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WOUND HEALING: AN OVERVIEW OF ACUTE, FIBROTIC AND DELAYED HEALING

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1. ABSTRACT

Acute wounds normally heal in a very orderly and efficient manner characterized by four distinct, but overlapping phases: *hemostasis*, *inflammation*, *proliferation* and *remodeling*. Specific biological markers characterize healing of acute wounds. Likewise, unique biologic markers also characterize pathologic responses resulting in fibrosis and chronic non-healing ulcers. This review describes the major biological processes associated with both normal and pathologic healing.

The normal healing response begins the moment the tissue is injured. As the blood components spill into the site of injury, the platelets come into contact with exposed collagen and other elements of the extracellular matrix. This contact triggers the platelets to release clotting factors as well as essential growth factors and cytokines such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-ß). Following *hemostasis*, the neutrophils then enter the wound site and begin the critical task of phagocytosis to remove foreign materials, bacteria and damaged tissue. As part of this *inflammatory* phase, the macrophages appear and continue the process of phagocytosis as well as releasing more PDGF and TGFß. Once the wound site is cleaned out, fibroblasts migrate in to begin the *proliferative* phase and deposit new extracellular matrix. The new collagen matrix then becomes cross-linked and organized during the final *remodeling* phase. In order for this efficient and highly controlled repair process to take place, there are numerous cell-signaling events that are required.

In pathologic conditions such as non-healing pressure ulcers, this efficient and orderly process is lost and the ulcers are locked into a state of chronic inflammation characterized by abundant neutrophil infiltration with associated reactive oxygen species and destructive enzymes. Healing proceeds only after the inflammation is controlled. On the opposite end of the spectrum, fibrosis is characterized by excessive matrix deposition and reduced remodeling. Often fibrotic lesions are associated with increased densities of mast cells. By understanding the functional relationships of these biological processes of normal compared to abnormal wound healing, hopefully new strategies can be designed to treat the pathological conditions.

2. INTRODUCTION

To understand the underlying mechanisms involved in pathologic conditions such as fibrosis and chronic non-healing ulcers, it is helpful to first review what is known about normal tissue response to injury. The human body can sustain a variety of injuries, including penetrating trauma, burn trauma and blunt trauma. All of these insults set into motion an orderly sequence of events that are involved in the healing response, characterized by the movement of specialized cells into the wound site. Platelets and inflammatory cells are the first cells to arrive at the site of injury and they provide key functions and "signals" needed for the influx of connective tissue cells and a new blood supply. These chemical signals are known as cytokines or growth factors (1).

The fibroblast is the connective tissue cell responsible for collagen deposition that is needed to repair the tissue injury (2). Collagen is the most abundant protein in the animal kingdom, accounting for 30% of the total protein in the human body (3). In normal tissues collagen provides strength, integrity and structure. When tissues are disrupted following injury, collagen is needed to repair the defect and restore anatomic structure and function. If too much collagen is deposited in the wound site, normal anatomical structure is lost, function is compromised and fibrosis occurs. Conversely, if an insufficient amount of collagen is deposited, the wound is weak and may dehisce (4). Therefore, to understand fully the process of wound

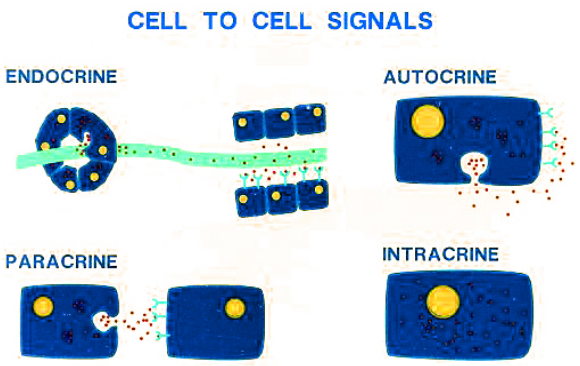

Figure 1. Cell signaling by cytokines.

Figure 2. The four possible responses following tissue injury

healing, it is essential to understand first the basic cell biology, immunology and biochemistry involved in the processes of inflammation and collagen metabolism, and how these pathways are regulated.

3. CELL SIGNALING

The many diverse activities taking place during wound healing are directed by chemical signals referred to as growth factors or cytokines (1, 5, 6). Originally these signals were named growth factors because that was their main observed function. As more information was developed it became apparent that many of these factors controlled more than just cell growth. Cell migration, matrix production, enzyme expression and differentiation can also be controlled by these factors. Therefore the term "cytokine" may be a better description for these chemical signals. A listing of some of the cytokines important for wound healing is presented in Table 1.

