Understanding wound healing today involves much more than simply stating there are three phases: inflammation, proliferation, and maturation. Wound healing is a complex series of reactions and interactions among cells and “mediators.” Each year, new mediators are discovered and our understanding of inflammatory mediators and cellular interactions grows. Many intrinsic and extrinsic factors affect wound healing, and an enormous industry provides the clinician with a huge and complex armamentarium to battle wound-healing problems. This article will provide the reader with a wide overview of wound healing and wound-healing problems and solutions.

WOUND HEALING

Wound healing has traditionally been divided into three distinct phases: inflammation, proliferation, and remodeling.1,2 A detailed review of the basic science of wound healing can be found in this Supplement. This discussion will serve as a broad overview of clinical wound healing. Table 1 summarizes the inflammatory mediators by source and function.3,4

Hemostasis and Inflammation (from Immediately upon Injury through Days 4 to 6)

The inflammatory phase is characterized by hemostasis and inflammation. Collagen exposed during wound formation activates the clotting cascade (both the intrinsic and extrinsic pathways), initiating the inflammatory phase. After injury occurs, the cell membranes release the potent vasoconstrictors thromboxane A2 and prostaglandin 2-α. The clot that forms is made of collagen, platelets, thrombin, and fibronectin, and these factors release cytokines and growth factors (Table 2) that initiate the inflammatory response.5 The fibrin clot serves as scaffolding for arriving cells, such as neutrophils, monocytes, fibroblasts, and endothelial cells.6 It also serves to concentrate the cytokines and growth factors.7

Chemotaxis and Activation

Immediately after the clot is formed, a cellular distress signal is sent out and neutrophils are the first responders. As the inflammatory mediators accumulate, and prostaglandins are elaborated, the nearby blood vessels vasodilate to allow for the increase cellular traffic as neutrophils are drawn into the injured area by interleukin (IL)-1, tumor necrosis factor (TNF)-α, transforming growth fac-
tor (TGF)-β, platelet factor-4 (PF4), and bacterial “products.”\textsuperscript{8,9} Monocytes in the nearby tissue and in the blood will be attracted to the area and transform into macrophages, usually around 48 to 96 hours after injury. Activation of the inflammatory cells is critical, especially for the macrophage. An activated macrophage is important for the transition into the proliferative phase. An activated macrophage will mediate angiogenesis, fibroplasia, and synthesize nitric oxide.\textsuperscript{10}

Neutrophils will enter into the wound site and begin clearing it of invading bacteria and cellular debris. The neutrophil releases caustic proteolytic enzymes that will digest bacteria and nonviable tissue. The next cells present in the wound are the leukocytes and the macrophages (monocytes). The macrophage is essential for wound healing. Numerous enzymes and cytokines are secreted by the macrophage, including collagenases, which débride the wound; ILs and TNF, which stimulate fibroblasts (produce collagen) and promote angiogenesis; and TGF, which stimulates keratinocytes.

**Table 1. Summary of Inflammatory Cytokines*\textsuperscript{*}

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Cell of Origin</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>EGF</td>
<td>Platelets, macrophages</td>
<td>Mitogenic for keratinocytes and fibroblasts, stimulates keratinocyte migration</td>
</tr>
<tr>
<td>FGF</td>
<td>Macrophages, mast cells, T lymphocytes, endothelial cells</td>
<td>Chemotactic and mitogenic for fibroblasts and keratinocytes, stimulates angiogenesis</td>
</tr>
<tr>
<td>IFNs (α, β, and γ)</td>
<td>Lymphocytes, fibroblasts</td>
<td>Activate macrophages, inhibit fibroblast proliferation</td>
</tr>
<tr>
<td>ILs (1, 2, 6, and 8)</td>
<td>Macrophages, mast cells, keratinocytes, lymphocytes</td>
<td>IL-1: induces fever and adrenocorticotropic hormone release; enhances TNF-α and IFN-γ, activates granulocytes and endothelial cells; and stimulates hematopoiesis IL-2: activates macrophages, T cells, natural killer cells, and lymphokine-activated killer cells; stimulates differentiation of activated B cells; stimulates proliferation of activated B and T cells; and induces fever IL-6: induces fever and enhances release of acute-phase reactants by the liver IL-8: enhances neutrophil adherence, chemotaxis, and granule release</td>
</tr>
<tr>
<td>KGF</td>
<td>Fibroblasts</td>
<td>Stimulates keratinocyte migration, differentiation, and proliferation</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelets, macrophages, endothelial cells</td>
<td>Cell chemotaxis, mitogenic for fibroblasts, stimulates angiogenesis, stimulates wound contraction</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Macrophages, T lymphocytes, keratinocytes</td>
<td>Mitogenic for keratinocytes and fibroblasts, stimulates keratinocyte migration</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Platelets, T lymphocytes, macrophages, endothelial cells, keratinocytes</td>
<td>Cell chemotaxis stimulates angiogenesis and fibroplasia</td>
</tr>
<tr>
<td>Thromboxane A2</td>
<td>Destroyed wound cells</td>
<td>Potent vasoconstrictor</td>
</tr>
<tr>
<td>TNF</td>
<td>Macrophages, mast cells, T lymphocytes</td>
<td>Activates macrophages, mitogenic for fibroblasts, stimulates angiogenesis</td>
</tr>
</tbody>
</table>

EGF, epidermal growth factor; FGF, fibroblast growth factor; IFN, interferon; IL, interleukin; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor.


**Proliferative Phase (Epithelization, Angiogenesis, and Provisional Matrix Formation; Day 4 through 14)\textsuperscript{4,11}**

Epithelialization, angiogenesis, granulation tissue formation, and collagen deposition are the principal steps in this building portion of wound healing. Epithelialization occurs early in wound repair. If the basement membrane remains intact, the epithelial cells migrate upward in the normal pattern. The epithelial progenitor cells remain intact below the wound (in skin appendages), and the normal layers of epidermis are restored in 2 to 3 days. If the basement membrane has been destroyed, then epithelial cells located on the skin edge begin proliferating and sending out projections to re-establish a protective barrier. Angiogenesis, stimulated by TNF-α, is marked by endothelial cell migration and capillary formation. The migration of capillaries into the wound bed is critical for proper wound healing. The granulation phase and tissue deposition require nutrients supplied by the capillaries, and failure of this to
occur results in a chronically unhealed wound. Epithelial cells located on the skin edge begin proliferating and sending out projections to re-establish a protective barrier against fluid losses and further bacterial invasion. The stimulus for epithelial proliferation and chemotaxis is epidermal growth factor (EGF) and TGF-α produced by activated platelets and macrophages (fibroblasts do not appear to synthesize TGF-α).\(^4\,11\) Epithelialization begins shortly after wounding and is first stimulated by inflammatory cytokines, IL-1 and TNF-α upregulate keratinocyte growth factor (KGF) gene expression in fibroblasts. In turn, fibroblasts synthesize and secrete KGF-1, KGF-2, and IL-6, which simulate neighboring keratinocytes to migrate in the wound area, proliferate, and differentiate in the epidermis.\(^12\,13\) It has been shown that, for humans, KGF-2 is most important for directing this process.\(^14\)

The final part of the proliferative phase is granulation tissue formation. Fibroblasts migrate into the wound site from the surrounding tissue, become activated, and begin synthesizing collagen and proliferate. Platelet-derived growth factor (PDGF) and EGF are the main signals to fibroblasts and are derived from platelets and macrophages. PDGF expression by fibroblasts is amplified by autocrine and paracrine signaling. Fibroblasts already located in the wound site (termed “wound fibroblasts”) will begin synthesizing collagen and transform into myofibroblasts for wound contraction (induced by macrophage-secreted TGF-β1); they have less proliferation compared with the fibroblasts coming in from the wound periphery.\(^15\,17\) In response to PDGF, fibroblasts begin synthesizing a provisional matrix composed of collagen type III, glycosaminoglycans, and fibronectin.\(^18\)

### Maturation and Remodeling (Day 8 through Year 1)

Clinically, the maturation and remodeling phase is perhaps the most important. The main feature of this phase is the deposition of collagen in an organized and well-mannered network. If patients have matrix deposition problems (from diet or disease), then the wound’s strength will be greatly compromised; if there is excessive collagen synthesis, then a hypertrophic scar or keloid can result.

Net collagen synthesis will continue for at least 4 to 5 weeks after wounding. The increased rate of collagen synthesis during wound healing is not only from an increase in the number of fibroblasts but also from a net increase in the collagen production per cell.\(^19\,20\) The collagen that is initially laid down is thinner than collagen in uninjured skin and is orientated parallel to the skin. Over time, the initial collagen threads are reabsorbed and deposited thicker and organized along the stress lines. These changes are also accompanied by a wound with an increased tensile strength, indicating a positive correlation between collagen fiber thickness/orientation and tensile strength.\(^5\) The collagen found in granulation tissue is biochemically different from collagen from uninjured skin. Granulation tissue collagen has a greater hydroxylation and glycosylation of lysine residues, and this increase of glycosylation correlates with the thinner fiber size.\(^21\) The collagen in the scar (even after a year of maturing) will never become as organized the collagen found in uninjured skin. Wound strength also never returns to 100 percent. At 1 week, the wound has only 3 percent of its final strength; at 3 weeks, 30 percent; and at 3 months (and beyond), approximately 80 percent.\(^22\)

### Table 2. Mediators Found in a Blood Clot

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrin, plasma fibronectin</td>
<td>Coagulation, chemoattraction, adhesion, scaffolding for cell migration</td>
</tr>
<tr>
<td>Factor XIII (fibrin-stabilizing factor)</td>
<td>Induces chemoattraction and adhesion</td>
</tr>
<tr>
<td>Circulatory growth factors</td>
<td>Regulation of chemoattraction, mitogenesis, fibroplasia</td>
</tr>
<tr>
<td>Complement</td>
<td>Antimicrobial activity, chemoattraction</td>
</tr>
<tr>
<td>Cytokines, growth factors</td>
<td>Regulation of chemoattraction, mitogenesis, fibroplasia</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Early matrix, ligand for platelet aggregation</td>
</tr>
<tr>
<td>Platelet-activating factor</td>
<td>Platelet aggregation</td>
</tr>
<tr>
<td>Thromboxane A₂ (via platelet COX-1)</td>
<td>Vasoconstriction, platelet aggregation, chemotaxis</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Induces vascular permeability, chemoattraction for neutrophils</td>
</tr>
<tr>
<td>Platelet factor IV</td>
<td>Chemotactic for fibroblasts and monocytes, neutralizes activity of heparin, inhibits collagenase</td>
</tr>
</tbody>
</table>

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FACTORS IN WOUND HEALING

The complexity of wound healing makes it vulnerable to interruption at many levels. Factors that affect physiologic responses and cellular function can potentially influence wound healing. This section will briefly highlight the effect several factors have on wound healing.

Local Factors

Several local factors can greatly influence wound healing. Ischemic tissues, wounds with foreign bodies, infection, contamination, and so on, will all affect wound healing. The Venn diagram (Fig. 1) highlights these obstacles.

