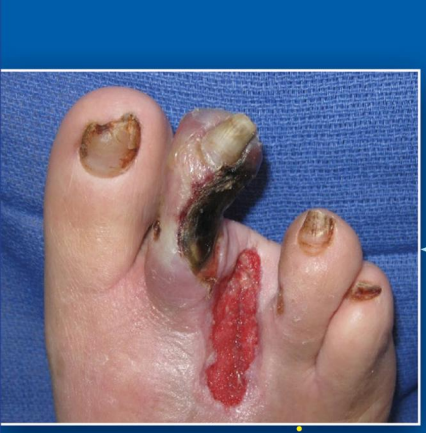
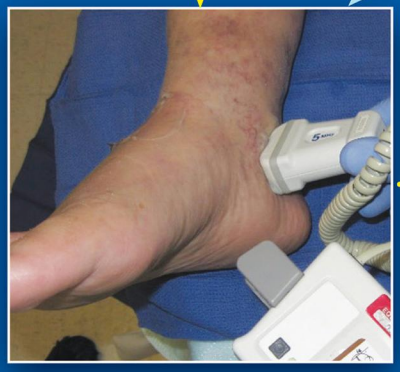
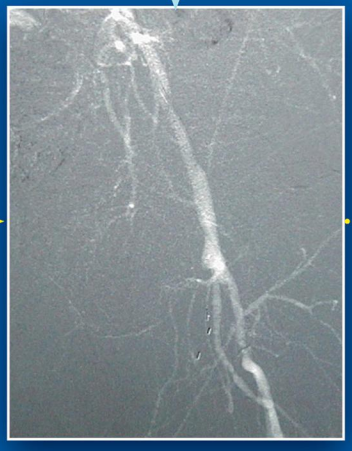
TEXT AND ATLAS OF

Wound Diagnosis and Treatment

SECOND EDITION







ROSE L. HAMM

Mc Graw Hill Education

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Second Edition

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Text and Atlas of Wound Diagnosis and Treatment

Second Edition

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The second edition of *Text and Atlas of Wound Diagnosis and Treatment* is dedicated to my nine awesome grandchildren, each of whom has inspired me in his/her own way. From eldest to youngest, their individual personalities, values, and passions have touched my heart and soul as follows:

Amy, whose unwavering faith has inspired me to stay focused on my mission of serving God's people, especially those who have nonhealing wounds.

Michael, whose passion for music and pursuing his role in the musical industry, while at the same time developing his own teaching skills as a hands-on massage therapist, has inspired me to share my passion for treating patients through teaching colleagues and physical therapy students.

Chelsea, whose innate love of adventure has inspired me to say "Yes!" to every adventure offered by my profession, including editing this second edition after being supposedly retired.

Ben, whose compassion and dedication to serving the marginalized population has inspired me to focus my professional efforts on a patient population that is often undertreated due to lack of understanding of the pathologies and social ramifications of their medical issues.

Garrett, my tennis buddy, who has inspired me to stay physically active, to always take time to play and travel, even when deadlines are looming.

Fabrizio, whose unabashed curiosity has inspired me to be open and unashamed to ask questions of my patients and colleagues in order to answer the question "Why isn't this wound healing?" because it is in asking that we learn.

Juliet, whose creativity has inspired me to be creative in my approach to patients, to make our time together fun and full of laughter, and to always be willing to think outside the box when the answers seem to allude me.

Zvi, whose unboundless energy has inspired me to keep my own energy level as high as possible for my family, friends, and profession, and whose desire to have more "GG time" has constantly reminded me of what is most important in my life.

Izzy, whose enthusiasm for and engagement in all of the world around her inspires me to be engaged with my profession, but only as much as engagement with family and friends will comfortably allow.

To each of my grandchildren, thank you for being yourselves and for all the inspiration you so generously, albeit unknowingly, give to me every day. I love you beyond words!!!

Grandma Rose/GG

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Foreword

"Imperfect action is better than perfect inaction."
Harry S. Truman

While the provenance of this quote from President Truman may be seen as a rationalization for the launch of the nuclear age, I would suggest that we might be able to salvage from it a remarkable bit of useful wisdom. For far too long, wound healing has been referred to strictly as "wound care." While caring for a wound (and the patient attached) is a noble pursuit, a good argument can be made for moving from "care" to "closure" and ultimately prevention of wound remission. Understanding what constitutes complete healing of both the wound and the patient is what makes this second edition of Rose Hamm's superb text so insightful both for entry-level students in the medical professions and for practicing clinicians.

The current edition of the *Text and Atlas of Wound Diagnosis and Treatment* contains updated diagnostic methods, interventions and tutorials to move toward the aforementioned goal of treating and healing the patient with a wound. Chapters authored by a world-class group of interdisciplinary clinicians take the reader on a step-by-step journey through assessment to diagnosis, to therapy and to prevention of recurrence.

In the following pages, the reader will find beautiful photographic illustrations of the causes and effects of a variety of wound diagnoses. The reader will learn about risk factors, clinical signs and timely treatment for the wound and for any underlying conditions that may be impeding wound healing. It is my firm belief that, with the collective wisdom shared in this text, we may all one day be able to heed the words of Truman—and be rightly accused of working to perfect our actions toward helping our patients with wounds move through the world with more confidence, better function, and a healthier life-style.

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Preface

At a recent Max Gaspar MD Symposium on limb salvage that focused especially on patients with diabetes and peripheral arterial disease, I was impressed by the panels that consisted of vascular surgeons, podiatrists, a physical therapist, a plastic surgeon, and a researcher—a real multi-disciplinary team all talking about the same patient population and how to preserve an ischemic limb, heal a wound, and restore optimal function and quality of life for the individual. The entire day reinforced what I so passionately wanted to capture in the first edition of *Text and Atlas of Wound Diagnosis and Management*, and have tried to enhance in this second edition. If we as a medical profession are going to be successful in caring for these patients, every profession has to be involved. And involvement starts with the education of our entry-level students in all of the medical professions, and continues throughout one's career, whether teaching students, colleagues, or patients.

Another pivotal moment for me at the Gaspar Symposium occurred during lunch, talking with two medical students about their impressions of the day thus far, and having them respond, "We don't get any of this in medical school." I encouraged them to ask for it from their professors, because as one supervisor said to me early on in my career, "Whirlpool and betadine don't get it anymore." While we are way past the whirlpool and betadine era of wound care, there are still antiquated ideas, financial constraints, and just plain lack of knowledge that prevent patients with wounds from getting the right diagnosis and appropriate medical care. In the long run, this costs not only the patient in terms of compromised care and all the anxiety and emotions that go along with having a nonhealing wound, but also costs the payers untold more dollars because of the wasted care that is neither evidence-based, appropriate, nor effective. As Dr. Robert Kirsner so eloquently stated in the foreword to the first edition, "While wound care has improved, practice gaps exist and chronic wounds will become a more significant public health concern as the US population ages and the incidence of risk factors for chronic wounds (such as diabetes) continues to rise. To combat the increasing number of patients with wounds and wound-healing problems, more and better-trained clinicians are needed."

The multi-disciplinary team of authors who have contributed to this book are outstanding clinicians and educators in their individual fields. Each of the original authors agreed to review and revise his/her chapter, bringing it up-to-date as reflected in current research and literature. They have willingly given innumerable hours from their busy schedules because they share that same vision—for all the disciplines to bring their unique expertise to patient care, but based on the same evidence-based principles of wound healing. My fervent prayer is that educators in all disciplines of patient care will be inspired to learn, to teach, and to care for patients with nonhealing wounds in such a way that the ripple effect will be far-reaching and non-ending. God bless you as you use the extensive information in this book to care for His people!

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Acknowledgments

First and foremost, my deepest appreciation goes to the incredible authors who contributed to the success of the first edition and to the revision for the second edition of this book. Their expertise in caring for patients with wounds and their dedication to education compelled them to say, "Yes," when asked to participate in this project. Having the sales representatives of McGraw-Hill say, "Don't change anything, just up-date it" made it easy to ask this busy group of professionals to help with the revision, and their suggestions, up-dates, corrections, and additions were all right on target for each chapter. Dr. David Armstrong, who has been an inspiration and a mentor to me, was enthusiastic and original in his preface, bringing his humor and commitment to limb salvage to life with his words. Thank you, everyone!!

The editorial staff at McGraw-Hill have been supportive and encouraging from the very beginning. Michael Weitz never stops dreaming with me, and Regina Brown is awesome with handling every detail. Anthony Landi, Arman Osvepyan, and the artistic team at McGraw-Hill were patient and understanding with me when we brain-stormed cover ideas to reflect the mission of the book, and were beyond successful in bringing the mission to life. And to each and every sales rep who has taken the textbook to the educational marketplace, you have my sincere thanks.

Poonam Bisht and the team at MPS Limited were awesome in catching every inconsistency, grammar and spelling mistake, and reference error. They were sensitive to my suggestions for figure and table placement in order to make the book as student-friendly as possible. The team exemplified professionalism and they made my task infinitely easier.

As I was writing both editions, there were two past professors from graduate school who were constantly sitting on my shoulder and acting as my conscience as I put the words, sentences, paragraphs, and pages together. Dr. Carolee Winstein was my first professor in graduate school, a most daunting experience I must say. She was the consummate professor who taught me, not just the material of the class, but HOW to be a good student, and specifically how to critically read journal articles for their credibility and applicability. Her lessons were my yardstick for every single reference in the book. Dr. Michael Schneir, a professor in the Herman Ostrow School of Dentistry at USC, taught me scientific writing, first in a class and then in one-on-one sessions as I prepared my first teaching document for publication. Every word, period, comma, phrase, sentence had to be placed and worded in such a way that it relayed the most correct information with the least amount of words; indeed every *a* or *the* before a noun could be a cause for discussion. To both of these magnificent educators, I say thank you for all that you taught me and for being ever-lasting mentors.

My family, including husband Bob Bothner, children and grandchildren, have been encouraging and understanding with the time, focus, energy, and messy office that any writing project demands. As it reaches completion, I can only be excited about the additional time I will now have to spend with them. They fill my life with joy, happiness, love and laughter for which I am so very grateful.

Any successful venture takes a team working together, each member with its own special skills and talents, and the **team** that created *Text and Atlas of Wound Diagnosis and Management* has been the best any author/editor could possibly have. May each and every reader feel the passion and expertise that is reflected in these pages, and use it as Dr. Armstrong so eloquently stated, "to help our patients with wounds move through the world with more confidence, better function, and a healthier life-style."

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PART ONE
Integumentary Basics

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Anatomy and Physiology of the Integumentary System

Rose L. Hamm, PT, DPT, CWS, FACCWS

CHAPTER OBJECTIVES

At the end of this chapter, the learner will be able to:

1. **Identify each layer of the skin and its components and discuss their functions.**
2. **Relate the function of each cell type to the overall function of the integumentary system.**
3. **Recognize the role of non-cellular components of skin in maintaining an integumentary system capable of healing.**
4. **Diagnose tissue injury based on the depth of skin loss.**

SKIN

Skin is an important part of one's personality and character; a lot can be learned by observing an individual's skin and its abnormalities. Wrinkles are an indication of one's mood, age, social habits, or overexposure to the sun. The skin color reflects one's ethnicity as a result of the melanin content; the skin texture can reflect one's life occupation that involves repeated mechanical forces or weather exposure. Skin reflects one's emotions as it moves fluidly with the underlying muscles and connective tissue. Skin abnormalities can be a response to a disease process, injury, allergy, or medication. But what does the skin have to do with wound healing? In order to be considered closed, a wound has to have full re-epithelialization, defined as new skin growth, and no drainage or weeping from the pores. An appreciation for the anatomy and physiology of the integumentary system and the skin's role in healing is needed to understand wound closure, complete with optimal aesthetics and function.

ANATOMY OF THE SKIN

The skin is a complex, dynamic, multilayered organ that covers the body, making it the largest single organ. It comprises 15–20% of the total body weight; if laid out flat, the skin would cover a surface of 1.5–2 m^2.[1] Embedded in the layers are a plethora of cells, vessels, nerve endings, hair follicles, glands, and collagen matrixes, each performing a specific task that as a whole enables the skin to protect and preserve the rest of the body. Both the cellular and non-cellular components of the epidermis and dermis are described in **TABLES 1-1** and **1-2**.

The layers of the skin are organized into the outermost *epidermis* and the underlying *dermis*. Beneath the dermis is a structure called the *hypodermis* or subcutaneous layer, although it is not a true part of the skin (**FIGURE 1-1**). The junction of the epidermis and dermis is reticular, with an individualized pattern that forms dermatoglyphs, or the fingerprints and footprints, of the hands and feet.[1] The reticular structure allows the skin to withstand the repeated friction and shear forces that occur with activities of daily living; however, as the skin ages the ridges flatten out and the skin is more susceptible to frictional tears and blistering. Between the epidermis and dermis is a laminar adhesive layer termed the *basement membrane* that binds the two layers of the skin.

Epidermis

The layers of the epidermis are, from innermost to the surface, *stratum basale, stratum spinosum, stratum granulosum, stratum lucidum,* and *stratum corneum*; in totality the layers are 50–150 μm in thin skin, 400–1400 μm in thick skin[1,2] (**FIGURE 1-2**). The primary cells composing the epidermal layers are keratinocytes, with melanocytes, Langerhans cells, and Merkel cells embedded in layers. The keratinocytes are mitotically active in the stratum basale, but through a process defined as *stratification*, they migrate outward to the avascular stratum spinosum and begin to flatten out and become less active. When they reach the outer stratum corneum, the keratinocytes are termed *corneocytes*, dead flat cells that form the outer protective layer of the skin.

The keratinocytes are composed of keratin protein filaments that are present in greater concentrations as the cells migrate toward the stratum corneum. In the stratum basale, the keratinocytes are bound to the basal lamina by *hemidesmosomes*; and in all the epidermal layers, to each other by *desmosomes*. These cell-to-cell adherent discs are composed of transmembrane glycoproteins, termed *cadherins*, and include four desmoglein proteins (**FIGURE 1-3**).[3]

As the keratinocytes move into the stratum spinosum, they become active in keratin or protein synthesis. The keratin forms filament bundles called *tonofibrils* that converge on the hemidesmosomes and desmosomes to give the skin strength to withstand friction or shear force. As the keratinocytes migrate into the stratum granulosum, *filaggrin* (derived from "filament-aggregating protein") binds to the tonofibrils,

TABLE 1-1 Cellular Components of the Skin

Cell Name	Description	Location	Function
EPIDERMIS			
Keratinocytes—dead	Flat, elongated, nonnuclear	Stratum corneum of epidermis	Provide mechanical strength[2]
Squames	Horny, cornified cells	Surface of stratum corneum	Sloughing layer of epidermis
Keratinocytes—living	Polyhedral, slightly flattened	Stratum spinosum of epidermis	Synthesize keratin filaments; phagocytose the tips of melanocytes to release melanin; hold large amounts of water
Keratinocytes—living	Flattened polygonal	Stratum granulosum	Keratinization at terminal differentiation of the epithelial cells
Eosinophilic cells	Flat, no nuclei or organelles; densely packed keratin filaments embedded in a dense matrix	Stratum lucidum (only in soles of the feet and palms of the hands)	Provide dense, thick layer of skin
Melanocytes	Round cell bodies with long irregular dendritic extensions	Between the stratum basal and stratum spinosum; in hair follicles[1]	Produce melanin, the pigment that gives color to the skin
Langerhans cells (dendritic cells)	Round cell bodies with long dendritic extensions into intercellular spaces	Stratum spinosum with cytoplasmic processes extending between the keratinocytes of all the epidermal layers	Bind, process, and present antigens to the T-lymphocytes
Lymphocytes	Dendritic	Epidermis	Recognize epitopes; produce cytokines
Merkel cells	Dendritic; have contact with unmyelinated sensory fibers in basal lamina	Stratum basale of highly sensitive areas; base of hair follicles	Mechanoreceptors for touch
Basal cells	Cuboid or columnar, contain keratin in progressively increasing amounts as the cells migrate toward the stratum corneum	Stratum basale	Continuous production of epidermal cells
DERMIS			
Stem cells		Stratum basale, bulge of the hair follicle	Production of keratinocytes
Fibroblasts	Elongated, irregular shape with large, ovoid nucleus	In the connective tissue of the papillary layer of dermis	Synthesize collagen, elastin, GAGs, proteoglycans, and glycoproteins
Mast cells	Large, oval or round; filled with basophilic secretory granules	Near the capillaries in the dermis	Produce histamine and heparin
Macrophages	Large cells with off-center, large nuclei	In the connective tissue of the papillary layer of dermis; become Langerhans cells in the epidermis	Phagocytosis, produce enzymes and cytokines that facilitate wound healing; immune processes
Leukocytes	Spherical white blood cells	Papillary layer of dermis	Phagocytose foreign material and dead cells
Free nerve cell endings	Unencapsulated receptors	Papillary layer of dermis into lower epidermal layers	Detect temperature changes, pain, itching, light touch
Tactile discs	Unencapsulated receptors	Papillary layer of dermis	Receptors for light touch
Root hair plexus	Web of sensory fibers	Reticular layer of dermis	Detect movement of the hairs
Meissner corpuscles (tactile corpuscles)	Elliptical encapsulated nerve endings	Reticular layer of dermis	Detect texture and slow vibrations[10]
Pacinian corpuscles (lamellated)	Large oval nerve endings with outer capsule and concentric lamellae of flat Schwann-type cells and collagen around an unmyelinated axon	Reticular layer of the dermis, hypodermis	Detect coarse touch, deep pressure, fast vibration[10]
Ruffini corpuscles	Enlarged dendritic endings with elongated capsules	Reticular layer of the dermis	Detect sustained pressure[10]
Adipocytes	Globular cells containing fat molecules	Hypodermis	Produce and store lipids that can be used for energy, provide insulation, produce cytokines for cell-to-cell communication (restin, leptin, adiponectin)

Data from Mescher AL. *Junqueira's Basic Histology: Text & Atlas.* 15th ed. New York, NY: McGraw Hill; 2018.