These cytokines range in weight from 4 to 60 kD and they direct cellular activity when they are present in very small quantities. In general these factors are very stable. However in the chronic wound environment where there are increased numbers of neutrophils releasing proteolytic enzymes, such as neutrophil elastase, they can be destroyed. Cytokines can regulate cellular activities and functions via endocrine, paracrine, autocrine and intracrine mechanisms (Figure 1). In order for a particular cytokine to modulate a cellular activity the target cell must have a receptor. Once receptor binding takes place then a series of intracellular signals are activated and eventually result in a specific response. Many of the signal pathways are mediated via activation of tyrosine kinase (7). The number of receptors expressed on the target cell can also regulate the cell-signaling cascade to some extent (8).

4. NORMAL AND PATHOLOGICAL RESPONSES TO INJURY

The term wound has been defined as a disruption of normal anatomical structure and, more importantly, function. Therefore, healing is the complex and dynamic process that results in the restoration of anatomical continuity and function (4). There are four basic responses that can occur following an injury (Figure 2). Normal repair is the response where there is a re-established equilibrium between scar formation and scar remodeling. This is the typical response that most humans experience following injury. The pathological responses to tissue injury stand in sharp contrast to the normal repair response. In excessive healing there is too much deposition of connective tissue that results in altered structure and, thus, loss of function (9). Fibrosis, strictures, adhesions and contractures are examples of excessive healing. Keloids and hypertrophic scars in the skin are examples of fibrosis (10, 11). Contraction is part of the normal process of healing but if excessive, it becomes pathologic and is known as a contracture (12). Deficient healing is the opposite of fibrosis; it exists when there is insufficient deposition of connective tissue matrix and the tissue is weakened to the point where it can fall apart. Chronic non-healing ulcers are examples of deficient healing. Regeneration is the elegant process that occurs when there is loss of structure and function but the organism has the sophisticated capacity to replace that structure by replacing exactly what was there before the injury. Lower forms of life, such as the salamander and crab, can regenerate tissues in this manner. As man has evolved, we have lost this capacity and can only replace a limited amount of damaged tissues by the process of regeneration. In humans the liver, epidermis and, to some extent, nerves can be partially regenerated after injury. In addition, our laboratory has examined the process of fetal tissue repair, and it appears that the fetus has the capability to repair tissue by a process that closely resembles true regeneration (13).

Basically, all dermal wounds heal by three basic mechanisms: connective tissue matrix deposition, contraction and epithelization. Wounds that are simple and can be closed by sutures, tape or staples heal by Primary Intention (14). The main mechanism of healing during Primary Intention is connective tissue matrix deposition, where collagen, proteoglycans and attachment proteins are deposited to form a new extracellular matrix. In contrast, wounds that remain open heal mainly by contraction; the interaction between cells and matrix results in movement of tissue toward the center of the wound. The underlying mechanisms responsible for contraction are not fully understood but there appears to be a complex interaction between contractile fibroblasts sometimes referred to as "myofibroblasts" and the matrix components (15). Some

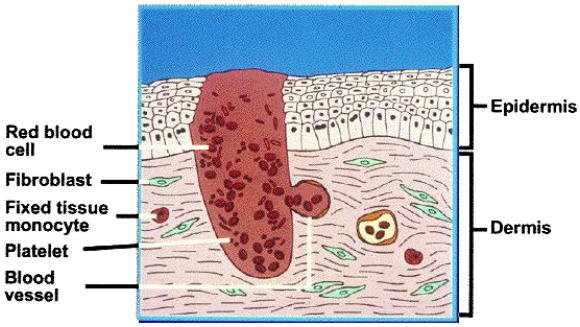

Figure 3. At the time of injury, the tissue is disrupted and the platelets adhere to the exposed collagen and to each other. The platelets release clotting factors, PDGF and TGF-ß to initiate the repair process.

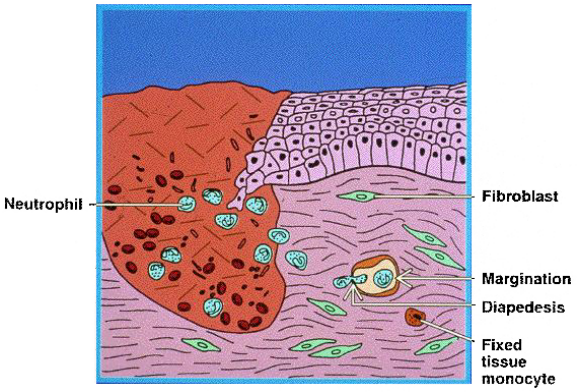

Figure 4. By the first day following injury, neutrophils attach to endothelial cells in the vessel walls surrounding the wound (margination), then change shape to move through the cell junctions (diapedesis) and migrate to the wound site (chemotaxis). This is the beginning of the inflammatory phase.

work has indicated that nerve growth factor and IL-8 can modulate the contraction response (16). Epithelization is the process where epithelial cells around the margin of the wound or in residual skin appendages such as hair follicles and sebaceous glands lose contact inhibition and begin to migrate into the wound area by the process termed "epiboly." (17) As migration proceeds, cells in the basal layers begin to proliferate to provide additional epithelial cells.

