Emad Tawfik Ahmed
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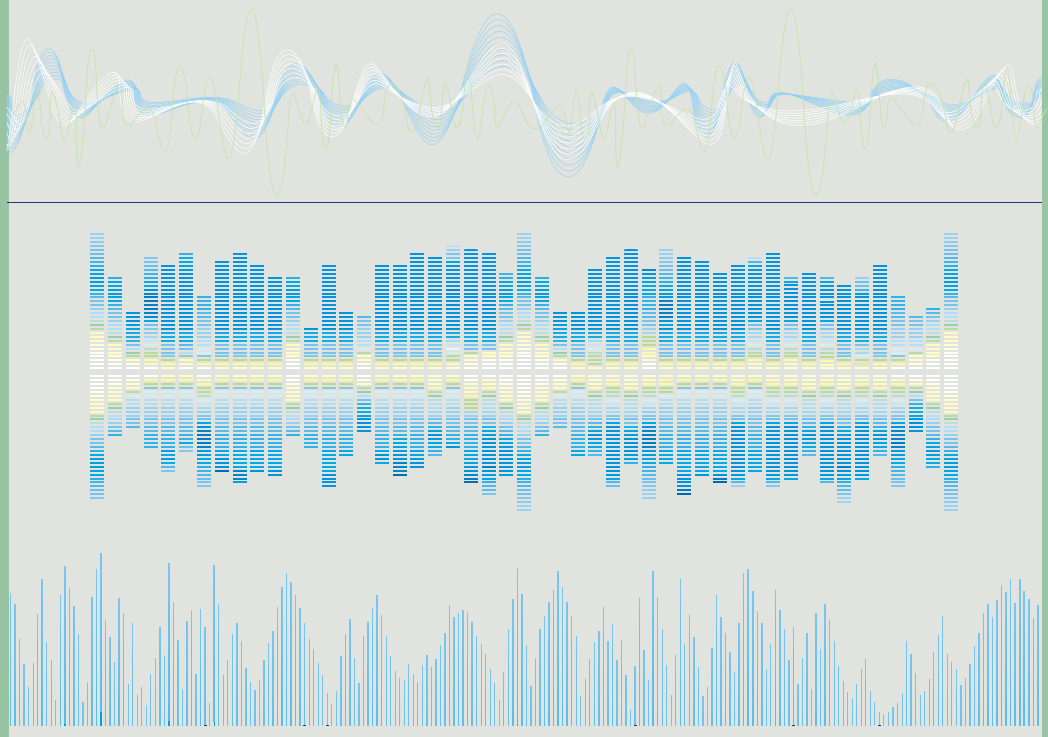
Electrical Stimulation in Wound Healing



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Preface

Electrical stimulation in wound healing is designed as a text on the management of burned patients, not only for surgeons, anesthesiologists, and residents but also nurses and allied health professionals. This book has served as a sophisticated instruction manual to guide those with less experience through difficult experiences in burn care. We have allowed some repetition of concepts and techniques throughout the text so that each chapter can be self-contained in its discussion of its main topic. Themes covered elsewhere in the literature have been condensed and the bibliographies selected to assure the reader ready access to the expanded literature on current burn care. The scope of this book is so specific in getting all physical therapy paractioner about the effectiveness of diverse electrical stimulation modalities on wound healing and giving them finally a clear cut point about the appropriate parameters for each modality if it is effective.

I would like to express my deep appreciation to many respected colleagues and friends for their contributions to the First edition of *electrical stimulation in wound healing*. Grateful acknowledgment is given to the many authors whose time and expertise made this book possible.

Finally, I would like to thank my wife, Safa, for her invaluable support.

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Chapter 1

Anatomy of Skin

Chapter 1

Anatomy of Skin

The skin is the largest organ of the body, accounting for about 15% of the total adult body weight. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body and a role in thermoregulation. The skin is continuous, with the mucous membranes lining the body's surface.[1] The integumentary system is formed by the skin and its derivative structures (see Figure 1). The skin is composed of three layers: the epidermis, the dermis, and subcutaneous tissue[1].

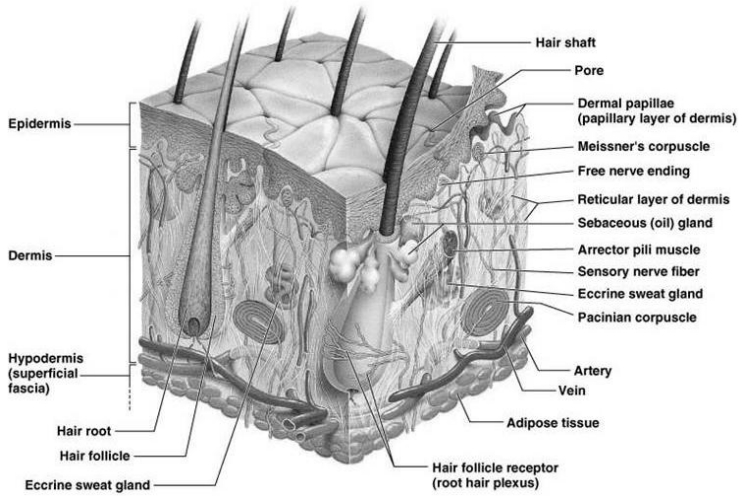

Figure 1. *Cross section of the skin.*

1.1 Skin Performs the Following Functions:[2–4]

Protection: an anatomical barrier from pathogens and damage between the

internal and external environment in bodily defense; Langerhans cells in the skin are part of the adaptive immune system.

Sensation: contains a variety of nerve endings that react to heat and cold, touch, pressure, vibration, and tissue injury.

Heat regulation: the skin contains a blood supply far greater than its requirements which allows precise control of energy loss by radiation, convection and conduction. Dilated blood vessels increase perfusion and heatloss, while constricted vessels greatly reduce cutaneous blood flow and conserve heat.

Control of evaporation: the skin provides a relatively dry and semi-impermeable barrier to fluid loss. Loss of this function contributes to the massive fluid loss in burns.

Aesthetics and communication: others see our skin and can assess our mood, physical state and attractiveness.

Storage and synthesis: acts as a storage center for lipids and water, as well as a means of synthesis of vitamin D by action of UV on certain parts of the skin.

Excretion: sweat contains urea, however its concentration is 1/130 that of urine, hence excretion by sweating is at most a secondary function to temperature regulation.

Absorption: the cells comprising the outermost 0.25–0.40 mm of the skin are "almost exclusively supplied by external oxygen", although the "contribution to total respiration is negligible" In addition, medicine can be administered through the skin, by ointments or by means of adhesive patch, such as the nicotine patch or iontophoresis. The skin is an important site of transport in many other organisms.

Water resistance: The skin acts as a water resistant barrier so essential

nutrients aren't washed out of the body.

1.2 Structure of the Skin

The integumentary system is formed by the skin and its derivative structures (see Figure 1). The skin is composed of three layers: the epidermis, the dermis, and subcutaneous tissue[1].

1.2.1 Epidermis[5]

The epidermis contains no blood vessels and is entirely dependent on the underlying dermis for nutrient delivery and waste disposal via diffusion through the dermoepidermal junction. The epidermis is a stratified, squamous epithelium that consists primarily of keratinocytes in progressive stages of differentiation from deeper to more superficial layers. The named layers of the epidermis include the stratum germinativum, stratum spinosum, stratum granulosum, and stratum corneum.

Keratinocytes

The stratum germinativum, or the basal layer, is immediately superficial to the dermoepidermal junction. This single cell layer of keratinocytes is attached to the basement membrane via hemidesmosomes.

As keratinocytes divide and differentiate, they move from this deeper layer to the more superficial layers. Once they reach the stratum corneum, they are fully differentiated keratinocytes devoid of nuclei and are subsequently shed in the process of epidermal turnover. Cells of the stratum corneum are the largest and most abundant of the epidermis. This layer ranges in thickness from 15-100 or more cells depending on anatomic location and is the primary protective barrier

from the external environment.

Melanocytes

Melanocytes, derived from neural crest cells, primarily function to produce a pigment, melanin, which absorbs radiant energy from the sun and protects the skin from the harmful effects of UV radiation. Melanin accumulates in organelles termed melanosomes that are incorporated into dendrites anchoring the melanosome to the surrounding keratinocytes. Ultimately, the melanosomes are transferred via phagocytosis to the adjacent keratinocytes where they remain as granules. Melanocytes are found in the basal layer of the epidermis as well as in hair follicles, the retina, uveal tract, and leptomeninges. These cells are the sites of origin of melanoma.

In areas exposed to the sun, the ratio of melanocytes to keratinocytes is approximately 1:4. In areas not exposed to solar radiation, the ratio may be as small as 1:30. Absolute numbers of melanosomes are the same among the sexes and various races. Differing pigmentation among individuals is related to melanosome size rather than cell number. Sun exposure, melanocyte-stimulating hormone (MSH), adrenocorticotropic hormone (ACTH), estrogens, and progesterones stimulate melanin production. With aging, a decline is observed in the number of melanocytes populating the skin of an individual. Since these cells are of neural crest origin, they have no ability to reproduce.

Langerhans Cells

Langerhans cells originate from the bone marrow and are found in the basal, spinous, and granular layers of the epidermis. They serve as antigen-presenting cells. They are capable of ingesting foreign antigens, processing them into small peptide fragments, binding them with major histocompatibility complexes, and

subsequently presenting them to lymphocytes for activation of the immune system. An example of activation of this component of the immune system is contact hypersensitivity.

Merkel Cells

Merkel cells, also derived from neural crest cells, are found on the volar aspect of digits, in nail beds, on the genitalia, and in other areas of the skin. These cells are specialized in the perception of light touch.

1.2.2 Dermis[6]

The primary function of the dermis is to sustain and support the epidermis. The dermis is a more complex structure and is composed of 2 layers, the more superficial papillary dermis and the deeper reticular dermis. The papillary dermis is thinner, consisting of loose connective tissue containing capillaries, elastic fibers, reticular fibers, and some collagen. The reticular dermis consists of a thicker layer of dense connective tissue containing larger blood vessels, closely interlaced elastic fibers, and coarse bundles of collagen fibers arranged in layers parallel to the surface.

The reticular layer also contains fibroblasts, mast cells, nerve endings, lymphatics, and epidermal appendages. Surrounding the components of the dermis is the gel-like ground substance, composed of mucopolysaccharides (primarily hyaluronic acid), chondroitin sulfates, and glycoproteins. The deep surface of the dermis is highly irregular and borders the subcutaneous layer, the panniculus adiposus, which additionally cushions the skin.

Fibroblasts

The fibroblast is the major cell type of the dermis. These cells produce and

secrete procollagen and elastic fibers. Procollagen is terminally cleaved by proteolytic enzymes into collagen that aggregates and becomes cross-linked. These tightly cross-linked collagen fibers provide tensile strength and resistance to shear and other mechanical forces. Collagen makes up 70% of the weight of the dermis, primarily Type I (85% of the total collagen) and Type III (15% of the total collagen). Elastic fibers constitute less than 1% of the weight of the dermis, but they play an enormous functional role by resisting deformational forces and returning the skin to its resting shape.