Ischemia

Healing is an energy-dependent process and requires an adequate supply of the energy currency, adenosine triphosphate (ATP). The initial anaerobic conditions following injury stimulate cells to adopt anaerobic production of ATP via glycolysis.23 The proliferative phase of healing is characterized by increased metabolism and protein synthesis requiring much larger quantities of ATP via oxidative phosphorylation. This demands a rich blood supply to provide glucose and oxygen. Hypoxia (or decreased glucose) has the potential to slow or halt the healing process.24

The physiologic response of the vascular endothelium to localized hypoxia in the early phase of wound healing is to precipitate vasodilation, stimulate fibrin deposition, and increase proinflammatory activity, capillary leak, and neovascularization. The endothelial cell response to sustained hypoxia is a TNF-α induction of apoptosis. Sustained hypoxia will result in endothelial cell apoptosis.25

Wound neutrophil activity has been demonstrated to be impaired at lower oxygen tensions identified in wounds. Low temperature, low pH, and elevated glucose concentrations also limit leukocyte function.26 Fibroblasts exposed to longer periods of hypoxia may not participate in the formation of the extracellular matrix, thus delaying healing.27

Infection

Local wound infection and foreign bodies affect healing by prolonging the inflammatory phase. If the bacterial count in the wound exceeds 10⁵ organisms per gram of tissue, or if any beta-hemolytic Streptococcus is present, the wound will not heal by any means, including flap closure, skin graft placement, or primary sutures.28 The bacteria prolong the inflammatory phase and interfere with epithelialization, contraction, and collagen deposition. The endotoxins themselves stimulate phagocytosis and the release of collagenase, which contributes to collagen degradation and destruction of surrounding, previously normal tissue. Wound contamination in association with tissue hypoxia potentially suppresses macrophage-regulated fibroblast proliferation.29

Foreign Bodies

Foreign bodies (which also include nonviable tissue) are a physical obstacle to wound healing and an asylum for bacteria. Foreign bodies prolong the inflammatory phase. Wounds with foreign bodies cannot contract, repopulate the area with capillaries, or completely epithelize (depending on the size and location of the foreign body). Wounds with necrotic tissue will not heal until all the necrotic tissue is removed.30

Edema/Elevated Tissue Pressure

The edema and locally raised pressures associated with ischemic tissue injury can potentially further compromise perfusion. The inflammatory response to wounding may be prolonged as a result, thus delaying the healing process. This is dramatically demonstrated in the development of compartment syndrome in limb skeletal muscles following ischemia and reperfusion.31 Mast cells in skeletal muscle have been demonstrated to produce most of the nitric oxide associated with ischemia-reperfusion injury.32 Mast cells are resident...
tissue inflammatory cells that, when stimulated, release numerous cytokines and histamines responsible for an intense inflammatory reaction and edema.

Raised tissue pressure, either externally (pressure) or internally (compartment syndrome), induces increased capillary closure through its effect on critical closing pressures. Compromised cellular function due to prolonged, severe hypoxia may rapidly progress to cell death, with necrosis of skin, adipose tissue, and muscle, thus precipitating ulceration and further contributing locally to impaired healing. In critical illness, additional risk factors, including depletion of soft-tissue adipose and protein components, tissue edema due to lowered plasma oncotic pressures, leaky endothelium, and potentially compromised peripheral perfusion, may further compromise tissue perfusion by raising interstitial pressures.31

**SYSTEMIC OBSTACLES**

A patient may already have characteristics that predispose him or her to soft-tissue wound-healing dysfunction. This has been studied in surgical patients in an attempt to predict wound complications in abdominal and breast reconstructive surgery.35-35

These characteristics include obesity, cardiovascular disease affecting tissue perfusion, respiratory disease affecting adequate blood oxygenation, metabolic disease, endocrine disease, and renal and hepatic failure. Diabetes mellitus affects soft-tissue healing via metabolic, vascular, and neuropathic pathways, as typified in diabetic foot disease. Increasing age is associated with delayed healing, but it is difficult to separate the effects of age alone from those diseases commonly associated with age.37 Supplementation using topical estrogen has been demonstrated to improve healing in elderly women.38

Malignancy and its treatments can have profound effects on the ability of a patient to mount a response to injury and infection. Poor nutrition, specific organ compromise, ectopic hormone production, and immune and hematological effects, together with aggressive therapies, including potent antimetabolic, cytotoxic, and steroid agents and radiation, are all associated with compromised immunity, increased susceptibility to sepsis, and failure of tissue repair.39-41

The stresses associated with critical illness may further significantly affect healing. Critical illness places higher demands on tissue oxygen supply because of the stresses of acute illness and the requirements of increased cellular activity associated with wound healing.42

**Diabetes Mellitus**

Increased serum glucose has a major effect on wound healing. This is most likely a multifactorial process due to hyperglycemia-related deleterious effects on molecular and cellular physiology. Although traditional teaching is that the etiology of diabetic complications is microvascular occlusive disease, recent research does not support this.43 Several defects that may influence the healing process are now known. Sorbitol, a toxic byproduct of glucose metabolism, accumulates in tissues and is implicated in many of the renal, ocular, and vascular complications associated with diabetes.44 Increased dermal vascular permeability results in pericapillary albumin deposition, which impairs the diffusion of oxygen and nutrients.45 Hyperglycemia-associated nonenzymatic glycosylation inhibits the function of structural and enzymatic proteins. In addition, glycosylated collagen is resistant to enzymatic degradation and less soluble than the normal protein product.44

Experimental studies of diabetic animal models and wound healing show decreased granulation, decreased collagen in granulation tissue, and defects in collagen maturation.45 Human studies of healing in diabetic patients parallel these findings, showing slow wound maturation and decreased numbers of dermal fibroblasts. Growth factor abnormalities have also been shown in recent studies.

**Hypothyroidism**

Experimental data and anecdotal series show that hypothyroidism causes delayed wound healing.46,47 Rats pharmacologically rendered hypothyroid have decreased collagen production, as measured by extractable soluble hydroxyproline levels.48 Hypothyroidism also significantly decreases wound tensile strength in rats.49 Irradiation of hypothyroid pigs resulted in poor healing.50 These studies indicate the deleterious effect of inadequate thyroid hormone levels on fibroblast function and subsequent wound strength. Compounding these local effects are the systemic complications (i.e., higher risk of heart failure, risk of intraoperative hypotension, neuropsychiatric disturbances, and lack of postoperative fever) for which the postoperative hypothyroid patient is at risk.51

**Age**

It is well accepted that aging patients have higher rates of complications and mortality than do younger persons undergoing major surgery.52
It also is widely accepted that elderly patients heal more slowly than younger patients.\(^53,54\) This information should be balanced with the fact that older patients generally have more comorbidities than younger patients. Coronary artery disease, peripheral vascular disease, diabetes, and pulmonary compromise are more common with advanced age and may affect the healing process independent of age. Experimental studies show that the inflammatory and proliferative phases are less efficient in older animals, particularly compared with very young subjects.\(^54\) A longitudinal study of hamster fibroblast cultures over the span of the animal’s life showed progressively decreasing proliferative capacity over time.\(^55\) Studies of healthy human volunteers do not completely support these findings. Skin graft donor sites have slower reepithelialization rates in older persons, although the deposition of dermal collagen is equivalent between young and older, healthy volunteers. The fact that proteins other than collagen are less abundant in older subjects’ wounds may indicate age-related differences in the production of the extracellular matrix.\(^56\) Studies suggest that the defect in age-related wound healing is related to abnormal initiation of healing as a result of insufficient presence of growth factors.\(^57\)

Fetal Wound Healing

Tissue repair in the mammalian fetus is fundamentally different from normal postnatal healing. “In adult humans, injured tissue is repaired by collagen deposition, collagen remodeling, and eventual scar formation. [In contrast], fetal wound healing seems to be more of a regenerative process with minimal or no scar formation.”\(^58\)

Siebert et al.\(^59\) examined healing fetal wounds histologically and biochemically and found that they contained a small amount of collagen identical to that found in the exudate from wounds in adults (i.e., type III collagen but no type I). The fetal wound matrix was also rich in hyaluronic acid, which has been associated experimentally with decreased scarring postnatally. The authors proposed a mechanism of a hyaluronic acid/collagen/protein complex acting in fetal wound healing to check scar formation, and concluded that healing in fetuses involved a much more efficient process of matrix reorganization than that which takes place after birth. True regeneration apparently does not play a role in fetal healing, based on the few appendage elements seen.

Rowse\(^60\) suggests that the collagen present in fetal wounds is “structural” rather than “scar tissue.”

In their review of scarless wound healing in the mammalian fetus, Mast and coworkers\(^58\) state that “a striking difference between postnatal and fetal repair is the absence of acute inflammation in fetal wounds,” and offer several hypotheses to explain this phenomenon. Epithelialization occurs at a much faster rate in fetal wounds, but adult-like angiogenesis is absent. More importantly, the fetal wound matrix is markedly different from the adult’s in that it lacks collagen and instead contains predominantly hyaluronic acid.\(^25,62\) The fetal wound contains a persistent abundance of hyaluronic acid, while collagen deposition is rapid, nonexcessive, and highly organized,\(^53\) so that the normal dermal structure is restored and scarring does not occur. The authors speculate about the applications of scarless fetal healing, namely, for intrauterine repair and in the treatment of pathologic, postnatal processes.

Whitby and Ferguson\(^61\) conclude that it may be possible to manipulate the adult wound to produce more fetal-like, scarless wound healing by therapeutically altering the levels of growth substances and their inhibitors. This hope is shared by other groups,\(^64-\text{69}\) though it has not yet emerged in the clinical setting. Bone morphogenetic protein-2,\(^70\) hypoxia-inducible factor 1α,\(^71\) decorin (a TGF-β modulator),\(^72\) α- and β-fibroblast growth factor,\(^73-74\) IL-6,\(^74\) and IL-8\(^69\) are also under study.

Tenascin (cytotactin) is a large, extracellular matrix glycoprotein synthesized by fibroblasts that is present during embryogenesis but only sparsely distributed in the connective tissue papillae of adults. Tenascin is present earlier in fetal wounds, compared with adult wounds, and may be responsible for initiating cell migration and the rapid epithelialization of fetal wounds.\(^75\) Some investigators\(^25,76\) believe that tenascin could be a modulator of cell growth and movement and that it may influence the deposition and organization of other extracellular matrix glycoproteins during tissue repair.
Tissue Perfusion
Disruption of tissue perfusion can be categorized as local (from small vessel occlusion-emboli or external compression) or general (from decreases in circulatory volumes, cardiac insufficiency, inotropic infusions, or large vessel disruption). Inadequate tissue perfusion (the definition of shock) results in tissue hypoxia.

Hypothermia and Pain
Hypothermia also has a profound effect on cutaneous perfusion by inducing peripheral vasoconstriction; therefore, the thermal insulation of wounds is important. Painful stimuli cause a diffuse adrenergic discharge, leading to cutaneous vasoconstriction; thus, adequate pain control can improve cutaneous perfusion and potentially improve healing.