5. THE HEALING CASCADE

The healing cascade begins immediately following injury when the platelets come into contact with exposed collagen (Figure 3). As platelet aggregation proceeds, clotting factors are released resulting in the deposition of a fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing (18). Platelets not only release the clotting factors needed to control the bleeding and loss of fluid and electrolytes but they also provide a cascade of chemical signals, known as cytokines or growth factors, that initiate the healing response. The two most important signals are platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-ß) (5). The PDGF initiates the chemotaxis of neutrophils, macrophages, smooth muscle cells and fibroblasts. In addition it also stimulates the mitogenesis of the fibroblasts and smooth muscle cells.

TGF-ß adds another important signal for the initiation of the healing cascade by attracting macrophages and stimulates them to secrete additional cytokines including FGF (fibroblast growth factor), PDGF, TNFa (tumor necrosis alpha) and IL-1 (interleukin-1). In addition, TGF-ß further enhances fibroblast and smooth muscle cell chemotaxis and modulates collagen and collagenase expression. The net result of these redundant signals is a vigorous response of the matrix producing cells to ensure a rapid deposition of new connective tissue at the injury site during the *Proliferative* phase that follows the *Inflammato*ry phase.

Neutrophils are the next predominant cell marker in the wound within 24 hours after injury (Figure 4). The major function of the neutrophil is to remove foreign material, bacteria and non-functional host cells and damaged matrix components that may be present in the wound site (19, 20). Bacteria give off chemical signals, attracting neutrophils, which ingest them by the process of phagocytosis. During bacterial protein synthesis a waste product represented by a tri-peptide called f-Met-Leu-Phe is released which in turn attracts inflammatory cells (21). Neutrophils will engorge themselves until they are filled with bacteria and constitute what is called "laudable pus" in the wound (22).

The mast cell is another marker cell of interest in wound healing. Mast cells release granules filled with enzymes, histamine and other active amines and these mediators are responsible for the characteristic signs of inflammation around the wound site (23). The active amines released from the mast cell, causes surrounding vessels to become leaky and thus allow the speedy passage of the mononuclear cells into the injury area. In addition fluid accumulates at the wound site and the characteristic signs of inflammation begin. The signs of inflammation have been well recognized since ancient times: *rubor* (redness), *calor* (heat), *tumor* (swelling) and *dolor* (pain).

By 48 hours after injury, fixed tissue monocytes become activated to become wound macrophages (Figure 5). These specialized wound macrophages are perhaps the most essential inflammatory cells involved in the normal healing response (24). Inhibition of macrophage function will delay the healing response (25). Once activated these wound macrophages also release PDGF and TGF-ß that further attracts fibroblasts and smooth muscle cells to the wound site. These highly phagocytic macrophages are also responsible for removing nonfunctional host cells, bacteria-filled neutrophils, damaged matrix, foreign debris and any remaining bacteria from the wound site. The presence of wound macrophages is a marker that the *Inflammatory* phase is nearing an end and that the *Proliferative* phase is beginning. Lymphocytes come into the wound area at a later stage but are not considered to be major inflammatory

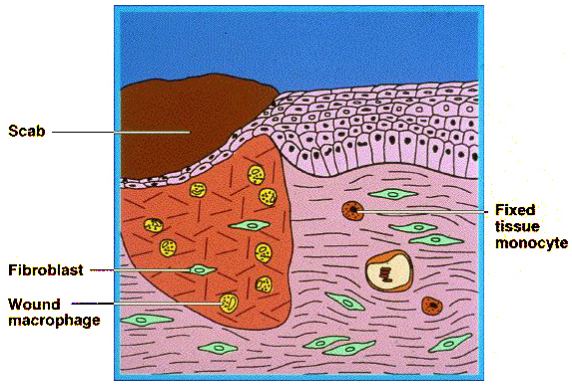

Figure 5. The inflammatory phase continues as fixed tissue macrophages become active and move into the site of injury and transform into very active wound macrophages. These highly phagocytic cells also release PDGF and TGF-ß to recruit fibroblasts to the site and thus begin the proliferative phase.