1.3 Epidermal Appendages[7, 8]

Epidermal appendages are intradermal epithelial structures lined with epithelial cells with the potential for division and differentiation. These are important as a source of epithelial cells, which accomplish reepithelialization should the overlying epidermis be removed or destroyed in situations such as partial thickness burns, abrasions, or split-thickness skin graft harvesting.

Epidermal appendages include the following:

- Sebaceous glands
- Sweat glands
- Apocrine glands
- Mammary glands
- Hair follicles

They often are found deep within the dermis and in the face may even lie in the subcutaneous fat beneath the dermis. This accounts for the remarkable ability of the face to reepithelialize even the deepest cutaneous wounds.

1.3.1 Sebaceous Glands

Sebaceous glands, or holocrine glands, are found over the entire surface of the body except the palms, soles, and dorsum of the feet. They are largest and most concentrated in the face and scalp where they are the sites of origin of acne. The normal function of sebaceous glands is to produce and secrete sebum, a group of complex oils that include triglycerides and fatty acid breakdown products, wax esters, squalene, cholesterol esters, and cholesterol. Sebum lubricates the skin to protect it against friction and makes the skin more impervious to moisture.

1.3.2 Sweat Glands

Sweat glands, or eccrine glands, are found over the entire surface of the body except the vermillion border of the lips, the external ear canal, the nail beds, the labia minora, and the glans penis and the inner aspect of the prepuce. They are most concentrated in the palms and soles and the axillae.

Each gland consists of a coiled secretory intradermal portion that connects to the epidermis via a relatively straight distal duct. The normal function of the sweat gland is to produce sweat, which cools the body by evaporation. The thermoregulatory center in the hypothalamus controls sweat gland activity through sympathetic nerve fibers that innervate the sweat glands. Sweat excretion is triggered when core body temperature reaches or exceeds a set point.

1.3.3 Apocrine and Mammary Glands

Apocrine glands are similar in structure, but not identical, to eccrine glands. They are found in the axillae, in the anogenital region, and, as modified glands, in the external ear canal (ceruminous glands), the eyelid (Moll's glands), and the

breast (mammary glands). They produce odor and do not function prior to puberty, which means they probably serve a vestigial function. The mammary gland is considered a modified and highly specialized type of apocrine gland.

1.3.4 Hair Follicles

Hair follicles are complex structures formed by the epidermis and dermis. (See the image below.) They are found over the entire surface of the body except the soles of the feet, palms, glans penis, clitoris, labia minora, mucocutaneous junction, and portions of the fingers and toes. Sebaceous glands often open into the hair follicle rather than directly onto the skin surface, and the entire complex is termed the pilosebaceous unit.

1.4 Cutaneous Blood Supply

Cutaneous vessels ultimately arise from underlying named source vessels. Each source vessel supplies a 3-dimensional vascular territory from bone to skin termed an angiosome. Adjacent angiosomes have vascular connections via reduced caliber (choke) vessels or similar caliber (true) anastomotic vessels. The cutaneous vessels originate either directly from the source arteries (septocutaneous or fasciocutaneous perforators) or as terminal branches of muscular vessels (musculocutaneous perforators).

During their course to the skin, the cutaneous vessels travel within or adjacent to the connective tissue framework and supply branches to each tissue with which they come into close contact (bone, muscle, fascia, nerve, fat). They emerge from the deep fascia in the vicinity of the intermuscular or intramuscular septa or near tendons and travel toward the skin, where they form extensive subdermal and dermal plexuses. The dermis contains horizontally arranged superficial and deep

plexuses, which are interconnected via communicating vessels oriented perpendicular to the skin surface. Cutaneous vessels ultimately anastomose with other cutaneous vessels to form a continuous vascular network within the skin. Clinically, this extensive horizontal network of vessels allows for random skin flap survival[9–11].

1.5 Lymphatic's

Skin lymphatics parallel the blood supply and function to conserve plasma proteins and scavenge foreign material, antigenic substances, and bacteria. Blind-ended lymphatic capillaries arise within the interstitial spaces of the dermal papillae. These unvalved, superficial dermal vessels drain into valved deep dermal and subdermal plexuses. These then coalesce to form larger lymphatic channels, which course through numerous filtering lymph nodes on their way to join the venous circulation near the subclavian vein – internal jugular vein junction bilaterally[6].

1.6 Skin Innervation

Sensory perception is critically important in the avoidance of pressure, mechanical or traumatic forces, and extremes of temperature. Numerous specialized structures are present in the skin to detect various stimuli. As previously mentioned, Merkel cells of the epidermis detect light touch. Meissner corpuscles also detect light touch. These are found in the dermal papillae and are most concentrated in the fingertips. Pacini corpuscles are found deep within the dermis or even in the subcutaneous tissue. These structures are specialized to detect pressure.

Pain is transmitted through naked nerve endings located in the basal layer of the epidermis. Krause bulbs detect cold, whereas Ruffini corpuscles detect heat. Heat, cold, and proprioception also are located in the superficial dermis. Cutaneous nerves follow the route of blood vessels to the skin. The area supplied by a single spinal nerve, or a single segment of the spinal cord, is termed a dermatome. Adjacent dermatomes may overlap considerably, which is important to note when performing field blocks with local anesthesia[12].

Chapter 2

Wound Healing

Chapter 2

Wound Healing

2.1 Introduction

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.

2.2 Wound Healing

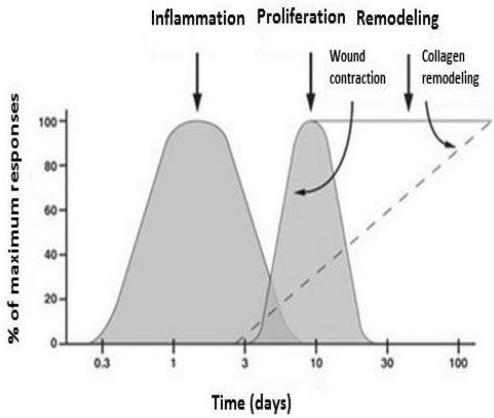

Figure 2. *Phases of wound healing.*

Wound healing has traditionally been divided into three distinct phases:

inflammation, proliferation, and remodeling[13, 14]. 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 (see figure 2).

2.2.1 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 that initiate the inflammatory response[15]. The fibrin clot serves as scaffolding for arriving cells, such as neutrophils, monocytes, fibroblasts, and endothelial cells[16]. It also serves to concentrate the cytokines and growth factors[17].

2.2.2 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 factor (TGF), platelet factor-4 (PF4), and bacterial "products."[18, 19] 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[20].

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 debride the wound; ILs and TNF, which stimulate fibroblasts (produce collagen) and promote angiogenesis; and TGF, which stimulates keratinocytes.

2.2.3 Proliferative Phase (Epithelization, Angiogenesis, and Provisional Matrix Formation; Day 4 through 14)

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[21]. 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 reestablish 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 don't appear to synthesize TGF-α)[22]. Epithelization begins shortly after wounding and is first stimulated by inflammatory cytokines. IL-1 and TNF 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[23, 24]. It has been shown that, for humans, KGF-2 is most important for directing this process[25].

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[26–28]. In response to PDGF, fibroblasts begin synthesizing a provisional matrix composed of collagen type III, glycosaminoglycans, and fibronectin[29].

2.2.4 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[30]. 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[15].

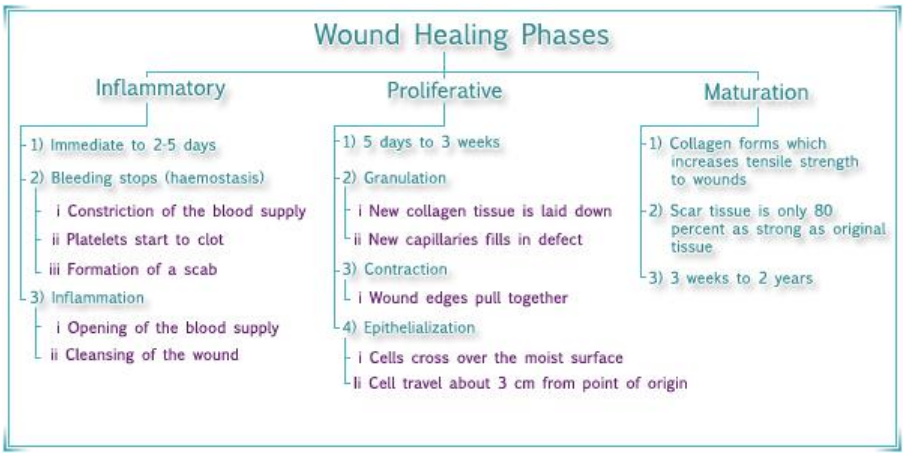

Figure 3. *Summary of events for wound healing phases.*

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[31]. 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[32]. Finally the events of wound healing can be summarized in figure (3).

2.3 Skin Metabolism and Physiology

The blood supply of the skin is far greater than it requires metabolically. Blood vessels in the skin are capable of carrying 20–100X the amounts of oxygen and nutrients that are needed for cellular survival and function. (Cells above the basal layer of the epidermis have largely lost their mitochondria and respire mainly through glycolysis, contributing little to the metabolic needs of the skin.) Despite the abundant blood supply, skin perfusion is insufficient to support wound healing, which requires granulation tissue.

Ryan[33] summarizes this paradox as follows: Although the skin can resist many hours of compression and obliteration of its blood supply but non-healing of the skin is one of the most common of problems and is often blamed on impairment of blood supply. The dilemma is explained by the fact that exchange between blood vessels and the supplied tissue services the functions of that tissue, and, although it is often stated that richness of the skin vasculature exceeds nutritional need, this statement is a misconception that is why the frequent stimuli of scratching, stretching, compressing, heating, or cooling of the skin require restoration of skin stiffness to a status quo. In restoring itself to the status quo, the mechanical properties of the skin must be instantly repaired and this repair requires a luxurious blood supply to maintain not merely cell metabolism but the physical properties of the interstitium.

2.3.1 Collagen

Collagen is the principal building block of connective tissue, accounting for one third of the total protein content of the body. Collagen is an unusual protein in that it is almost devoid of the sulfur containing amino acids cysteine and tryptophan. In their stead, collagen contains hydroxyproline and hydroxylysine, two amino acids with very limited distribution otherwise - only in collagen, elastin, the C1q subcomponent of the complement system, and the tail structure of acetylcholinesterase[34, 35]. Collagen has a very complex tertiary and quaternary molecular structure consisting of three polypeptide chains, each chain wound upon itself in a left handed helix and the three chains together wound in a right-handed coil to form the basic collagen unit. The polypeptide chains are held in their relative configurations by covalent bonds. Each triple helical structure is a tropocollagen molecule. Tropocollagen units associate in a regular fashion to form collagen filaments; collagen filaments in turn aggregate as collagen fibrils, and collagen fibrils unite to form collagen fibers, which are visible under the light microscope (Fig 4).

Five types of collagen have been identified in humans on the basis of amino acid sequences. Their relative distribution in connective tissues varies, hinting at individual properties valuable for specific functions (Table 1). Type I collagen is abundant in skin, tendon, and bone. These tissues account for more than 90% of all collagen in the body. Normal skin contains Type I and Type III collagen in a 4:1 ratio, the latter mainly in the papillary dermis. In hypertrophic and immature scars the percentage of Type III collagen may be as high as 33% (a 2:1 Type I:III ratio)[36].