Major Trauma and Burns
Severe trauma and tissue loss can result in hypovolemic shock as a consequence of circulatory volume loss or compromised cardiac function. The systemic inflammatory response syndrome that occurs in major trauma states is characterized by elevation of circulating cytokines and inflammatory mediators, including TNF-α, resulting in activation and consumption of clotting factors and platelets potentially leading to clotting anomalies, disseminated intravascular coagulation, and subsequent delays in wound healing. The infusion of large volumes of packed red blood cells also will affect coagulation, largely by dilutional effects, as will cold intravenous fluid replacement. The development of a posttraumatic immunoparalysis has implications in developing sepsis and subsequent delays in wound healing. Maintenance of a normovolemic state and body temperature together with good analgesia will facilitate adequate peripheral perfusion to soft-tissue injuries. Deep soft-tissue burns, in particular, elicit a major and often prolonged local inflammatory response associated with local tissue ischemia, thrombosis, and an intense vasoconstriction resistant to the effects of nitric oxide. There is evidence that peripheral vasoconstriction persists for up to 60 hours after hypovolemia, despite adequate resuscitation and a normal mean arterial pressure. Mild or moderate anemia does not appear to deleteriously affect healing in a well-perfused wound, with collagen deposition being proportional to wound tissue oxygenation and perfusion. The reperfusion of injured tissue itself can be deleterious to wound healing, with the release of anaerobic metabolites and reactive oxygen species creating additional oxidative stresses. Fat emboli and disseminated intravascular coagulation following severe trauma can cause respiratory complications and occlusion of both larger and smaller cutaneous vessels, as well as general and focal tissue hypoxia and multiple-organ dysfunction syndrome.

Sepsis
The sepsis-associated mortality rate in an intensive care setting remains high. The endotoxin-related excessive secretion of proinflammatory mediators is believed to be important in the development of whole-body inflammation and septic shock. Systemic inflammation in sepsis appears to have a biphasic pattern, which includes a proinflammatory state associated with immunodeficiency characterized by monocyte deactivation and immunoparalysis. A compromised leukocytic activity and deranged inflammatory response will have inhibitory effects on wound healing.

Septicemia can have profound effects on coagulation. Activation of the clotting cascade, via plasma- and endothelial-related inflammatory changes, can lead to disseminated intravascular coagulation with a profound decrease in platelets and loss of a functional, organized clotting system. Disseminated intravascular coagulation in sepsis, as in major trauma, is associated with microcirculatory thrombosis, with potentially widespread effects on tissue perfusion in the soft tissues and major organs leading to multiple-organ failure. These factors will compromise hemostasis and, therefore, healing. The systemic inflammatory responses in sepsis affect plasma viscosity and erythrocyte rheologic characteristics. This may have important implications in tissue perfusion, particularly in situations where the capillary blood velocities are reduced. Hypothermia, potentially made worse by the infusion of cold intravenous fluid and fresh-frozen plasma (or an equivalent), will compromise clotting and healing.

Specific Organ Failure
Gut Failure
A critically ill patient may have impaired gut absorption, either directly as a result of abdominal catastrophe or gastrointestinal surgery or indirectly as a result of dysfunctional bowel secondary to severe metabolic anomaly, trauma, or denervation causing a paralytic ileus. Bacterial translocation of gut bacteria and toxins to the portal cir-
calculation can occur, causing sepsis and increased oxidative stress.

**Hepatic Failure**

Decreased clotting factors, low plasma proteins, decreased bactericidal activity, and failure of glucose regulation can all contribute to soft-tissue wound failure.

**Renal Failure**

Acute or chronic renal impairment may require dialysis treatment. Raised levels of uremic toxins and metabolic acidosis will affect wound healing by influencing both the innate and adaptive immune systems. Hemodialysis and peritoneal dialysis are associated with increased susceptibility to infections. Downregulation of circulating neutrophils due to their repeated activation triggering by dialysis membranes has been demonstrated. Circulating reactive oxygen species are also increased as a result of dialysis membrane activation. Deficient responses of both B and T lymphocytes also are associated with renal dialysis.

**Respiratory Failure**

Adequate gaseous exchange is essential for all biological systems, including wound healing. Tissue hypoxia secondary to hypoxemia will have profound effects on healing at all levels.

**Nutrition**

Septic, surgical, and trauma patients in hypermetabolic states associated with the release of endogenous cytokines from activated leukocytes experience excessive protein loss in an effort to maintain normoglycemia; these patients require additional caloric input to counter a negative nitrogen balance. Such patients consume body stores of fat and protein, particularly skeletal muscle, more rapidly than patients with normal metabolism. This, together with depletion of micronutrients and immunonutrients, has implications in immune system function and healing. Elevated steroid levels because of stress are intimately involved in muscle catabolism. Insulin administration may help modulate muscle protein losses in patients with severe burns. Growth hormone stimulates wound healing, but its effects in critical illness need further study.

Glucose is the main fuel for wound repair. Protein malnutrition and particularly deficiencies in the amino acids arginine and methionine are associated with compromised wound healing because of prolonged inflammation and disruption of matrix deposition, cellular proliferation, and angiogenesis. Malnutrition is associated with decreased deposition of collagen in skin wounds. Glutamine has been reported to enhance the actions of lymphocytes, macrophages, and, in particular, neutrophils, and may be of particular benefit in severe infection and trauma. Glycine has inhibitory effects on leukocytes and may have an important role in reducing inflammation-related tissue injury. Micronutrients such as vitamins and minerals are critically important in immune function and wound healing. Many trace metals, including manganese, magnesium, copper, calcium, and iron, are cofactors in collagen production, and deficiencies influence collagen synthesis. Zinc influences reepithelialization and collagen deposition. Zinc has also been demonstrated to greatly influence B and T lymphocyte activity, but many other nutrients, including copper, selenium, several other metals, and several vitamins, including A, B, C, and E, have been implicated in immune dysfunction. Vitamin C is the main vitamin associated with poor healing, because of its influence on collagen modification. L-Arginine is required in a variety of metabolic functions, wound healing, and endothelial function. It is important in the synthesis of nitric oxide, and deficiency is linked to immune dysfunction and failure of wound repair. Maintenance of plasma oncotic pressure is dependent on adequate production of plasma proteins and is important in maintaining body water distribution and preventing soft-tissue edema. Vitamins and minerals, particularly ascorbic acid, zinc, and selenium, are essential for wound repair. Copper recently has been linked to the production of vascular endothelial growth factor.

**Smoking**

Clinicians have long suspected that smoking has a poisonous effect on healing wounds, especially postsurgical flaps and grafts. In 1977, Mosely and Finseth demonstrated the detrimental effect of smoking on healing hand wounds. Many studies have since confirmed that smoking is harmful to a healing wound. Goldminz and Bennett reviewed 916 flaps and full-thickness grafts and found that 1-pack-per-day smokers had three times the frequency of necrosis as nonsmokers and that 2-pack-per-day smokers had necrosis six times more frequently than nonsmokers did. The mechanism of these harmful effects is likely multifactorial. Nicotine is an addictive and vasoconstrictive substance that decreases proliferation of erythrocytes, macrophages, and fibroblasts. Hydrogen cyanide is inhibitory to oxidative metabolism enzymes. Carbon monoxide decreases the
oxygen-carrying capacity of hemoglobin by competitively inhibiting oxygen binding. In one study using human volunteers, subcutaneous partial pressure of oxygen decreased significantly after 10 minutes of cigarette smoking. The effect lasted for almost 1 hour. Taken together, this triad has obvious implications for reduction of the cellular response and efficiency of the healing process.

Smoking also increases platelet aggregation and blood viscosity and decreases collagen deposition and prostacyclin formation, all of which negatively affect wound healing. Vasconstriction associated with smoking is not a transient phenomenon. Smoking a single cigarette may cause cutaneous vasconstriction for up to 90 minutes; hence, a pack-a-day smoker sustains tissue hypoxia for most of each day.

Smoking also affects the cosmetic appearance of wounds, which has serious ramifications for the smoker who desires facial cosmetic surgery.

Corticosteroids

Anti-inflammatory steroid medications globally inhibit cell growth and production. They are also well known to have widespread negative effects on the wound-healing process. This is seen clinically and experimentally. A decreased inflammatory infiltrate is the most obvious effect of steroids. The macrophage response to chemotactic factors is inhibited. Effective phagocytosis by polymorphonuclear neutrophils and macrophages also is decreased as a result of the stabilizing effect of steroids on lysosomes. Because of the lack of an appropriate initial inflammatory response, these cells do not produce the typical growth factor profile. This has been shown experimentally. Glucocorticoids also inhibit epithelial regeneration. The application of hydrocortisone to epidermal cultures decreases cell proliferation.

Steroids have a direct inhibitory effect on the fibroblast genome, which has been shown in cell culture. Corticosteroid-treated rat fibroblasts have minimal endoplasmic reticulum, indicating a low-secretory state. Without the appropriate deposition and maturation of collagen, there is less wound strength and wound dehiscence is likely. Vitamin A restores the inflammatory response and promotes epithelialization and the synthesis of collagen and ground substances. Vitamin A does not reverse the detrimental effects of glucocorticoids on wound contraction and infection. The recommended dose of vitamin A is 25,000 IU by mouth daily preoperatively and for 3 days postoperatively. Vitamin A supplementation should not be given to pregnant women.

Ionizing Radiation and Chemotherapy

Ionizing radiation either directly damages the genome or injures the DNA through the production of free radicals. This effect has short- and long-term consequences for radiated tissue. In patients with acute radiation injury, the skin becomes erythematous and edematous. Histologically, dilation of fine blood vessels, endothelial edema, and lymphatic obliteration are seen. As the acute response abates, thrombosis and fibrinoid necrosis of capillaries occur. In this setting, although perfusion of radiation-damaged skin is seen with fluorescein injection, effective tissue oxygenation is inadequate. Blood vessel appearance is varied, either thick-walled, telangiectatic, or thrombosed. The deposition of dense, hyalinized collagen characterizes radiated dermis. The endpoint of chronic radiation damage is the nonhealing ulcer. Obvious necrotic tissue is seen, and there is loss of epithelial coverage. Electron microscopy of human radiation ulcers reveals varying degrees of vascular injury and few myofibroblasts.

The mechanisms of radiation damage and inhibited wound healing are currently being studied. Experimentally, healing immediately after irradiation is hampered by slowed fibroblast proliferation, migration, and contraction. Impairment of the acute inflammatory response and granulation formation also occurs. The production of mRNA coding for collagen is delayed up to 2 weeks. Clinically, few surgical procedures are performed on patients with acutely radiated tissues. The greater concern lies with the patient who has chronic radiation damage.

Fibroblast cultures from radiation ulcers proliferate at a slower rate compared with cultures of cells from nearby unirradiated tissue. Effects vary between patients. Each person has a differing sensitivity to radiation injury. Human fibroblasts cultured from patients with severe radiation reactions have poor survival rates relative to those from patients who have a less severe injury. Thus, fibroblast defects have emerged as a central problem in the inhibited healing of chronic radiation injury.

Cellular mechanisms of phagocytosis and bacteriocidal metabolic functions in polymorphonuclear neutrophils harvested from tissue with chronic radiation damage also are impaired. The effect increases as time passes after therapy, which
cannot be a result of a direct effect of radiation on the neutrophils because their life span is too short. The local wound environment is the spotlight of the problem. Irradiated tissue may not “prime” the neutrophils with the appropriate cytokines and growth factors needed for activation, which has a major effect on the incidence of postoperative infection in previously irradiated patients.

The association between radiation damage and postoperative complications has been debated. In retrospective studies, patients undergoing surgery after failing primary radiotherapy for early-stage tumors do not show an increase in severity of complications or length of hospital stay. One prospective trial comparing antibiotic regimens did not show any increase in wound infection rate in irradiated patients, although other investigators have shown increased rates of postoperative infections and major wound complications. With the advent of more aggressive radiation regimens, concern regarding increased operative complications has surfaced. Twice-a-day hyperfractionation protocols versus once-a-day radiation do not seem to increase the surgical morbidity.