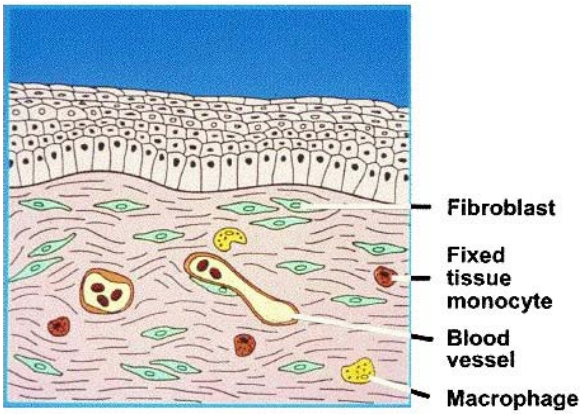

Figure 6. The remodeling phase is characterized by continued synthesis and degradation of the extracellular matrix components trying to establish a new equilibrium.

cells involved in the healing response; their precise role in the wound healing process remains unclear.

As the *Proliferative* phase progresses, the TGF-ß released by the platelets, macrophages and T lymphocytes becomes a critical signal. TGF-ß is considered to be a master control signal that regulates a host of fibroblast functions (26). TGF-ß has a three-pronged effect on extracellular matrix deposition (27). First, it increases transcription of the genes for collagen, proteoglycans and fibronectin thus increasing the overall production of matrix proteins. At the same time TGF-ß decreases the secretion of proteases responsible for the breakdown of the matrix and it also stimulates the protease inhibitor, tissue inhibitor of metallo-protease (TIMP) (28). Other cytokines considered to be important are interleukins, fibroblast growth factors and tumor necrosis factor-alpha (Table 1).

As healing progresses several other important biological responses are activated. The process of epithelization is stimulated by the presence of EGF (epidermal growth factor) and TGFa (transforming growth factor alpha) that are produced by activated wound macrophages, platelets and keratinocytes (Figure 6) (29, 30, 31). Once the epithelial bridge is complete, enzymes are released to dissolve the attachment at the base of the scab resulting in removal. Due to the high metabolic activity at the wound site, there is an increasing demand for oxygen and nutrients. Local factors in the wound microenvironment such as low pH, reduced oxygen tension and increased lactate actually initiate the release of factors needed to bring in a new blood supply (32, 33). This process is called angiogenesis or neovascularization and is stimulated by vascular endothelial cell growth factor (VEGF), basic fibroblast growth factor (bFGF) and TGFß (34, 35). Epidermal cells, fibroblasts, macrophages and vascular endothelial cells produce these factors. One interesting signaling pathway involves the role of low oxygen tension that in turn stimulates the expression of a nuclear transcription factor termed "hypoxia-inducible factor" (HIF) by vascular endothelial cells (36). The HIF in turn binds to specific sequences of DNA that regulate the expression of VEGF thus stimulating angiogenesis. As new blood vessels enter the wound repair area and the oxygen tension returns to a normal level, oxygen binds to HIF and blocks its activity leading to a decreased synthesis of VEGF.

As the Proliferative phase progresses the predominant cell in the wound site is the fibroblast. This cell of mesenchymal origin is responsible for producing the new matrix needed to restore structure and function to the injured tissue. Fibroblasts attach to the cables of the provisional fibrin matrix and begin to produce collagen (18). At least 23 individual types of collagen have been identified to date but type I is predominant in the scar tissue of skin (3). After transcription and processing of the collagen messenger ribonucleic acid, it is attached to polyribosomes on the endoplasmic reticulum where the new collagen chains are produced. During this process, there is an important step involving hydroxylation of proline and lysine residues (37). The collagen molecule begins to form its characteristic triple helical structure and the nascent chains undergo further modification by the process of glycosylation (38). The procollagen molecule is then secreted into the extracellular spaces where it is further processed (39). Hydroxyproline in collagen is important because it gives the molecule its stable helical conformation (40). Fully hydroxylated collagen has a higher melting temperature. When hydroxyproline is not present, for example in collagen produced under anaerobic or Vitamin C-deficient conditions (scurvy), the collagen has an altered structure and can undergo denaturation much more rapidly and at a lower temperature (37, 41). Finally, the collagen released into the extracellular space undergoes further processing by cleavage of the procollagen N and C-terminal peptides. In the extra-cellular spaces an important enzyme, lysyl oxidase, acts on the collagen to form stable cross-links. As the collagen matures and becomes older, more and more of these intramolecular and intermolecular cross-links are placed in the molecules. This important cross-linking step gives collagen its strength and stability over time (42).