Collagen synthesis takes place extracellularly as well as intracellularly. Certain substances inhibit the formation of collagen either by interfering with its

synthesis or activating its degradation (Fig 5). Normal connective tissue is in a state of dynamic equilibrium balanced between synthesis and degradation, and this makes it vulnerable to local stimuli such as mechanical forces on the tissue. While excessive collagen degradation results from unchecked collagenase synthesis, not enough collagenase gives rise to tissue fibrosis. Homeostasis is achieved through *activation* of collagenase by parathyroid hormone, adrenal corticosteroids, and colchicine; and *inhibition* of collagenase synthesis by serum alpha-2 macroglobulin, cysteine and progesterone[37].

Table 1. *Types and distribution of collagen.*

Type	Structure	Distribution
I	Hyprid of two chains low in hydroxylysine and glycosylated hydroxylysine.	Bone, tendon, skin, dentin, ligament, fascia, arteries, and uterus
II	Relatively high in hydroxylysine and glycosylated hydroxylysine.	Hyaline cartilage, eye tissues
III	High in hydroxylysine and low in hydroxylysine contain interchains disulfide bonds.	Skin, arteries, uterus, and bowel wall.
IV	High in hydroxylysine and glycosylated hydroxylysine. May contain large globular regions.	Basement membrane.
V	Similar to Type IV	Basement membrane and perhaps other tissues

(Annotated from Prockop DJ et al.: The biosynthesis of collagen and its disorders. N Engl J Med 301:13, 1979.)

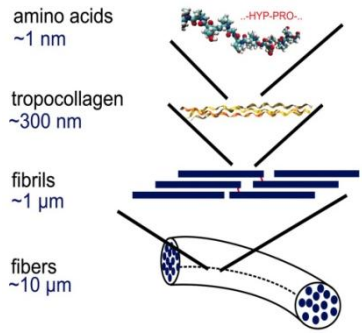

Figure 4. *Molecular and fibrillar structure of collagen.*

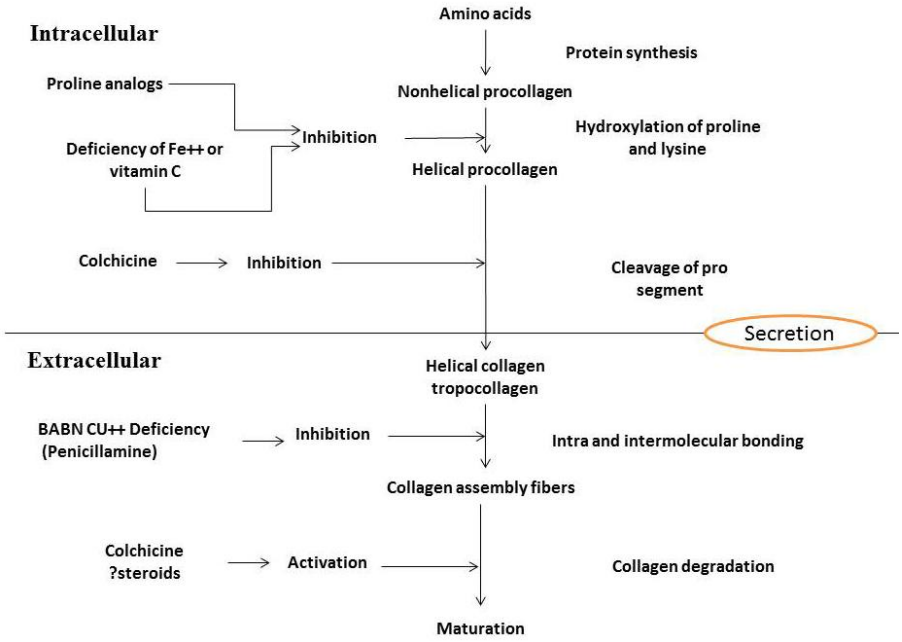

Figure 5. *Collagen synthesis and site of action of common inhibitors.*

2.3.2 The Myofibroblast and Wound Contraction

Contraction is an essential part of the repair process by which the organism closes a gap in the soft tissues. *Contracture*, on the other hand, is an undesirable result of healing, at times due to the process of contraction and at other times due to fibrosis or other tissue damage[38].

In 1971 Gabbiani, Ryan, and Majno[39] first noted a type of fibroblast in granulation tissue that bore some structural similarities to smooth muscle cells. Myofibroblasts differ from ordinary fibroblasts by having cytoplasmic microfilaments similar to those of smooth muscle cells. Within the filamentous system are areas of "dense bodies" that serve as attachments for contraction. The nuclei demonstrate numerous surface irregularities such as those of smooth

muscle cells but unlike those of ordinary fibroblasts. Myofibroblasts are also different from normal fibroblasts in that they have well-formed intercellular attachments such as desmosomes and maculae adherens. Myofibroblasts are the source of contraction within a wound[40–43].

Rudolph[44, 45] found a direct relationship between the rate of wound contraction and the number of myofibroblasts within a wound. Rudolph[45]also demonstrated the presence of myofibroblasts throughout the wound, not just adjacent to the wound margins. McGrath and Hundahl[46] confirmed the parallel paths of wound contraction and number of myofibroblasts in the wound and the relatively even distribution of myofibroblasts in granulation tissue except at the wound bed (fewer) and adjacent to foci of inflammation (more). Their findings support the "pull theory" of wound contraction, which holds that the entire granulating surface of the wound acts as a contractile organ. This concept implies contraction of individual myofibroblasts to shorten the wound, followed by collagen deposition and crosslinking to maintain the shortening, in a lock-step mechanism. Prostaglandin inhibitors do not inhibit myofibroblast production, therefore wound contraction is not altered[47]. Although present in a number of contracture disorders like Dupuytren's disease[48], Peyronie's, and lederhosen disease[41], myofibroblasts have not been implicated in their etiology.

2.3.3 Tensile Strength

The *tensile strength* of a wound is a measurement of its load capacity per unit area. A wound's *breaking strength* is defined as the force required to break it regardless of its dimensions. Depending solely on different skin thicknesses, breaking strength can vary several folds; tensile strength, on the other hand, is constant for wounds of similar size.

Experimental studies give evidence that collagen fibers are largely responsible for the tensile strength of wounds[49]. The rate at which a healing wound regains strength varies not only among species, but also among individuals and even among different tissues in the same individual[50]. The healing pattern of the various tissues, however, is remarkably similar within a phylogenetic family.

All wounds gain strength at approximately the same rate during the first 14–21 days, but thereafter the curves may diverge significantly according to the tissue involved. In skin, the peak tensile strength is achieved at approximately 60 days after injury[51] (Fig 6). Given optimal healing conditions, the tensile strength of a wound never reaches that of the original, unwounded skin, leveling off at about 80%.

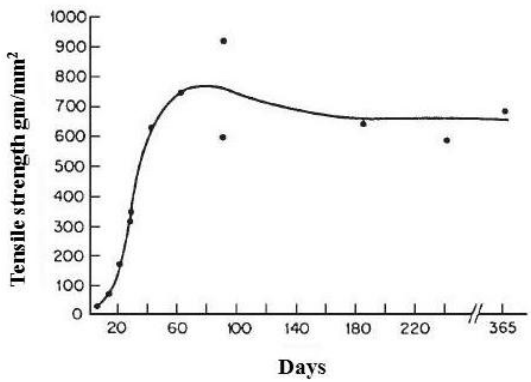

Figure 6. *Tensile strength of a healing skin incision as a function of time. (Reprinted with permission from Levenson SM et al.: The healing of rat skin wounds. Ann Surg 161:293, 1965.)*

2.4 Factors Affecting Wound Healing

Impaired wound healing can be caused by multiple factors. This can be categorized into local and systemic factors.

2.4.1 Local Factors That Influence Healing

(a) Oxygenation

Oxygen is important for cell metabolism, especially energy production by means of ATP, and is critical for nearly all wound-healing processes. It prevents wounds from infection, induces angiogenesis, increases keratinocyte differentiation, migration, and re-epithelialization, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction[52]. In addition, the level of superoxide production (a key factor for oxidative killing pathogens) by polymorphonuclear leukocytes is critically dependent on oxygen levels.

The microenvironment of the early wound is depleted of oxygen and is quite hypoxic. Several systemic conditions, including advancing age and diabetes, can create impaired vascular flow, thus setting the stage for poor tissue oxygenation. In the context of healing, this overlay of poor perfusion creates a hypoxic wound. Chronic wounds are notably hypoxic; tissue oxygen tensions have been measured transcutaneously in chronic wounds from 5 to 20 mm Hg, in contrast to control tissue values of 30 to 50 mm Hg[53].

Healing is impaired in wounds where oxygenation is not restored,. Temporary hypoxia after injury triggers wound healing, but prolonged or chronic hypoxia delays wound healing[52]. In acute wounds, hypoxia serves as a signal that stimulates many aspects of the wound-healing process. Hypoxia can induce cytokine and growth factor production from macrophages, keratinocytes, and fibroblasts. Cytokines that are produced in response to hypoxia include PDGF, TGF-β, VEGF, and tumor necrosis factor-α (TNF-α), are crucial promoters of cell proliferation, migration and chemotaxis, and angiogenesis in wound healing[54].

In normally healing wounds, ROS such as hydrogen peroxide (H_2O_2) and superoxide (O^2) are thought to act as cellular messengers to stimulate key processes associated with wound healing, including cell motility, cytokine action (including PDGF signal transduction), and angiogenesis. Both hypoxia and hyperoxia increase ROS production, but an increased level of ROS transcends the beneficial effect and causes additional tissue damage[54].

In summary, the proper oxygen level is crucial for optimum wound healing. Hypoxia stimulates wound healing such as the release of growth factors and angiogenesis, while oxygen is needed to sustain the healing process[52]. One therapeutic option that can sometimes overcome the influence of tissue hypoxia is hyperbaric oxygen therapy (HBOT)[54]. While HBOT can be an effective treatment for hypoxic wounds, its availability is limited.

(b) Infections

After skin injury, micro-organisms that are normally sequestered at the skin surface obtain access to the underlying tissues. The state of infection and replication status of the micro-organisms determines whether the wound is classified as having contamination, colonization, local infection/critical colonization, and/or spreading invasive infection. Contamination is the presence of non-replicating organisms on a wound, while colonization is defined as the presence of replicating micro-organisms on the wound without tissue damage. Local infection/critical colonization are an intermediate stage, with micro-organism replication and the beginning of local tissue responses. Invasive infection is defined as the presence of replicating organisms within a wound with subsequent host injury[55].