Chemotherapy now is included in many “organ preservation” protocols, in an attempt to cure patients with cancer without disfiguring or functionally devastating surgery. If chemotherapy is given peroperatively, the resultant bone marrow suppression inhibits the formation of an adequate inflammatory response needed for the initiation of the healing process. Experimental studies show the inhibition of fibroblast collagen production and a decreased ability to fight off wound infection with the application of antineoplastic agents. These effects appear to be transient, as opposed to the progressive nature of radiation damage. The combination of radiation and chemotherapy increases the risk of serious postoperative complications. This risk appears to decrease if surgery is delayed for more than 1 year.

Surgery for patients with previous radiotherapy should be approached cautiously.

**SYNDROMES ASSOCIATED WITH ABNORMAL WOUND HEALING**

There are several genetic syndromes that can adversely affect wound healing. The more common of the rare syndromes are discussed below (Table 3).

**Cutis Laxa**

There are two broad classifications of cutis laxa: congenital and acquired (Table 4). The congenital form can be autosomal dominant, recessive, or X-linked recessive. Acquired cutis laxa can result from drug ingestions, some solid and hematogenous neoplasms, and inflammatory skin conditions.

**Ehlers-Danlos Syndrome**

There are 10 identifiable phenotypes/clinical subtypes of Ehlers-Danlos syndrome. The different types have autosomal dominant and recessive inheritance. Individuals with the syndrome demonstrate connective tissue abnormalities as a result of defects in the inherent strength, elasticity, integrity, and healing properties of the tissues. All of the subtypes share varying degrees of the four major clinical features: skin hyperextensibility, joint hypermobility, tissue fragility, and poor wound healing. As many as 50 percent of patients with the syndrome do not have a type or form that can be classified easily on the clinical basis alone, which complicates the diagnostic process for the clinician because specific molecular diagnosis or confirmation (if available) may not be possible until a clinical subtype has been defined. Beighton et al. revised the nosology to “facilitate an accurate diagnosis of the Ehlers-Danlos syndrome and allow a clearer distinction of disorders that

| Table 3. Syndromes Associated with Abnormal Wound Healing |
|-----------------------------------------------|---------------------------------------|
| **Syndrome** | **Brief Description** | **Wound Result** |
| Cutis laxa | Defective elastin fibers; can be acquired or congenital | Thick, stretchy skin; easy bruising; hernias are common |
| Ehlers-Danlos | Deficit in collagen metabolism; has autosomal dominance and recessive inheritance | Total failure of the mechanical properties of the skin; poor wound healing, leading to wide, thin scars (classically described as “cigarette paper scars”); skin tears easily |
| Homocystinuria | Cystathionine synthetase deficiency | Advanced arteriosclerosis; associated arterial and venous thrombosis; platelet malfunction |
| Osteogenesis imperfecta | Defective gene for type I collagen | Wide scars |
overlap with the syndrome. The new classification scheme includes the following:

- Classic type (formerly types I and II)
- Hypermobility type (formerly type III)
- Vascular type (formerly type IV)
- Kyphoscoliosis type (formerly type VI)
- Arthrochalasia type (formerly type VII B)
- Dermatosparaxis type (formerly type VII C)

This new classification scheme does not include types V, VIII, IX, and X, all of which have only been described in one family. Most current literature still refers to Ehlers-Danlos syndrome patients as types I, II, or III (the most common). Elective surgery is considered contraindicated in patients with Ehlers-Danlos syndrome, although some would disagree.

**Homocystinuria**

Patients with homocystinuria have wound-healing problems because of poor wound perfusion secondary to thrombosis. These patients have defects in methylation and/or transsulfuration enzymes that cause the accumulation of homocysteine. Homocysteine is formed from ingested methionine. Homocysteine has cytotoxic effects on vascular endothelium and causes intimal thickening, lipid-laden macrophages, and smooth cell proliferation. Homocysteine initiates the coagulation pathway by enhancing factor V activity, increasing factor Xα-catalyzed prothrombin activation, suppressing protein C activation, and inducing endothelial cell tissue activity. Reduced antithrombin III activity has been seen in some patients, but this association has not been established. Patients with homocystinuria are also at very high risk for coronary vascular disease.

**Osteogenesis Imperfecta**

Osteogenesis imperfecta is a heritable disorder of connective tissue affecting bone and soft tissues. The general clinical features of this disorder include bone fragility, neonatal dwarfism, deformities of the long bones, scoliosis, ligamentous laxity, blue sclerae, defective dentinogenesis, and deafness. There are four major forms, but all have mutations in the genes that encode type I collagen.

**ADJUNCTS TO WOUND HEALING**

Failures of wounds to heal are generally the result of wound hypoxia, infection, edema, and metabolic abnormalities. Wounds require a min-
imum oxygen tension of 30 mmHg for normal cell division. Oxygen also increases fibroblast migration and replication, normal collagen production, and leukocyte killing. Infection keeps the wound in the inflammatory phase. Wound edema acts as a barrier to oxygen and nutrients by increasing the distance that they must diffuse across. Metabolic derangements affect wound healing in a variety of ways, depending on the abnormality. “Time heals all wounds” is a well-known proverb; Bennet and Schultz introduced the acronym TIME to highlight the four key clinical scenarios the clinician should check through when a patient’s wound healing stalls: tissue nonviable or deficient, infection or inflammation, moisture imbalance, and edge of wound nonadvancing or nonmigrating. Adjuncts to wound healing attempt to address and correct these obstacles to wound healing. They include bioengineered skin, electrostimulation, growth factors, hydrotherapy, hyperbaric oxygen, lasers, light-emitting diodes, negative pressure therapy, and ultrasound.

### Bioengineered Skin

Living bioengineered skin equivalents are “food on the hoof” for the wound, providing a living supply of growth factors and cytokines and a collagen matrix to build upon. A summary of the bioengineered skin replacements and the pro-healing factors found in living products are shown Tables 5 and 6. The mechanism of action is not fully understood on how these skin equivalents initiates wound healing, but it has been studied extensively with the Apligraf product. The cells contained in the Apligraf grow and proliferate, producing growth factors, collagens, and extracellular matrix proteins, which stimulate reepithelialization, formation of granulation tissue, angiogenesis, and neutrophil and monocyte chemotaxis. Extensive clinical experience suggests that Apligraf acts as a potent cellular remedy that can provide a different and adaptable response in acute and chronic wounds. After Apligraf is placed in chronic wounds, outgrowth of previously dormant keratinocytes at the wound edge is observed, suggesting that Apligraf releases factors that activate keratinocytes and stimulate migration and reepithelialization.

It is believed that Apligraf stimulates wound healing in two phases. During the initial phase, the release of growth factors from Apligraf cells results in healing from the skin edge. Apligraf covers the wound, provides a barrier, and interacts with the wound bed to stimulate wound healing. In the second phase, there is cellular communication between the tissue-engineered product and the cells in the wound and other cells attracted to the wound site through the release of wound-healing-related growth factors and cytokines.

### Electrostimulation

Electrical current in skin was first described as far back as 1860 by DuBois-Reymond. In 1945, it was observed that wounds had a positive potential compared with the surrounding skin. In 1982, Barker described the “skin battery.” He found that the skin surface was always negatively charged (compared with the deeper skin layers), and he measured transcutaneous voltages up to 40 mV.

### Table 5. Bioengineered Skin Replacements*

<table>
<thead>
<tr>
<th>Composition</th>
<th>Structure</th>
<th>Living</th>
<th>Trade Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured keratinocytes autografts</td>
<td>Epidermal</td>
<td>Yes</td>
<td>Epicel</td>
</tr>
<tr>
<td>Treated cadaver skin allograft</td>
<td>Dermal</td>
<td>No</td>
<td>AlloDerm</td>
</tr>
<tr>
<td>Bovine collagen/glycosaminoglycan/Silastic</td>
<td>Dermal</td>
<td>No</td>
<td>Integra</td>
</tr>
<tr>
<td>Neonatal fibroblasts/polyglactin mesh allograft</td>
<td>Dermal</td>
<td>Yes</td>
<td>Dermagraft</td>
</tr>
<tr>
<td>Neonatal fibroblasts/keratinocytes collagen allografts</td>
<td>Composite</td>
<td>Yes</td>
<td>Apligraf, OrCel†</td>
</tr>
</tbody>
</table>

†OrCel is a product made by Ortec International that is very similar to Apligraf. It was formerly known as Composite Cultured Skin.

### Table 6. Pro-Wound Healing Factors Found in Living Bioengineered Skin Equivalents*

<table>
<thead>
<tr>
<th>Cytokines and Growth Factors</th>
<th>Extracellular Matrix Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-α</td>
<td>Collagen, types IV and VII</td>
</tr>
<tr>
<td>Amphiregulin</td>
<td>Laminin</td>
</tr>
<tr>
<td>IL-1, IL-3, IL-6, IL-8</td>
<td>Kolinin</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Fibronectin</td>
</tr>
<tr>
<td>Granulocyte-macrophage CSF</td>
<td>Thrombospondin</td>
</tr>
<tr>
<td>PDGF</td>
<td>Glycosaminoglycans</td>
</tr>
<tr>
<td>Basic FGF</td>
<td>Plasminogen activator</td>
</tr>
<tr>
<td>TGF-β</td>
<td></td>
</tr>
</tbody>
</table>

Electrostimulation is believed to restart or accelerate the wound-healing process by imitating the natural electrical current that occurs in skin when it is injured.\textsuperscript{142–145} This current of injury was found to vary in specific ways during the regeneration process, with current ceasing to flow as healing is completed or arrested.\textsuperscript{146} Electrical current applied to wounded tissue increases the migration of cells vital to the wound-healing process (i.e., neutrophils, macrophages,\textsuperscript{147–149} and fibroblasts).\textsuperscript{146,150,151} Investigators have found that electrostimulation resulted in a 109 percent increase in collagen,\textsuperscript{150} a 40 percent increase in tensile strength,\textsuperscript{152} and a 36 percent increase in tensile strength.\textsuperscript{153} Electrostimulation may also play a role in wound healing through improved blood flow.\textsuperscript{154,155}

These findings have sparked investigators to use electrostimulation to promote wound healing in chronic wounds. There are four primary types of stimulation use: direct current, low-frequency pulsed current, high-voltage pulsed current, and pulsed electromagnetic fields (Table 7).\textsuperscript{70,126,133,156–171}

Current polarity has been found to have some unique actions on wound healing. These effects are summarized in Table 8.\textsuperscript{133}