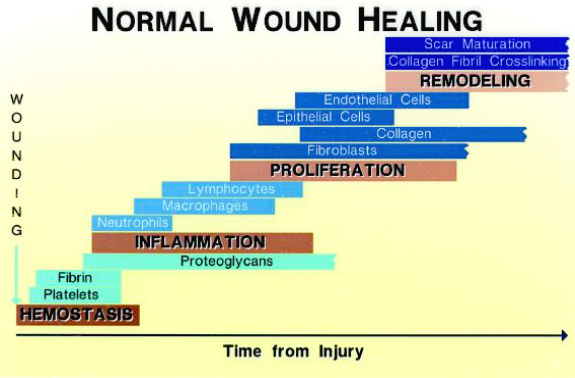

Figure 7. The sequence of events during normal wound healing. Reprinted with permission (45).

Dermal collagen on a per weight basis approaches the tensile strength of steel; in normal tissue it is a strong and highly organized molecule. In contrast, collagen fibers formed in scar tissue are much smaller and have a random appearance; scar tissue is always weaker and will break apart before the surrounding normal tissue. The regained tensile strength in a wound will never approach normal. In fact the maximum tensile strength that a wound can ever achieve is approximately 80% of normal skin.

Finally, in the process of collagen remodeling, collagen degradation also occurs (43, 44). Specific collagenase enzymes in fibroblasts, neutrophils and macrophages clip the molecule at a specific site through all three chains, and break it down to characteristic three-quarter and one-quarter pieces. These collagen fragments undergo further denaturation and digestion by other proteases.

In summary, the normal healing cascade begins with an orderly process of hemostasis and fibrin deposition, which leads to an inflammatory cell cascade, characterized by neutrophils, macrophages and lymphocytes within the tissue (45). This is followed by attraction and proliferation of fibroblasts and collagen deposition, and finally remodeling by collagen cross-linking and scar maturation (Figure 7). Despite this orderly sequence of events responsible for normal wound healing, pathologic responses leading to fibrosis or chronic ulcers may occur if any part of the healing sequence is altered.

6. FIBROSIS

Fibrosis can be defined as the replacement of the normal structural elements of the tissue by distorted, non-functional and excessive accumulation of scar tissue. This is perhaps the most significant biological marker for fibrosis. Many clinical problems are associated with excessive scar formation (46). For example, keloids and hypertrophic scars in the skin, tendon adhesions, transmission blockage following nerve injury, scleroderma, Crohn's disease, esophageal strictures, urethral strictures, capsules around breast implants, liver cirrhosis, atherosclerosis and fibrotic non-union in bone.

Keloids can be used as a clinical example of fibrosis to define some of the biochemical and cellular markers characteristic of fibrosis (11, 47). Fibroblasts isolated from keloids produce about 2 to 3 times more collagen compared to fibroblasts isolated from normal skin in the same patients (48). It appears that keloids have increased expression of TGFß and also an up-regulation of receptors for TGFß (49, 50). Hypertrophic scars are also characterized by excessive accumulation of scar collagen and are frequently misdiagnosed as keloids. There is one very significant biological marker that distinguishes keloids from hypertrophic scars and that is the absence of myofibroblasts in keloids and an abundance of these contractile cells in hypertrophic scars (51). It is also interesting to note that most conditions of fibrosis are characterized by an increased density of mast cells (52, 53). Mast cells contain specialized enzymes capable of processing procollagen and it has been suggested that abnormal peptides are produced that can actually stimulate collagen synthesis thus producing fibrosis (54).

7. CHRONIC ULCERS

Chronic non-healing dermal ulcers such as pressure ulcers contribute significantly to the morbidity and even mortality of many patients (55). Pressure ulcers are a serious and frequent occurrence among the immobile and debilitated patients. Spinal cord injury patients are particularly vulnerable to pressure ulcer formation. There are approximately 225,000 spinal cord injury patients in the United States, with approximately 9,000 new patients each year. Approximately 60% of these patients develop pressure ulcers, and the annual cost estimate ranges from $14,000 to $25,000 per patient for medical, surgical, and nursing care. If the elderly nursing home population with pressure ulcers is added to the spinal cord injury population, then the figure for the care of all pressure ulcers is enormous. The national expenditure for costs related to the care of patients with pressure ulcers is over $1.3 billion per year (56)! In addition, it is estimated that in the next 15 years the population over age 85 will increase from 4 million to over 17 million individuals! Therefore, this health care problem is increasing at a dramatic rate.