TABLE 1-2 Noncellular Components of the Skin

Structure Name	Description	Location	Function
EPIDERMIS			
Basement membrane	Composite structure of basal lamina and reticular lamina	Between the stratum basale and the papillary layer of the dermis	Binds the dermis and epidermis; allows diffusion of nutrients from the dermis to the epidermis
Basal lamina	Felt-like sheet of extracellular matrix composed of laminin, Type IV collagen, and entactin	In the basement membrane	Attach to reticular fibers in the connective tissue to bind the layers of skin
Reticular lamina	Fibers composed of Type III collagen	Below the basal lamina in the basement membrane	Attach to the basal lamina with reticular fibers
Vitamin D_3	Also known as cholecalciferol; technically a hormone and not a vitamin	Keratinocytes	Metabolizes calcium, bone formation; up-regulates antimicrobial peptide synthesis for immune system
Desmosomes	A disk-shaped structure on the surface of one cell that connects with an identical structure on an adjacent cell	Between epithelial cells	Provide a strong bond between the cells
Hemidesmosomes	Half of a desmosome structure; contain integrins	Between the cells of the stratum basale and the basement membrane	Bind the basal cells to the basement membrane
Corneodesmosomes	Specialized protein structures modified from desmosomes	Between the keratinocytes, or corneocytes, in the stratum corneum	Bind the cells in the outer layer of skin, are degraded as the cells migrate to the epithelial surface so that outer keratinocytes are sloughed
Integrins	Transmembrane proteins	In the hemidesmosomes	Receptor sites for macromolecules of laminin and for Type IV collagen
Keratins	Filament proteins	In all epithelial cells and hard structures (eg, nails)	Strengthen epidermis, protect against abrasion, prevent water loss
Tonofibrils	Bundles of keratin filaments	Converge and terminate at desmosomes located in areas subject to continuous mechanical forces, eg, soles of the feet	Protect the skin from effects of continuous friction and pressure
Keratohyalin granules	Contain dense masses of filaggrin	In the cytoplasm of the stratum granulosum cells	Link with the keratins of tonofibrils to facilitate keratinization
Filaggrin	Protein monomers that bind to keratin fibers in the stratum corneum	In the keratohyalin granules of stratum granulosum cells	Help regulate epidermal homeostasis; assist in water retention in the skin
Lamellar granules	Small ovoid structures containing lamellae of lipids	In the stratum granulosum cells	Form lipid envelopes around the cells to prevent water loss from the skin
Melanin	A brownish-black pigment produced by the melanocytes	In the stratum basale cells and hair follicles	Provides color to the skin, protects from UV exposure
Carotene	Unsaturated hydrocarbon absorbed from the diet and stored in the fat	In the stratum basale cells	Stores vitamin A, provides pigment
Melanosomes	Mature elliptical-shaped protein vesicles	In the melanocytes of the stratum basale and stratum spinosa	Store melanin
DERMIS			
Connective tissue	Extracellular matrix composed of protein fibers and ground substance	Dermal layer and hypodermis	Gives form to all organs, connects and binds tissues and cells, allows diffusion of nutrients and waste products
Anchoring fibrils	Filaments of Type VII collagen	Between the basal lamina and the papillary dermis	Bind the dermis to the epidermis
Elastic fibers	Thin collagen fibers that form networks with other collagen bundles	Dermis	Provide elasticity and flexibility to the skin

Data from Mescher AL. *Junqueira's Basic Histology: Text & Atlas*. 15th ed. New York, NY: McGraw Hill; 2018.

thereby forming an insoluble keratin matrix that "acts as a protein scaffold for the attachment of cornified-envelope proteins and lipids that together form the stratum corneum."[4] Also in the stratum granulosum, *lamellar granules* containing many lamellae of lipids undergo exocytosis, releasing a lipid-rich material into the intercellular spaces and forming envelopes around the protein-filled cells that are undergoing keratinization.[1] This combination of tightly adhered filaments and lipid-rich envelopes is what gives the skin its ability to serve as both a barrier to loss of water from the body and protection from extrinsic foreign material.

The stratum lucidum is present primarily in the thick, hairless skin of the palms and soles (termed *glabrous skin*) and consists of dead, clear keratinocytes, thus the term "clear layer."

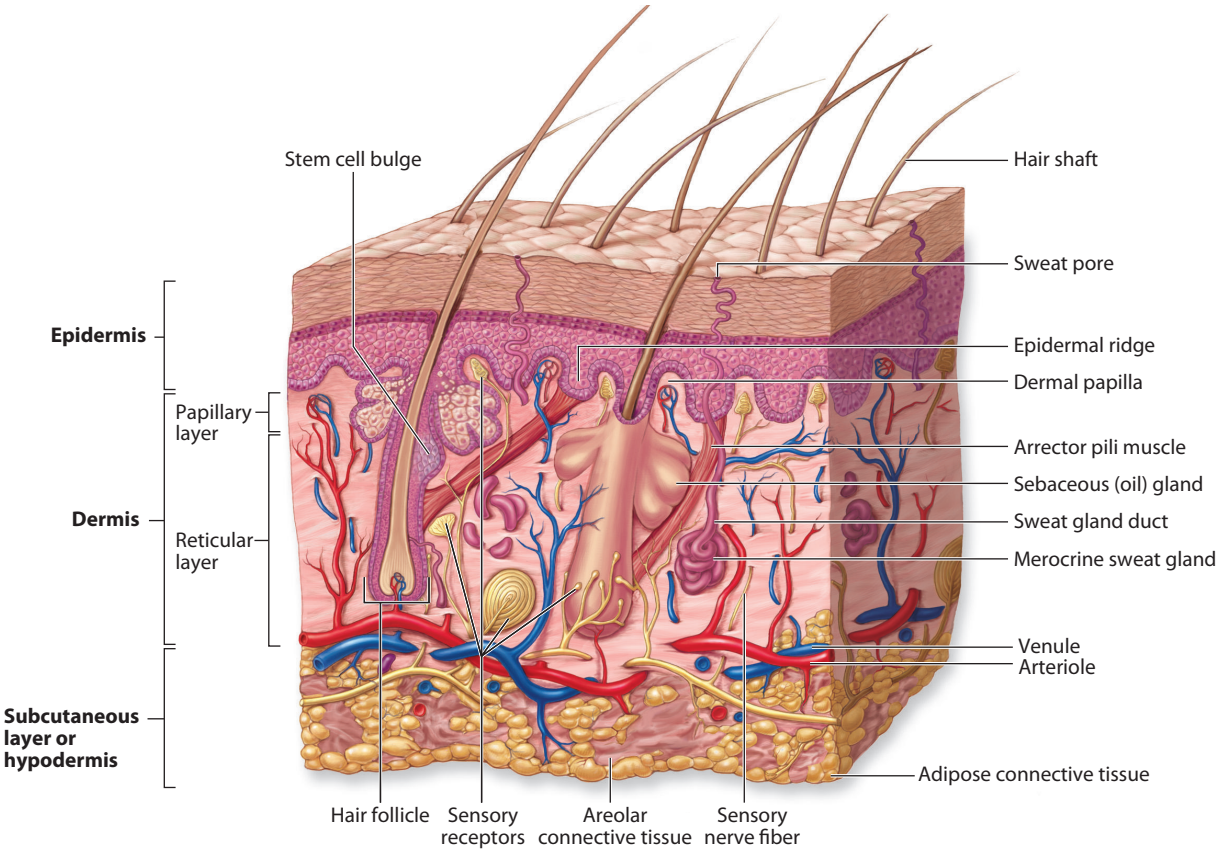

FIGURE 1-1 Anatomy of the skin (Used with permission from Mescher AL, ed. *Junqueira's Basic Histology: Text and Atlas.* 12th ed. New York, NY: McGraw Hill; 2010.)

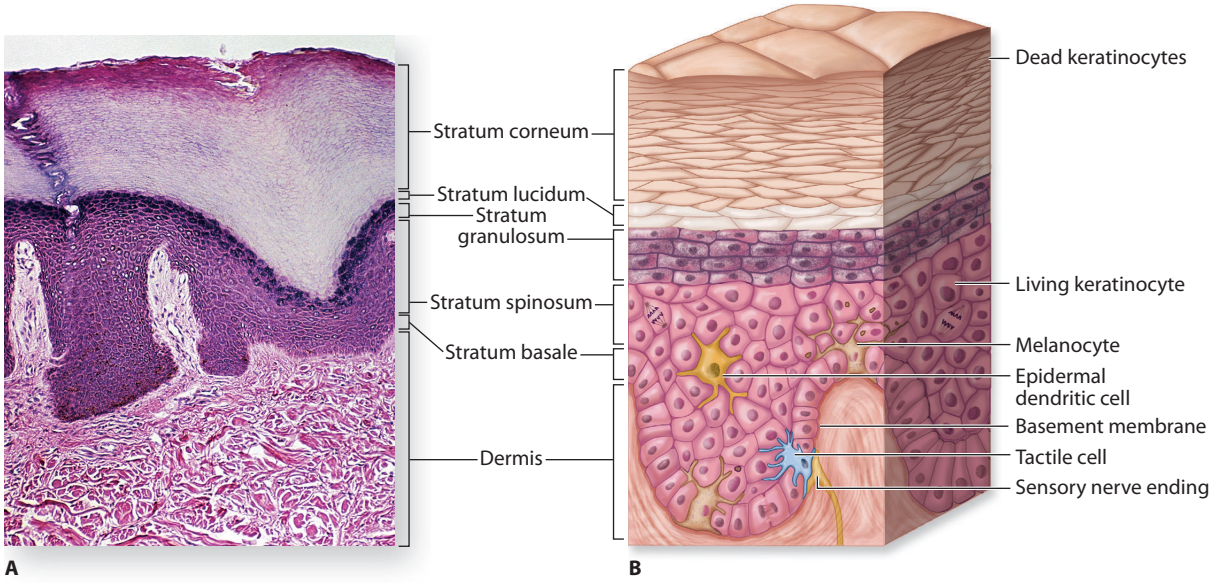

FIGURE 1-2 Layers of the epidermis

Stratum basale—composed of a single layer of cuboid cells, attached to the underlying dermis by the basement membrane. The stratum basale is constantly producing epidermal cells (keratinocytes) from stem cells located in both the basal layer and the bulge of the hair follicles in the dermis.

Stratum spinosum—composed of slightly flattened cells that are responsible for protein synthesis, primarily keratin that forms bundles called *tonofibrils*. This is the thickest layer of the epidermis.

Stratum granulosum—composed of 3–5 layers of flattened cells that are undergoing terminal differentiation as they approach the outermost layer of skin. The intercellular spaces are filled with a lipid-rich material that forms a sheet or envelope around the cells, thereby making skin a barrier to both water loss and extrinsic foreign material.

Stratum lucidum—composed of flattened eosinophilic cells, creating a clear or translucent layer located only in the soles of the feet and palms of the hands. Cells contain densely packed keratin and are connected by desmosomes. Provides thickness and strength to withstand friction to the soles and palms.

Stratum corneum—composed of 15–20 layers of dead keratinized cells that are continuously being shed in a process called *desquamation*.

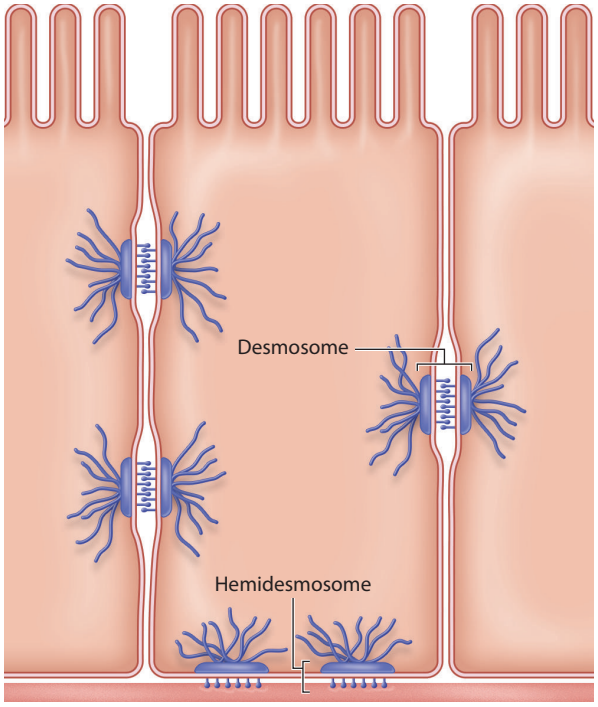

FIGURE 1-3 Cell adherence with desmosomes and hemidesmosomes *Desmosomes* are adherent glycoprotein discs that bind keratinocytes to each other. *Hemidesmosomes* are adherent glycoprotein half-discs that bind keratinocytes to the basement membrane between the stratum basale and the dermis.

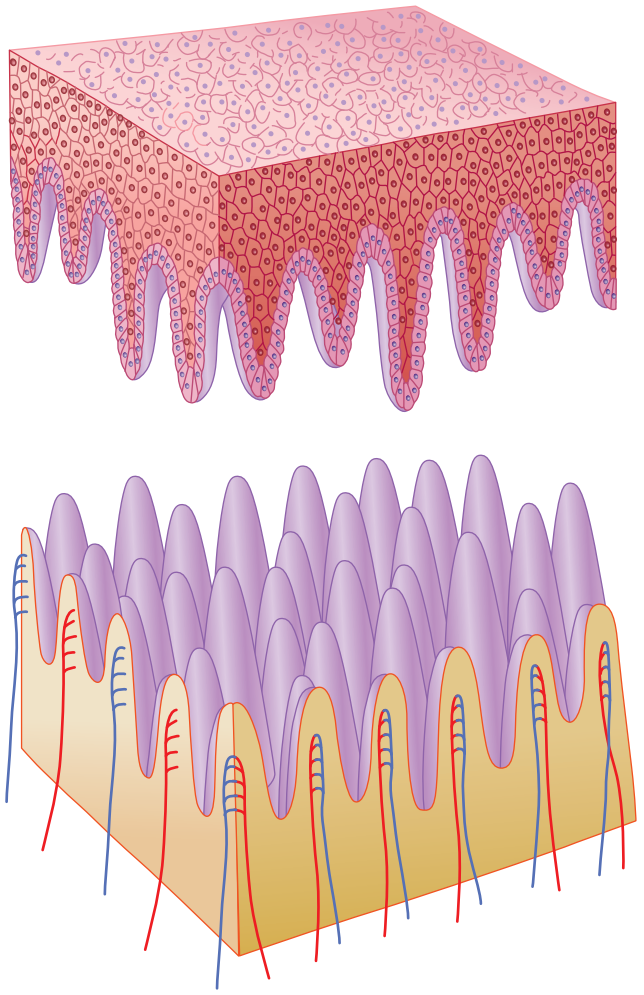

FIGURE 1-4 Dermal/epidermal junction The epidermal/dermal junction is composed of dermal papillae and epidermal pegs that interdigitate to create a bond that will withstand friction and shear forces on the skin. The junction flattens with age, making geriatric skin more susceptible to skin tears.

The stratum lucidum is between the stratum granulosum and the stratum corneum and provides the palms and soles more protection from friction and serves as a greater moisture barrier.

When the keratinocytes enter the stratum corneum, they are flat, stacked, and embedded in lipid layers to form the main protective shield of the skin. The keratinocytes are held together with modified adhesive desmosomes, termed *corneodesmosomes*.[5] As the keratinocytes migrate to the surface they are termed corneocytes, the corneodesmosomes are degraded in a process carefully controlled by a number of proteases and their inhibitors, and as a result the cells desquamate or slough off.[6] Over a period of 30 days, the entire process of migration and desquamation is completed and the epidermis is renewed.

Dermis

The dermis is composed of connective tissue and binds the epidermis to the hypodermis or subcutaneous tissue. The extracellular matrix of the dermis is composed of collagen (mostly Type I), elastic fibers, and ground substances such as glycosaminoglycans (GAGs) and proteoglycans. The uppermost surface of the dermis is reticular and interdigitates with the ridges of the epidermis; the structures are termed *epidermal pegs* and *dermal papillae* (**FIGURE 1-4**). Between the dermis and epidermis is the basement membrane, consisting of the basal lamina and the reticular lamina. Besides holding the two layers together, the basement membrane allows the nutrients from the dermal vasculature to pass through to the avascular epidermis.