**Table 7. Electrostimulation Modalities in Clinical Practice**

<table>
<thead>
<tr>
<th>Description</th>
<th>Clinical Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Direct current</strong></td>
<td></td>
</tr>
<tr>
<td>A negative or positive electrode is placed in the wound with the other one distant to the wound. A current of 0.03 to 1 mA is passed across the wound for 1 to 3 hours, until the wound is healed. As healing plateaus, the polarity is reversed. This mode is rarely used today, because there are more efficient forms of electrostimulation.</td>
<td>Ischemic ulcers,\textsuperscript{153} venous ulcers,\textsuperscript{156} nonunion bone healing\textsuperscript{157,158}</td>
</tr>
<tr>
<td><strong>Low-frequency pulsed current (tetanizing current)</strong></td>
<td></td>
</tr>
<tr>
<td>Physical therapists have been using this modality for more than 25 years to treat muscular pain as transcutaneous electrical nerve stimulation. A current of 50 mA, with a frequency of 2 to 100 Hz, is delivered in pulses of 45 to 500 μsec and causes contraction of surrounding muscle, resulting in increased blood flow.\textsuperscript{135}</td>
<td>Pressure ulcers\textsuperscript{159–161} (especially in spinal cord injury patients)</td>
</tr>
<tr>
<td><strong>High-voltage pulsed current</strong></td>
<td></td>
</tr>
<tr>
<td>The negative electrode is placed in the wound and the positive electrode is placed on the wound edge. A current of 100 to 150 V (usually &lt;200 V), with short pulse duration and a low current (15–40 mA), is applied. Once the healing rate plateaus, the polarity is reversed.\textsuperscript{155}</td>
<td>Pressure ulcers,\textsuperscript{162–165} mixed-chronic ulcers\textsuperscript{159, 166}</td>
</tr>
<tr>
<td><strong>Pulsed electromagnetic field</strong></td>
<td></td>
</tr>
<tr>
<td>A pulsed electromagnetic field delivers 27.12 MHz of energy at a pulse rate of 80 to 600 pulses per second and a per-pulse power range of 293 to 975 peak Watts. The therapy is given twice a day for 30 minutes per session until the wound is healed.\textsuperscript{133}</td>
<td>Nonunion bone healing,\textsuperscript{70,126,167,168} pressure ulcers,\textsuperscript{169} venous ulcers\textsuperscript{170,171}</td>
</tr>
</tbody>
</table>
taminants precisely, without the collateral trauma associated with traditional surgical modalities (heat and aggressive sharp débridement).184

Hyperbaric Oxygen

Oxygen therapy has its roots dating back to 1662, with the construction of a pressurized room, or “domicillium,” by an English physician named Henshaw. The room was pressurized by bellows that raised the air pressure a few pounds per square inch above ambient pressure. This, claimed Henshaw, was good for most afflictions of the lungs and bowels. Junod treated pulmonary diseases in 1834 by placing patients in a chamber with 2 to 4 atmospheres of pressure. Dr. Orval Cunningham constructed the largest hyperbaric chamber in 1928; it was five stories high, 64 feet in diameter, and had multiple floors, each holding 12 beds. Cunningham never recorded his therapies, and the American Medical Association condemned his treatments in 1942 for a lack of scientific proof.185

Atmospheric pressure at sea level is 1 ATA. At 1 ATA, the oxygen dissolved in blood is 1.5 ml/dl; at 3 ATA, dissolved oxygen in blood is 6 ml/dl. Normal subcutaneous tissue oxygen tension is 30 to 50 mmHg. Dividing cells in a wound require an oxygen tension of at least 30 mmHg. Tissues in wounds that are not healing have partial pressure of oxygen values of 5 to 20 mmHg. A “dive” of 2.4 ATA will result in a wound partial pressure of oxygen of 800 to 1100 mmHg.183

Many reports in the literature have demonstrated benefit from hyperbaric oxygen treatment for a variety of conditions, including amputations,186 osteoradionecrosis,187,188 surgical flaps, and skin grafts.185,189,190 The success of hyperbaric oxygen treatment for necrotizing soft-tissue infections has been controversial. Studies have failed to show statistically significant outcome differences with respect to mortality rates and length of hospitalization. Two studies found that the mean number of débride-

ments was higher in patients treated with hyperbaric oxygen.191,192 There is, however, a bias for treated patients to be younger and have positive clostridial wound cultures and more severe infection.193 In addition to simply providing more oxygen to the wound site, hyperbaric oxygen therapy also increases expression of nitric oxide, which is crucial for wound healing.194 In an ischemic rabbit ear model, hyperbaric oxygen therapy in combination with PDGF or TGF-β1 had a synergistic effect that totally reversed the healing deficits caused by ischemia.13

Lasers

Light amplification by stimulated emission of radiation (or laser) management of open wounds has been used for more than 35 years in Europe and Russia. Only in the past 10 years has laser therapy for wounds been used in the United States. Lasers for wound management are low-energy lasers capable of raising tissue temperature 0.1°C to 0.5°C.195 Low-energy laser treatment is called biostimulation.196 Low-energy lasers appear to function according to the Arndt-Schulz law, which states that “less is more.” In other words, weak biostimulation excites physiological activity, moderate favors physiologic effect, strong impedes physiological processes, and very strong stimuli arrests physiological stimulation and is destructive. Low-energy stimulation of tissue results in increased cellular activity in wounded skin.197,198 The mechanism for enhanced physiologic activity is not fully understood. It is believed to be caused by the stimulation of ascorbic acid uptake by cells, stimulation of photoreceptors in the mitochondria respiratory chain, changes in cellular ATP or cyclic AMP levels, and cell membrane stabilization.199–201

The common types of low-energy lasers are shown in Table 10. The two most common lasers used clinically are the helium-neon laser and the gallium-arsenide (or infrared) lasers.

Lasers have been shown to increase the healing (especially when combined with hyperbaric oxygen treatments) of ischemic, hypoxic, and infected

<table>
<thead>
<tr>
<th>Positive Current</th>
<th>Negative Current</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promotes epithelial growth and organization</td>
<td>Decreases edema around the electrode</td>
<td>Stimulates neovasculature</td>
</tr>
<tr>
<td>Acts as a vasoconstrictor and induces clumping Denatures protein Aids in preventing postischemic lipid peroxidation Decreases mast cells in Attracts macrophages</td>
<td>Lyses or liquefies necrotic tissue Stimulates growth of granulation tissue Increases blood flow Causes fibroblasts to proliferate and make collagen Induces epidermal cell migration Attracts neutrophils Stimulates neurite growth directionally</td>
<td>Has a bacteriostatic effect Stimulates receptor sites for certain growth factors</td>
</tr>
</tbody>
</table>

Table 8. Effect of Polarity on Wound Healing

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Lasers affect wound healing at many different levels. The helium-neon laser light at 633 nm was optimal for wound healing using 1 mW exposures and greater to provide 4 J/cm² energy density. Later studies of in vitro skin fibroblast stimulation demonstrated that consecutive exposures to 660 nm and 780 nm of laser light at 24-hour intervals increased collagen production fourfold compared with untreated cultures. It appears that Regranex can be useful in a variety of other types of wounds, such as pressure ulcers, and currently trials for venous insufficiency ulcers are underway. There have also been select instances of case reports utilizing Regranex in other chronic and acute wounds, such as pyoderma gangrenosum, ulcers of vasculitis, and acute surgical defects. Macrophages and inflammation play an important role in the first phase of wound healing; this material has been tried in some chronic wounds as an injectable material around the area of the ulcers and topically as an aqueous solution. Results have been promising, but it is not currently approved for use in chronic wounds. Shown to significantly increase healing and epithelialization over the wound bed. The initial trial in venous ulcers has shown very promising results, and it is currently in phase 2 clinical development.

### Light-Emitting Diodes

The National Aeronautics and Space Administration has developed a light-emitting diode that...
can be made to produce multiple wavelengths and be arranged in large, flat arrays to treat large wounds. The treatment area for a laser is limited—large areas must be treated in a grid-like pattern. The agency developed the light-emitting diode in response to their research on wound healing in a weightless environment. Studies on cells exposed to microgravity and hypergravity indicate that human cells need gravity to stimulate growth. As the gravitational force increases or decreases, the cell function responds in a linear fashion. This poses significant health risks for astronauts in long-term space flight. Work done on shuttle missions and on the space station has shown significant improvements of wound healing using light-emitting diodes alone or in combination with hyperbaric oxygen treatment. Light-emitting diode therapy is done at wavelengths of 680, 730, and 880 nm simultaneously. The array was tested by the U.S. Special Operations Command on submariners. Reports indicated a 50 percent faster healing of lacerations in crew members treated with a light-emitting diode array with three wavelengths combined in a single unit (670, 720, and 880 nm) compared those who underwent untreated control healing (7 days compared with approximately 14 days). This is significant because wound healing is slow aboard submarines due to the higher carbon dioxide and lower oxygen levels. Light-emitting diode therapy is also being investigated as therapy for neural cancers, leukemia, lymphomas, and Barrett’s esophagus.

Negative Pressure Therapy

Negative pressure therapy (or vacuum-assisted closure), developed at the Bowman Gray School of Medicine, uses a subatmospheric pressure dressing to convert an open wound into a controlled closed wound. The negative pressure exerts many effects on both gross and microscopic levels. The negative pressure removes interstitial fluid and edema to improve tissue oxygenation. It also removes inflammatory mediators that suppress the normal progression of wound healing. Granulation tissue forms more rapidly (103.4 ± 35.5 percent) than in controls, and 5 days of therapy decreases the wound bacterial count to less than 105 organisms per gram of tissue. The negative pressure dressing is convenient to use; it requires changing every 48 to 72 hours and has few complications. The dressing is used in a variety of wound types, involving soft-tissue loss, exposed bone and hardware, and weeping wounds, and as a skin graft bolster. Negative pressure therapy is contraindicated when (1) wounds contain necrotic tissue, (2) osteomyelitis is untreated, (3) body cavity or organ fistulas are present, (4) malignancy is present in the wound, or (5) treatment would place the foam dressing directly over exposed arteries and veins. Negative pressure therapy should be used cautiously when there is active bleeding in the wound, when hemostasis is difficult after débridement, and when anticoagulant therapy is used. The vacuum dressing is a great temporizer and gives the patient and surgeon time to transform a hostile wound into a manageable one.

Ultrasound

The therapeutic effects of ultrasound therapy are from its thermal and nonthermal properties. Ultrasound results when electrical energy is converted to sound waves at frequencies greater than 20,000 Hz. Sound waves are transmitted to the tissue through a hydrated medium sandwiched between the tissue and the transducer. The depth of penetration depends on the frequency; the lower the frequency, the deeper the penetration. Ultrasound therapy has traditionally been used to relieve muscular spasm for musculoskeletal pain, but the thermal component (a setting of 1 to 1.5 W/cm²) of ultrasound has also been used to improve scar outcome. The nonthermal component of ultrasound (at a setting of 0.3 to 1 W/cm²) produces two effects: cavitation and streaming. Cavitation is defined as the formation of gas bubbles, and streaming is a unidirectional, steady mechanical force. These effects cause changes in the cell membrane permeability and enhance diffusion of cellular metabolites.

Ultrasound’s effect on wound healing in the laboratory include cellular recruitment, collagen synthesis, increased collagen tensile strength, angiogenesis, wound contraction, fibroblast and macrophage stimulation, fibrinolysis, reduction of the inflammatory healing phase, and promotion of the proliferative phase.

