells through a kinase dependent mechanism. VEGF promotes angiogenesis and granulation tissue formation in conjunction with FGFs, PDGF and TGF-β[103,104].

--Tumor Necrosis Factor α (TNF-α)

TNF-α is secreted primarily by macrophages and lymphocytes. It was originally described to cause hemorrhagic necrosis of certain transplanted tumors in mice. TNF-α modulates acute inflammation, fibroblast activity, and angiogenesis during wound healing[30,105].

--Insulin-like Growth Factor I (IGF-I)

IGF-I is a plasma bound growth factor that acts in synergy with pituitary growth hormones to promote cell division and differentiation. During wound healing, IGF-I is released from platelets or produced by fibroblasts. It is a potent chemotactic agent for endothelial cells. IGF-I also stimulates fibroblast and epithelial cell proliferation[30,106].

Remodeling

Collagen hemostasis in normal connective tissue represents a dynamic balance between collagen synthesis and degradation. Wound remodeling can be achieved thru the modulation of collagen degradation thru the activity of collagenase enzymes such as MMPs[107]. Remodeling also occurs in response to mechanical forces within the tissue[108].

Contraction represents the centripetal movement of peripheral components of a healing open wound. Contracture refers to the contraction of a large wound across a joint surface, leading to restricted joint motion. There are two main theories describing the process of wound contraction. The first implicates a specialized cell, the myofibroblasts, found in large quantities during wound contraction. Myofibroblasts are described as specialized fibroblasts that contain actin microfilaments seen on electron microscopy. They are thought to be differentiated from normal wound fibroblasts by the cytokine stimulation in the wound. This theory suggests that the myofibroblasts at the wound edges apply a centripetal force that leads to wound contraction[109,110]. The alternative theory suggests that fibroblasts are the primary contributors to wound contraction through their interactions with the ECM. According to this theory, as fibroblasts elongate and migrate through the matrix they retract collagen fibrils. This theory further suggests that myofibroblasts are merely quiescent fibroblasts with stress fibers in their cytoplasm[111,112].

Contraction of the collagen matrix is stimulated by TGF-β and PDGF and inhibited by FGF and interferon-γ[113]. Wound contraction cannot be eliminated, but it may be modulated with the choice of wound coverage. For instance, full thickness skin graft by

virtue of containing more dermis exhibit less contraction than split thickness skin grafts. Moreover, earlier grafting of a wound results in less contraction compared with delayed grafting[114].

Collagen content three weeks after the onset of injury is the maximal concentration of collagen found in the wound. The wound tensile strength however is only 15% compared with normal skin. During the following 3 weeks, as scar remodeling progresses, the wound reaches approximately 80-90% of its eventual strength. By six months, the wound tensile strength will be 90% of normal skin and it will not reach normal levels. These effects are due to changes in matrix cross-linking. During wound remodeling, both the intramolecular and intermolecular cross-links between collagen fibers are significantly increased. This increase in cross-linking accounts for the observed increase in wound tensile strength. As collagen maturation proceeds, the amount of type III collagen is decreased in relation to type I collagen. The ratio of type I to type III reverts back to 4:1 as in the pre-injury state. During remodeling the quantity of water and GAG's in the ECM are decreased. Angiogenesis reaches homeostasis and no net new blood vessels are formed. The process of remodeling continues for 12-18 months during which the texture, thickness and color of the wound continue to be in a dynamic state[1,115,116].

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