Normal part of the wound-healing process is Inflammation, and is important to the removal of contaminating micro-organisms. In the absence of effective

decontamination, however, inflammation may be prolonged, since microbial clearance is incomplete. Both bacteria and endotoxins can lead to the prolonged elevation of pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α and elongate the inflammatory phase. If this continues, the wound may enter a chronic state and fail to heal. This prolonged inflammation also leads to an increased level of matrix metalloproteases (MMPs), a family of proteases that can degrade the ECM. In tandem with the increased protease content, a decreased level of the naturally occurring protease inhibitors occurs. This shift in protease balance can cause growth factors that appears in chronic wounds to be rapidly degraded[56]. Similar to other infective processes, the bacteria in infected wounds occur in the form of biofilms, which are complex communities of aggregated bacteria embedded in a self-secreted extracellular polysaccharide matrix (EPS)[55]. Mature biofilms develop protected microenvironments and are more resistant to conventional antibiotic treatment. *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and β-hemolytic *streptococci* are common bacteria in infected and clinically non-infected wounds[57].

Many chronic ulcers probably do not heal because of the presence of biofilms containing *P. aeruginosa*, thus shielding the bacteria from the phagocytic activity of invading polymorphonuclear neutrophils (PMNs). This mechanism may explain the failure of antibiotics as a remedy for chronic wounds[58].

2.4.2 Systemic Factors That Influence Healing

(a) Age

Increased age is a major risk factor for impaired wound healing. Many clinical and animal studies at the cellular and molecular level have examined age-related changes and delays in wound healing. It is commonly recognized that, in healthy

older adults, the effect of aging causes a temporal delay in wound healing, but not an actual impairment in terms of the quality of healing[59]. Delayed wound healing in the aged is associated with an altered inflammatory response, such as delayed T-cell infiltration into the wound area with alterations in chemokine production and reduced macrophage phagocytic capacity[60]. Delayed re-epithelialization, collagen synthesis, and angiogenesis have also been observed in aged mice as compared with young mice[60]. Overall, there are global differences in wound healing between young and aged individuals. A review of the age-related changes in healing capacity demonstrates that every phase of healing undergoes characteristic age-related changes, including enhanced platelet aggregation, increased secretion of inflammatory mediators, delayed infiltration of macrophages and lymphocytes, impaired macrophage function, decreased secretion of growth factors, delayed re-epithelialization, delayed angiogenesis and collagen deposition, reduced collagen turnover and remodeling, and decreased wound strength[59].

Age-related impairment of healing can be reduced by several treatments. Interestingly, exercise has been reported to improve cutaneous wound healing in older adults as well as aged mice, and the improvement is associated with decreased levels of pro-inflammatory cytokines in the wound tissue. The improved healing response may be due to an exercise-induced anti-inflammatory response in the wound[61].

(b) Sex Hormones in Aged Individuals

Aged males have been shown to have delayed healing of acute wounds when compared with females. A partial explanation for this is that the female estrogens (estrone and 17β-estradiol), male androgens (testosterone and 5α-dihydrotestosterone), and their steroid precursor dehydroepiandrosterone

(DHEA) appear to have significant effects on the wound-healing process[62]. It was recently found that the differences in gene expression between elderly male and young human wounds are almost exclusively estrogen-regulated[63]. Estrogen affects wound healing by regulating a variety of genes associated with regeneration, matrix production, protease inhibition, epidermal function, and the genes primarily associated with inflammation[63]. Studies indicate that estrogen can improve the age-related impairment in healing in both men and women, while androgens regulate cutaneous wound healing negatively[62].

(c) Stress

Stress has a great impact on human health and social behavior. Many diseases - such as cardiovascular disease, cancer, compromised wound healing, and diabetes - are associated with stress. Numerous studies have confirmed that stress-induced disruption of neuroendocrine immune equilibrium is consequential to health[64]. The pathophysiology of stress results in the deregulation of the immune system, mediated primarily through the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal medullary axes or sympathetic nervous system (SNS)[65].

Studies in both humans and animals have demonstrated that psychological stress causes a substantial delay in wound healing. Caregivers of persons with Alzheimer's and students undergoing academic stress during examinations demonstrated delayed wound healing[66]. The hypothalamic-pituitary-adrenal and the sympathetic-adrenal medullary axes regulate the release of pituitary and adrenal hormones. These hormones include the adrenocorticotrophic hormones, cortisol and prolactin, and catecholamines (epinephrine and norepinephrine). Stress up-regulates glucocorticoids (GCs) and reduces the levels of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α at the wound site. Stress also

reduces the expression of IL-1α and IL-8 at wound sites - both chemoattractants that are necessary for the initial inflammatory phase of wound healing[65]. Furthermore, GCs influence immune cells by suppressing differentiation and proliferation, regulating gene transcription, and reducing expression of cell adhesion molecules that are involved in immune cell trafficking[67]. The GC cortisol functions as an anti-inflammatory agent and modulates the Th1-mediated immune responses that are essential for the initial phase of healing. Thus, psychological stress impairs normal cell-mediated immunity at the wound site, causing a significant delay in the healing process[65].

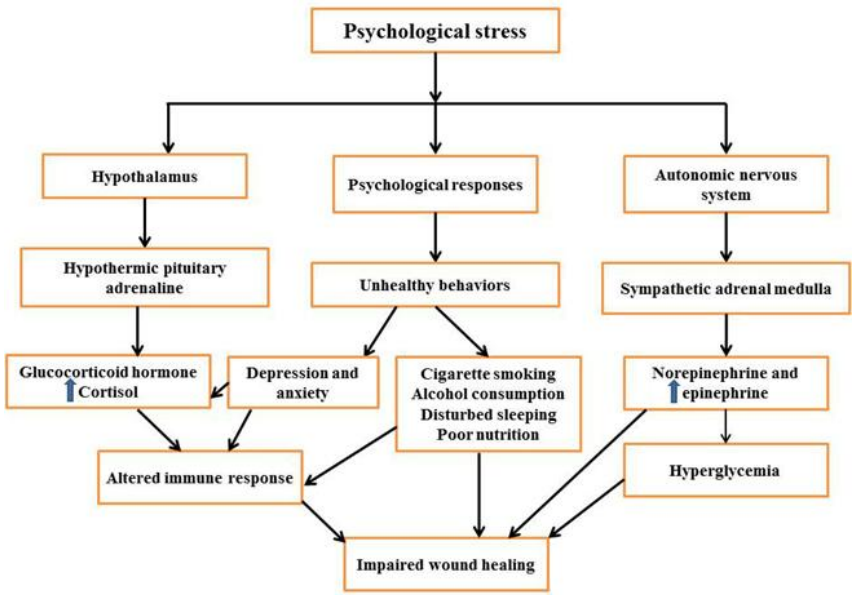

Figure 7. *The effects of stress on wound healing.*

Stressors can lead to negative emotional states, such as anxiety and depression, which may in turn have an impact on physiologic processes and/or behavioral patterns that influence health outcomes. In addition to the direct influences of anxiety and depression on endocrine and immune function, stressed individuals are more likely to have unhealthy habits, which include poor sleep patterns,

inadequate nutrition, less exercise, and a greater propensity for abuse of alcohol, cigarettes, and other drugs. All of these factors may come into play in negatively modulating the healing response. The effects of stress on wound healing are summarized in Fig. 7.

(d) Diabetes

Diabetes affects hundreds of millions of people worldwide. Diabetic individuals exhibit a documented impairment in the healing of acute wounds. Moreover, this population is prone to develop chronic non-healing diabetic foot ulcers (DFUs), which are estimated to occur in 15% of all persons with diabetes. DFUs are a serious complication of diabetes, and precede 84% of all diabetes-related lower leg amputations[68]. The impaired healing of both DFUs and acute cutaneous wounds in persons with diabetes involves multiple complex pathophysiological mechanisms. DFUs, like venous stasis disease and pressure-related chronic non-healing wounds, are always accompanied by hypoxia[53]. A situation of prolonged hypoxia, which may be derived from both insufficient perfusion and insufficient angiogenesis, is detrimental for wound healing. Hypoxia can amplify the early inflammatory response, thereby prolonging injury by increasing the levels of oxygen radicals[69]. Hyperglycemia can also add to the oxidative stress when the production of ROS exceeds the anti-oxidant capacity[70]. The formation of advanced glycation end-products (AGEs) under hyperglycemia and the interaction with their receptors (RAGE) are associated with impaired wound healing in diabetic mice as well[71]. High levels of metalloproteases are a feature of diabetic foot ulcers, and the MMP levels in chronic wound fluid are almost 60 times higher than those in acute wounds. This increased protease activity supports tissue destruction and inhibits normal repair processes[69].

Several dysregulated cellular functions are involved in diabetic wounds, such

as defective T-cell immunity, defects in leukocyte chemotaxis, phagocytosis, and bactericidal capacity, and dysfunctions of fibroblasts and epidermal cells. These defects are responsible for inadequate bacterial clearance and delayed or impaired repair in individuals with diabetes[72].

As mentioned above, hypoxia contributes to the compromised healing of DFUs, and diabetic wounds exhibit inadequate angiogenesis. Several studies that have investigated the mechanisms behind the decreased restoration of vasculature in diabetic wounds have implied that EPC mobilization and homing are impaired, and that the level of VEGF, the primary pro-angiogenic factor in wounds, is decreased in the diabetic state[68]. Stem-cell-based therapies aimed at inducing EPCs or BM-MSCs have shown a promising outcome in diabetic non-healing wounds, both in animals and in clinical trials[73]. In animal studies, therapeutic restoration of VEGF has been shown to improve repair outcomes significantly[74].

The neuropathy that occurs in diabetic individuals probably also contributes to impaired wound healing. Neuropeptides such as nerve growth factor, substance P, and calcitonin gene-related peptide are relevant to wound healing, because they promote cell chemotaxis, induce growth factor production, and stimulate the proliferation of cells. A decrease in neuropeptides has been associated with DFU formation. In addition, sensory nerves play a role in modulating immune defense mechanisms, with denervated skin exhibiting reduced leukocyte infiltration[75].

In summary, the impaired healing that occurs in individuals with diabetes involves hypoxia, dysfunction in fibroblasts and epidermal cells, impaired angiogenesis and neovascularization, high levels of metalloproteases, damage from ROS and AGEs, decreased host immune resistance, and neuropathy. The influence of these factors on wound healing is summarized in Fig. 8.

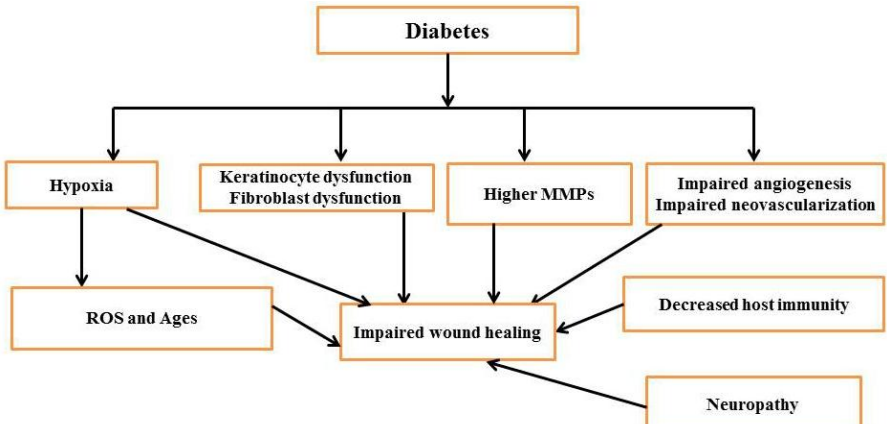

Figure 8. *The potential effects of diabetes on wound healing.*

(e) Medications

Many medications, such as those which interfere with clot formation or platelet function, or inflammatory responses and cell proliferation have the capacity to affect wound healing. Here we review only the commonly used medications that have a significant impact on healing, including glucocorticoid steroids, non-steroidal anti-inflammatory drugs, and chemotherapeutic drugs.