Table 10. Common Low-Energy Lasers

<table>
<thead>
<tr>
<th>Laser System</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argon (Ar)</td>
<td>488–514</td>
</tr>
<tr>
<td>Carbon dioxide (CO2)</td>
<td>10,600</td>
</tr>
<tr>
<td>Dyne</td>
<td>Variable</td>
</tr>
<tr>
<td>Gallium-arsenide (GaAs) or infrared (IR)</td>
<td>904</td>
</tr>
<tr>
<td>Gallium-aluminum-arsenide (GaAlAs)</td>
<td>830</td>
</tr>
<tr>
<td>Helium-neon (HeNe)</td>
<td>632.8</td>
</tr>
<tr>
<td>Neodymium:yttrium-aluminium-garnet (Nd:YAG)</td>
<td>1064</td>
</tr>
<tr>
<td>Ruby</td>
<td>694</td>
</tr>
</tbody>
</table>
ative healing phase. Clinically, the results are not as comparable. Ultrasound is used most often to treat venous stasis ulcers and pressure sores. Some have shown a demonstrable reduction in wound size compared with placebo. Mirastchijski et al. were unable to show any improvement in wound healing with ultrasound versus placebo. Ultrasound treatment of pressure ulcers showed a similar division; some showed improvement and others did not.

**KELOIDS AND HYPERTROPHIC SCARS**

Hypertrophic scars are raised scars that remain confined to the limits of the incision (Fig. 2). Keloids are scars that have overgrown their boundaries (Fig. 3).

**Differences between Keloids and Hypertrophic Scars**

**Histologic Differences**

Light microscopy reveals large collagen bundles in keloidal scars but not in hypertrophic scars. Keloidal scars may have few macrophages, but they have abundant eosinophils, mast cells, plasma cells, and lymphocytes and are associated with a mucopolysaccharide ground substance; hypertrophic scars have only scant amounts. No morphologic differences in keloidal and hypertrophic scar fibroblasts have been found, but their biological behavior is different. Hypertrophic scars have nodules containing cells and collagen within the middle to deep part of the scar. Within these nodules are α-smooth muscle actin–staining myofibroblasts, which are absent from normal dermis, normal scars, and 88 percent of keloidal scars.

**Differences Observed with Electron Microscopy**

Ehrlich et al. found an amorphous substance around keloidal fibroblasts that separated them from the collagen bundles. This substance was not seen in hypertrophic scars. Kischer and Hendrix found that collagen bundles were “crisp” in hypertrophic scars and more “glazed” in keloidal scars. In addition, differences were found in the degree of microvascular injury seen in the keloidal scars.

**Differences in Metabolic Activity**

Ueda et al. found that keloidal scars had higher levels of adenosine triphosphate and fibroblasts up to 10 years after formation, compared with hypertrophic scars. Nakaoka et al. found a higher density of fibroblasts in both keloidal scars and hypertrophic scars, but keloidal scars had a higher expression of proliferating cell nuclear antigen, which the authors believed may help explain the tendency of keloidal scars to grow beyond the boundary of the original wound.

**Other Differences**

Antinuclear antibodies against fibroblasts and epithelial and endothelial cells have been found in patients with keloidal scars but not in those with hypertrophic scars.

**Epidemiology**

Keloids tend to occur during periods of physical growth and most often form between the ages of 10 and 30 years. A keloid rarely forms in an
elderly person. Keloids occur in persons of all races; darkly pigmented skin is affected 15 times more often than is lighter skin. No reports exist of the occurrence of a keloid in an albino person. In a rural African population, the incidence was noted to be approximately equal in males and females (5.4 and 6.2 percent, respectively). The teenage population has a higher incidence, with keloids occurring in 12.2 percent of boys and 14.4 percent of girls. Although the three most common causes in Oluwasanmi’s field survey were unspecified trauma, vaccinations, and tattoos, the most common predisposing factor in a hospital review was earlobe piercing. In a predominantly black and Hispanic population, Cosman and Wolff noted the incidence of keloid formation to be between 4.5 and 16 percent in a review of three large series. Data regarding the incidence of recurrence based on ethnicity or degree of skin pigmentation are nonexistent.

**Pathogenesis**

The etiology of keloid formation is not entirely clear. Porter et al. compiled a list of possible causes derived from numerous treatment strategies that appear to have some benefit in the treatment and management of keloids.

**Trauma**

Trauma seems to be the most common antecedent factor to developing a keloid. Random (e.g., laceration, burn, vaccination, bug bite) and planned (e.g., surgery, tattoos, ear piercing) traumatic events were reported to cause the formation of a keloid. Keloids may develop within 1 year of the traumatic event, but occasionally they can form many years later. After a traumatic injury, a keloid forms because fibroblasts haphazardly lay down collagen. Keloids can be seen on all parts of the body, but the sternum and supradeltoid regions are the most often affected. Keloids of the ears, penis, female genitalia, corneum, intraoral region, and plantar region have also been reported.

**Abnormal Fibroblast Activity**

Fibroblasts from hypertrophic scars and keloidal scars have different properties. Hypertrophic scar fibroblasts have a moderate increase in their basal level of collagen production, but they still respond normally to growth factors. In contrast, fibroblasts from keloidal scars produce high levels of collagen, elastin, fibronectin, and proteoglycan and show abnormal responses to stimulation. Collagen type I is predominantly produced by keloidal fibroblasts. These fibroblasts have been shown to have a greater capacity to proliferate.

**Increased Levels of Growth Factor and Other Cytokines**

TGF-β has three subtypes: 1, 2, and 3. Levels of types 1 and 2, which stimulate fibroblasts, are increased in keloidal scars, whereas levels of type 3 TGF-β are not increased. TGF-β type 1 has been associated with increased collagen and fibronectin synthesis by fibroblasts. IFN-α and IFN-γ have been shown to reduce both collagen synthesis from fibroblasts and fibroblast proliferation.

**Decreased Apoptosis**

Programmed cell death, or apoptosis, is believed to play a role in wound healing and possibly keloidal scar formation. Lower rates of apoptosis have been observed in keloidal fibroblasts. It has been suggested that keloidal fibroblasts resist physiological cell death, continuing to proliferate and produce collagen.

**Increased Levels of Plasminogen Activator Inhibitor-1**

Fibrin plays an important part in wound healing, leading to migration of inflammatory cells, formation of granulation tissue, and collagen synthesis. Regulation of fibrin is important in wound healing; it is degraded by plasmin, which in turn is regulated by a number of activators (urokinase and plasminogen activator) and inhibitors (tissue plasminogen activator inhibitor 1). Keloidal fibroblasts have increased levels of plasminogen activator inhibitor-1 and low levels of urokinase. This may lead to reduced collagen removal and contribute to scar formation.

**Abnormal Immune Reactions**

Theories that keloidal scars are generated by specific immune reactions led researchers to examine the levels of immunoglobulins within keloidal scars. The overall results are conflicting. It has been suggested that antigenic substances may be present in keloidal scars.

**Hypoxia as an Etiologic Factor**

Tissue hypoxia has been implicated in keloidal scar formation. Histologic examination shows occluded microvessels with plump endothelial cells lining the vessel wall. The mechanism by which hypoxia may lead to keloidal scar formation is unclear. Vascular endothelial growth factor (VEGF) has been shown to be released from fibroblasts in response to hypoxia. Gira et al. found that VEGF production was abundant in keloids and that the source of the VEGF was the overlying epidermis. Steinbrech et al., however,
found no difference in VEGF levels between keloidal fibroblasts and normal dermal fibroblasts.

**Other Theories Regarding Etiology**

There is a theory that keloidal scars are caused by an immune reaction to sebum. This theory is supported by the following observations: keloidal scars are more common in adolescence; they rarely occur on the palms and soles; spontaneous keloidal scars occur in skin areas with sebaceous activity; and one scar may be keloidal, whereas an adjacent scar may be normal. Researchers positing this theory suggest that the development of keloidal scars is dependent on random damage to pilosebaceous structures.

Keloids can be considered a mesenchymal neoplasm. Keloid fibroblasts have been shown to contain the oncogene gli-1 and express the protein Gli-1. Immunohistochemistry staining for gli-1 showed a strong presence in the cytoplasm, similar to basal cell carcinoma, which also expresses the gli-1 oncogene. This oncogene is not expressed in fibroblasts from normal tissue and nonhypertrophic scars. Rapamycin (sirolimus) and tacrolimus (FK506) have been shown to be beneficial for keloid regression, and both drugs bind the cellular receptor (FKBP12), which is a cis-trans-prolyl isomerase—the same target of the Gli-1 protein. Table 11 summarizes the biochemical alterations seen in keloids and hypertrophic scars.

**Clinical Presentation**

Hypertrophic scars may regress with time and occur earlier after injury (usually within 4 weeks). Most keloids form within 1 year of wounding, but some may begin to grow years after the initial injury. Surgical incision, trauma, burns, inflammation, infection, and puncture wounds are the most common inciting events. Symptoms associated with keloid formation include pain, pruritus, hyperpigmentation, disfigurement, and decreased self-esteem (which appears most commonly in the teenage population). Pruritus is experienced transiently in normal healing wounds, but persistent pruritus is associated with keloid formation. Lesions can be located in various places but predominate on the head, neck, and earlobe in those patients who present for treatment. Areas of the head and neck that are spared include the eyelids and the mucous membranes.

**Treatment Options**

The availability of so many treatment options speaks to the fact that none of them has proved to be completely effective. The primary problem with treatment of keloidal scars is the high rate of recurrence. Keloid recurrence rates range from 0 to 100 percent. The literature is replete with retrospective, uncontrolled studies with small sample sizes. The few randomized prospective studies that do exist also have small sample sizes, so statistical significance may be lacking. Beyond the basic problems of study design, these studies are not uniform with regard to outcome measures and duration of follow-up. To add further confusion, there is little standardization of analysis that takes into account previous treatments that subjects have had before the current study’s new and improved keloid treatment strategy. Finally, many studies include both hypertrophic scars and keloids in the subject population. A summary of the most common treatment strategies is provided below.

<table>
<thead>
<tr>
<th>Table 11. Biochemical Alternations Seen in Excessive Scar Formation*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemical Alteration</strong></td>
</tr>
<tr>
<td>Prolyl hydroxylase</td>
</tr>
<tr>
<td>Total collagen</td>
</tr>
<tr>
<td>Collagen type I</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>Chondroitin-4-sulfate</td>
</tr>
<tr>
<td>Glycosaminoglycans</td>
</tr>
<tr>
<td>Fibronectin</td>
</tr>
<tr>
<td>Elastin</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
</tr>
<tr>
<td>Apoptosis</td>
</tr>
<tr>
<td>TGF-β</td>
</tr>
<tr>
<td>VEGF</td>
</tr>
<tr>
<td>gli-1 oncogene</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
</tr>
</tbody>
</table>

Excision Alone

Excision alone has not been successful in eliminating keloids. Recurrence rates range from 45 to 93 percent.\textsuperscript{234,260} Excision is the most obvious treatment, but it has met with little success when it is performed alone. Apfelberg et al.\textsuperscript{261} proposed using the keloid epidermis as an autograft for wound closure after keloid excision. Using the keloidal skin avoids donor-site morbidity, decreases the amount of tension on the closure, and lessens the cosmetic deformity. Only five patients were reported in his series, which had a 40 percent recurrence rate. Weimar and Ceilley\textsuperscript{262} incorporated the autograft technique for use with adjunctive measures, pressure therapy, and steroid injections, but results were not provided. Adams\textsuperscript{126} described a suprakeloid flap with postoperative radiation therapy for the successful treatment of an earlobe keloid.

