

Dermal ulcer healing: Advances in understanding

Thomas Mustoe

Dermal wound healing has been perhaps better studied than wound healing in other organs because of its accessibility for experimentation, and the frequency of wound healing problems. Most dermal wounds heal in uncomplicated fashion, but there are a large number of chronic wounds that fall into three major categories: venous ulcers, pressure sores, and diabetic foot ulcers, all with an occurrence rate of tens of thousands and an aggregate multibillion dollar health problem. Research efforts and therapeutic efforts have focused on these problem chronic wounds. In order to understand these focused research efforts it is necessary to understand normal wound healing.

NORMAL WOUND HEALING

Wound healing is the body's response to injury. Injury can be acute or chronic and involve multiple tissues, but wound healing is most clearly illustrated by examining the response to full-thickness injury (eg, a cut or an incision) to the epidermis and dermis. This injury sets in motion a sequence of interrelated reparative forces. Although the events overlap in time, it is helpful to consider the process as stages or phases of wound healing; these will be presented as separate events. This provides for clear conceptualization of the individual events and conforms with standard conventions. These events, however, do not occur independently, and the degree of temporal overlap is significant.

Inflammatory Phase

The inflammatory phase of acute wound healing begins immediately after injury. The initial response to the disruption of blood vessels is bleeding. The homeostatic response to this is clot formation to stop hemorrhage. Platelet plug formation initiates the hemostatic process along with clotting factors activated by collagen and basement membrane proteins exposed by the injury. Fibrin, produced by the clotting cascade, binds the platelet plug and forms a matrix for the cellular responses that follows. Platelet degranulation, the emptying of the granules into the extracellular space, provides the contents of α granules and dense granules, most notably platelet-derived growth factor (PDGF) and transforming growth factor β (TGF- β). These substances initiate chemotaxis and proliferation of inflammatory cells, beginning the inflammatory response that will ultimately heal the wound. Transient vasoconstriction is necessary to decrease blood loss at the time of the initial wounding and also to allow clot formation. Once a clot has been formed and active bleeding has stopped, vasodilation increases local blood flow to the wounded area, supplying cells and substrate necessary for further wound repair. The vascular endothelial cells also deform, increasing vascular permeability. The vasodilation and increased endothelial permeability are mediated by histamine, prostaglandin E_2 (PGE $_2$), and prostaglandin I_2 (prostacyclin; PGI $_2$) as well as vascular endothelial cell growth factor released by injured endothelial cells among other cells.

At this stage, the wound is full of debris from the initial injury. This material consists of a mixture of injured, devitalized tissue (fat, muscle, epithelium), clot (platelets, erythrocytes, fibrinogen), bacteria (from the skin surface and external environment), extravasated serum proteins (glycoproteins and mucopolysaccharides), and foreign material introduced at the time of injury (suture, dirt). During the next several days, the wound is cleared of bacteria, devitalized tissue, and foreign material by recruited and activated phagocytic cells. Polymorphonuclear leukocytes (PMNs) begin to arrive immediately, attaining large numbers within 24 hours. The

process of clearing the wound of debris usually takes several days, but the time varies depending on the amount of material to be cleared. The PMNs are followed temporarily by macrophages, which appear in wounds in significant numbers within 2 or 3 days. The macrophages are mononuclear phagocytic cells derived from circulating monocytes or resident tissue macrophages. They complete the process of removing all material not necessary for the ensuing steps of wound healing. The number of PMNs are regulated, and evidence from several kinds of experiments indicate that an excess of PMNs depress wound healing, probably due to an excess of reactive oxygen species (ROS) released by the PMNs, as well as excess proteases.

In the absence of significant bacterial contamination, macrophages promptly replace PMNs as the dominant cell type during the inflammatory phase. The role of the macrophages is not limited to phagocytosis.¹ In addition, macrophages are the sources of more than 30 different growth factors and cytokines. These growth factors induce fibroblast proliferation, endothelial cell proliferation (angiogenesis), and extracellular matrix production, and they recruit and activate additional macrophages. The result is the induction of a wound healing amplification cycle as growth factors recruit macrophages and elicit additional growth factor release. It has become clear in recent years that several chemokines are also important in attracting cells into the wound.

Proliferative Phase

The proliferative phase begins with the formation of a provisional matrix of fibrin and fibronectin as part of initial clot formation. Initially, the provisional matrix is populated by macrophages; however, by day 3, fibroblasts appear in the fibronectin–fibrin framework and initiate collagen synthesis. Fibroblasts proliferate in response to growth factors to become the dominant cell type during this phase. Growth factors produced by macrophages simultaneously induce angiogenesis, which induces the ingrowth and proliferation of endothelial cells, forming new capillaries. This neovascularity is visible through the epithelium and gives the wound a pink or purple-red appearance. Capillary ingrowth provides the fibroblasts with oxygen and nutrients to sustain cell proliferation and support the production of the permanent wound matrix. This is composed of collagen and proteoglycans or ground substance and replaces the provisional fibronectin–fibrin matrix.