Excessive infiltration of these ulcers by neutrophils appears to be a significant biological marker. The over-abundant neutrophil infiltration is responsible for the chronic inflammation characteristic of non-healing pressure ulcers. The neutrophils release significant amounts of enzymes such as collagenase (matrix metalloproteinase-8) that is responsible for destruction of the connective tissue matrix (57, 58). In addition, the neutrophils release an enzyme called elastase that is capable of destroying important healing factors such as PDGF and TGF-ß (59). Another marker of these chronic ulcers is an environment containing excessive reactive oxygen species that further damage the cells and healing tissues (60). These chronic ulcers will not heal until the chronic inflammation is reduced. These wounds will not respond to the current high tech materials such as skin substitutes and topical cytokines such as PDGF until the wound bed is properly prepared by the skills of the wound care specialist (61, 62).

8. CONCLUSION

As we continue to develop new information about the unique biological markers associated with normal and pathologic wound healing responses, the better prepared we will be to develop new strategies to treat these costly clinical problems. In addition, understanding this basic biological information will allow wound care specialists greater insight into the importance of how their skills can impact the healing response.

9. REFERENCES

1. Lawrence, W. T., and Diegelmann, R. F., Growth factors in wound healing, *Clin Dermatol, 12*, 157 (1994)
2. Ross, R., Wound healing, *Sci Am, 220*, 40 (1969)
3. Prockop, D. J., and Kivirikko, K. I., Collagens: molecular biology, diseases, and potentials for therapy, *Annu Rev Biochem, 64*, 403 (1995)
4. Lazarus, G. S., Cooper, D. M., Knighton, D. R., Margolis, D. J., Pecoraro, R. E., Rodeheaver, G., and Robson, M. C., Definitions and guidelines for assessment of wounds and evaluation of healing, *Arch Dermatol, 130*, 489 (1994)
5. Kim, W. J., Gittes, G. K., and Longaker, M. T., Signal transduction in wound pharmacology, *Arch Pharm Res, 21*, 487 (1998)
6. Gillitzer, R., and Goebeler, M., Chemokines in cutaneous wound healing, *J Leukoc Biol, 69*, 513 (2001)
7. Friesel, R. E., and Maciag, T., Molecular mechanisms of angiogenesis: fibroblast growth factor signal transduction, *Faseb J, 9*, 919 (1995)
8. Ito, Y., Bringas, P., Jr., Mogharei, A., Zhao, J., Deng, C., and Chai, Y., Receptor-regulated and inhibitory Smads are critical in regulating transforming growth factor beta-mediated Meckel's cartilage development, *Dev Dyn, 224*, 69 (2002)
9. van Zuijlen, P. P., Angeles, A. P., Kreis, R. W., Bos, K. E., and Middelkoop, E., Scar assessment tools: implications for current research, *Plast Reconstr Surg, 109*, 1108 (2002)
10. Bock, O., and Mrowietz, U., Keloids. A fibroproliferative disorder of unknown etiology, *Hautarzt, 53*, 515 (2002)
11. Rahban, S. R., and Garner, W. L., Fibroproliferative scars, *Clin Plast Surg, 30*, 77 (2003)
12. Nedelec, B., Ghahary, A., Scott, P. G., and Tredget, E. E., Control of wound contraction. Basic and clinical features, *Hand Clin, 16*, 289 (2000)
13. Krummel, T. M., Nelson, J. M., Diegelmann, R. F., Lindblad, W. J., Salzberg, A. M., Greenfield, L. J., and Cohen, I. K., Fetal response to injury in the rabbit, *J Pediatr Surg, 22*, 640 (1987)