The acellular dermal components are the extracellular matrix, anchor fibrils of Type VII collagen linking the dermal papillae and the basal lamina, and the elastic fibers that are intertwined with the other collagen fibers to give flexibility and elasticity to the skin. Hyaluronan (previously known as hyaluronic acid and renamed because it is not an acid) is an anionic nonsulfated GAG located in the extracellular matrix that contributes to cellular proliferation and migration; it is thereby an integral part of the wound healing process.[7,8]

The cellular components of the dermis are listed in **TABLE 1-1** and their role in dermal physiology is discussed

in the section on function of the skin. The papillary layer contains the fibroblasts, mast cells, and macrophages, as well as some extravasated leukocytes.[1] The reticular layer is composed of dense, mainly Type I collagen and contains the vasculature, nerve endings, glands, hair follicles, and more elastic fibers.

The hypodermis, or subcutaneous layer, is not anatomically part of the skin; however, it is the structure that binds the skin to the underlying structures. It is composed of loose connective tissue, vascular supply, and adipose cells that vary in number at different body areas and also among individuals. The hypodermis allows the skin to move freely over the underlying structures, thereby facilitating fluid muscle and joint movement.

SKIN PHYSIOLOGY

Vascular Supply

The dermis contains several microvascular blood vessel plexuses and lymphatic vessels that are parallel to the skin surface (see **FIGURE 1-1**). The larger arterioles and venules are in the deep reticular layer with smaller vessels extending into the papillary layer and terminating in capillary loops. Blood flow through the capillary loops is controlled by highly innervated arterioles,[9] and their close proximity to the basement membrane allows the blood supply to feed the deep keratinocytes of the epidermis. Between the larger deep plexus and the capillary loops are numerous *arteriovenous anastomoses* or shunts that play a major role in maintaining constant body temperature during hot and cold weather conditions. Lymphatic terminal vessels are little sacs interspersed with the capillary loops, controlled by a filament anchored to the connective tissue. As the filament moves, it opens a flap to the lymphatic vessels, thereby facilitating transport of excess interstitial fluid, protein molecules, and fat molecules out of the dermis. (Refer to Chapter 5, Lymphedema.)

Nerve Supply

Because of its large and superficial surface area, the skin contains the sensory receptors necessary for the body to process the external environment. The nerve endings are either unencapsulated (have no glial or collagenous covering) or encapsulated (have a covering of glia and connective tissue capsules).[1,10] When the nerves cross the dermal/epidermal junction, they lose the Schwann cell covering and exist in the epidermal pegs as free nerve endings. Also in the granulosum basale are unencapsulated mechanoreceptors termed *tactile* or *Merkel cells*. It is thought that in addition to external stimuli, the keratinocytes have a role in stimulating the nerve receptors by the release of neuropeptides.[11]

Skin Nutrition

Much has been written, and even more spent, on nutrients, supplements, and topicals to maintain skin nutrition and ergo youth. While there are no double-blind, placebo-controlled studies to support what is called the "inside-out" approach to maintaining skin integrity, there are certain vitamins and antioxidants that are known to play a role in skin health, in large part by their antioxidant effects.[12] These substances and their functions are listed in **TABLE 1-3**.

Skin Renewal

The skin is continuously renewing itself through synthesis of new keratinocytes in the stratum basale and sloughing of the corneocytes from the stratum corneum. The major cells responsible for skin renewal are the fibroblasts, located in

TABLE 1-3 Nutrients Important to Maintenance of Skin Health

Nutrient	Function	Source
Vitamin D	Maintains the bony structure Maintains calcium hemostasis May help modulate the skin's immune response	Exposure to sunlight Enriched milk Fatty fish
Vitamin C (ascorbic acid)	Eliminates free radicals (antioxidant) Promotes wound healing May promote fibroblast proliferation	Vegetables Citrus fruits
Vitamin E	Lipid-soluble, membrane-bound antioxidant May protect against UVB effects	Vegetables, oils, seeds, corn, soy, whole wheat flour, margarine, nuts, some meat and dairy products
Vitamin A (derived carotenoids)	Antioxidants	Yellow and orange vegetables Salmon Leafy green vegetables
Vitamin F (essential fatty acids)	Formation of cell walls	Oils from flaxseed, canola, hemp seed, walnuts, sesame seeds, avocados, salmon, albacore tuna
Coenzyme Q10	Endogenous antioxidant	Synthesized by the body, present in all cells
Plant-derived antioxidants	Prevent lipid peroxidation	Soy, curcumin, silymarin, ginkgo, green tea, pomegranate

Adapted from Draelos ZD. Nutrition and enhancing youthful-appearing skin. *Clinics in Dermatology*. 2010;28:400–408.

the dermis, which are capable of producing the remodeling enzymes (eg, proteases and collagenases).[13] The collagen needed for cell synthesis is produced by both fibroblasts and myofibroblasts. All of the cells involved in this process are discussed in detail in Chapter 2, Healing Response in Acute and Chronic Wounds; however, it is important to realize that this is an ongoing process that can be inhibited by disease processes or facilitated and upregulated by tissue injury.

FUNCTIONS OF THE SKIN

Protection from Environment

The dense, adhered structure of the skin provides protection from the environment by preventing the penetration of some microbes and other foreign bodies, by absorbing shock as a result of the cushioning hypodermis, by serving as a barrier to excessive water absorption or loss, and by containing specialized structures and cells with other protective functions (eg, lymphocytes and antigen-presenting cells that respond to invading microorganisms and thereby mount an immune response).[1] When the skin is damaged by disease or lost as a result of injury, its functions are compromised and can have detrimental, even fatal, effects on the body.

Sensation

Sensation is both informative and protective. Stimuli received in the skin and transmitted to the brain can initiate a motor response that moves the person away from noxious stimuli. Embedded in the dermis are numerous nerve endings, illustrated and summarized in **FIGURE 1-5**. The most prevalent diagnosis resulting in the loss of tactile, pressure, and pain sensation in the skin is diabetic polyneuropathy, a major contributing factor to the formation of diabetic foot wounds. The lack of sensation allows trauma, even repeated trauma, to occur unnoticed and thereby results in wounds that are difficult to heal. This is just one example of how the failure of the skin sensory function may be a primary cause of wounds.

Prevention of Fluid Loss

The dense, extensively cross-linked lipid and protein matrix in the stratum corneum serves as a barrier to fluid loss, thereby helping to maintain homeostasis. This protection is enhanced in the palms and soles by the presence of the stratum lucidum. In addition, "natural moisturizing factors" (including free amino acids, lactic acid, urea, and salts) attract and hold water in the stratum corneum which is normally approximately 30% water.[14] This property of maintaining the water content is termed *hygroscopy*. The amino acids are a result of filaggrin degradation by proteolytic enzymes.[14] Injury to the skin or atmospheric conditions that result in loss of water can cause dry skin or irritant dermatitis, and moisturizers that rehydrate and repair the skin can use some of the same compounds that are in normal skin (eg, hyaluronan).[11]

Immunity

In addition to the physical barrier to environmental microbes, the skin has three properties that contribute to its role in the body's immune system: Langerhans cells, an acidic pH, and antimicrobial peptides and lipids.

Langerhans cells are dendritic cells primarily in the stratum spinosum that are alerted by any foreign microbes that enter the epidermis. Subsequently they bind, process, and present the antigens to the T-lymphocytes that are also in the epidermis, thereby initiating an immune response.[1] Antimicrobial peptides are innate protein fragments that prick the microbe cell membrane and destroy its integrity, rendering it inactive. Some antimicrobial peptides are present in both healthy and infected tissue (eg, human β-defensin or HBD 1 and RNase 7), whereas others are present only in the event of epidermal penetration by the microbes (eg, psoriasin S100A7, HBD 2, and HBD 3). Lysozyme, dermcidin, and LL-37 are antimicrobial peptides found in the hair follicles and eccrine glands.[2] These same peptides signal and recruit the immune cells (T-lymphocytes, macrophages, neutrophils, and other dendritic cells) needed to phagocytose the attacked microbes or present antigens to the host immune system. See Chapter 2 for a more detailed discussion of peptides and their role in wound healing.

The skin has a slightly acidic pH (4.2–6) that serves as a barrier to exogenous bacteria. The "acid mantle" of the stratum corneum is a combined result of free fatty acids, oils (sebum) produced by the sebaceous glands, secretions from the eccrine sweat glands, and proton pumps (by pumping H^+ ions out of cells onto the skin).[15] This acidic layer is a hostile environment for the bacteria, inhibiting their replication and thus serving as a natural immune mechanism.

Thermoregulation

Thermoregulation as a response to changes in the environmental temperature is maintained by the dermal vasculature and by the sweat glands. When a person is inactive, normal skin blood flow is 30–40 mL/min/100 g of skin. During cold stress, the arterioles and the arteriovenous anastomoses (AVAs) constrict and thereby reduce the flow of blood to the skin and preserve inner body heat. In extreme conditions, the flow can be reduced almost to zero, at which point the AVAs will dilate to maintain tissue temperature and viability. (Examples are when the skin turns erythematous upon application of a cold pack or when the nose turns red in extremely cold weather.) On the contrary, during times of heat stress, the same vessels will dilate to allow more blood to circulate near the skin surface and thereby dissipate the heat. The catalyst for the vasoconstriction or vasodilation is a dual sympathetic neural control. Glabrous (non-hairy) skin arterioles have sympathetic, norepinephrine innervation; nonglabrous (hairy) skin has both noradrenergic and cholinergic innervation. Nonglabrous skin vasculature also responds to the effects of local temperature changes (eg, with application of hot or cold packs).[9]

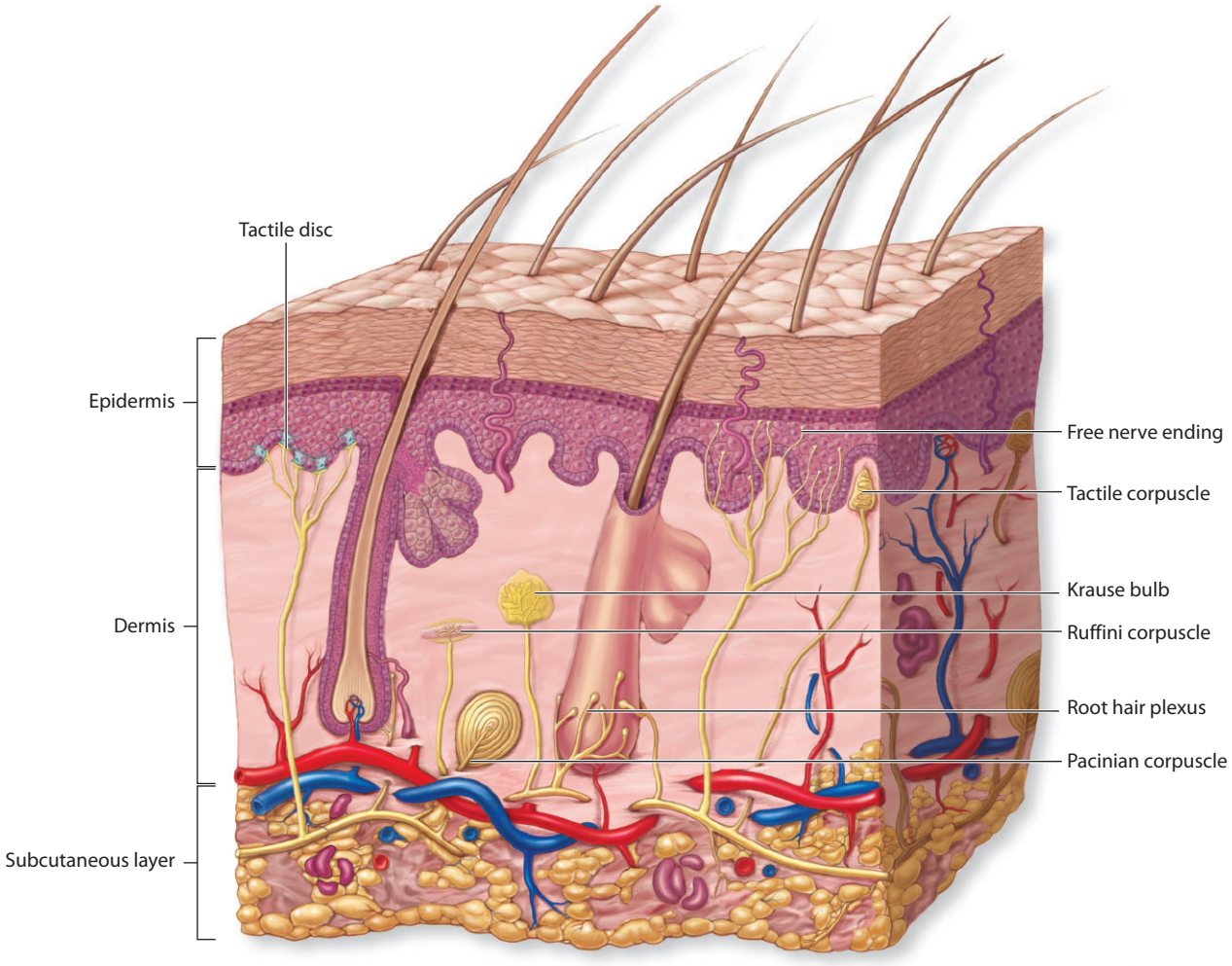

FIGURE 1-5 Sensory nerves within the dermal reticular layer (Used with permission from Mescher AL. Skin. In: Mescher AL. eds. *Junqueira's Basic Histology: Text and Atlas*, 15th ed. New York, NY: McGraw-Hill; 2018. Available at: http://accessmedicine.mhmedical.com/content.aspx?bookid=2430&sectionid=190285972. Accessed June 12, 2018.)

Free nerve endings—unencapsulated nerve endings resembling the roots of a tree that are in the stratum basale of the epidermis; function as thermoreceptors, nociceptors, or cutaneous mechanoreceptors. The nerves lose the Schwann cell covering when they cross the dermal/epidermal junction into the stratum basale.
Tactile or Merkel disc—unencapsulated nerve ending close to the dermal/epidermal junction that is a receptor for light touch.
Meissner corpuscle—encapsulated unmyelinated nerve ending in the dermal papillae that responds to texture and slow vibrations. The corpuscle is a single nerve fiber surrounded by lamella of flattened connective tissue cells, giving it a bulbous appearance. Meissner corpuscles are located most densely in glabrous skin.
Pacinian corpuscle—oval-shaped mechanoreceptor that consists of a single unmyelinated nerve fiber in a fluid-filled cavity surrounded by lamella of thin, flat, modified Schwann cells and wrapped in a layer of connective tissue, giving it the appearance of an onion. Pacinian corpuscles detect deep pressure and high-frequency, fast vibration.
Krause bulb—encapsulated nerve fiber located in the middle dermal layer; both a mechanoreceptor and a thermoreceptor, detecting light pressure, soft low vibrations, and cold.
Ruffini corpuscle—encapsulated elongated dendritic nerve ending located in the deep dermis and hypodermis; both a mechanoreceptor and thermoreceptor, detecting sustained pressure, stretching, and heat.
Root hair plexus—a network of sensory fibers around the root of the hair follicles in the deep dermis; detects and transmits any hair movement.

During periods of heat stress due to exercise or when the environmental temperature is higher than the blood temperature, thermoregulation is enhanced by the evaporation of fluid from the eccrine sweat glands. Initially the fluid produced is isotonic, but as it progresses toward the outer layer of the skin it becomes hypotonic by the reabsorption of the Na^+ ions.[14]

Protection from Ultraviolet Rays

The presence of melanin in the skin provides color variation among individuals and protects the underlying tissue from the effects of ultraviolet rays. This is accomplished through the activity of the epidermal-melanin unit, composed of the melanocytes that *produce* melanin and keratinocytes that *store* melanin.

In the stratum basale, there is one melanocyte for every 5–6 keratinocytes, located within 600–1200/mm^2 of skin surface.[1] Melanocytes synthesize melanin through a multistep process in which tyrosinase converts tyrosine into dihydroxyphenylalanine (DOPA) that is further transformed into melanin. The melanin migrates into the dendrites of the melanocytes. The dendritic ends of the keratinocytes phagocytose the melanocyte tips, allowing the melanin to enter into the keratinocyte where it is stored as *melanosomes* in quantities sufficient to absorb and reflect UV rays, thereby protecting the cellular DNA from the harmful effects of UV radiation. Increases in both melanin production and accumulation result from increased exposure to sunlight, and is evidenced by the darker color of ethnic groups who originated in geographical areas near the equator.[1,16]

Synthesis and Storage of Vitamin D

Vitamin D is necessary for calcium metabolism and bone formation; vitamins D_2 and D_3 are both secosteroids (vitamin D_2 is ergocalciferol; vitamin D_3 is cholecalciferol). The skin is the primary source of vitamin D_3 synthesis in the stratum basale and stratum spinosum.[17] Keratinocytes express vitamin D hydroxylase enzymes that convert provitamin D_3 (7-dehydrocholesterol) to vitamin D_3. This process is stimulated by exposure to sunlight, occurs rapidly, and peaks within hours of exposure. Vitamin D_3 is bound to a vitamin D–binding protein that carries it from the epidermis through the bloodstream and to the liver and kidneys where it is hydroxylated into an active form for calcium metabolism.