A major recent focus of research has been on the potential importance of bone marrow derived endothelial progenitor cells and fibrocytes. Previously it had been believed that all mesenchymal cells were derived from the local tissue, but transgenic mouse studies have suggested that in some experimental conditions as many as 30% of local wound healing cells may be derived from the bone marrow. Under most circumstances that number is probably far lower, but their importance and potential role therapeutically are an important area for future research.

Remodeling Phase

The transition from the proliferative phase to the remodeling phase is defined by reaching collagen equilibrium. Collagen accumulation within the wound reaches a maximum within 2 to 3 weeks after wounding. Although supranormal rates of synthesis and degradation continue throughout remodeling, there is no further change in total collagen content. Tensile strength gradually increases as random collagen fibrils are replaced by organized fibrils with more intermolecular bonds. Collagenase activity is balanced against new production of collagen to produce a steady state. As equilibrium is achieved, the collagen fibrils align themselves in a longitudinal arrangement as dictated by stress placed on the wound. Scars never achieve the degree of order achieved by collagen in normal skin or tendons, but they do increase in strength for 6 months or longer, eventually reaching 70% of the strength of unwounded skin.

Epithelialization

The skin is composed of two layers, the epidermis and the dermis. The outermost layer, the epidermis, is the protective barrier that forms the external interface between the body and the environment. The epidermis protects against water loss, allowing the other cells of the body to live in a liquid environment, as well forming a barrier to bacteria and other environmental factors. Reconstitution of the epithelial barrier (epithelialization) begins within hours of the initial injury. As an initial step, epithelial cells from the basal layer at the wound edge flatten and migrate across the wound, completing wound coverage within 18 to 24 hours in a coated surgical wound. The cells along the margin are also dividing to reform the characteristic basilar to apical differentiation of multilayered mature epithelium.

In an open wound the epidermis must migrate across a surface of granulation tissue. If there is a crust or eschar, then collagenases and other metalloproteinases must be released to allow the epidermis to burrow underneath and lift the eschar. A logical conclusion, and one of the tenets of therapy for dermal ulcers is that moist wound healing (which prevents crusts and eschars from forming) speeds epithelialization. If the provisional matrix on the surface of the granulation tissue is degraded by proteases (with excess inflammation), then epithelialization does not occur, and the epithelial cells heap up at the edge of the ulcer. Epithelialization occurs only from the margins of the wound, at a maximal rate of 1 to 2 mm/d. In practice, adequate coverage of sizable wounds is rarely achieved. Thus, lower leg ulcers rarely heal faster than 1 cm/mo; that is, a 2-cm diameter ulcer typically takes 2 months to heal under ideal conditions.

After the first layer of cells restores the epithelial barrier, additional layers develop, restoring the basilar-to-apical order. As the cells mature, they resume keratin formation. This regenerates the stratum corneum of the epidermis and completes the restorative process of epithelialization and provides stable coverage. Reconstitution of the epidermis induces apoptosis of inflammatory cells, and mesenchymal cells in the underlying dermis with initiation of the maturation process. Delayed epithelialization in a younger patient with prolonged inflammation often results in hypertrophic excessive scarring. The importance of epithelial mesenchymal cell interactions is an active area of investigation. There are number of suggestions that growth factors released by the epidermis are important in regulating the scarring process, and until a mature stratum corneum completely restores the normal barrier to evaporation, homeostasis is not achieved, and the epidermis continues to release growth factors which stimulate collagen production and scar.

Wound Contraction

Wound contraction is an important event that contrasts the healing of open wounds with closed incisions. When open wounds contract, the surrounding skin is pulled over the wound to reduce its size. This can occur much faster than epithelialization. In addition to increasing the speed of wound closure, another advantage is that the open wound is resurfaced by the normal sensate skin surrounding the wound. Most animals are loose skinned—meaning that the skin (epithelium, dermis, subcutaneous fat) is only loosely attached to the underlying muscle fascia. Some animal wounds heal almost entirely by contraction; for example, a 2-cm ulcer heals to a 3-mm point in a loose skinned animal. Humans however, do not have this degree of skin mobility in most sites; the skin is tightly adherent and less elastic, especially in the lower leg. Therefore, although contraction may account for 90% of the reduction in wound size on the perineum, it accounts for at most 30% to 40% of the healing of a lower leg ulcer. This is one important reason why leg ulcers are so slow to heal. All healing wounds generate a strong contractile force. When this contractile force is exerted across a joint, such as the neck, axilla, or elbow, it may result in a scar contracture. A scar contracture is a scar that limits the functional range of motion of a joint.

At the cellular level, the forces that drive wound contraction come from the fibroblasts. Fibroblasts, like muscle cells, contain actin microfilaments. When these filaments increase in number, the cells take on the morphologic appearance of myofibroblasts. Myofibroblasts are seen in increased numbers in contracting wounds, but their role is unclear. It is unknown whether the fibroblasts that attach to the collagen fibrils by means of integrin receptors move collagen fibers together using a locomotor action or whether the contraction comes from intrinsic cellular contraction.