Glucocorticoid Steroids

Systemic glucocorticoids (GC), which are frequently used as anti-inflammatory agents, are well-known to inhibit wound repair *via* global anti-inflammatory effects and suppression of cellular wound responses, including fibroblast proliferation and collagen synthesis. Systemic steroids cause wounds to heal with incomplete granulation tissue and reduced wound contraction[76]. Glucocorticoids also inhibit production of hypoxia-inducible factor-1 (HIF-1), a key transcriptional factor in healing wounds[77]. Beyond effects on repair itself, systemic corticosteroids may increase the risk of wound infection. While systemic corticosteroids inhibit wound repair, topical application produces quite

different effects. Topical low-dosage corticosteroid treatment of chronic wounds has been found to accelerate wound healing, reduce pain and exudate, and suppress hypergranulation tissue formation in 79% of cases. While these positive effects are striking, careful monitoring is necessary to avoid a potential increased risk of infection with prolonged use[78].

Non-steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen are widely used for the treatment of inflammation and rheumatoid arthritis and for pain management. Low-dosage aspirin, due to its anti-platelet function, is commonly used as a preventive therapeutic for cardiovascular disease, but not as an anti-inflammatory drug[79]. There are few data to suggest that short-term NSAIDs have a negative impact on healing. However, the question of whether long-term NSAIDs interfere with wound healing remains open. In animal models, systemic use of ibuprofen has demonstrated an anti-proliferative effect on wound healing, resulting in decreased numbers of fibroblasts, weakened breaking strength, reduced wound contraction, delayed epithelialization[80], and impaired angiogenesis[81]. The effects of low-dose aspirin on healing are not completely clear. Clinical recommendations suggest that, to avoid anti-platelet effects, individuals should discontinue NSAIDs for a time period equal to 4 to 5 times the half-life of drugs before surgery. Thus, the majority of surgical patients do not have significant NSAID activity at the time of wound repair. The exception may be those cardiac patients who must be maintained on low-dose aspirin due to severe risk of cardiovascular events[79]. In terms of the topical application of NSAIDs on the surfaces of chronic wounds, the local use of ibuprofen-foam provides moist wound healing, reduces persistent and temporary wound pain, and benefits chronic venous leg ulcer healing[82].

Chemotherapeutic Drugs

Most chemotherapeutic drugs are designed to inhibit cellular metabolism, rapid cell division, and angiogenesis and thus inhibit many of the pathways that are critical to appropriate wound repair. These medications inhibit DNA, RNA, or protein synthesis, resulting in decreased fibroplasia and neovascularization of wounds[76]. Chemotherapeutic drugs delay cell migration into the wound, decrease early wound matrix formation, lower collagen production, impair proliferation of fibroblasts, and inhibit contraction of wounds[76]. In addition, these agents weaken the immune functions of the patients, and thereby impede the inflammatory phase of healing and increase the risk of wound infection. Chemotherapy induces neutropenia, anemia, and thrombocytopenia, thus leaving wounds vulnerable to infection, causing less oxygen delivery to the wound, and also making patients vulnerable to excessive bleeding at the wound site.

Impaired wound healing due to chemotherapeutic drugs such as adriamycin is most common when the drugs are administered pre-operatively or within 3 weeks post-operatively[83]. Additionally, low post-operative albumin levels, low post-operative hemoglobin, advanced stage of disease, and electrocautery use have all been reported as risk factors for the development of wound complications[84].

A newer generation of tumor chemotherapeutics is the angiogenesis inhibitors, such as bevacizumab, which is an antibody fragment that neutralizes VEGF. These therapies work in conjunction with traditional chemotherapeutics to limit the blood supply to tumors, reducing their ability to grow. Wound-healing complications, including an increase in wound dehiscence, have been described in patients on angiogenesis inhibitors[85]. A caveat is that most patients on angiogenesis inhibitors are also on traditional chemotherapeutics, making it

difficult to sort out whether angiogenesis inhibitors alone would perturb repair[86]. Nevertheless, current recommendations include discontinuation of angiogenesis inhibitors well in advance of any surgical procedures.

(f) Obesity

The prevalence of obesity continues to increase among adults, children, and adolescents in the United States, with more than 30% of adults and 15% of children and adolescents classified as obese in a recent survey (Centers for Disease Control and Prevention, CDC). Obesity is well-known to increase the risk of many diseases and health conditions, which include coronary heart disease, type 2 diabetes, cancer, hypertension, dyslipidemia, stroke, sleep apnea, respiratory problems, and impaired wound healing. Obese individuals frequently face wound complications, including skin wound infection, dehiscence, hematoma and seroma formation, pressure ulcers, and venous ulcers[87]. An increased frequency of wound complications has been reported for obese individuals undergoing both bariatric and non-bariatric operations[88]. In particular, a higher rate of surgical site infection occurs in obese patients. Many of these complications may be a result of a relative hypoperfusion and ischemia that occurs in subcutaneous adipose tissue. This situation may be caused by a decreased delivery of antibiotics as well. In surgical wounds, the increased tension on the wound edges that is frequently seen in obese patients also contributes to wound dehiscence. Wound tension increases tissue pressure, reducing microperfusion and the availability of oxygen to the wound[87].

The increase in pressure ulcers or pressure-related injuries in obese individuals is also influenced by hypovascularity, since poor perfusion makes tissue more susceptible to this type of injury. In addition, the difficulty or inability of obese individuals to reposition themselves further increases the risk of pressure-related

injuries. Moreover, skin folds harbor micro-organisms that thrive in moist areas and contribute to infection and tissue breakdown. The friction caused by skin-on-skin contact invites ulceration. Together, these factors predispose obese individuals to the development of impaired wound healing[89].

In addition to local conditions, systemic factors also play an important role in impaired wound healing and wound complications in obese patients. Obesity can be connected to stress, anxiety, and depression, all situations which can cause an impaired immune response[87].

The function of adipose tissue used to be considered as primarily caloric storage. However, more recent findings have documented that adipose tissue secretes a large variety of bioactive substances that are collectively named adipokines. Both adipocytes themselves as well as macrophages inside the adipose tissue are known to produce bioactive molecules including cytokines, chemokines, and hormone-like factors such as leptin, adiponectin, and resistin. Adipokines have a profound impact on the immune and inflammatory response[90]. The negative influence of adipokines on the systemic immune response seems likely to influence the healing process, although direct proof for this is lacking. Impaired peripheral blood mononuclear cell function, decreased lymphocyte proliferation, and altered peripheral cytokine levels have been reported in obesity. Importantly, though, many of the obesity-related changes in peripheral immune function are improved by weight loss[91].

(g) Alcohol Consumption

Clinical evidence and animal experiments have shown that exposure to alcohol impairs wound healing and increases the incidence of infection[92]. The effect of alcohol on repair is quite clinically relevant, since over half of all emergency room trauma cases involve either acute or chronic alcohol exposure[93]. Alcohol

exposure diminishes host resistance, and ethanol intoxication at the time of injury is a risk factor for increased susceptibility to infection in the wound[94]. Studies have demonstrated profound effects of alcohol on host-defense mechanisms, although the precise effects are dependent upon the pattern of alcohol exposure (*i.e.*, chronic *vs*. acute alcohol exposure, amount consumed, duration of consumption, time from alcohol exposure, and alcohol withdrawal). A recent review on alcohol-induced alterations on host defense after traumatic injury suggested that, in general, short-term acute alcohol exposure results in suppressed pro-inflammatory cytokine release in response to an inflammatory challenge. The higher rate of post-injury infection correlates with decreased neutrophil recruitment and phagocytic function in acute alcohol exposure[95].

Beyond the increased incidence of infection, exposure to ethanol also seems to influence the proliferative phase of healing. In murine models, exposure to a single dose of alcohol that caused a blood alcohol level of 100 mg/dL (just above the legal limit in most states in the US) perturbed re-epithelialization, angiogenesis, collagen production, and wound closure[96]. The most significant impairment seems to be in wound angiogenesis, which is reduced by up to 61% following a single ethanol exposure. This decrease in angiogenic capacity involves both decreased expression of VEGF receptors and reduced nuclear expression of HIF-1alpha in endothelial cells. The ethanol-mediated decrease in wound vascularity causes increased wound hypoxia and oxidative stress. Connective tissue restoration is also influenced by acute ethanol exposure, and results in decreased collagen production and alterations in the protease balance at the wound site. In summary, acute ethanol exposure can lead to impaired wound healing by impairing the early inflammatory response, inhibiting wound closure, angiogenesis, and collagen production, and altering the protease balance at the wound site[97].

As mentioned previously, the host response to chronic alcohol exposure

appears to be different from that of acute alcohol exposure. Analysis of clinical data indicates that chronic alcohol exposure causes impaired wound healing and enhanced host susceptibility to infections, but the detailed mechanisms that explain this effect need more investigation.

(h) Smoking

Post-operatively, patients who smoke show a delay in wound healing and an increase in a variety of complications such as infection, wound rupture, anastomotic leakage, wound and flap necrosis, epidermolysis, and a decrease in the tensile strength of wounds[98]. Approximately over 4000 substances in tobacco smoke have been identified, and some have been shown to have a negative impact on healing[99]. Most studies have focused on the effects of nicotine, carbon monoxide, and hydrogen cyanide from smoke. Nicotine probably interferes with oxygen supply by inducing tissue ischemia, since nicotine can cause decreased tissue blood flow *via* vasoconstrictive effects[100].

Several cell types and processes that are important to healing have been shown to be adversely affected by tobacco smoke. In the inflammatory phase, smoking causes impaired white blood cell migration, resulting in lower numbers of monocytes and macrophages in the wound site, and reduces neutrophil bactericidal activity. Lymphocyte function, cytotoxicity of natural killer cells, and production of IL-1 are all depressed, and macrophage-sensing of Gram-negative bacteria is inhibited. These effects result in poor wound healing and an increased risk of opportunistic wound infection[99].

During the proliferative phase of wound healing, exposure to smoke yields decreased fibroblast migration and proliferation, reduced wound contraction, hindered epithelial regeneration, decreased extracellular matrix production, and upset in the balance of proteases[99].

Despite the overall negative effects of smoking, some recent studies have suggested that low doses of nicotine enhance angiogenesis and actually improve healing[101, 102].

(i) Nutrition

Carbohydrates, Protein, and Amino Acids

Fats and carbohydrates are the primary source of energy in the wound-healing process. Glucose is the major source of fuel used to create the cellular ATP that provides energy for angiogenesis and deposition of the new tissues[103]. The use of glucose as a source for ATP synthesis is essential in preventing the depletion of other amino acid and protein substrates[104].