Vitamin D also contributes to the role of the epidermis in immunity by upregulating the expression of antimicrobial peptides, and when the vitamin is lacking in the epidermis, there is a concordant increase in infection.[18]

Aesthetics and Communication

Skin color, texture, and hyper/hypopigmentation are a major component of an individual's appearance and contribute to sexual attraction. Apocrine sweat glands, located primarily in the axillary and perineal regions, are dependent upon sex hormones for development and their secretions contain sex pheromones that can influence social behavior.

DEFINITIONS OF SKIN LOSS

Regardless of its etiology, every wound can be classified by the depth of tissue injury or loss as defined by the following terms.

Erosion is the loss of the superficial epidermis only, with no involvement of the dermis (**FIGURE 1-6**). These wounds will probably not bleed, although there may be increased redness of the skin due to proximity to the dermal vasculature and the capillary loops in the dermal papillae. Examples of erosion are superficial burns (previously termed *first degree*), Stage I pressure ulcers, and abrasions. Repair is accomplished by a local inflammatory response and epidermal replacement by migrating keratinocytes.

Partial thickness wounding is the loss of the epidermis and part of the dermis (**FIGURE 1-7**). These wounds will bleed due to interference with the microvascular structure in dermal tissue. Examples of partial thickness skin loss are Stage II pressure ulcers, superficial and deep partial thickness burns (previously termed *second degree*), skin tears, and deep abrasions. Repair is accomplished by re-epithelialization as a result of epithelial cell migration from the wound edges, hair follicles, and sebaceous glands.

Full thickness wounding is the loss of the epidermis and dermis, extending into the subcutaneous tissue and in some cases involving bone, tendon, or muscle (**FIGURES 1-8** and **1-9**). Examples are full thickness burns (previously termed

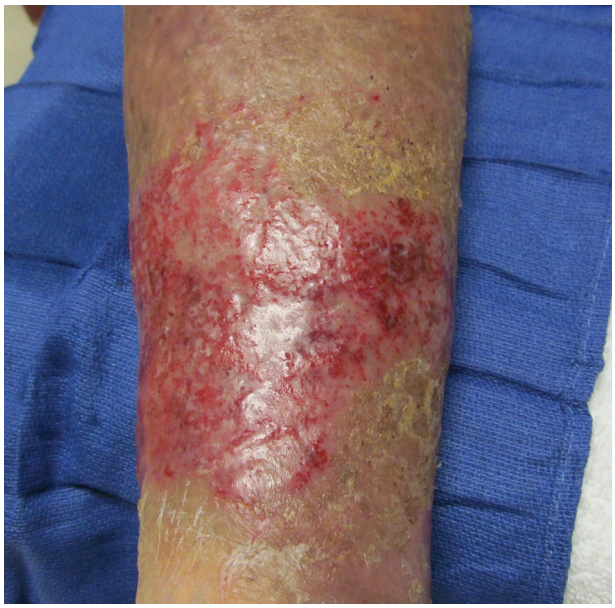

FIGURE 1-6 Erosion The loss of the superficial epidermis only, with no involvement of the dermis

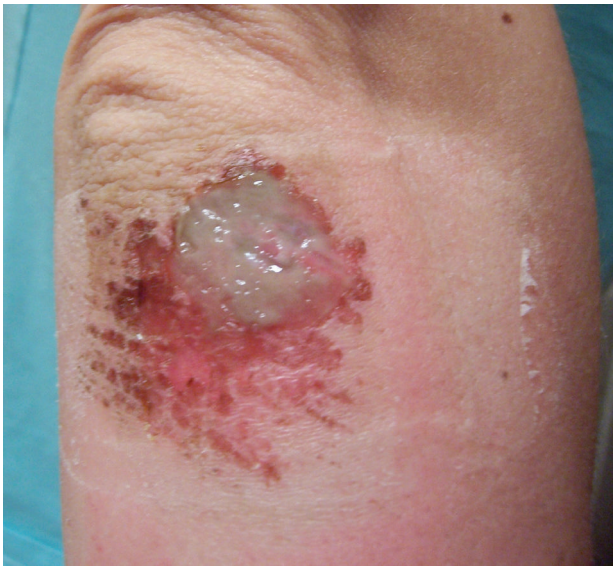

FIGURE 1-7 Partial thickness skin loss The loss of the epidermis and part of the dermis

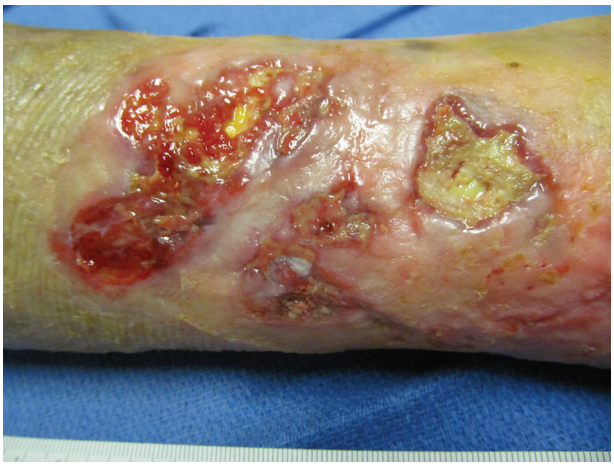

FIGURE 1-8 Full thickness skin loss The loss of the epidermis and dermis, extending into the subcutaneous tissue or hypodermis

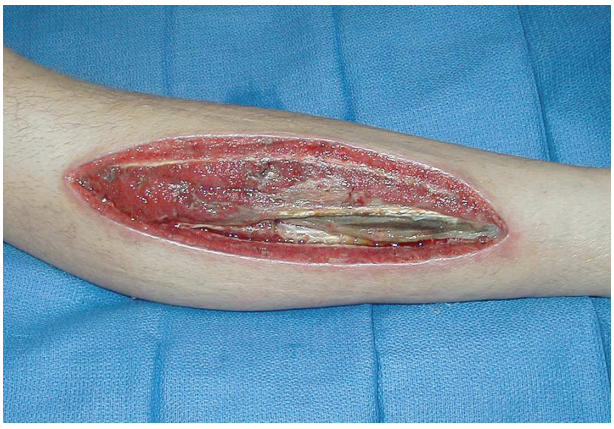

FIGURE 1-9 Full thickness skin loss with involvement of muscle, bone, and tendon

third degree), Stage III and IV pressure ulcers, surgical incisions, traumatic wounds that are full thickness, and wounds that require debridement of necrotic tissue into the subcutaneous tissue. Repair occurs through the process of secondary intention, discussed at length in the next chapter.

SUMMARY

The skin is a complex, multilayered organ that functions both independently to provide the body with its protective functions, and interactively with other structures and organs to ensure total health. These cellular and acellular skin components exist at all times to maintain homeostasis; however, injury or disease processes can stimulate these cells to be present in greater numbers, to be more active, and to have greater influence on other processes in order to accomplish repair and regeneration. These adaptive processes that lead to wound healing after tissue injury are the focus of Chapter 2.

STUDY QUESTIONS

1. The primary characteristic of the skin that enables it to withstand friction and shear forces is
   a. The number of layers in the epidermis.
   b. The amount of water and lipids in the interstitial spaces.
   c. The reticular formation of the dermal/epidermal junction.
   d. The nerve supply that alerts the body to abnormal mechanical forces.
2. Fibroblasts, mast cells, and macrophages, all necessary for skin renewal and regeneration, are located primarily
   a. In the stratum basale of the epidermis.
   b. In the papillary layer of the dermis.
   c. In the reticular layer of the dermis.
   d. Throughout all the layers of the dermis and epidermis.
3. The epidermal layer that is located in the palms and soles, giving them additional strength and thickness, is the
   a. Stratum basale.
   b. Stratum granulosum.
   c. Stratum corneum.
   d. Stratum lucidum.
4. The epidermis prevents loss of fluid because of its dense matrix of
   a. Lipids and proteins.
   b. Corneocytes.
   c. Dermosomes.
   d. Vitamin complexes.
5. Langerhans cells contribute to innate immunity by
   a. Pricking the bacteria cell wall and causing cytoplasmic leaks.
   b. Presenting antigens to the T-leukocytes.
   c. Phagocytosis of dead tissue that feeds bacteria.
   d. Creating an acidic environment on the skin surface.
6. Which cells are responsible for storing melanin in the form of melanosomes?
   a. Corneocytes
   b. Keratinocytes
   c. Melanocytes
   d. Monocytes
7. The structures that bind the keratinocytes and are subsequently degraded to allow desquamation of the stratum corneum are termed
   a. Desmosomes.
   b. Hemidesmosomes.
   c. Corneodesmosomes.
   d. Melanosomes.

Answers: 1-c; 2-b; 3-d; 4-a; 5-b; 6-b; 7-c

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Healing Response in Acute and Chronic Wounds

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Illustrations by Shelby Luttrell

CHAPTER OBJECTIVES

At the end of this chapter, the learner will be able to:

1. **Describe the sequence of normal acute wound healing.**
2. **Identify the cells that direct activity in the healing cascade.**
3. **Describe the chemical messengers necessary for timely wound healing (including the cells of origin, target cells, and actions).**
4. **Classify the primary enzymes produced during healing (including the cells of origin and actions).**
5. **Describe the inate and adaptive immune responses that occur during wound healing.**
6. **Describe the functions of platelets, monocytes, and fibroblasts and how they change during the course of wound healing.**
7. **Explain the differences between normal acute and chronic wound healing.**

INTRODUCTION

Perhaps the hamartia, or the flaw, in the study of wound healing is the tendency to oversimplify the truly elegant system that ensures healing, both anatomically and functionally. The multitude of processes that ensure wound closure and the commensurate return of function are equally marvelous.

The illustrations in this chapter introduce the interplay of cellular and molecular signaling in conjunction with vascular events that occur during the healing process. The figures demonstrate how cells involved in the repair process are directed, based upon global and local stimuli that may be cytokine, chemokine, pH, or galvanically driven.[1,2] If invaders or pathogens (eg, bacteria, fungi, viruses, or debris) are present, innate immune cells migrate and proliferate to the site of injury.[3] These cells include macrophages, neutrophils, natural killer (NK) cells, and gamma delta T cells. If the invader is a repeat offender that the host has successfully fended off previously, adaptive immune responses (B cell clonal expansion) are triggered.[3–5] Simultaneously, debris (necrotic and/or injured cells) is removed and a new wound bed excavated via proteases and extracellular matrix (ECM) degradation. This serves two purposes: (1) the clearance of cell and invader refuse and (2) the provision of pathways for cellular migration and proliferation, which constitute repair.[2]

The signaling in wound healing, renewal, and regeneration is a product of many factors, including the concentration and timing of chemical signal delivery, target cell receptor availability, active form after cleavage, degradation rate, messenger half-life, pH and presence of enzymes (eg, proteases) in the wound milieu, hydrophobicity, and hydrophilicity. Scaffold-binding (via heparin activation or other mechanisms), fiber type (whether fibrin or collagen), cell shape, adhesion interfaces (via integrins), and storage of growth factors all contribute to the timing and intensity of cell signaling during wound healing. These factors work together to drive growth factor, cytokine, and chemokine bioavailability, thus resulting in wound healing.

Vascular changes occur as a result of endothelial cell activation, migration, and capillary expansion in response to tissue hypoxia and increased lactic acid concentration.[6–8] Phenotypical changes in prominent cells (platelets, macrophages, and fibroblasts) are important in directing healing through the influence and production of many of the chemical messengers. The macrophages have a pronounced cellular functional metamorphosis and are the central orchestrators of the healing process. Cellular roles pertinent to wound healing are depicted in **TABLE 2-1** along with the cellular communication and signaling that occur to effect progression through the healing process.

The pivotal cells and phases of healing are depicted in **TABLE 2-2**, which provides a cross-reference of healing phases with key cells and signals, as well as a chronological timeline, thus providing the reader an appreciation of the overlapping and essential functions directed in sequence as opposed to an isolated view of cell function. Although exceedingly complex, the elegance of the healing response lies both in the ability of multiple systems to evoke healing and in the use of paracrine, autocrine, and juxtacrine mediators to effect expedient resolution of tissue injury using the resources immediately available.

TABLE 2-1 Important Cells in Wound Healing

Endothelial Cells Description:		**Cell Graphic**
PRIMARY ACTION	EFFECT	MECHANISM/SIGNAL
Reestablishment of ECM	Fibroblast proliferation	Acidic fibroblast growth factor (aFGF)
Facilitate in angiogenesis	Facilitation of the reestablishment of the vascular base including the reabsorption of excess capillaries	Basic fibroblast growth factor (bFGF)
	VEGF is a potent stimulator of angiogenesis	VEGF—upregulated in the presence of nitric oxide
Angiogenesis	Angiogenesis is further facilitated by the secretion of PDGF and the upregulation of target cell receptors for PDGF (PDGFRs). Cells primed with PDGFRs include circulating progenitor cells, both endothelial and pericyte cells[9]	PDGF platelet-derived growth factor. (This is a family of growth factors, with five members)[10]
Epithelial Cells Description:		**Cell Graphic**
PRIMARY ACTION	EFFECT	MECHANISM/SIGNAL
Attracts platelets	Chemo attraction to injury site	PDGF
Increases vascular permeability	In response to injury, increased vascular permeability allows movement of other key cells (neutrophils, macrophage) into the interstitial space	VEGF
Increase other cells' motility and proliferation	Pleiotropic cell motility and proliferation. Regeneration of the epidermis and other mesenchymal cells	TGF-α
Stimulates angiogenesis	Facilitation of the reestablishment of the vascular base including the reabsorption of excess capillaries	Basic fibroblast growth factor (bFGF) VEGF TNF-α
Formation of granulation tissue during proliferation	Increased granulation tissue in wound bed/base	Insulin-like growth factor (ILGF)
Final re-epithelialization	Reestablishes epithelial barrier	ILGF
Fibroblasts Description:		**Cell Graphic**
PRIMARY ACTION	EFFECT	MECHANISM/SIGNAL
Pro-inflammatory	Stimulate neutrophil development	IL-1—amplifies inflammatory response by increasing synthesis of itself (IL-1) and IL-6
Site-specific migration	Respond to aFGF and bFGF	
Both a constructor and a component of granulation tissue	Elastin production GAGs Adhesive glycoproteins produced on the cell surface anchor the fibroblasts to other cells and proteins in the extracellular matrix	Connective tissue growth factor (CTGF)[11,12]
Change phenotype	During late stage proliferation, fibroblasts morph into myofibroblasts to help bridge the "gap" between the wound edges[13–15]	Differentiation initiated by TGF-β[13]
Collagen production	Direct collagen matrix	Production of fibrin, fibronectin
Recruitment of other key cells	Endothelial cells Keratinocytes[16]	Activated macrophage induce in vitro keratinocyte growth factor (KGF)
Epithelialization	Directs epithelialization and enables cellular migration	KGF2
Differentiate into Myofibroblasts	Epidermal cell motility and proliferation to reestablish intact skin	KGF
Scar contraction	The myofibroblasts pull the newly formed regranulated/scar base together	Actin (REF)
Granular tissue formation and remodeling	Remodeling of the ECM Inhibits and shuts down the tissue MMPs	ILGF-1 TGF-β

(*Continued*)

TABLE 2-1 Important Cells in Wound Healing (*Continued*)

Keratinocytes Description: ▪ Basal cells = Basal Keratinocytes		**Cell Graphic**
PRIMARY ACTION	EFFECT	MECHANISM/SIGNAL
Entry to site a few hours after injury	Migrate over wound bed at the interface between the wound dermis and the fibrin clot	Facilitated by production of specific proteases (eg, collagenase by epidermal cells, which degrades the ECM)[17–19]
↑ Keratinocyte recruitment	Stimulate keratinocytes and induce keratinocyte site specific proliferation	IL-6
Vitamin D_3 synthesis	Key to antimicrobial peptide production	Only cell in the body, which can complete both hydroxylation steps to activate Vitamin D_3
De Novo Hair Follicle Formation	Can contribute to hair follicle formation	Site of epidermal stem cells continued proliferation of keratinocytes
Recruit macrophage	Migration to and cross talk with macrophage	Cytokines, chemokines, interleukins, growth factors[20,21]
Migration and proliferation	Cross talk with macrophage	Activation of epithelial growth factor receptor (EGFR) expressed on keratinocytes Macrophage-produced EGF
Neo-angiogenesis	Provide nutrients and oxygen for new tissue synthesis	Production of VEGF (↑ VEGF indirectly promoted by macrophage secretion of TNF-α and TGF-β)[22]
Macrophage Description: ▪ Mononuclear phagocytes ▪ Mature continuously from monocytes[5,23]		**Cell Graphic**
PRIMARY ACTION	EFFECT	MECHANISM/SIGNAL
Early surveillance	Bacterial replication activates Binding of bacterial components via membrane proteins, for example, toll-like receptor 4 (TLR4), and causes the release of pro-inflammatory cytokines[24]	Release of IL-1β, IL-6, TNF-α17
Phagocytosis	Binding of bacterial components Binding of immunoglobulins[24]	Presence of pathogens, apoptotic cells (including neutrophils)[4]
Wound debridement	Clearing of damaged vessels, necrotic cells, and ECM Pro-inflammatory	Granulocyte-macrophage colony-stimulating factor (GM-CSF) Granulate colony-stimulating factor (G-CSF) Enzymes produced by the macrophage include collagenase and elastase
Recruitment of other cells	Essential for entry of angioblasts, keratinocytes, endothelial cells, and fibroblasts	Cytokines, chemokines, fibronectin, IL-1, INF-γ, TNF-α, and growth factors including PDGR, TGF-β, EGF, and IGF16
Inflammation	Wound Associated Macrophage Central role in the control of inflammation. Upregulation of MMP transcription and nitric oxide (NO) synthesis. TNF-α induces MMP transcription and stimulates the production of NO. Promotes wound closure in normal conditions but are also associated with fibrosis and scar formation[5]	IL-1β IL-6 TNF-α17
Plastic cells	Switch from one functional subpopulation to another depending on the stimulus received[25]	Bacteria, quorum sensing, wound milieu[25]
Coordination of neo-angiogenesis	New vessel formation in the wound bed and surrounding periphery	Stimulates VEGF production by keratinocytes[22]
Stimulate matrix production and regulation	Initially collagen type III is deposited in the wound; however, macrophages are key in each step as listed below. 1. Enzymatically—collagenase and elastase are produced to degrade the ECM 2. Cytokines TNF-α, IL-1, and INF-γ are produced, all of which are pro-inflammatory 3. Growth factor production TGF-β, EGF, and PDGF 4. Prostaglandin production PGE2	Growth factor TGF-β1 and TGF-β2 are associated with inflammation and TGF-β3 is associated with scar-free wound healing[26]
Remodeling	Re-epithelialization from the very first day! Wound activated macrophage (WAM) promotes key cell (keratinocyte and endothelial and epithelial cells) migration via the release of proteases to selectively degrade the ECM[27]	Collagenase secretion Lytic enzyme secretion TGF-β