Core excision of a dumbbell keloid located on the earlobe with anterior and posterior components has excellent cure rates when it is used along with steroids. Core excision results in a full-thickness defect; the anterior wound is closed primarily and the posterior wound is allowed to granulate close. Six patients were treated successfully in this manner without recurrence at more than 1 year.\textsuperscript{263}

Excision alone is not recommended as definitive therapy. Adjuvant therapy has become the standard of care to improve outcomes.

Laser Excision

Different forms of laser therapy for keloids have been evaluated over the years. Lasers, which cause a range of nonspecific to specific thermal tissue reactions, are believed to wound in such a way so as to minimize scar contraction compared with scalpel wounds. Both carbon dioxide and argon lasers showed early promise in excision, creating a dry and bloodless surgical environment with limited damage in surrounding tissue. These lasers are not frequently used at present, however, because long-term studies revealed recurrence rates of up to 92 percent when they were used as a single modality.\textsuperscript{233,234,264–266}

The most promising form of laser therapy seems to be the 585-nm flashlamp-pumped pulsed-dye laser, which has been effective in reducing pruritus, erythema, and the height of keloids, with improvement in 57 to 83 percent of cases.\textsuperscript{264–268}

Laser excision yields the best results when combined with adjunctive therapy. It is unclear if there is truly any variation in results of laser versus scalpel excision performed in isolation. Prospective, randomized, controlled trials would benefit the understanding of whether lasers provide more benefit than scalpel excision.

Steroids

Intralesional steroids often are used for initial treatment of the keloid. More commonly, they are the adjunctive treatment of choice perioperatively, because they are readily available and easily used. Steroids suppress the inflammatory phase of wound healing, decrease collagen production by the fibroblast, and decrease fibroblast proliferation. Triamcinolone acetonide seems to be the steroid of choice. Studies vary significantly regarding the strength of the steroid preparation used and at what point in the treatment regimen it is administered. Of the range of dose strengths, 40 mg/ml is the most common strength used to treat keloids. With regard to timing of administration, it is occasionally used preoperatively at one or several sessions\textsuperscript{269,270}; this is followed by excision and further postoperative treatment. Alternatively, some physicians use it preoperatively, intraoperatively, postoperatively, or in some combination of the three. No one regimen proved to be more beneficial. Adverse reactions with the use of intralesional steroids include localized problems, such as depigmentation, hypopigmentation, epidermal atrophy, telangiectasias, and skin necrosis. Occasionally, these changes may be only temporary. Systemic side effects and Cushing’s syndrome are rare and are associated with improper steroid use.

One of the first studies to report results of triamcinolone acetonide injection found that 88 percent of keloids (n = 22) injected with steroids regressed to varying degrees and that pruritus disappeared within 3 to 5 days of injections.\textsuperscript{271} The best responses were noted when the injection was performed at times of excision and with follow-up visits. High doses, up to 120 mg, were injected. Complications included atrophy, depigmentation, and recurrence. Currently, most practitioners do not administer such high doses. Monthly doses of approximately 12 mg are recommended.\textsuperscript{270} This modality continues to be a mainstay of treatment. Perioperative steroids generally are considered the standard of care, because most controlled studies use this as the control arm.\textsuperscript{233,270}

Radiation Therapy

Radiation therapy has been used to treat keloids since 1906\textsuperscript{234} and has been successful in erad-
indicating this benign lesion. When used alone, it was noted to have a wide range of cure rates, from 15 to 94 percent.\textsuperscript{260} When the lesions are first incised and subsequently radiated, however, the response rates increase to 33 to 100 percent.\textsuperscript{260} The results of studies performed in the recent past (1974 to 1990) have shown that the response rates are even better, from 64 to 98 percent.\textsuperscript{260} Success seems to depend on the number of rads delivered to the surgical sites and the institution of the radiation treatment in the immediate postoperative period.

In a retrospective study of 24 patients treated with postexcisional superficial radiation therapy, patients had a dosage of 800 to 1200 rads divided into one to three doses delivered to the bed of the wound.\textsuperscript{272} A recurrence rate of 53 percent was reported, one of the highest in the literature for this modality.

Controversy abounds regarding the safety of delivering radiation to a benign tumor.\textsuperscript{273} There are anecdotal reports regarding the development of malignancy after treatment of a keloid with radiation.\textsuperscript{273} Patients treated with radiation for acne in the past have an increased incidence of skin cancer, which may develop up to 30 to 40 years after the radiation exposure. Currently, radiation is no longer used to treat acne. Although adequate data are lacking, it is estimated that the risk of developing skin cancer from radiation exposures of less than 1000 Gy is low.\textsuperscript{237} Although the dose administered for the treatment of keloids is low, long-term follow-up is needed to determine the actual risk for development of malignancy; however, this modality seems to be a highly effective adjunctive measure for eradicating the keloid.

### Pressure Therapy

Pressure therapy is effective in the treatment of hypertrophic scars and keloids, especially after burn injury. This therapeutic strategy is used in combination with other treatment modalities (e.g., silicone gels or sheets to enhance the pressure treatment). Maximum benefit is achieved by wearing the pressure appliance for 18 to 24 hours per day for at least 4 to 6 months.\textsuperscript{233,274,275} Good response rates were seen in 90 to 100 percent of patients treated with keloid excision followed by pressure therapy, especially when the keloid was located on the earlobe.\textsuperscript{235,274,275} The amount of pressure delivered should be between 24 and 30 mmHg, to avoid decreased peripheral blood circulation. Pressure earrings have been developed for postoperative use on keloids involving the earlobe. Intralosional verapamil combined with 6 months of pressure therapy used after keloid excision resulted in a 55 percent cure rate.\textsuperscript{276}

### Magnetic Disks

Chang et al.\textsuperscript{275} treated 47 patients (91 auricles) with “hypertrophic scarring of the earlobe” (keloids) with compressive therapy using magnetic disks after surgical excision. With a mean follow-up of 18.1 months, they had only one recurrence 13 months after surgery. This was treated with additional disk compression for 3 months. Thirty-one of the 47 patients complained of pain with the disk therapy. The pain was treated by reducing the compression intensity, by attaching a silicone gel sheet to the magnetic disk, or by reducing the amount of time patients had to wear the disk. The authors offer no explanation for why the magnetic disks work, except that they are a form of compression therapy.

### Cryosurgery

Serial cryotherapy treatments using spray or contact methods have traditionally been useful for managing hypertrophic scars and keloids. Zouboulis et al.\textsuperscript{277} introduced an intralosional needle cryosurgery device for both hypertrophic scars and keloids The authors advocate this method as more effective for treating deeper parts of scars and keloids than spray or contact methods, requiring fewer treatments. Twelve lesions were frozen with a cryoneedle inserted along the long axis of the lesion; the needle was connected to a liquid nitrogen source. They found an average 51 percent reduction in lesion volume, with significantly improved subjective (pain, itching) and objective (hardness, color) ratings. Pretreatment and posttreatment histologic analysis demonstrated improved scar organization after needle cryosurgery.

### Interferon

Interferons interfere with fibroblasts’ ability to synthesize collagen. Specifically, IFN-\(\alpha\)-2b normalizes the collagen and glycosaminoglycan of the keloid.\textsuperscript{235} Complications with IFN-\(\alpha\)-2b injection include flu-like symptoms, headache, fever, and myalgias. These symptoms may be tolerable with the prophylactic administration of acetaminophen. In a retrospective study, Berman and Flores\textsuperscript{270} found that the recurrence rate with postexcisional use of IFN-\(\alpha\)-2b (18.7 percent) was superior to that for excision alone (51.1 percent) and for postexcisional steroid (triamcinolone) injections (58.4 percent). IFN-\(\alpha\)-2b was injected into the wound at a dosage of 1 million U in 0.1 ml per
linear centimeter per treatment. Twelve of the 16 patients treated with IFN were reinjected 1 week later with 5 million U. Follow-up was only 7 months on average, but the differences were statistically significant. Conejo-Mir et al.278 achieved a 3-year 0 percent recurrence rate with the combination of carbon dioxide laser excision and IFN-α-2b injections for earlobe keloids. IFN-α-2b seems to be effective in decreasing the keloid recurrence rate. In a recent, smaller prospective study,37 of 13 keloids treated with postoperative intralosomal IFN-α-2b, seven recurred (54 percent). In contrast, 26 keloids treated with triamcinolone (control group) had a 15 percent recurrence rate. IFN-γ is believed to work in manner similar to IFN-α-2b. There have been several anecdotal reports regarding the benefits of IFN-γ in treating keloids. More recently, a pilot study examined the treatment of keloids with this modality.279 In a placebo-controlled, randomized, double-blind study, patients with two keloids had one keloid randomized to excision and postoperative placebo injections and the other to IFN-γ injections. In the experimental group, patients were given a series of 10 injections of 10 µg of IFN-γ. Only seven patients were enrolled in the study, three of whom dropped out at the 1-year follow-up examination. Experimental and control groups had uniformly poor results, with an approximate 75 percent recurrence rate. Because of the small sample size, conclusions could not be drawn.

Imiquimod is a relatively new agent, an immune response modifier that indirectly acts to stimulate innate and cell-mediated immune pathways, thereby enhancing the body’s natural ability to heal.280 Along with inducing and activating natural killer cells, macrophages, and Langerhans cells, imiquimod also induces the local synthesis and release of cytokines, including IFN-α, IFN-γ, TNF-α, and IL-1, IL-6, IL-8, and IL-12, when topically applied.281 A variety of case reports and clinical studies have documented recent successes associated with the use of imiquimod and its antiviral, antitumor, and immunoregulatory properties, especially in conditions in which interferons have also been used successfully in treatment. Berman and Kaufman282 examined the effects of topical application of imiquimod 5% cream after surgical excision of 13 keloids from 12 patients. Imiquimod 5% cream was applied nightly directly to the suture line and to the surrounding area beginning on the day of the surgery and continuing for a total of 8 weeks. At 24 weeks, no recurrence of keloidal growth was noted among the 11 keloids of the 10 patients who completed the study, a recurrence rate lower than those previously reported in the literature for surgery alone.

Silicone Gel Sheets
The mechanism of action of silicone gel sheeting, also known as hydrocolloid dressing, is not fully known. Scar hydration is facilitated by the presence of this occlusive dressing. Hydrocolloid dressings have been deemed safe for treating wounds in the initial stages of healing.283 Silicone gel probably acts as an impermeable membrane that keeps the skin hydrated.27 In vitro experiments have shown that silicone is inert and does not affect fibroblast function or survival; however, enhanced keratinocyte hydration alters growth factor secretion, which in turn affects fibroblast function and collagen production.284 The clinical effects of silicone gel do not appear to be mediated by changes in pressure, temperature, or tissue oxygenation.37 In an animal study, no detrimental effect was found when silicone gel sheets were compared with Nu-Gauze dressings in the immediate postoperative period. Histologic examination of the wounds revealed no evidence of silicone leakage into the tissues. Prevention or improvement of keloids was not addressed in the study. In human trials, topical silicone gel was used to treat 22 keloids in 18 patients, with an 86 percent significant objective response rate.285 Silicone gel sheets may reduce recurrence rates after excision. It is a benign intervention that does not increase the rate of recurrence, and it may be useful as an adjunctive measure.

A brief summary of keloid treatments is shown in the Table 1.286–299 The reader should be aware that most of the studies include a mixture of patients with keloidal scars and hypertrophic scars, and they are rarely discussed separately.