14. Summers, B. K., and Siegle, R. J., Facial cutaneous reconstructive surgery: general aesthetic principles, *J Am Acad Dermatol, 29*, 669 (1993)
15. Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C., and Brown, R. A., Myofibroblasts and mechano-regulation of connective tissue remodelling, *Nat Rev Mol Cell Biol, 3*, 349 (2002)
16. Iocono, J. A., Colleran, K. R., Remick, D. G., Gillespie, B. W., Ehrlich, H. P., and Garner, W. L., Interleukin-8 levels and activity in delayed-healing human thermal wounds, *Wound Repair Regen, 8*, 216 (2000)
17. Stenn, K. S., Epibolin: a protein of human plasma that supports epithelial cell movement, *Proc Natl Acad Sci U S A, 78*, 6907 (1981)
18. Clark, R. A., Fibrin and wound healing, *Ann N Y Acad Sci, 936*, 355 (2001)
19. Hart, J., Inflammation. 1: Its role in the healing of acute wounds, *J Wound Care, 11*, 205 (2002)
20. Sylvia, C. J., The role of neutrophil apoptosis in influencing tissue repair, *J Wound Care, 12*, 13 (2003)
21. Tschaikowsky, K., Sittl, R., Braun, G. G., Hering, W., and Rugheimer, E., Increased fMet-Leu-Phe receptor expression and altered superoxide production of neutrophil granulocytes in septic and posttraumatic patients, *Clin Investig, 72*, 18 (1993)
22. Thurston, A. J., Of blood, inflammation and gunshot wounds: the history of the control of sepsis, *Aust N Z J Surg, 70*, 855 (2000)
23. Artuc, M., Hermes, B., Steckelings, U. M., Grutzkau, A., and Henz, B. M., Mast cells and their mediators in cutaneous wound healing--active participants or innocent bystanders?, *Exp Dermatol, 8*, 1 (1999)
24. Diegelmann, R. F., Cohen, I. K., and Kaplan, A. M., The role of macrophages in wound repair: a review, *Plast Reconstr Surg, 68*, 107 (1981)
25. Leibovich, S. J., and Ross, R., The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum, *Am J Pathol, 78*, 71 (1975)
26. Roberts, A. B., and Sporn, M. B., Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta), *Growth Factors, 8*, 1 (1993)
27. Roberts, A. B., McCune, B. K., and Sporn, M. B., TGF-beta: regulation of extracellular matrix, *Kidney Int, 41*, 557 (1992)
28. Hall, M. C., Young, D. A., Waters, J. G., Rowan, A. D., Chantry, A., Edwards, D. R., and Clark, I. M., The comparative role of activator protein 1 and Smad factors in the regulation of Timp-1 and MMP-1 gene expression by transforming growth factor-beta 1, *J Biol Chem, 278*, 10304 (2003)
29. Yates, R. A., Nanney, L. B., Gates, R. E., and King, L. E., Jr., Epidermal growth factor and related growth factors, *Int J Dermatol, 30*, 687 (1991)
30. Schultz, G., Rotatori, D. S., and Clark, W., EGF and TGF-alpha in wound healing and repair, *J Cell Biochem, 45*, 346 (1991)
31. Hunt, T. K., Knighton, D. R., Thakral, K. K., Goodson, W. H., 3rd, and Andrews, W. S., Studies on inflammation and wound healing: angiogenesis and collagen synthesis stimulated in vivo by resident and activated wound macrophages, *Surgery, 96*, 48 (1984)
32. LaVan, F. B., and Hunt, T. K., Oxygen and wound healing, *Clin Plast Surg, 17*, 463 (1990)
33. Knighton, D. R., Hunt, T. K., Scheuenstuhl, H., Halliday, B. J., Werb, Z., and Banda, M. J., Oxygen tension regulates the expression of angiogenesis factor by macrophages, *Science, 221*, 1283 (1983)
34. Tonnesen, M. G., Feng, X., and Clark, R. A., Angiogenesis in wound healing, *J Investig Dermatol Symp Proc, 5*, 40 (2000)