Collagen is the major protein component of connective tissue and is composed primarily of glycine, proline, and hydroxyproline. Collagen synthesis requires hydroxylation of lysine and proline, and co-factors such as ferrous iron and vitamin C. Impaired wound healing results from deficiencies in any of these co-factors[105].

Arginine is a semi-essential amino acid that is required during periods of maximal growth, severe stress, and injury. Arginine has many effects in the body, including modulation of immune function, wound healing, hormone secretion, vascular tone, and endothelial function. Arginine is also a precursor to proline, and, as such, sufficient arginine levels are needed to support collagen deposition, angiogenesis, and wound contraction[103]. Arginine improves immune function, and stimulates wound healing in healthy and ill individuals[106]. Under psychological stress situations, the metabolic demand of arginine increases, and its supplementation has been shown to be an effective adjuvant therapy in wound healing[105].

Glutamine is the most abundant amino acid in plasma and is a major source of metabolic energy for rapidly proliferating cells such as fibroblasts, lymphocytes, epithelial cells, and macrophages[104]. The serum concentration of glutamine is reduced after major surgery, trauma, and sepsis, and supplementation of this amino acid improves nitrogen balance and diminishes immunosuppression[105].

Fatty Acids

In surgical or critically ill patients lipids are used as nutritional support for to help meet energy demands and provide essential building blocks for wound healing and tissue repair. They have been reported to affect pro-inflammatory cytokine production, cell metabolism, gene expression, and angiogenesis in wound sites[107]. The true benefit of omega-3 fatty acids may be in their ability to improve the systemic immune function of the host, thus reducing infectious complications and improving survival[104].

Vitamins, Micronutrients, and Trace Elements

Vitamin C has many roles in wound healing, and a deficiency in this vitamin has multiple effects on tissue repair. Vitamin C deficiencies result in impaired healing, and have been linked to decreased collagen synthesis and fibroblast proliferation, decreased angiogenesis, and increased capillary fragility. Also, vitamin C deficiency leads to an impaired immune response and increased susceptibility to wound infection[104].

Similarly, vitamin A deficiency leads to impaired wound healing. The biological properties of vitamin A include anti-oxidant activity, increased fibroblast proliferation, modulation of cellular differentiation and proliferation, increased collagen and hyaluronate synthesis, and decreased MMP-mediated extracellular matrix degradation[108].

Vitamin E, an anti-oxidant, maintains and stabilizes cellular membrane integrity by providing protection against destruction by oxidation. Vitamin E also has anti-inflammatory properties and has been suggested to have a role in decreasing excess scar formation in chronic wounds. Animal experiments have indicated that vitamin E supplementation is beneficial to wound healing[104], and topical vitamin E has been widely promoted as an anti-scarring agent. However, clinical studies have not yet proved a role for topical vitamin E treatment in improving healing outcomes[109].

Chapter 3

Assessment of Wound Healing

Chapter 3

Assessment of Wound Healing

3.1 Wound Assessment

The pace of change in wound management is placing an emphasis on the development of more objective tools by which to assess and evaluate wound healing. At present, there are no predictive factors to guide clinicians to differentiate patients who will heal readily from those who will have prolonged courses of treatment[110].

3.2 Importance of Wound Measurement

Recording wound area and volume is considered a routine part of patient assessment and provides information on the progress of healing[111]. A thorough initial wound assessment provides baseline data about the status of the wound and valuable information that can assist in identifying short- and long-term goals of care and help to determine appropriate interventions at each stage[112].

Accurate wound measurement is an integral and objective component of the assessment process and is required for comparative results and analysis of treatment regimens[113]. However, in two studies of documentation of wound assessment, statements such as ‘healing well’ were commonly used whereas actual wound size was only recorded in six out of 40 patients’ notes with the method of wound measurement never mentioned[114, 115].

Being able to predict whether wounds will heal readily with conventional

treatment and deciding which patients are candidates for often expensive new treatments isimportant[114, 116]. Continuous monitoring of changes in wound size is key to the outcome of this process. Knowing which ulcer will probably fail to heal within a 24-week period allows the clinician to consider alternative and perhaps more aggressive treatment strategies after only four weeks of therapy, using simple measurements accessible to any practitioner[116].

The value of knowing wound size is demonstrated by Margolis et al.[116]. In a retrospective cohort study of 260 patients they were able to predict ulcer healing in venous leg ulcers at 24 weeks in 95% of cases when compression therapy was used. To predict this outcome they devised a scoring system. This system allocated one point to wounds greater than 5 cm^2 and one point to those greater than 6 months in duration. A total of 93–95% of whose with a score of 0 healed at 24 weeks compared with 13–37% of those with a score of two.

Predicting ulcer healing is especially important in the current managed care environment in which cost-containment and the need for referral to a specialist have assumed great importance[117]. If further referral and investigation are warranted then the measured ulcer area is an important piece of medical information[118].

3.3 Wound Measurement Methods

Methods used to determine the area of a wound can be subdivided into contact and non-contact methods. Of the methods listed in Table 2, ruler technique, tracing overlays and planimetry are most commonly used in routine clinical practice[118].

3.3.1 Ruler Method

The ruler method measures the maximal length by the maximal perpendicular width using a disposable paper ruler to calculate area.

The ruler method is the simplest method for measuring the sizes of skin lesions and determines their area through a manual measurement of their length and width (L x W) with a ruler or tape measure. A number of different measurement strategies can be used for the L x W method, including the longest head-to-toe length and longest perpendicular width of a lesion; the longest length and width perpendicular to one another; the longest head-to-toe length and greatest width at any angle; and the longest length and greatest width at any angle. A study done by Langemo et al.[119]. demonstrated that measurement of the longest head-to-toe length and greatest perpendicular width of a lesion was the most accurate method for all of the three wound shapes (symmetrical, L-shaped, and pear-shaped) in the study. However, although it is simple to use and inexpensive to implement, the RT method is accurate mathematically only for rectangular or square lesions. Consequently, the more the shape of a lesion deviates from a rectangle or square, the greater is the potential for overestimation of its true surface area

3.3.2 Acetate Method

The acetate method involves applying a two-layer transparent acetate over the wound and tracing the perimeter with a permanent pen. The contact layer is then discarded into clinical waste and the top layer stored within the patient notes. For most cutaneous lesions, measuring the wound area from contour tracings estimates healing reliably despite the errors introduced by flattening a curvedsurface[120].

Most acetates are provided preprinted with 1 cm^2 measures, and the number of

squares half or more within the perimeter are calculated as 1 cm^2. Some acetates are preprinted in 1 mm^2 areas but these take too long to count and are not suited to routine practice. In addition to providing an area outline of the wound, the acetate can be used to identify areas of slough or epithelialisation and can be dated and stored within patient notes. Computerised systems, such as digital planimetry, can be used in conjunction with acetate[121].

3.3.3 Digital Planimetry

Digital planimetry incorporates the same method to obtain the wound border as the acetate method, but rather than counting squares the tracing is placed on a digital tablet, and the border is re-traced using a stylus. The underlying sensor then calculates the wound area. The literature reports some studies that have compared methods to obtain wound area for superficial wounds. Oien et al.[122] compared the measurement of 50 leg ulcers in 20 patients by three physical therapists using four methods of area measurement: maximal perpendicular diameter; grid tracing and square counting; mechanical planimetry; and digital planimetry. The results demonstrated that all four methods had a high degree of agreement with each other, at least for ulcers with an area up to approximately 10 cm^2.

3.3.4 Photographic Method

At each ulcer measurement session, three photographs were taken using 35-mm color slide film* and an OlympusOM-2s camera. A metric ruler wastaped adjacent to and in the plane of the ulcer prior to photography. The distance between ulcer and camera was 27.9 to 30.5 cm (11-12 in). To obtain a tracing from each slide, the slide was projected onto paper, and the projector-to-paper distance and focus were adjusted until the image of the metric ruler in the slide exactly matched the ruler used in the original photograph, as described by

Bulstrode et al.[123]. The ulcer margins were then traced with a pen. One tracing was generated from each of the three slides for each measurement session of each individual ulcer.

3.3.5 Wound Depth

(a) Depth Gauge

Internal wound dimensions are commonly measured by placing a depth gauge at the “deepest” aspect of the wound[6] Use of a depth gauge leaves considerable room for measurement error because the value obtained from each measurement depends on the technician's determination of the “deepest” portion of the wound[124].

(b) Dental Impression Material or Saline Method

A technique that has been used clinically to assess wound volume involves filling the wound cavity with a substance such as alginate. A mold is made of the wound, and either the volume of the alginate cast can be measured directly with the use of a fluid displacement technique or the cast can be weighed and that weight divided by the density of the casting materials, which represents the wound volume. A variation of this technique for measuring wound volumes involves using saline. A quantity of saline is injected into the wound; the volume of fluid needed to fill the wound is recorded as the volume of the wound. These techniques have been described in various articles in the literature, and the relative merits and relative accuracy of each have been the subject of several studies reported in the literature[125].

Chapter 4

Electrical Stimulation and Wound Healing

Chapter 4

Electrical Stimulation and Wound Healing

4.1 Theoretic and Scientific Basis

A number of interesting and thought-provoking studies of electrotherapy and tissue repair have been done in recent years. In light of this research, possible mechanisms attributed to electrotherapy and the stimulation of tissue repair are examined here, starting with the theory of skin battery voltage, and healing.

Various living organisms, including humans, have a skin battery potential that is negatively charged on the surface (skin) and positively charged in deeper tissues[126, 127]. Research has demonstrated the presence of an electrical current in the wounds and injuries of human beings and other living organisms. Intact skin has a small negative charge, and wounds are positively charged[128]. The strength of the endogenous wound EFs measured in animals and humans that have been observed to direct cell migration (electrotaxis) after wounding have been quantified between 10 and 100 μA/cm[129]. In summary, there appears to be a relationship between the electrical "current of injury" and the repair, regeneration, and growth of tissue. Electrotherapy may mimic the body's own bioelectrical signal and promote healing in chronic wounds that have an impaired or insufficient "current of injury" [130].