Practical Guidance
Prevention is the best keloid therapy. Proposals for prevention include avoiding nonessential cosmetic surgery in keloid-forming patients, closing wounds with minimal tension, using cuticular, monofilament, synthetic permanent sutures in an effort to decrease tissue reaction, avoiding Z-plasties or any wound-lengthening techniques, and avoiding, as often as possible, making incisions that cross joints and follow skin creases.259

Despite a broad range of therapeutic possibilities, there is no universally effective treatment for keloids. A few treatment options, such as corticosteroid injections and perhaps compression, are
used as monotherapy. Polytherapy or a "shotgun approach" is used most often, and specific treatments are chosen on a patient-by-patient basis.²³⁷ For example, although injected triamcinolone is considered to be efficacious as a first-line therapy, silicone gel sheets may be more useful in children who cannot tolerate the pain of other therapies.²³⁰

### Table 12. Comparison of Treatment Modalities and Results*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Patients</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU + steroids (intralesional)²⁹⁰</td>
<td>10</td>
<td>All subjects showed significant flattening compared with control for all treatment arms. The most flattening was observed in the steroid treatment group, followed by steroid + 5-FU, 5-FU alone, and PDL treatment. Scar texture was better with PDL treatment.</td>
</tr>
<tr>
<td>5-FU²⁹⁶</td>
<td>10</td>
<td>All subjects showed significant flattening compared with control for all treatment arms. The most flattening was observed in the steroid treatment group, followed by steroid + 5-FU, 5-FU alone, and PDL treatment. Scar texture was better with PDL treatment.</td>
</tr>
<tr>
<td>Bleomycin²⁹⁷</td>
<td>13</td>
<td>Complete flattening, 42.8%</td>
</tr>
<tr>
<td>Cryosurgery + steroid (intralesional)²⁹⁸</td>
<td>58</td>
<td>61.3% had excellent or good results, 29% had poor response, 9.7% had no response</td>
</tr>
<tr>
<td>Cryotherapy²⁹⁵</td>
<td>93 (38 HS, 55 KS)</td>
<td>All 10 scars flattened, five out of 10 flattened greater than 50% of original height</td>
</tr>
<tr>
<td>IFN-γ (monotherapy)²⁹⁷</td>
<td>10</td>
<td>Recurrence was 18% after surgery and postoperative IFN treatment compared with 51% of patients treated with surgery + triamcinolone</td>
</tr>
<tr>
<td>IFN-2α-2β (monotherapy)²⁹¹</td>
<td>22</td>
<td>Only three out of 22 patients (14%) showed a reduction in size; nine patients withdrew from study (seven because of pain)</td>
</tr>
<tr>
<td>IFN-2α-2β + surgery²⁹⁸</td>
<td>124</td>
<td>33% recurrence rate after IFN + laser excision; 19% recurrence rate after IFN + surgery</td>
</tr>
<tr>
<td>IFN-2α-2β + surgery²⁷⁸</td>
<td>30</td>
<td>5% cream was applied to surgical scar after keloid was excised. At 24 weeks, there was no recurrence in 11 keloids of the 10 patients who completed the study.</td>
</tr>
<tr>
<td>Imiquimod²⁹²</td>
<td>13 keloids in 12 patients</td>
<td>55% excellent or good improvement (softening, flattening, lack of progression/recurrence), 11% poor</td>
</tr>
<tr>
<td>Laser²⁹² (77 with Argon, 5 with CO₂)</td>
<td>82</td>
<td>Effective in reducing pruritus and erythema and flattening scar in 57-83%</td>
</tr>
<tr>
<td>Pulsed dye laser²⁹⁷</td>
<td>16</td>
<td>All subjects showed significant flattening compared with control for all treatment arms. The most flattening was observed in the steroid treatment group, followed by steroid + 5-FU, 5-FU alone, and PDL treatment. Scar texture was better with PDL treatment.</td>
</tr>
<tr>
<td>Radiation (postsurgical)²⁹³</td>
<td>393</td>
<td>Cosmetic results excellent in 92%, favorable in 6%; only a 2.4% recurrence</td>
</tr>
<tr>
<td>Radiation (postsurgical)²⁷²</td>
<td>24</td>
<td>Irradiation given after keloid excised; 54% recurrence at 24 months</td>
</tr>
<tr>
<td>Radiation (postsurgical)²⁷⁴</td>
<td>147 KS</td>
<td>32.7% total recurrence rate within 18 months; the highest (41.7%) over high tension areas (chest wall, scapular region, upper limb) versus a 13.5% recurrence at low tension areas (neck, earlobes, lower limb)</td>
</tr>
<tr>
<td>Silicone²⁹⁵</td>
<td>94 (80 HS, 14 KS)</td>
<td>Overall, 84% were “greatly improved,” 11% were “somewhat improved,” and 5% had no improvement. Silicone “greatly improved” scar in 92.5% of hypertrophic scars and 35.7% of keloidal scars. “Somewhat improved” were 6.25% of hypertrophic scars and 35.7% of keloids. “No improvement” was seen in 1.25% of hypertrophic and 28.8% of keloidal scars.</td>
</tr>
<tr>
<td>Silicone²⁹⁶</td>
<td>46</td>
<td>Excellent, 13%; good, 47.8%; fair, 26%; poor, 13%</td>
</tr>
<tr>
<td>Steroids (intralesional) after excision²⁹⁰</td>
<td>58 (21 HS, 37 KS)</td>
<td>No recurrence, 93.1%; partial recurrence, 5.2%; full recurrence, 1.7%</td>
</tr>
<tr>
<td>Steroids (intralesional)²⁹⁶</td>
<td>10</td>
<td>All subjects showed significant flattening compared with control for all treatment arms. The most flattening was observed in the steroid treatment group, followed by steroid + 5-FU, 5-FU alone, and PDL treatment. Scar texture was better with PDL treatment.</td>
</tr>
<tr>
<td>Surgery + silicone + verapamil²⁷⁸</td>
<td>22</td>
<td>Complete result, 54%; partial result, 56%; absence of results, 9%</td>
</tr>
<tr>
<td>Surgery + silicone²⁷⁶</td>
<td>22</td>
<td>Complete result, 6%; partial result, 18%; absence of results, 82%</td>
</tr>
<tr>
<td>Verapamil (intralesional) + excision²⁷⁸</td>
<td>22</td>
<td>Verapamil was injected intralesionally and into the donor site (when skin graft was used) after excision of keloid with W-plasty. At 2-year follow-up, there were two recurrences (9%) (recurrent keloids were smaller than the originals), and four (18%) had hypertrophic-appearing scars; one patient developed a keloid at the skin donor site.</td>
</tr>
</tbody>
</table>

5-FU, 5-fluorouracil; HS, hypertrophic scar; KS, keloidal scar; PDL, pulsed dye laser.

*Nearly all of the above studies contain a mixture of hypertrophic and keloidal scars. When differences were noted by the author, those differences were included in the table. Otherwise, both types of scar were treated equally.

### FUTURE WOUND-HEALING THERAPIES

Gene therapy is the future for wound-healing strategies. Current research is aimed at inserting growth factor genomes into the wound. Petrie et al.³⁰¹ have written an excellent review of gene therapy in wound healing, and it is recommended to the reader who wants more detailed information.

Inserting the desired genome can be accomplished through several different vehicles: biologic (viral) techniques, chemical methods via cationic liposomes, and physical insertion.

#### Viral Techniques

Many different viruses have been tried in gene therapy. There are four popular virus types: retroviruses, adenoviruses, adenoassociated viruses, and herpes simplex virus. The principles behind
creating a recombinant viral vector involve deleting essential parts of the viral genome to render viral replication defunct and inserting the gene of interest. The transgene inserted is limited by the packaging capacity of the vector. Although the endogenous viral promoter may be able to drive the expression of the inserted gene, this process is usually optimized by cloning the target gene under the control of a more powerful promoter sequence, such as the cytomegalovirus promoter. Two complications of retroviral vectors limit their use. One is the theoretical risk of inducing neoplastic transformation in the target cell—an event termed insertional mutagenesis—which could occur should the viral genome integrate in proximity to a cellular proto-oncogene, driving its production, or by disrupting a tumor suppressor gene. The other complication is the risk of generating a replication-competent virus, as evidenced by reports of several outbreaks of wild-type virus from recombinant virus–producing cell lines.302

**Cationic Liposomes**

Cationic liposomes are lipid bilayer spheres that are rendered cationic (positively charged) and associate in a noncovalent fashion with negatively charged DNA to form liposome-DNA complexes. The rationale for coating nucleic acid with lipids is to allow highly negatively charged nucleic acid molecules to traverse the plasma membrane of the target cell. Lipofection is the term used to refer to gene transfer by this mechanism. It involves the addition of the complexes to the target cells, following which the molecules adsorb to the cell membrane and deliver the bound DNA. The advantages of lipofection include repeated application, lack of toxicity, and ability to carry large amounts of DNA. Although currently used in 13 percent of all gene therapy clinical trials, lipofection is limited by a low rate of stable integration into the target cell and a lack of specificity.301

**Physical Insertion Methods**

There are several methods of physically delivering the plasmid DNA directly into the cell301: direct injection using a hypodermic needle, microseeding, particle-mediated transfer (using a gene gun), and electroporation.

**Direct Injection Using a Hypodermic Needle**

Direct injection involves injecting naked plasmid DNA solution directly into the target tissue to enable transgene expression. It is a desirable mode of transfection because it is versatile and can be used for plasmid DNA solution and liposome-DNA complexes. It has a lower incidence of effects exhibited by some viral vectors, such as elicitation of an adverse immune response and insertional mutagenesis. This technique has the disadvantage of low levels of transfection.303

**Microseeding**

Microseeding is a technique for in vivo gene transfer. A plasmid DNA solution of choice is delivered directly to the target cells of the skin by a set of oscillating solid microneedles driven by a modified tattooing device. Because no special preparation is required, recombinant viral vectors can be delivered efficiently. No foreign material is deposited, as can occur with particle-mediated gene transfer, and microseeding has a much higher transfection efficiency compared with single injection, possibly because the DNA is delivered by smaller, finer solid microneedles.

**Particle-Mediated Transfer (Gene Gun)**

Particle-mediated gene transfer involves bombarding cells and tissues with particles or microparticles coated with DNA. The microparticles are composed of gold or tungsten and are 1 to 5 μm in diameter. The particles are coated with the DNA of interest and loaded into a device known as the gene gun before being accelerated by a force (either an electrical discharge or high-pressure helium) that drives the particles into the target cell, where the DNA dissociates from the particles and is expressed. Advantages of this technique include its broad spectrum for being able to transfect a wide variety of target tissues in vivo and in vitro, including skin, liver, pancreas, kidney, muscle, and cornea, and the high loading capacity of the microparticles, which permits the introduction of multiple genes. Limitations include a relatively low level of transfection, estimated in monolayer culture as being 3 to 15 percent. EGF,304 PDGF,305 and TGF-β306 are among the growth factors that have been delivered by particle-mediated gene transfer, and all have been shown to accelerate the wound-healing response.

**Electroporation**

The principle of electroporation rests on the ability of electrical field pulses to create pores in the cell membrane. Cells suspended in a medium containing plasmid DNA and treated with electrical field pulses take up and express DNA from the medium.
Volume 117, Number 7S • Wound Healing

SUMMARY
Successful wound management requires a thorough understanding of wound healing and the factors that influence it.

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