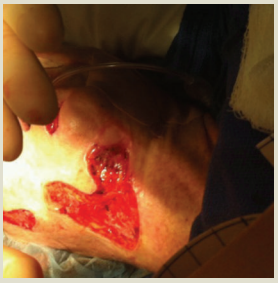
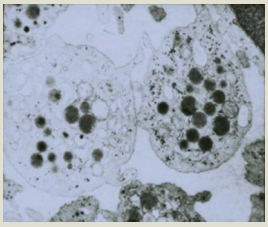
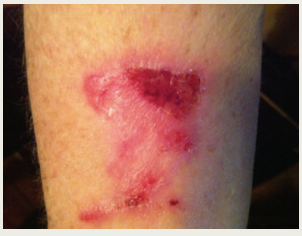
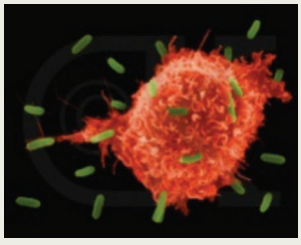
(*Continued*)

TABLE 2-1 Important Cells in Wound Healing (*Continued*)

Platelets Description: ▪ Anucleate cellular fragments ▪ Synthesis controlled by IL-6, IL-3, IL-11, and thrombopoietin ▪ Circulate in the INACTIVE form ▪ Once stimulated undergo major shape changes and develop cell surface receptors for clotting factors. This allows binding to themselves (platelet–platelet) and with the subendothelium		**Cell Graphic**
PRIMARY ACTION	EFFECT	MECHANISM/SIGNAL
Immediate entry into site	Release prothrombin and thrombin to bind-free floating fibrin (from the liver that is found in circulating plasma)	Change in platelet cell shape Change in cell receptors—Receptors displayed on the platelet surface for fibrin and clotting factors, specifically the von Willebrand adhesion factor (Factor VIII).
Entry to site a few hours after injury	Migrate over wound bed at the interface between the wound dermis and the fibrin clot	Facilitated by production of specific proteases (eg, collagenase by epidermal cells, which degrades the ECM)[17–19]
Increases chemotaxis of neutrophils, macrophages, and fibroblasts	Recruitment of macrophage to site of injury to clear and contain invader	Platelet-derived growth factor (PDGF) Tissue growth factors (TGF-β1 and TGF-β2 from platelets)
Delays new vessel formation until clot stable and debris cleared	Inhibits angiogenesis	Endostatin[28]
Proliferation	Extracellular matrix (ECM) synthesis and remodeling	Tissue growth factors (TGF-β1 and TGF-β2 from platelets)
Increased epidermal cell motility	Important in neo-angiogenesis and proliferation phase for reestablishment of epidermal barrier	TGF-β1 and TGF-β2 (from platelets)
Significant source of growth factors	Platelet-derived growth factor (PDGF) TGF-β1 and TGF-β2 Keratinocyte growth factor (KGF) Epidermal growth factor (EGF) Insulin-like growth factor (IGF)	
Polymorphic Neutrophilic Leukocytes (PMNs) Description: ▪ Neutrophils—classically underappreciated professional phagocytes[4]		**Cell Graphic**
PRIMARY ACTION	EFFECT	MECHANISM/SIGNAL
Short life span	Survive <24 hours[4,31]	Migrate from capillaries to interstitial space in response to chemokines[29]
Site-specific migration	Respond to infection ↑ in adhesiveness ↑ in cell motility ↑ in chemotactic response	↑ Vascular permeability Local prostaglandin release Presence of chemotactic substances (complement IL-1, TNF-α, TGF-β, platelets)[30–34]
First inflammatory cells recruited to the clot	Emigrate to the new wound and soon after enter apoptosis	Cytokine release[35,36]
Phagocytosis	Free radical production Scavenging of necrotic debris, bacteria, and foreign bodies	Release of oxygen radicals including H_2O_2, O_2^-, OH^- Super oxidase, NADH[29,37–43] Nitric oxide
Entrapment	Trap invading bacteria for phagocytosis by macrophage. DNA NETs contain decondensed chromatin, bound histones, azurophilic granule proteins, and cytosolic proteins[44,45]	DNA neutrophil extracellular traps (NETs)[45]
Lysis of invaders	Major source of proteases	Release proteases
Recruitment of other key phagocytic cells Resolution of inflammation	Particularly recruit and intensely stimulate macrophage.[46] In fact, the final stage of neutrophil differentiation is the induction of apoptosis, which causes the recognition by phagocytes/macrophages. This assists with clearing invaders and promotes inflammation, endothelial activation,[47] and eventually the resolution of inflammation[48–50]	Apoptosis (programmed cell death) of neutrophils[49–51] TNF-α (cachectin)

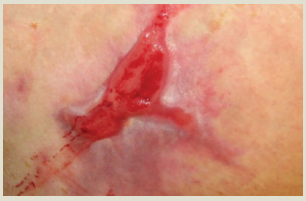
The primary cells that drive the wound healing cascade of cellular and acellular processes, their effects, and their mechanisms of action or of signaling other cells to act are summarized.

TABLE 2-2 Phases of Wound Healing

Clinical Presentation	Normal	Predominant Cell/Tissue Type
Hemostasis (<1 hour) 	Cellular Activity Clot formation ▪ Stop bleeding ▪ Contain invader ▪ Begin attracting phagocytes	Predominant Tissue Type/Cell Platelet 
Vascular events	▪ Transient arteriole constriction ▪ Fibrin from liver transported ▪ Vascular permeability increases after bleeding is controlled to allow passage of other key cells including neutrophils and macrophage into the interstitial space	
Cellular events	▪ Neutrophil influx ▪ Platelet aggregation in collagen ▪ Release of platelet α-granules and dense bodies	
Cell signaling	▪ Clotting cascade: von Willebrand adhesion factor (glycoprotein)—binds factor VIII, which initiates the clotting cascade via prothrombin and thrombin conversion ▪ TGF-β1 and TGF-β2 are released from platelets and stimulate the chemotaxis of fibroblast and macrophage ▪ Increased release of IL-1 from antigen presenting cells (APCs), those cells that are key to identifying the invader (REF). The increased release of IL-1 from APCs (dendritic cells, macrophage) and monocytes stimulates those same cells (autocrine) to produce IL-8. The chemical messenger, IL-8, attracts neutrophils and increases the "sticky" or adhesion factors along the endothelium to assist in this process	
Clinical signs	▪ Clot formation ▪ Hemostasis achieved ▪ Fibrous scab formation ▪ Peri-injury including inflammation and edema	
Inflammation (1 hour–4 days) 	Reactive Chemotaxis/Scavenge ▪ Damage control ▪ Recruit immune system ▪ ↑ Circulation to injury site ▪ Initiate healing sequence	Predominant tissue type/cell macrophage (WAM) 
Vascular events	▪ Vasodilation ▪ ↑ Permeability ▪ Stasis	
Cellular events	▪ Migration and accumulation of leukocytes, PMNs, and macrophage ▪ ↑↑ Tissue permeability ▪ ↑ Neutrophils ▪ ↑ Macrophage	

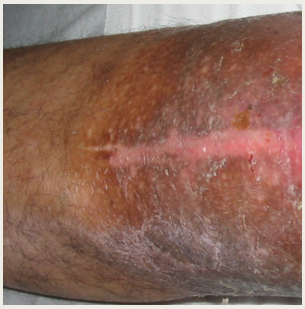
(*Continued*)

TABLE 2-2 Phases of Wound Healing (*Continued*)

Clinical Presentation	Normal	Predominant Cell/Tissue Type
Cell signaling	▪ Platelet α-granules released, which contain PDGF, TGF-β, IGF-1, fibronectin, fibrinogen, thrombospondin, and vWF ▪ Platelet dense bodies (vasoactive amines, serotonin) release ▪ IL-1 (produced by macrophage) ▪ Activates B cells (adaptive immune system/memory cells) • ↑ Clonal expansion of appropriate B cells • ↑ Transmigration from the blood to the tissues • ↑ Expression of adhesion molecules in endothelium, which helps neutrophils "stick" to the endothelium near the site of injury • ↑ Body temperature via the hypothalamus "endogenous pyrogen" ▪ IFN-γ production elevated (produced by Th1/innate immune system neutrophils) • ↑ Creates "angry" macrophages, which ↑ phagocytosis • ↑ Antigen identification/presentation by APCs • ↑ Macrophage phagocytosis • ↑ INHIBITS fibroblast and ECM production	Predominant tissue type/cell macrophage (WAM)
Clinical signs	▪ Rubor (redness) ▪ Tumor (swelling) ▪ Calor (heat) Locally AND ↑ Body temperature ▪ Dolor (pain)	
Proliferation (4–12 days) **Extracellular matrix** 	Repair 80% completed Laminin ▪ Anatomical cover ▪ ECM ▪ Endothelium ▪ New epithelial barrier in place ▪ Key protein in basal lamina (one of the layers of the basement membrane) ▪ Family of glycoproteins integral part of structural scaffolding ▪ Form independent networks via type IV collagen, entactin, fibronectin, and perlecan ▪ Bind to cell membranes, contribute to cell attachment and differentiation ▪ Specific peptide sequence promotes adhesion of endothelial cells[52]	▪ Extracellular matrix ▪ Fibroblast to myofibroblast ▪ Epithelial cells ▪ Anti-inflammatory macrophage (M2)
Vascular events	▪ Proliferation of new small blood vessels ▪ Proliferation of new ECM and epidermal cells	
Cellular events	▪ Increased mitotic activity (cellular division) of basal epithelial layer ▪ Fibroblast and vascular endothelial proliferation ▪ Proteoglycan, collagen, and ultimately ECM (fibronectin and laminin) synthesis	
Cell signaling	▪ VEGF—vasoendothelial growth factor-angiogenesis (acts through VEGFR-2 primarily) ▪ Proliferation and motility of endothelial cells ▪ aFGF/bFGF—acidic and basic fibroblast growth factor facilitates both fibroblast proliferation and wound vascularization and angiogenesis ▪ Endothelial precursor/progenitor cells ▪ TGF-β1 and TGF-β2 (from platelets)—important for extracellular matrix (ECM) synthesis and remodeling. Also, important in neo-angiogenesis and increased epidermal cell motility to reestablish the epidermal barrier IL-10 (produced by macrophage and keratinocytes) • ↓↓ Synthesis of IL-6 (pro-inflammatory cytokine) • ↓ Neutrophil/leukocyte migration • ↓ Macrophage cytokine production • Down-regulation of neutrophils (Th1 innate immune system cells) • Down-regulation of MHC II on APCs—because the invader has presumably been "cleared" and there is no need to continue to stimulate the innate and adaptive immune system cells TNF-α (from neutrophils)	

(*Continued*)

TABLE 2-2 Phases of Wound Healing (*Continued*)

Clinical Presentation	Normal	Predominant Cell/Tissue Type
Clinical signs	▪ Formation of granulation tissue ▪ Silvery clear covering of wound (new epithelium)	▪ Fibroblast—myofibroblast ▪ Anti-inflammatory macrophage
Maturation and remodeling 	Contraction Fibroblasts differentiate to myofibroblast Migration of melanocytes functional/scar remodel ▪ Function ▪ Thermoregulatory ▪ Range of motion ▪ No reoccurrence of wound	
Vascular events	▪ Removal/reabsorption of extraneous capillaries	
Cellular events	▪ Macrophages secrete collagenase and lytic enzymes ▪ Fibroblasts secret TIMPs which inhibit MMPs, the enzymes that degrade the ECM ▪ ↑ Tensile strength/fibrosis occurs ▪ Collagen type III replaced by collagen type I, which increases wound strength	
Cell signaling	▪ Tissue inhibitors of metalloproteinases (TIMPs) counteract MMPs so remodeling proceeds in concert ▪ TGF-β1 and TGF-β2 (produced by the platelets) • ↑ Fibroblast synthesis • ECM synthesis and remodeling	
Clinical signs	Blanching	

The phases of wound healing include hemostasis, inflammation, proliferation, and maturation and remodeling. Each phase is shown with the primary cells that are pertinent in communication, signaling, and/or tissue production. Vascular events, cellular events, cell signaling, and clinical symptoms that occur in each phase are described.

Cells exhibit various levels of activity in response to many factors. **FIGURE 2-1** illustrates four recognized levels of cellular activity and the associated effect on local tissue environment. Cells that exist in a *senescent state* (defined as resistant to apoptosis or programmed cell death) disrupt normal tissue differentiation, drain the metabolism, and secrete cell products that negatively impact the wound environment. Cells in a *baseline state* have normal mitotic and metabolic activity, actively survey and monitor adjacent tissues, and do not impact surrounding tissues negatively. An *upregulated state* has a higher level of metabolic activity and purposefully responds in concert with other cells in reaction to injury, presence of pathogens, or both. A cell that is *out of control* exhibits an overproduction of cellular byproducts, is not coordinated with any other cells, and does not respond to feedback inhibition. The cartoons that represent each level of cell activity are overlaid in important diagrams to help the reader discern the cellular state in normal and disrupted wound healing. *Both the correct cells and the appropriate level of cellular activity are required to ensure wound healing.*

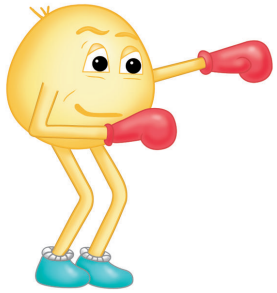
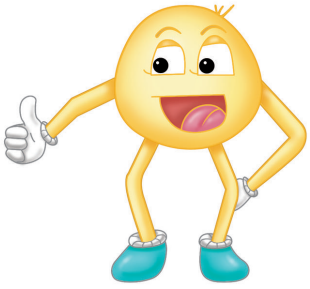
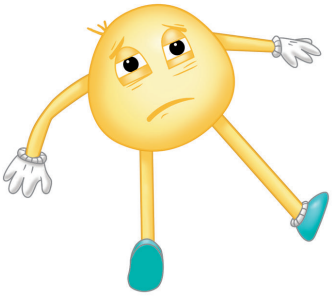
FIGURE 2-2 provides an illustration of the intricate and exquisite sequence of cell migration, proliferation, and signaling in the context of cell signaling and vascular events, all working in unison to culminate in tissue healing.

HEALING RESPONSE

The healing response occurs by one of the following four mechanisms: (1) continuous cell cycling, (2) cell proliferation, (3) regeneration, or (4) fibroproliferative response. Normal intact skin is representative of *continuous cell cycling* whereby labile cells are constantly undergoing a balance of proliferation and programmed apoptosis throughout life, thereby resulting in a steady state. The basal keratinocytes continuously undergo mitosis (cell division), followed by migration to the skin surface and subsequent desquamation or sloughing. *Cell proliferation* occurs when the damaged or lost tissue is replaced by the expansion of remaining healthy cells that undergo mitosis. The structure is not completely duplicated; however, function is approximated.