Research findings have provided a basis for the use of electrotherapy to augment the healing process. Many effects of electrotherapy on the healing of wounds have been reported. An extensive review of the electrotherapy and tissue healing literature is not provided here, but a synopsis of pertinent human and animal

research on the effects of electrotherapy appears in Table (2). And figure (9)

Table 2. *The general cellular effect of electrical stimulation under cathode and anode.*

Cellular effects	
Epidermal cell migration[132]	
Increased fibroblast proliferation[133]	
Anode	Cathode
Neutrophils attracted[134]	Neutrophils attracted[134]
Macrophages attracted[135]	Fibroblasts attracted[139]
Mast cells (associated with abnormal fibrotic healing) migration inhibited[136]	Epidermal cell migration[140]
Leukocytes attracted[137]	Leukocytes attracted (when infection/inflammation present)[141]
Thrombosis of small vessels[137]	Increased blood flow[141]
Bactericidal[138]	Decreased edema[142]
	Bactericidal[143]

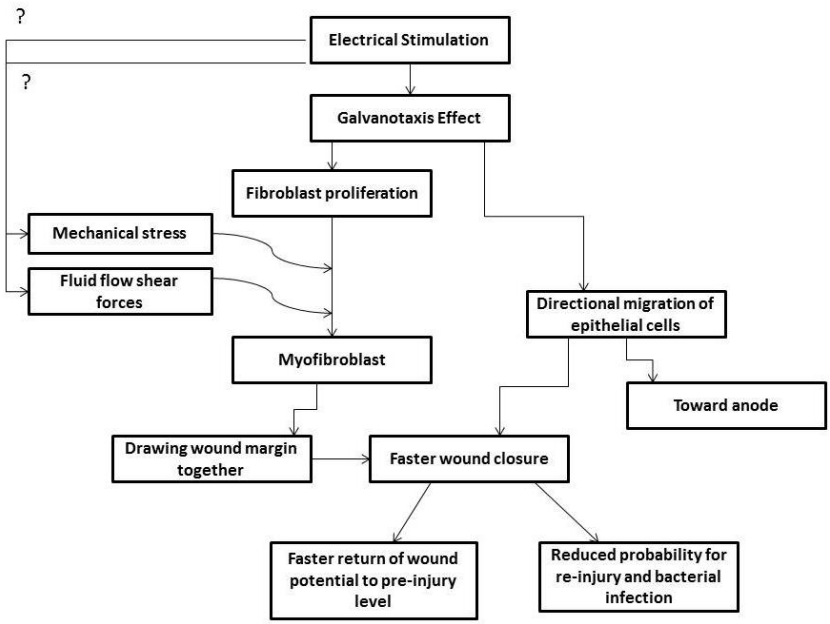

Figure 9. *Schematic illustration showing the effect of ES on wound closure.*

4.2 Types of Electrical Stimulation Current

Three main types of electrical stimulation can be used to induce the above cellular effect

- Direct current
- Pulsed current
- Alternating current

The differences between these currents are illustrated in the following figure (figure 10)[131].

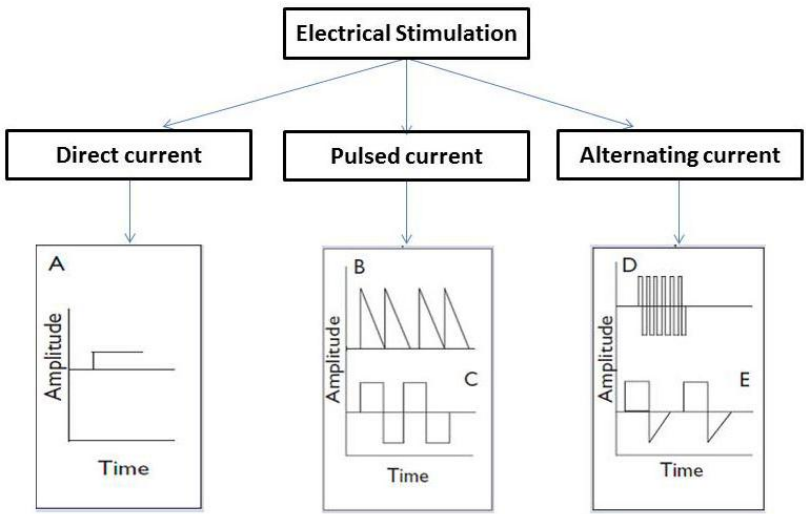

Figure 10. *The different types of electrical stimulation currents.*

4.2.1 HVPC for Wound Healing

A trial concerning the effects of HVPC was reported by Kloth and Feedar[144]. A

group of 16 patients with stage IV decubitus ulcers were recruited for the trial and all had lesions that had been unresponsive to previous treatment. Patients were allocated randomly to a treatment group (n=59) or sham treatment) group (n=57). The ES consisted of monophasic twin-pulse stimulation at 105 pps. delivered at a voltage just below that required to achieve visible muscle contraction (typically 100–175V). These stimulation parameters are reported as being arbitrarily set. ES was given for one 45 minute session a day for 5 days a week. Sham group patients had electrodes placed in the same way, but the machine output was set to zero. Electrode polarity was set initially for the wound electrode to be positive, with the negative electrode placed on the skin surface proximally. If a healing plateau was reached during the trial, the wound electrode was made negative and the treatment continued. If a second plateau was reached, the electrode polarity was reversed daily thereafter. Whichever electrode was placed at the wound site, the relative arrangement was maintained in that the positive electrode was always placed cephalad in relation to the negative electrode. All patients in the treatment group achieved complete healing of their ulcers (on average over 7.3 weeks at a mean healing rate of 44.8% per week). The control group patients did less well with an increase in mean wound size of almost 29% between the first and last treatments. A subgroup of patients who were in the control group went on to complete a course of ES following the main trial; the three patients achieved full healing of their ulcers over 8.3 weeks with an average healing rate of 38% per week.

Griffin et al.[145] assessed the effects of HVPC on pressure ulcer healing in a group of patients with spinal cord injury. Seventeen patients were assigned randomly to either a treatment or a control (sham treatment) group. ES treatments were carried out for 1 hour a day for 20 consecutive days with repeated wound assessments during this period. HVPC was delivered by means of a negative wound electrode with the stimulator delivering 100 pps. at an intensity of 200

volts using similar twin pulses to the previous study. The percentage change (decrease) in ulcer size for the treatment group was significantly greater at days 5, 15 and 20 and the average change for all ulcers in the treatment group was an 80% size reduction compared with a 52% decrease for the control group.

A more recent study by Houghton et al.[146] involved 27 patients with a total of 42 chronic leg ulcers of varying etiology (diabetic, arterial, venous) and employed a placebo controlled RCT design. Following initial assessment, there was a stable (baseline) period during which only 'conventional' therapy was employed, followed by a 4 week treatment phase with the patients divided into treatment or sham groups. The high voltage pulses were delivered at 150V, 100 pps and 100μsec duration, using 45 minute treatment periods, 3 times a week for the 4 weeks. The wound electrode was made negative throughout the treatment period i.e. no polarity reversal. Assessment included a one month follow up period. The treatment group wounds significantly reduced in size (mean 44% of original) compared with the sham group (mean 16%). The significant differences were not maintained at the 1 month follow up assessment, though there was a clear trend seen in the results.

Goldman et al.[147] aimed to evaluate the ability of high voltage pulsed current (HVPC) to increase microcirculation in critically ischemic wounds and, as a result, to improve wound healing. The diabetic patients presented with maleolar ischaemic lesions and serial measures were made of wound parameters, including oxygen tension. The results indicated that the use of electrical stimulation with these patients objectively improved tissue oxygenation and improved the anticipated wound healing profile.

In addition to the wound healing / wound closure studies[148, 149], HVPC has been shown (with other stimulation modalities) to have both a germicidal and

antibacterial effect.

Finally the parameters of application of high voltage galvanic stimulation can be summarized in (table 3).

Table 3. *High voltage pulsed galvanic stimulation proposed parameters on the basis of protocols used in studies.*

Inflammation and proliferation phase: • Cathode on wound. • Frequency: 30 pps. • Intensity: 100-150 V. • 60 min once a day 5-7 times/wk.
Epithelialization phase: • 3 days cathode followed by 3 days anode; continue the 3 days alternations. • Frequency: 100-128 pps. • Intensity: 100-150 V. • 60 min once a day 5-7 times/wk.
Remodeling /maturation phase: • Alternate polarity daily. • Frequency: 60-64 pps. • Intensity: 100-150 V. • 60 min once a day 5-7 times/wk.

4.2.2 Micro-current and Wound Healing

Low-intensity electric currents or microcurrents (MCs) are currents of an intensity less than or equal to 1 mA (1000 μA, μA = microampere). The current may be direct or alternating of varying - mainly rectangular - waveforms, frequency, and pulse duration. Low-intensity currents were formerly known as MC electrical neuromuscular stimulators, but were later named microcurrent electrical stimulator (MES) (MC electrical stimulator)[150].

Microcurrents are produced by low-voltage generators or combined electrotherapy units. Such generators or units can produce a range of waveforms,

from monophasic to square or rectangular biphasic, with a range of frequencies from 0.3 to 50 Hz. Electrotherapeutic units of low voltage may produce currents of intensities up to a few milliamperes in which case sensory stimulation or muscular stimulation results. Pulse duration may also be modified from 1 to 500 milliseconds at low frequencies or may be preselected when pulsed current is utilized[150].

4.2.3 Low-intensity Direct Current

Low-intensity direct current (LIDC) is the most common type of LIC studied in research. Wolcott et al.[151] studied wound healing resulting from application of LIDC in 83 patients with ischemic wounds. Three sessions per day took place, each lasting 2 hours. Intensity ranged from 200 to 800 μA, the negative electrode was placed on the wound and the positive electrode proximally. After 3 days, polarity was reversed provided that no infection had appeared. In the event of presence of infection, reversal was postponed until infection had subsided and was then delayed for an additional 3 days. Afterward, polarity was reversed each time healing reached a plateau. The rationale of the delay of polarity reversal may be attributed to the study of Rowley et al.[152], where by placing the negative electrode on the wound in similar parameters, the current presented with antimicrobial effects. Forty-five percent of wounds healed completely around a mean of 9.6 weeks, and the rest reached partial healing up to 64.7% over 7.2 weeks. Direct comparison of 2 treatments, standard treatment versus LIC, on the same subjects also took place, a fact that eliminated confounding factors stemming from differences among individuals such as age, sex, general health, and underlying pathology (eg, diabetes). Eight of the patients presented with bilateral wounds. One side was treated with LIDC (n = 8) and the other received standard care (n = 8). Six of 8 LIDC-treated ulcers completely healed, while the

rest 2 of 8 healed up to 70%. In the other side, 3 of 8 ulcers did not heal, 3 of 8 healed less than 50%, and 2 out of 8 healed no more than 75%. In another clinical study[153], LIDC stimulation was applied to 6 patients with bilateral ischemic skin ulcers. The parameters of LIDC were same as in the study by Wolcott et al.[151], only polarity was reversed once. One side received standard treatment, whereas the other side ulcer received the same treatment plus LIDC stimulation. The healing rate of the non-LIDC side was 14.7% compared with 30% in the LIDC-treated side. A significant enhancement of healing was observed. A total of 100 patients also received LIDC treatment on ischemic wounds including the six patients previously mentioned. Mean healing rate amounted to 28.4% per week.

The positive effect of LIDC on chronic leg ulcers nonresponsive to other treatment has also been supported in a case study by Assimacopoulos et al.[154], in which, LIDC was applied on 3 patients with venous leg ulcers. Healing occurred in all 3 patients in 6 weeks, by applying a current of 100 μA. No control group was available, and being a case study, the strength of the results is somewhat limited.

Carley and Wainapel[155] applied LIDC (200–800 μA) on 30 patients with ulcers of various pathologies located over the sacrum or the lower limb below the knee. Patients were assigned in an electrical stimulation treatment group (n = 15) or conventional treatment group (n = 15) matched according to age, diagnosis, etiology, and wound size, thus ensuring that confounding factors were controlled to a considerable extent. Both groups received standard conservative treatment. The treatment group received additional electrical stimulation of 200 to 800 μA for 2 hours, twice daily, with an interval of at least 2 to 4 hours, 5 days per week, for 5 weeks. The negative electrode was placed on the wound and the positive electrode proximally. Reversal of polarity took place, as in the study by Wolcott et al.[151], and treatment was continued until full wound healing was reached. Finally the parameters of low intensity direct current used in accelerating wound

healing can be summarized in table (4).