35. Battegay, E. J., Angiogenesis: mechanistic insights, neovascular diseases, and therapeutic prospects, *J Mol Med, 73*, 333 (1995)
36. Gerber, H. P., Condorelli, F., Park, J., and Ferrara, N., Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia, *J Biol Chem, 272*, 23659 (1997)
37. Peterkofsky, B., Ascorbate requirement for hydroxylation and secretion of procollagen: relationship to inhibition of collagen synthesis in scurvy, *Am J Clin Nutr, 54*, 1135S (1991)
38. Blumenkrantz, N., Assad, R., and Peterkofsky, B., Characterization of collagen hydroxylysyl glycosyltransferases as mainly intramembranous microsomal enzymes, *J Biol Chem, 259*, 854 (1984)
39. Prockop, D. J., Sieron, A. L., and Li, S. W., Procollagen N-proteinase and procollagen C-proteinase. Two unusual metalloproteinases that are essential for procollagen processing probably have important roles in development and cell signaling, *Matrix Biol, 16*, 399 (1998)
40. Zanaboni, G., Rossi, A., Onana, A. M., and Tenni, R., Stability and networks of hydrogen bonds of the collagen triple helical structure: influence of pH and chaotropic nature of three anions, *Matrix Biol, 19*, 511 (2000)
41. Woodruff, C. W., Ascorbic acid--scurvy, *Prog Food Nutr Sci, 1*, 493 (1975)
42. Hornstra, I. K., Birge, S., Starcher, B., Bailey, A. J., Mecham, R. P., and Shapiro, S. D., Lysyl oxidase is required for vascular and diaphragmatic development in mice, *J Biol Chem, 278*, 14387 (2003)
43. Pilcher, B. K., Wang, M., Qin, X. J., Parks, W. C., Senior, R. M., and Welgus, H. G., Role of matrix metalloproteinases and their inhibition in cutaneous wound healing and allergic contact hypersensitivity, *Ann N Y Acad Sci, 878*, 12 (1999)
44. Parks, W. C., Matrix metalloproteinases in repair, *Wound Repair Regen, 7*, 423 (1999)
45. Mast, B. A., The Skin, in *Wound Healing; Biochemical and Clinical Aspects*, Cohen, I. K., Diegelmann, B., and Lindblad, W. J., Eds., W.B. Saunders Company, Philadelphia, pp. 344 (1992)
46. Kovacs, E. J., Fibrogenic cytokines: the role of immune mediators in the development of scar tissue, *Immunol Today, 12*, 17 (1991)
47. Shaffer, J. J., Taylor, S. C., and Cook-Bolden, F., Keloidal scars: a review with a critical look at therapeutic options, *J Am Acad Dermatol, 46*, S63 (2002)
48. Diegelmann, R. F., Cohen, I. K., and McCoy, B. J., Growth kinetics and collagen synthesis of normal skin, normal scar and keloid fibroblasts in vitro, *J Cell Physiol, 98*, 341 (1979)
49. Babu, M., Diegelmann, R., and Oliver, N., Keloid fibroblasts exhibit an altered response to TGF-beta, *J Invest Dermatol, 99*, 650 (1992)
50. Chin, G. S., Liu, W., Peled, Z., Lee, T. Y., Steinbrech, D. S., Hsu, M., and Longaker, M. T., Differential expression of transforming growth factor-beta receptors I and II and activation of Smad 3 in keloid fibroblasts, *Plast Reconstr Surg, 108*, 423 (2001)
51. Ehrlich, H. P., Desmouliere, A., Diegelmann, R. F., Cohen, I. K., Compton, C. C., Garner, W. L., Kapanci, Y., and Gabbiani, G., Morphological and immunochemical differences between keloid and hypertrophic scar, *Am J Pathol, 145*, 105 (1994)
52. Gruber, B. L., Mast cells in the pathogenesis of fibrosis, *Curr Rheumatol Rep, 5*, 147 (2003)
53. LeRoy, E. C., Trojanowska, M., and Smith, E. A., The pathogenesis of scleroderma (systemic sclerosis, SSc), *Clin Exp Rheumatol, 9*, 173 (1991)
54. Kofford, M. W., Schwartz, L. B., Schechter, N. M., Yager, D. R., Diegelmann, R. F., and Graham, M. F., Cleavage of type I procollagen by human mast cell chymase initiates collagen fibril formation and generates a unique carboxyl-terminal propeptide, *J Biol Chem, 272*, 7127 (1997)
55. Keller, B. P., Wille, J., van Ramshorst, B., and van der Werken, C., Pressure ulcers in intensive care patients: a review of risks and prevention, *Intensive Care Med, 28*, 1379 (2002)
56. Allman, R. M., The impact of pressure ulcers on health care costs and mortality, *Adv Wound Care, 11*, 2 (1998)
57. Nwomeh, B. C., Liang, H. X., Diegelmann, R. F., Cohen, I. K., and Yager, D. R., Dynamics of the matrix metalloproteinases MMP-1 and MMP-8 in acute open human dermal wounds, *Wound Repair Regen, 6*, 127 (1998)
58. Nwomeh, B. C., Liang, H. X., Cohen, I. K., and Yager, D. R., MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers, *J Surg Res, 81*, 189 (1999)
59. Yager, D. R., Zhang, L. Y., Liang, H. X., Diegelmann, R. F., and Cohen, I. K., Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids, *J Invest Dermatol, 107*, 743 (1996)
60. Wenk, J., Foitzik, A., Achterberg, V., Sabiwalsky, A., Dissemond, J., Meewes, C., Reitz, A., Brenneisen, P., Wlaschek, M., Meyer-Ingold, W., and Scharffetter-Kochanek, K., Selective pick-up of increased iron by deferoxamine-coupled cellulose abrogates the iron-driven induction of matrix-degrading metalloproteinase 1 and lipid peroxidation in human dermal fibroblasts in vitro: a new dressing concept, *J Invest Dermatol, 116*, 833 (2001)
61. Robson, M. C., Mustoe, T. A., and Hunt, T. K., The future of recombinant growth factors in wound healing, *Am J Surg, 176*, 80S (1998)
62. Falanga, V., Growth factors and wound healing, *Dermatol Clin, 11*, 667 (1993)

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