Regeneration occurs with the loss of a structure. The acute injury that undergoes regeneration stimulates complete duplication in both structure and function of the lost tissue. The liver, hematopoietic tissue, gastrointestinal tract

FIGURE 2-1 Levels of cellular activity

Figure Key	Numeric Code	Cell Activity Level	Key Events or Conditions
	2	Running out of control	State: • No feedback inhibition • Not coordinated with any other cells in wound milieu Result: • Overproduction of cell byproducts • Delayed execution of sequential steps for wound healing • Increase in cell necrosis ◦ Necrosis creates increased cell debris and and burden which must be removed by macrophage and neutrophils. This is compared to apoptosis, or programmed cell death, which does not increase cellular debris. ◦ Excess cell debris provides an additional food source for bacterial invaders.
	1	Upregulated	State: • Appropriate elevated response in concert with other cells • In response to injury or invader (bacterium, fungi, virus, microbe) Result: • Elevated cellular products • Facilitates sequential steps for wound healing • Decrease in cellular debris
		Baseline	State: • Surveilance state. Geographically localised and poised to respond to intruders or injury Result: • Actively monitoring milieu and adjacent cell states • Normal mitosis occurring
	–1	Senescent	State: • Depressed, unresponsive • Not able to be stimulated into appropriate action • Associated with aging or chronic wound environment Result: • Altered cell receptor display and secretion of cell products/ gene expression • Metabolic drain-senescent cells consume energy yet do not contribute to local cell health • Resistant to apoptosis

This figure provides an explanation of various cell activity levels and the observed cellular events associated with that level of cellular activity. The cartoons illuminate the cell state and are used throughout the chapter to illustrate whether the level of activity is appropriate or inappropriate, as well as the ramifications.

epithelium, and epidermis are examples of tissue that are capable of regeneration. *Fibroproliferative healing* typically occurs in dermal wound healing. The lost tissue is not replaced, but rather a "patch" is constructed that restores the skin covering, integrity, and function. Inflammed tissue that fails to progress to healing results in tissue fibrosis. Divisions of wound healing are graphically depicted in **FIGURE 2-3**.

Categories of Healing Responses

Wound healing can be classified by category or by depth; both assist clinicians in communicating clearly regarding patient needs. Four categories describe wound healing—categories 1 to 3 describe healing of full thickness wounds while category 4 refers to partial thickness skin wounds.[53,54]

Category 1 Category 1 (Primary Intention) healing occurs when a clean surgical incision is created and the resulting wound is free from contamination of bacteria, fungi, or foreign bodies. There is minimal tissue loss and the edges can be safely approximated and secured with sutures, staples, or surgical glue. The clotting cascade at the wound surface is largely not initiated and the resulting fibrous scab is absent because of the minimal mortality of cells central to wound healing. The cell signaling cascades usually launched in an acute penetrating injury are not activated. This incisional wound resolves in an orderly, sequenced manner over the course of approximately two weeks (**FIGURE 2-4**).[55]

Category 2 Category 2 (Delayed Primary Intention) healing occurs when wound edges are not approximated because of the concern for the presence of pathogens or debris, an existing abscess, or loss of extensive tissue (**FIGURE 2-5**). Delayed primary wound healing is set in motion by the release of multiple pro-inflammatory cytokines, chemokines, and growth factors. Foreign debris is walled off by macrophages that may metamorphose into epithelioid cells, which in turn become encircled by layers of mononuclear leukocytes. The layers of mononuclear leukocytes can be compared to the sequential layering of nacre on a pearl—the clam overlays the grain of sand with nacre, smooth and protecting. In a wound, the result is a granuloma, with the foreign body or pathogen at the center, walled off from the host tissue. In these wounds the inflammatory response is more intense and is accompanied by increased granular tissue formation.[55]

These wounds frequently undergo delayed surgical closure after surgical removal of the granuloma, abscess, or debris. Once the wound is determined to be ready for closure, surgical intervention (such as suturing, skin graft placement, or flap design) is performed, provided the wound edges can be approximated. If the host-initiated cleansing, termed *autolysis*, of the wound is incomplete, chronic inflammation can ensue. Without further intervention, the result is likely to be prominent scarring.

Category 3 Category 3 (Secondary Intention) healing is entirely accomplished through an appropriate inflammatory response, granulation tissue formation and re-epithelialization. Left to close without surgical intervention, wound contraction by myofibroblasts plays a significant role. The myofibroblasts have characteristics of smooth muscle cells and, when activated, they contract and thereby assist in consolidating the extracellular matrix and decreasing the distance between the dermal edges. The myofibroblasts are maximally present in the wound from approximately 10 to 21 days post-wounding.[14] Additional time may be required for these wounds to close depending on the surface area and depth (volume) of the wound (**FIGURE 2-6**).[55]

Category 4 Partial thickness wounding refers to partial loss of the epidermis or loss of the epidermis and superficial dermis (the basement membrane is intact and the hypodermis is not exposed). In this case healing is accomplished by epithelial cell mitosis and migration. Wound contracture is not an expected or common occurrence during the healing of partial thickness wounds, as the sub-dermal layers are not involved and minimal to no granulation tissue is formed (**FIGURE 2-7**).[55]

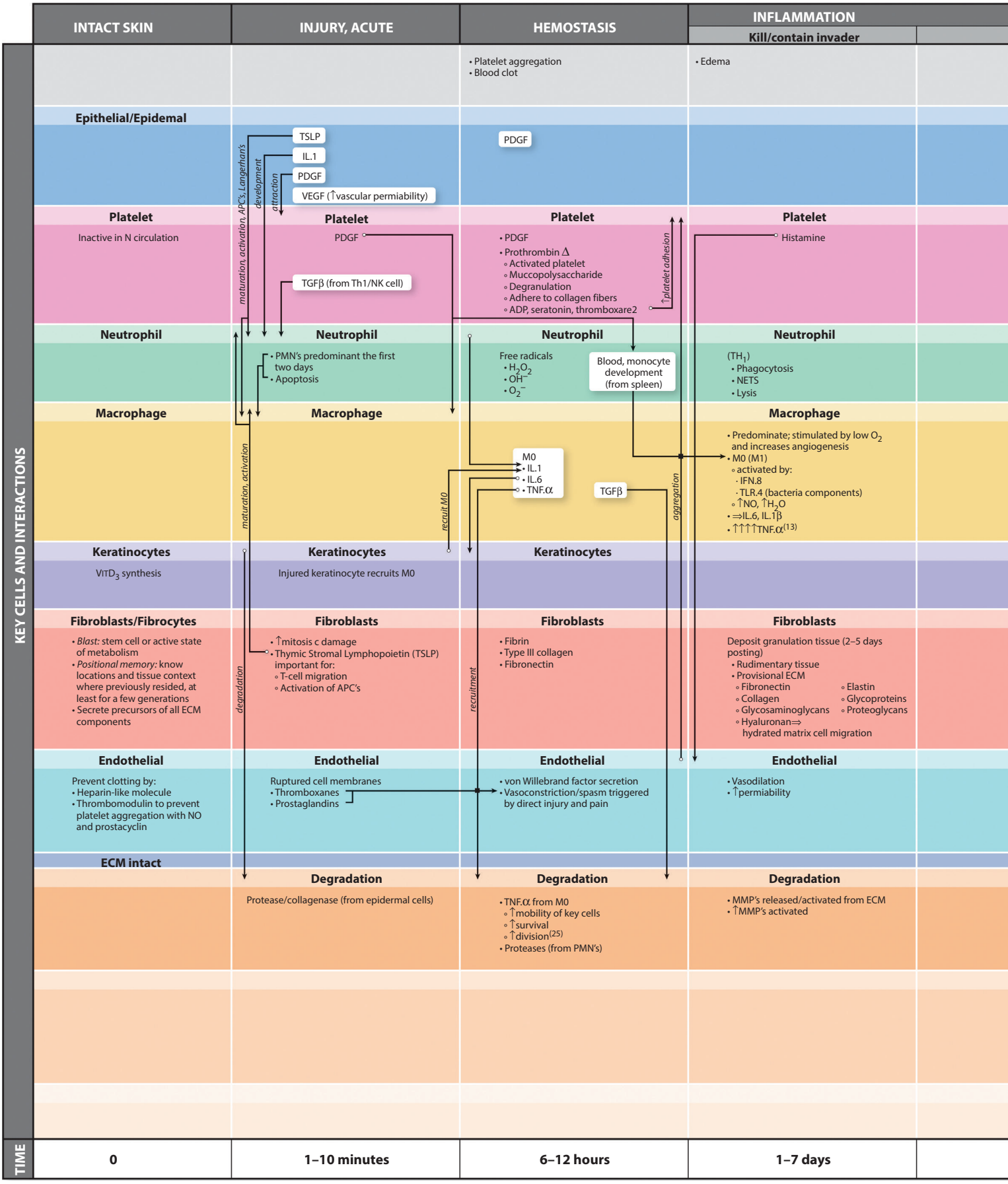
Wounding can also be categorized by the depth and involvement of tissue lost or damaged as a result of the injury. These classifications progress from the most superficial erosion, to partial thickness of the dermis, and full thickness extending into the hypodermis. (Refer to Chapter 1, Anatomy and Physiology of the Integumentary System.)

Overview of Healing

Acute wound healing is divided into the following phases: hemostasis, inflammation, proliferation, and remodeling. Inflammation is further subdivided into three overlapping phases: kill/contain the invader, inflammation, and neo-angiogenesis. **FIGURES 2-8** to **2-14B** provide a framework for each of the major healing phases in terms of four important events: (1) vascular, (2) cellular, (3) cell signaling, and (4) clinical signs. **FIGURES 2-8** and **2-9** depict normal intact skin prior to wounding; **FIGURE 2-10**, hemostasis; **FIGURES 2-11A** to **2-13B**, inflammation; and **FIGURES 2-14A** to **2-16**, proliferation. The interplay between each of the four phases is both complex and transitional such that within any wound, signs of more than one phase may be present. Vascular, cellular, and tissues changes that occur during the four phases include the following:

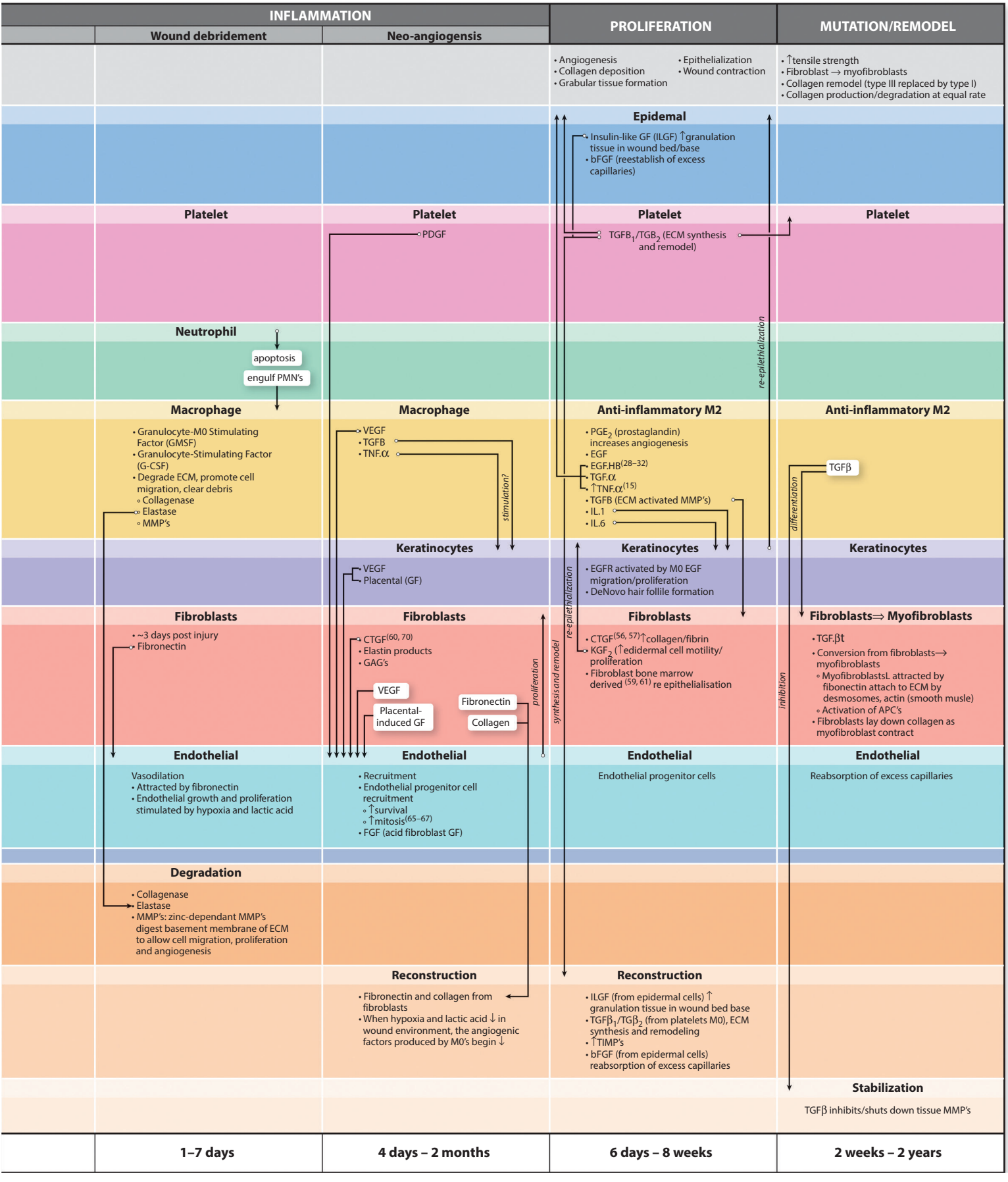
1. Vascular events include hemostasis, transient vasoconstriction, retrograde degradation of damaged vessels, and the transition to endothelial cell differentiation, migration, and proliferation—also termed *neo-angiogenesis*.
2. Cellular events include the directed migration and accumulation of cells known to be necessary for wound healing (eg, neutrophils and macrophages) to the site of injury.[5] Some cells (platelets, macrophages, and fibroblasts) morph in both phenotype and function, depending on the phase of healing and the surrounding stimuli, whether cytokine, chemokine, or ECM activation.[5]
3. Cell signaling orchestrates healing by the actions of cytokines, chemokines, growth factors, and receptor accessibility on target cells. It is accomplished through the binding of chemical messengers to cell receptors present on the target cell surface. The binding of the chemical messenger (eg, cytokine, chemokine, growth factor, or interleukin) activates or depresses target cell

FIGURE 2-2 **Cell migration, proliferation, and signaling in the wound healing process**



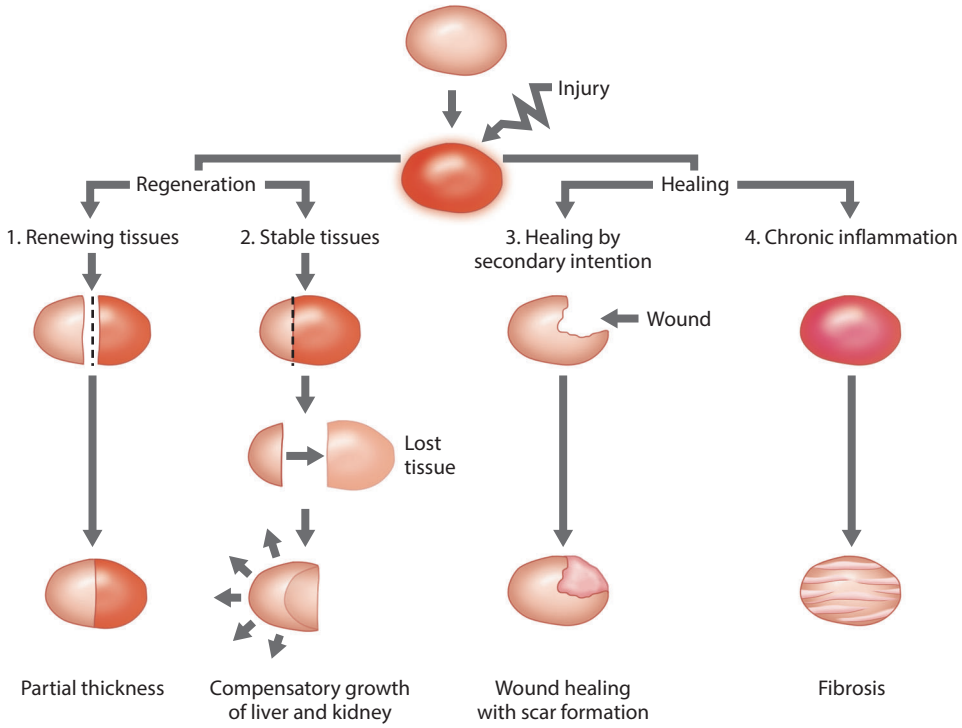
The healing map is a summary of both the acellular and cellular components of wound healing. This illustration is parsed by phases of healing along the horizontal axis, while the primary cells of importance are along the vertical axis.

FIGURE 2-2 (*Continued*)



The cytokines, chemokines, and growth factors important at each phase are depicted along with the directional impact exerted on healing by each of these components. The direction is indicated by color-keyed arrows.

FIGURE 2-3 Divisions of wound healing



The body responds to tissue injury in mechanisms that result in tissue regeneration, thus restoring both structure and function of that specific tissue. The four pathways of response are regeneration, compensatory growth, renewal, and fibrosis. When regeneration of epithelium or the underlying tissue fails to occur in a timely fashion, a state of chronic inflammation usually results.