CHRONIC WOUNDS:

A practical definition of a chronic wound is one that has failed to heal within 3 months. Although there are a variety of underlying causes, most can be categorized as pressure sores, diabetic foot ulcers or leg ulcers. An important question is whether chronic wounds are intrinsically different than acute wounds. For instance, does local tissue senescence make healing a chronic wound impossible? Or, do chronic wounds have the inherent potential to heal, but a combination of factors lead to delayed healing? Research in the past few years has begun to address the question of what is different about the environment in a chronic wound.

Clinicians have long recognized that most chronic wounds do indeed have the potential to heal. Healing usually does not occur because of inadequate attention to the basic principles of open wound care—adequate cleansing, débridement, edema control, avoidance and treatment of ischemia, and achievement of a moist wound healing environment. However, the wound environment in many chronic wounds does differ in important ways from acute wounds. Studies have examined wound fluid and biopsies collected from chronic wounds. These reveal significant increases in tissue levels in proteases and collagenases, which are capable of degrading matrix proteins and growth factors. Degradation of growth factors inhibits their crucial functions of proliferation and chemotaxis. When wound fluid from a chronic wound is compared with fluid from an acute surgical wound, there is indeed a decreased level of growth factors. It is

not clear what influence bacteria have in this process, although the direct release of bacterial proteases and the indirect effect of protease release from phagocytic cells are both relevant. It is unknown whether growth factor levels are depressed because of proteolysis, primary inhibition of release, or secondary phenotypic changes in the cells of the chronic wound.

Another important factor in the genesis of chronic wounds is the fact that their occurrence is generally in the aged who have an impaired response to the stress factors of local tissue hypoxia, the increase in reactive oxygen species generated by inflammatory cells, and the existing bacterial burden. Most chronic wounds are of three types: pressure sores, diabetic foot ulcers, and venous leg ulcers. In all three cases, the inciting injury is an ischemia reperfusion injury. In the first two, pressure exceeding tissue perfusion resulting in a period of ischemia is followed by reperfusion. Repeated cycles of ischemia and reperfusion are much more damaging than an ischemic insult by itself. Experimental evidence in animals supports the notion of an increase in tissue damage in response to an ischemia reperfusion injury.

In chronic wounds there is some evidence that cells in the periphery of the wound can become senescent after the prolonged stimulus of many months. This is likely a more problematic factor in the elderly, and provides a theoretical basis for the efficacy of surgical debridement and excision of the “rind” or callus of a chronic wound, converting it to an acute wound, and converting a nonhealing wound to a healing wound.

Therapeutic approaches:

The role of oxygen: The role of oxygen as a therapeutic agent has been in part stimulated by an increased understanding of the role of oxygen in intracellular signaling. Previously oxygen was thought to play an essential role in oxidative metabolism, but oxygen sensors were thought to be limited to the kidney (erythropoietin synthesis), the carotid body and other specialized organs. However, there has been a great deal of interest, in recent years, on the existence in every cell of signal transduction pathways in response to hypoxia. These pathways are mediated by hypoxia inducible factor which is transported to the nucleus and binds to the promoter region to activate synthesis of many genes including growth factors, VEGF (vascular endothelial cell growth factor), and TGF β . Therefore, there is a plausible role for intermittent oxygen therapy to induce signal transduction and improve wound healing. The amount of oxygen necessary to result in a therapeutic effect is unknown, but in the acute surgical situation there is data indicating a beneficial effect from supplemental oxygen in the immediate postoperative period. For the treatment of chronic wounds, placing the wound in an enriched oxygen environment has had some anecdotal interest.

Growth factors

Growth factors have been a focus of research as potential therapeutic agents since the early 1990s. Only one agent, however, has been approved for human use. After a seven year process, involving 1,000 patients, platelet derived growth factor (PDGF, Regranex[®]) was approved in 1997 by the Food and Drug Administration (FDA) for use in diabetic foot ulcers. An increase in complete healing from approximately 30% of patients to 40% was achieved over a 5 month course of therapy. The modest magnitude of this effect, and the large number of patients that had to be studied undoubtedly explain the failure of other growth factor clinical trials which have been performed on at least 6 other growth factors by multiple companies.

Bioengineered skin equivalents

Another product, an artificial skin equivalent made of a dermis like matrix covered with cultured epithelium from heterologous human foreskins (Apligraf[®]) was FDA approved in 1998 for use in venous ulcers of more than one years duration that had failed other therapies. This products main effect is most likely due to the delivery of growth factors produced by the neonatal cells. Other skin equivalents including dermagraft utilize variations on dermal, epithelial and composite tissues. Although some therapeutic success has been achieved, their use has been limited by their expense, and limited shelf life. In the USA, the product Integra which is composed of a synthetic collagen glycosaminoglycan matrix (artificial dermis) covered by a silicone sheet (water barrier) has been used as a temporary surface for chronic wounds as well as burns with some clinical success.

Conclusions: Although there has been a great deal advance in the understanding of underlying principles of normal and altered wound healing, the complex etiology of chronic wounds have made therapeutic advances a challenging problem.