Table 4. *Low-intensity direct current proposed parameters on the basis of protocols used in studies.*

Intensity	**200–800 μA (negative electrode on wound)**
Treatment time	2 h
Times/d	2 to 3 sessions with a 2- to 4-h interval
Times/wk	5 d/wk
Duration of treatment	5 to 9 wk

4.2.4 Low-intensity Pulsed Direct Current

Low-intensity current provides minor stimulation to the healing site, being an LIC. One might expect that by using a pulsed form of this current, effectiveness would probably decrease because stimulation might be even less.

In a double-blind study by Wood et al.[156], 74 patients with stages II and III chronic decubitus ulcers in 4 centers, were randomly allocated in a treatment group (n = 43) and a placebo (sham treatment) group (n = 31), which received standard treatment. Treatment composed of electrical stimulation using low-intensity pulsed direct current (LIPDC) of 300 to 600 μA. After 8 weeks of treatment, 58% of ulcers in the treatment group had healed, whereas in the placebo group only 1 healed, and in the rest of the ulcers, ulcer area increased. A statistically significant accelerated rate of healing (P< .0001) was observed.

Reversal of polarity of pulsed direct current during the healing period has been studied. Junger et al.[157] investigated the effect of LIPDC on venous leg ulcers of 15 patients who had not responded to standard compression treatment over 79 months. An intensity of 630 μA was selected initially (frequency: 128 pulses per second; pulse duration: 140 μs) with the cathode placed on the wound for 7 to 14 days. The following 3 to 10 days, the positive electrode was positioned on the

wound, and after that specific time frame polarity was reversed again. As soon as significant healing had occurred, intensity was reduced to 315μA (64 pulses per second). Treatment was performed on a daily basis, each session lasting 30 minutes. Mean ulcer area was reduced to 63% ($P< .01$). Furthermore, capillary density was increased to 43.5% ($P< .039$), and improvement of skin perfusion was observed ($PtCo_2$ = 13.5 increased to 24.7 to 40 mm Hg being normal). Finally the parameters of low intensity pulsed direct current used in accelerating wound healing can be summarized in table (5).

Table 5. *Low-intensity pulsed direct current proposed parameters on the basis of protocols used in studies.*

Intensity	300 to 630 μA (negative electrode on wound, stable polarity or reversal of polarity on 3 to 10 days or when on plateau)
Treatment time or times/wk	30 minutes minimum per day
Frequency	130 Hz
Duration of treatment	4 to 8 wk

The evidence available indicates that LIC appear to accelerate wound healing. Regarding the selection of intensity, LIDC (continuous or pulsed) appears to be effective in the range of 200 to 800 μA, and polarity may or may not be reversed. Further research is required to elucidate the effect of LIC on wound healing.

4.2.5 Alternating Current (AC)

AC has been applied to chronic wounds in two types of protocols: symmetric square -wave, most commonly delivered using a portable TENS device, or asymmetric biphasic pulsed wave. As opposed to DC or PC stimulators, AC stimulation is generally delivered by electrodes adjacent to the wound rather than directly overlying it.

(a) TENS

Initial case report[158] and uncontrolled case series[159] is treating patients with TENS applied to nerves in the vicinity of the wounds suggested this approach might be beneficial, The etiologies of the treated ulcers were varied, but included neurotrophic lesions, with the rationale that the neural stimulation provided by TENS would enhance healing. One interesting study by Kaada and Emru[160] used TENS therapy to treat 32 patients with longstanding lower leg ulcers secondary to leprosy. Patients received trains of 5 pulses (25 mA at 100 pps, 0,1 to 0-2 millisecond duration) for 30 minutes sessions, twice daily for 5 to 6 days per week. Twelve weeks post-treatment, 59% of the patients healed completely. All those who completed therapy healed completely with a mean healing time of 5.2 weeks. All the above TENS studies were uncontrolled studies, and all used different treatment regimens, making conclusions difficult to draw. Thus far there has been only one randomized controlled study of the effect of TENS on wound healing. Lundeberg et al. studied 161 the effect of TENS on diabetic patients with stasis ulcers. The patients received either TENS therapy (treatment parameters not given) for 20-minutes, twice daily for 12 weeks or sham treatment. The polarity was changed after each session. All patients received standard wound care, which was a compression dressing. After 12 weeks, 42% of the treated group healed compared to 15% of the control, with statistical significance. This study does support a role of TENS stimulation in the treatment of ulcers in diabetic patients.

(b) Biphasic Pulsed

Asymmetric biphasic pulsed waveforms have been used in some wound healing studies, presumably because the asymmetry of the waveform allows the polarity of one pole to predominate One case series[162] and one non-randomized control trial[163] have suggested that this modality may be useful in enhancing

healing in a wide array of chronic ulcers. However, only one randomized controlled trial has evaluated the efficacy of this modality.

Baker et al.[164] evaluated the effects of two stimulation waveforms on healing rates in patients with diabetic ulcers, Patients received stimulation with either an asymmetric biphasic or symmetric biphasic square-wave pulse both at 50 pps, at unreported amplitudes, A third group received a sham ES. All patients in the study received standard wound care. In this study, treatment with asymmetric biphasic ES showed a statistically significant 60% increase in the healing rate, as compared to controls. This study suggests that the asymmetric biphasic wavelength may be more advantageous in ulcers in diabetic patients. The rationale for this is not entirely clear.

It appears that most of the studies on the efficacy of AC stimulation for wound healing evaluated patients with decubitus ulcers, so no inferences may be comfortably extended to other types of non-healing wounds. The double-blind randomized controlled study by Lundeberg et al.[161] is particularly strong, and its results do support a role for AC therapy in decubitus ulcers, Its efficacy in other chronic wounds remains to be evaluated.

Reger et al.[165] reported changes in surface area and volume of induced pressure ulcers after using DC (current amplitude about 0.7 mA and current density of 30–200 lA/cm^2) and alternating current(AC; 300 l s pulse duration, 40 Hz, and current density of 1,189 – 219 lA/cm^2) stimulation. Wound contraction occurred more rapidly in stimulated animals than in the controls. No histological changes were noted between the AC and DC stimulated wounds in the early phase of healing. Tissue perfusion was enhanced more by DC than by AC stimulation. A shorter wound area time constant and a higher rate of wound area reduction were noted after DC compared with AC ES. AC stimulation reduced wound volume

more than DC.

4.3 Effect of Electrical Stimulation on Different Types of Wounds

4.3.1 Effect of Electrical Stimulation on Skin Flap Survival

Kjartansson et al.[166] investigated the effect of segmental and extra segmental transcutaneous electrical nerve stimulation (monophasic PC) with a different frequency and amplitude on survival of the dorsal musculocutaneous flap in rat. They raised the flaps (2 cm to 7 cm) from the deep fascia of the muscles and then sutured these back into position. ES was delivered as monophasic PC with a 0.2 ms pulse duration, a frequency of 80 or 2 pps, and an intensity of 20 or 5 mA. In the segmental mode, ES was delivered to the base of the flap; in the extra segmental method, ES was delivered at the base of the animal's tail. Preoperative ES did not increase flap survival area when compared with the untreated control group. The highest flap survival was obtained with repeated segmental ES applied postoperatively with a high intensity. The authors showed that flap survival was not related to the frequency used.

4.3.2 Effect of Electrical Stimulation on Hypertrophic Scar

Reich et al.[167] designed one study to examine the effects of ES on the proliferation of mast cells in acute wounds. They made 80 wounds in 4 pigs (20 wounds in each animal): The wounds of 2 animals were treated using positive monophasic pulsed ES (38 mA, 140mspulse duration, and 128 pps), twice a day, for 1.5 h in each treatment session. The number of mast cells was significantly lower in these ES treated wounds than in the control wounds at days 1, 2, and 3. They speculated that this decrease may be related to a reduction in either

proliferation or migration of mast cells. Positive pulsed ES reduces wound vascularity and may be responsible for mast cell reduction[168, 169]. Keloids and hypertrophic scars, in particular, are often associated with increased numbers of mast cells[170]. Surgical flap survival rate is increased in mast cell deficient rats[171], indicating that ES may affect scar formation and should be considered in future animal studies.

4.4 Contraindications and Precautions

Before finishing the review of electrotherapy and its effects on healing, a look at the adverse effects, precautions, and contraindications is needed. Few adverse effects are cited in the literature; except for complaints of tingling or prickly feelings and occasional skin irritation[172]. The possibility that electrotherapy may cause an increase in the pain of patients with peripheral vascular disease. As mentioned earlier, the clinician must ensure that there is no foreign material, medications, heavy metals, or other topical substances in the wound that may hinder the treatment or adversely affect the care of the wound. In light of these possibilities, electrotherapy should be utilized with caution[173]. Contraindications to the use of electrotherapy are listed in Table (6). Before a wound is treated electro therapeutically, the patient should be screened for these conditions.

Table 6. *The precaution and contraindication of electrical stimulation.*

Precaution of electrical stimulation	Contraindication to electrical stimulation
Pregnancy	Unstable cardiac condition
Epilepsy	Implanted pacemaker
Decreased sensation	Acute hemorrhage
No stimulation over carotid sinus	Acute thromboembolism
No stimulation over laryngeal nerve	
Do not stimulate over skin diseases	
Avoid grounding faults	

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Abbreviations

Abbreviation	Meaning
ES	Electrical stimulation
PPS	Pulse per second
wk	week
min	minute
V	Volt
UV	Ultraviolet
HIF	Hypoxia inducible factor
mm	millimeter
MSH	Melanocyte stimulating hormone
ACTH	Adrenocorticotropic hormone
IL	Interleukin
TNF	Tumor necrosis factor
TGF	Transforming growth factor
PF4	Platelet factor 4
EGF	Epidermal growth factor
KGF	Keratinocyte growth factor
PDGF	Platelet derived growth factor
ATP	Adenosine triphosphate
ROS	Reactive oxygen species
HPOT	Hyperbaric oxygen therapy
MMPs	Matrix metalloproteases
ECM	Extracellular matrix
EPS	Extracellular polysaccharide matrix

PMNs	Polymorphonuclear neutrophils
DHEA	Dehydroepiandrosterone
HPA	Hypothalamic pituitary adrenal
SNS	Sympathetic nervous system
GCs	Glucocorticoids
Th1	Types of cytokines
DFUs	Diabetic foot ulcer

This book entitled “Electrical Stimulation in Wound Healing” consists of 4 chapters, 6 tables and 16 figures, which talks about what is the epithelial wound healing and how it can be measured and treated by the use of different types of electrical stimulation currents and what is the proposed mechanism of action of electrical stimulation. The book also gives clear evidence about the effect of these types of currents and gives the readers the parameters needed to achieve such an effect. This book can be very important for physical therapist, physical therapist assistant, nurse, and all allied health professionals who are working in the field of burn and general surgery.

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