DNA transcription and protein translation of important activities that are performed by the target cell. These activities include (a) the production of additional chemical messengers, (b) a change in cell phenotype and function, or (c) binding to adjacent ECM sites. Cell signaling occurs between cells (cell to cell) and between the cell and wound matrix (cell to matrix).[56]

4. Clinical signs and symptoms include changes observed in the local periwound environment (pain, redness, and edema) or systemic symptoms detected in the patient (fever, chills, increased heart rate, or pain).

Cytokines are small proteins or glycoproteins that are secreted by numerous cells and alter the function of the target cell. The target cell for the cytokine/interleukin may be itself (autocrine) or a neighboring cell (juxtacrine). Cytokines can be either pro-inflammatory or anti-inflammatory.

Interleukins are a group of cytokines that were first observed being expressed by white blood cells (leukocytes).[1] The term *interleukin* derives from *inter* as a means of communication, and *leukin,* deriving from the fact that leukocytes produce many of these proteins and are the target of their action. The name is something of a relic as it has been determined that interleukins are in fact produced by a wide variety of body cells. The function of the immune system depends in a large part on interleukins, the majority of which are synthesized by helper CD4+ T-lymphocytes, as well as monocytes, macrophages, and endothelial cells.[3]

TABLE 2-3 lists the cytokines important to wound healing. The pro-inflammatory cytokines necessary for wound healing are TNF-α, IL-1, IL-2, IL-6, IL-8, and IFN-γ. In general, IL-4 and IL-10 are considered anti-inflammatory. Receptor expression on target cells can be either up- or down-regulated. Each of the signals and the complex interplay serves to enhance, depress, or change entirely the cell function while ensuring that the process culminates in functional wound healing.

Growth factors are soluble polypeptides, produced in both normal and wounded tissues, which stimulate cell migration, proliferation, and alterations in cellular function. They are extremely potent and can exert significant effects in nanomolar concentrations. Growth factors bind to specific cell receptors and have one of two different effects: (1) the stimulation of DNA transcription or (2) the regulation of cell entry in the cell cycle (mitosis). Growth factors that are important in wound healing are listed in **TABLE 2-4**.

Cell-to-wound matrix communication occurs extensively during debridement and angiogenesis. Matricellular proteins (defined as dynamically expressed non-structural proteins in the ECM that are rapidly turned over and have regulatory roles)[57] destabilize the cell–matrix bonds and interactions, in essence creating a more fluid environment for cell migration. Proteinases, both plasminogen activators and matrix

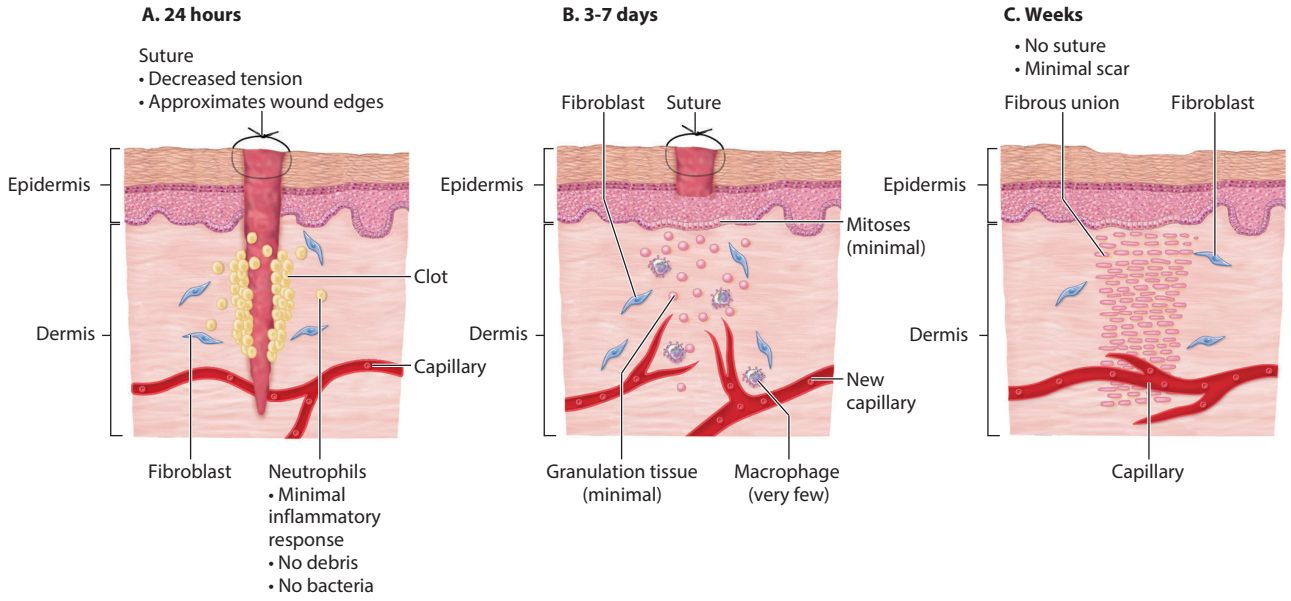
FIGURE 2-4 Healing by primary intention

Incision causes only focal disruption
- Epithelial basement membrane continuity largely maintained
- Death of a relatively few epithelial and connective tissue cells

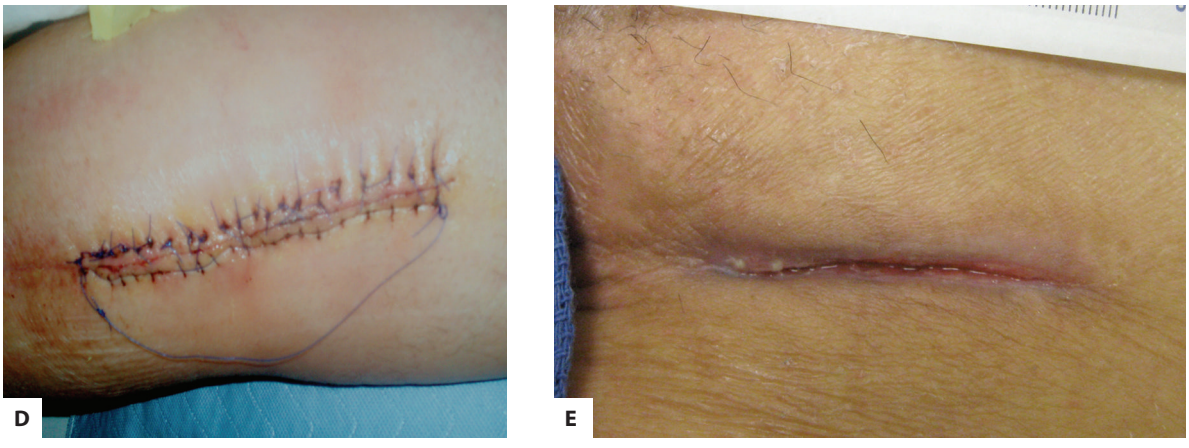
Epithelial regeneration predominates over fibrosis

Small scar is formed
- Minimal wound contraction occurs

Filling of narrow incisional space
- First fibrin- and clotted blood
- Followed by rapid invasion of granulation tissue
- Covered by new epithelium



A–C. The series of three images illustrate the tissue response to an incision. Focal disruption and tissue stabilization by suture result primarily in epithelial regeneration.



D. Sutures are used to close a surgical incision by primary intention. Healing is achieved when the new epithelium bridges the gap between the two edges, and minimal scar is formed. **E.** The left aspect of this groin incision illustrates closure by primary intention where the epithelium has bridged the incision. In the remaining part, the incision has separated in part because of the amount of moisture that has dissolved the superficial sutures without full skin growth. The incision is termed *separated* if the gap is less than 1 cm; *dehiscence*, if more than 1 cm.

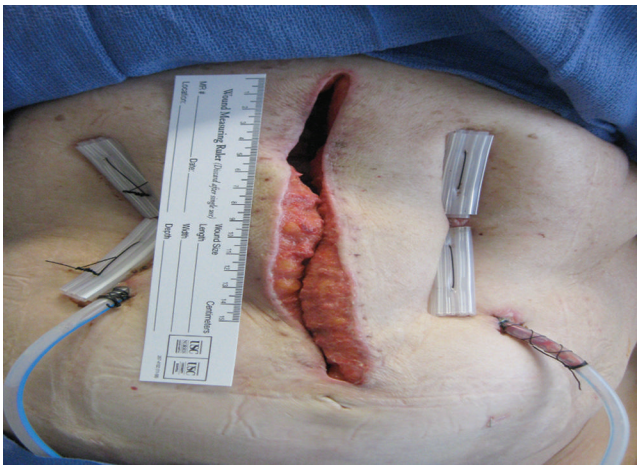


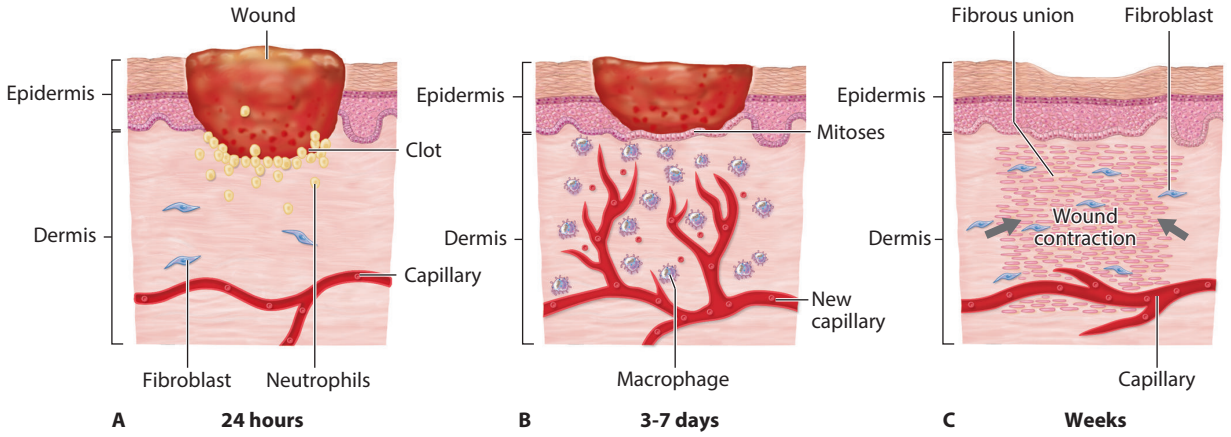
FIGURE 2-5 Healing by delayed primary intention Large wounds can be partially closed with retaining sutures, or in the case of this abdominal wound, with tension sutures. This technique is used if approximating the edges puts too much strain on the periwound skin and subcutaneous tissue or if there is concern for infection and drainage that needs to be removed in order to prevent abscess formation.

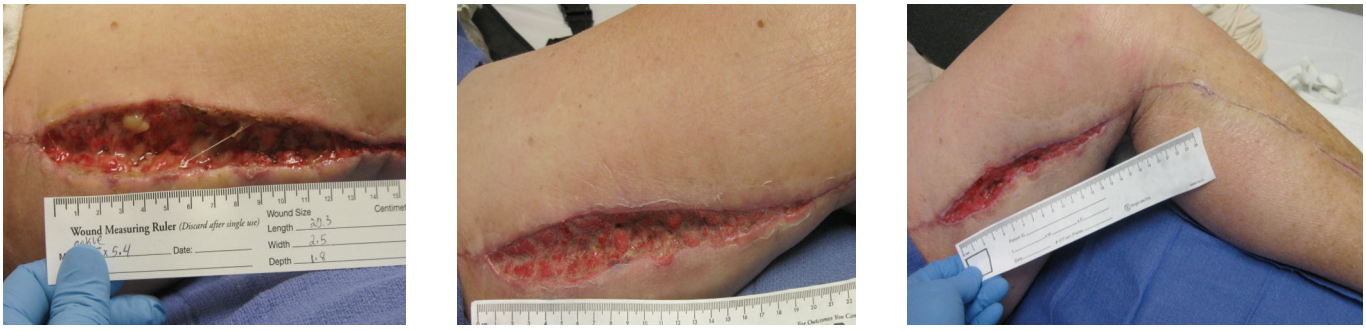
metalloproteinases (MMPs), act to dissolve the wound matrix both immediately after injury and during the proliferative and remodeling phases. This is necessary during angiogenesis for the directed migration of endothelial cells.

The intricacies of cellular communication involve the components of the extracellular matrix (ECM) including fibrous structural proteins, water-hydrated gels, and adhesive glycoproteins (**FIGURE 2-17**). The fibrous structural proteins include the collagens and elastins, which confer tensile strength and recoil to the tissue. Water-hydrated gels that permit resilience and lubrication are categorized as proteoglycans and hyaluronans. Adhesive glycoproteins connect the matrix elements to one another and to cells.

Collagen, one of the two fibrous structural proteins, is composed of three separate polypeptide chains braided into a rope like a triple helix (**FIGURE 2-18**). There are approximately 50 types of identified collagen. Some collagen types (eg, I, II, III, V) form fibrils by virtue of lateral cross-linking of the triple helix and are a major portion of connective tissue in healing wounds and particularly in scars. The cross-linking is a result of a covalent bond catalyzed by the enzyme lysyl oxidase, a process that is dependent on vitamin C. Types of collagen important to

FIGURE 2-6 Healing by secondary intention





When there is extensive tissue loss or contamination, the repair process increases in complexity as illustrated by a robust inflammatory response and an abundance of granulation tissue. A–C. The series of three illustrations highlights these attributes along with wound contraction through the action of myofibroblasts. **A.** Dehisced surgical incision on the medial thigh, approximately 24 hours after the site was irrigated and drained surgically. The wound is in the inflammatory phase of healing. **B.** The same wound two weeks later is in the proliferative phase with granulation visible throughout the wound bed. **C.** Two weeks later the wound is significantly smaller, and the incision along the lower leg is observed to be closed and remodeling. The wound completed closure by secondary intention without further surgical intervention.

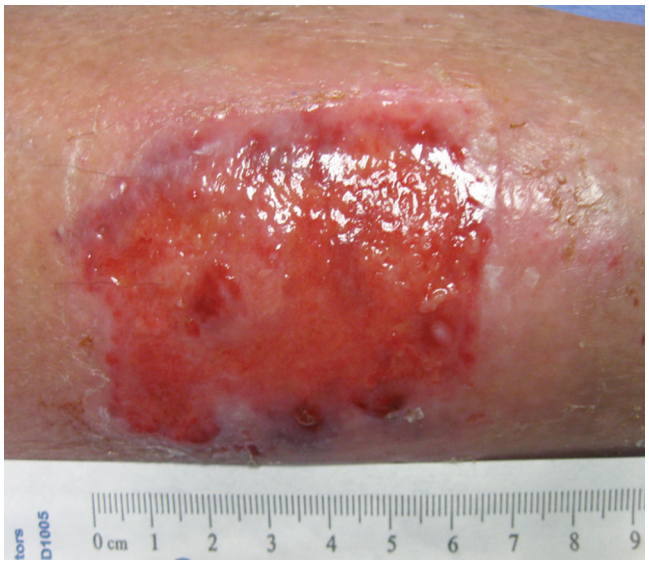


FIGURE 2-7 Healing of a partial-thickness wound
Re-epithelialization can be seen at the wound edges, as well as on the small "epithelial island" at the lower edge. The island indicates that the epithelial cells in that region are migrating from the hair follicle rather than the edge and is commonly seen in partial-thickness wounds.

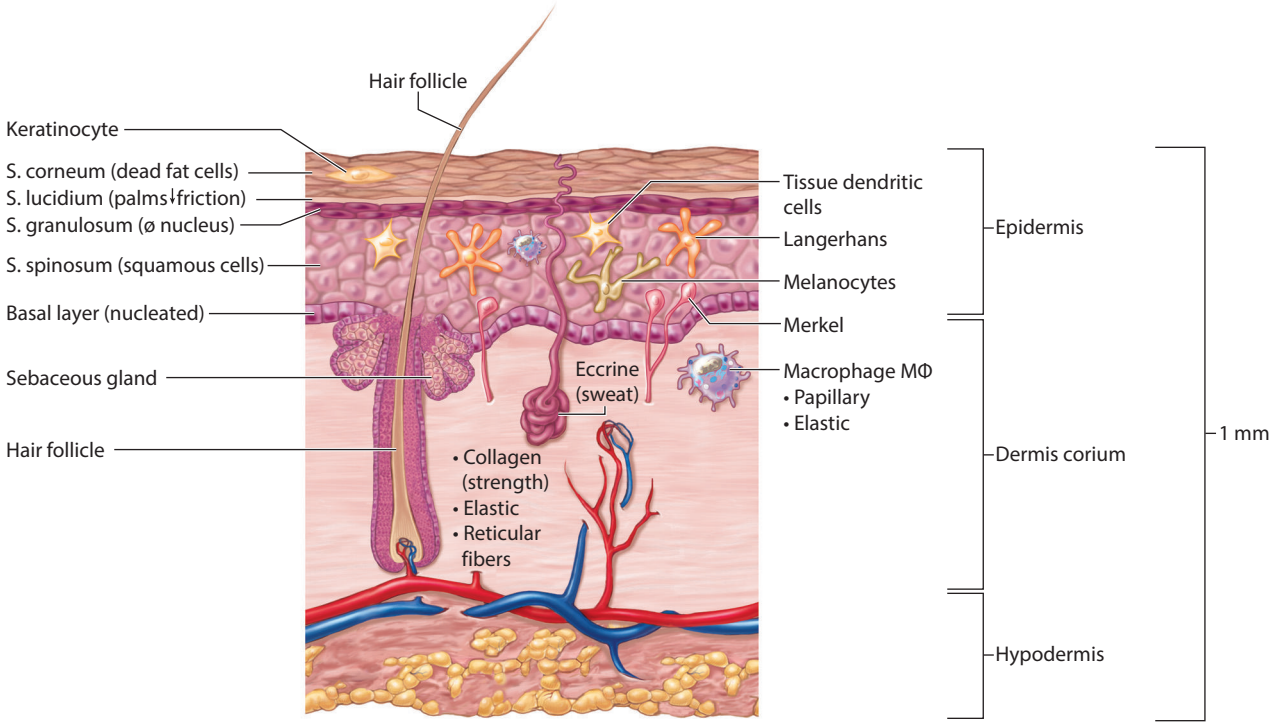
wound healing include Type I, skin and bone; Type IV, basement membrane; and Type VIII, dermal–epidermal junction.

Elastin (present mainly in the skin, large vessels, ligaments, and uterus) consists of a central core of elastin surrounded by a mesh-like network of fibrillin glycoprotein. Fibroblasts secrete fibrillin into the ECM where it becomes assimilated into insoluble microfibrils and provides a platform for elastin deposition (**FIGURE 2-19**).

Proteoglycans form extremely hydrated compressible gels that provide both resilience and lubrication (eg, in the skin, cartilage, and joints). Proteoglycans consist of glycosaminoglycans (GAGs) and hyaluronan. GAGs are long polysaccharide chains like heparin sulfate and dermatan sulfate. Hyaluronan binds water and forms a very viscous, gelatin-like matrix. Proteoglycans also function to provide compressibility and serve as a reservoir for growth factors that are secreted into the ECM. Proteoglycans are also an important component of cell membranes and as such have roles in proliferation, migration, and adhesion (**FIGURE 2-20**).

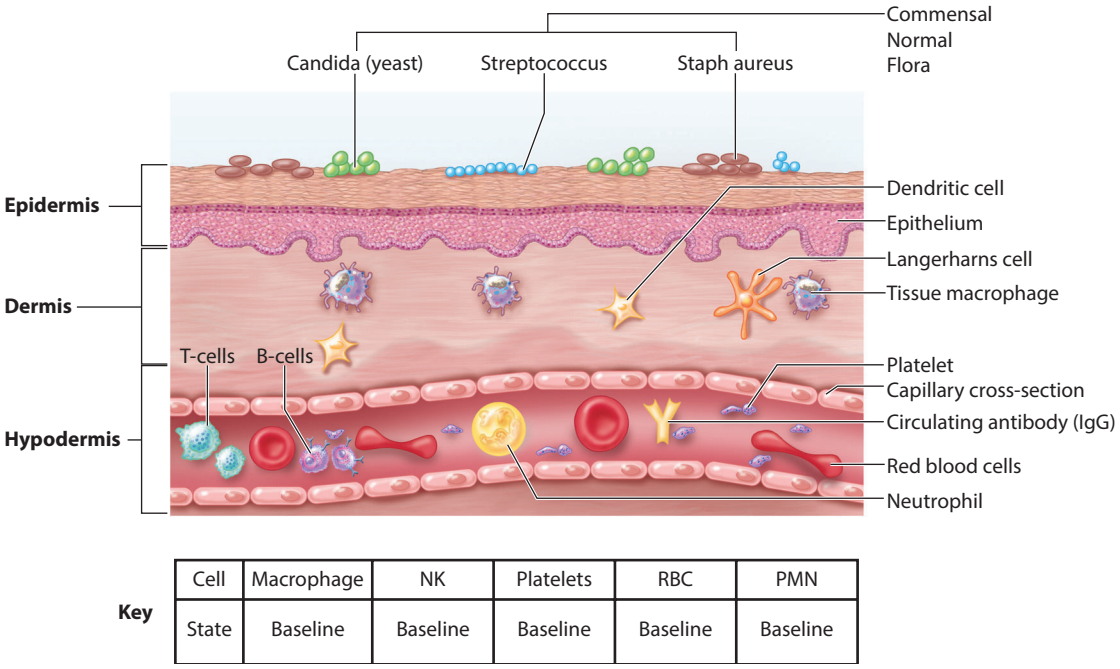
The adherent components of the ECM are the adhesive glycoproteins and adhesion receptors. The adhesive glycoproteins include fibronectin and laminin. The adhesion receptors include immunoglobulin, selectin, cadherins, and integrins. Together laminin and fibronectin, by adjoining to collagen and connecting to the cellular plasma membrane of cells primary to tissue healing, reestablish both strength and function to the new replacement tissue. Laminin and fibronectin also form the critical active junction providing both orientation and a dynamic functioning framework. **FIGURE 2-21** depicts the adhesion receptors, which are paramount to ECM function and structure.

FIGURE 2-8 Normal intact skin



Normal intact and uninjured skin showing the layers of epidermis, dermis, and hypodermis. The appropriate and significant cells are illustrated in the layer of skin as observed in the uninjured skin.

FIGURE 2-9 Baseline state of the skin prior to injury



Key	Cell	Macrophage	NK	Platelets	RBC	PMN
	State	Baseline	Baseline	Baseline	Baseline	Baseline

The cells of the skin involved in the immune system are constantly on the attack against invaders that may cause infection, and when injury occurs they are mobilized to be even more active.

PHASES OF ACUTE WOUND HEALING

Wound healing initially appears so very simple.[58–60] The human body is designed to heal, repair, and in some cases regenerate lost tissue through a well-orchestrated sequence of events. When it proceeds as planned—though infinitely complex—healing is elegant, rapid, and efficient. The following four phases of wound healing are delineated by the pertinent vascular, cell-signaling, cellular activity, and clinical response as previously defined (**FIGURE 2-10** to **2-14B**).

1. Hemostasis—Clot Formation

With an acute injury, the small blood vessels respond initially with vasoconstriction to stem further blood loss and tissue injury (**FIGURE 2-22**). Activated platelets adhere to the endothelium and eject adenosine diphosphate (ADP) which promotes the clumping of thrombocytes and further ensures clot formation. The clot, composed of various cell types (red blood cells, white blood cells, and platelets), is stabilized by fibers of fibrin.[17] (See **FIGURES 2-23** and **2-24**.)

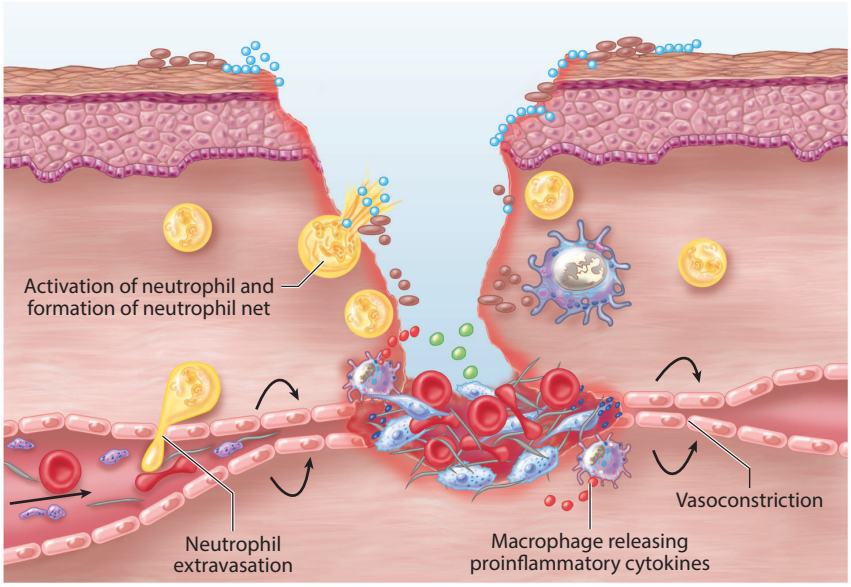
Alpha granules containing platelet-derived growth factor (PDGF), platelet factor IV, and transforming growth factor beta (TGF-β) are released from the platelets. Dense bodies contained within the thrombocytes release vasoactive amines, including histamine and serotonin. PDGF is chemotactic for fibroblasts, and in coordination with TGF-β modulates mitosis of fibroblasts,[61] thereby increasing the number of fibroblasts in close proximity to the wound. Fibrinogen is cleaved into fibrin which undergirds the structural support for the completion of the coagulation process and provides an active lattice for the important cellular components during the inflammatory phase. The fibrin will further serve as a scaffold for other infiltrating cells and proteins. (See **TABLE 2-5**.[3,10,62]) Clinically, a full-thickness wound bed with a stable clot functions to mitigate blood loss. Clear proteinaceous exudate may or may not be present.

2. Inflammation

Inflammation presents clinically as rubor (redness), tumor (swelling), calor (heat), and dolor (pain). The orchestrated arrival and departure of important cell mediators are depicted in **FIGURE 2-25**. Important changes that permit the initiation of inflammation and result in the clinical findings associated with inflammation are summarized in **TABLE 2-6**.

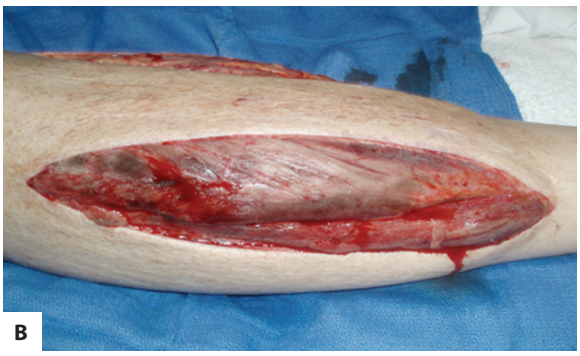
Kill and Contain Invader At the immune cellular level several cells arrive, depart, upregulate, or down-regulate during the phases of healing. Within the first six to eight hours after injury, polymorphonuclear leukocytes (PMNs) or neutrophils flood the wound. TGF-β (released from platelets) facilitates PMN migration and extrusion from surrounding intact blood vessels to the interstitial wound space. PMNs are phagocytic cells, functioning to cleanse the wound of debris, including both necrotic cells and pathogens (**TABLE 2-7**). The highest number of PMNs within the wound is observed between 24 and 48 hours post injury. By 72 hours, PMN numbers are significantly reduced, just as macrophages are infiltrating (**FIGURE 2-26**).[56,63,64] Factors that promote neutrophil adherence and migration are

FIGURE 2-10 Hemostasis



Key	Cell	Macrophage	NK	Platelets	RBC	PMN
A	State	Upregulated M1	Upregulated	Upregulated	Upregulated	Upregulated

A. Hemostasis is the first phase of wound healing and is characterized by vasoconstriction of the injured vessel followed by vasodilation of the adjoining vasculature. Platelets aggregate and, along with fibrin, form a stable clot. At the wound site, platelets release molecules to stimulate platelet aggregation and growth of tissues important to healing. In the dermis and epidermis, PMNs and macrophages aggregate to kill and contain pathogenic invaders. The brown cells represent the pathogens.



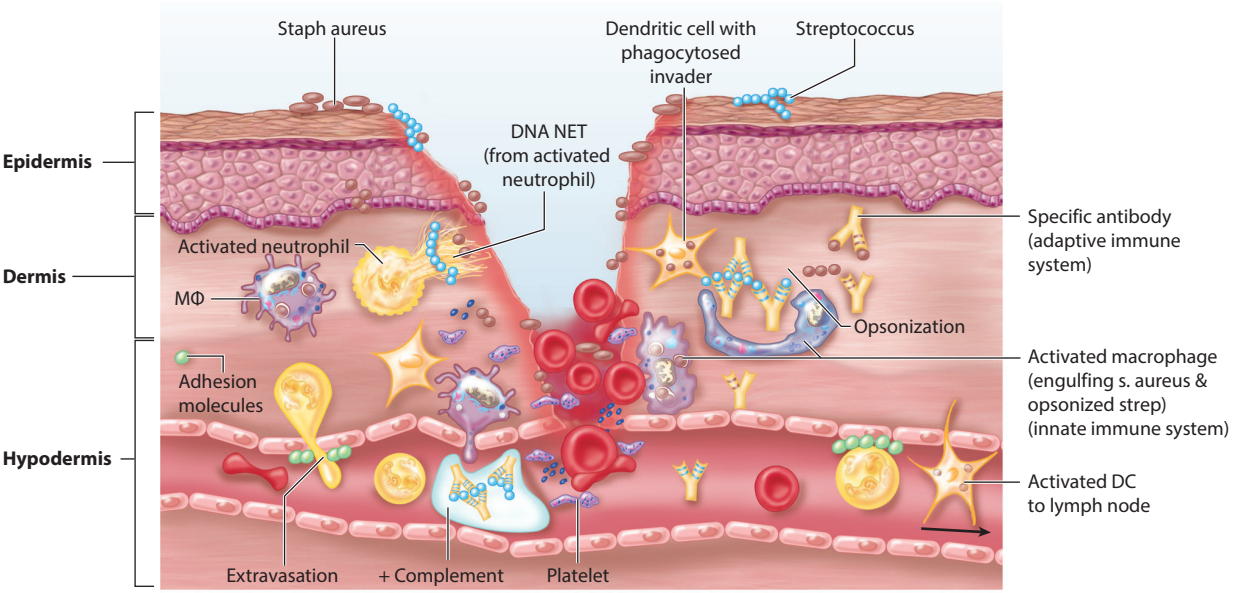
B. The fasciotomy wound has not yet achieved hemostasis, as evidenced by the bleeding occurring at the inferior undermining of the wound.

a combination of cellular activity, chemokines, cytokines, and proteases (**FIGURE 2-26**).

Mast cells are activated by antibodies and move rapidly from vascular circulation to the site of injury. Upon activation, mast cells become "sticky" and adhere to the endothelial surface (**FIGURE 2-27**). Mast cells can influence the local environment by degranulation or through the products that they synthesize. Histamine, a product of degranulation, increases the permeability of endothelial cells, thus facilitating extravasation of PMNs and macrophages. The three major product categories synthesized by mast cells are prostaglandins, thromboxanes, and leukotrienes.[3]

Neutrophils play an important role in the early phagocytosis of pathogens and in the recruitment of macrophages by programmed apoptosis. Neutrophils are also capable of expelling a neutrophil extracellular trap (NET) that is composed of DNA and loosely aggregated chromatin. The NET works somewhat like flypaper, trapping would-be invaders and allowing increased clearance by recruited macrophages or neighboring neutrophils.[45]

FIGURE 2-11A Inflammatory phase: contain and kill the invader



(1a) Clear invader (innate)
- Antimicrobial peptides & proteins
- Phagocytosis (macrophage / NK cells)
- Complement cascade
- Activation γ; α T-cells
- Neutrophil activator—DNA NET / Trap

(1b) Clear invader (adaptive)
- Dendritic cells
  - Pick up antigen (bacterium)
  - Traffic to lymph node to present Ag
  - Activate memory B cells—clonal expansion
- Antibodies released from B cells
  - (prior exposure to known invader)

(2a) Inflammation response iniated
- Platelets release aracadonic acid
- Clot formation fibroblast
- Wound debridement

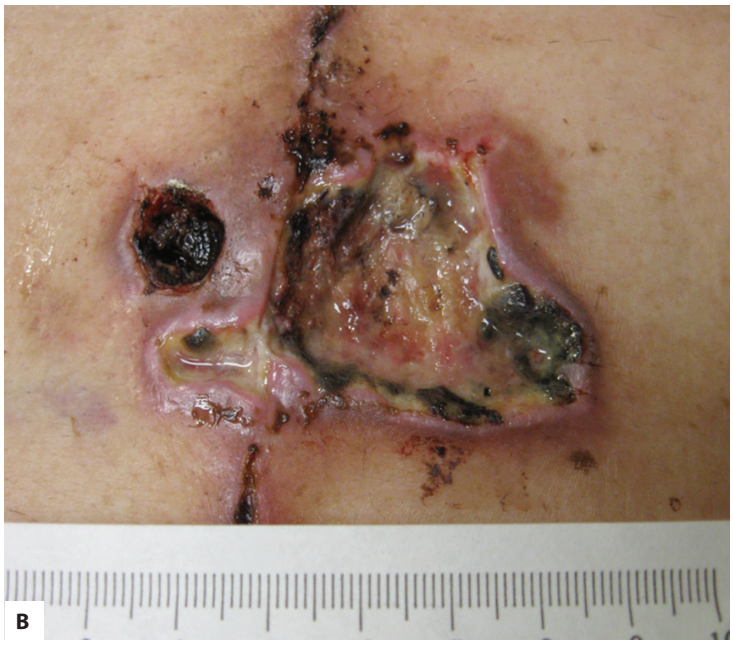
Key

Cell	Macrophage	NK	Platelets	RBC	PMN	Fibroblast
State	Upregulated M1	Upregulated	Baseline	Baseline	Upregulated	Upregulated

A

Inflammation is the second phase and has three sub-phases: killing and containing the invader, wound debridement, and neo-angiogenesis. During the process of killing and containing the invader both the innate and the adaptive immune systems are triggered and an inflammatory response initiated.

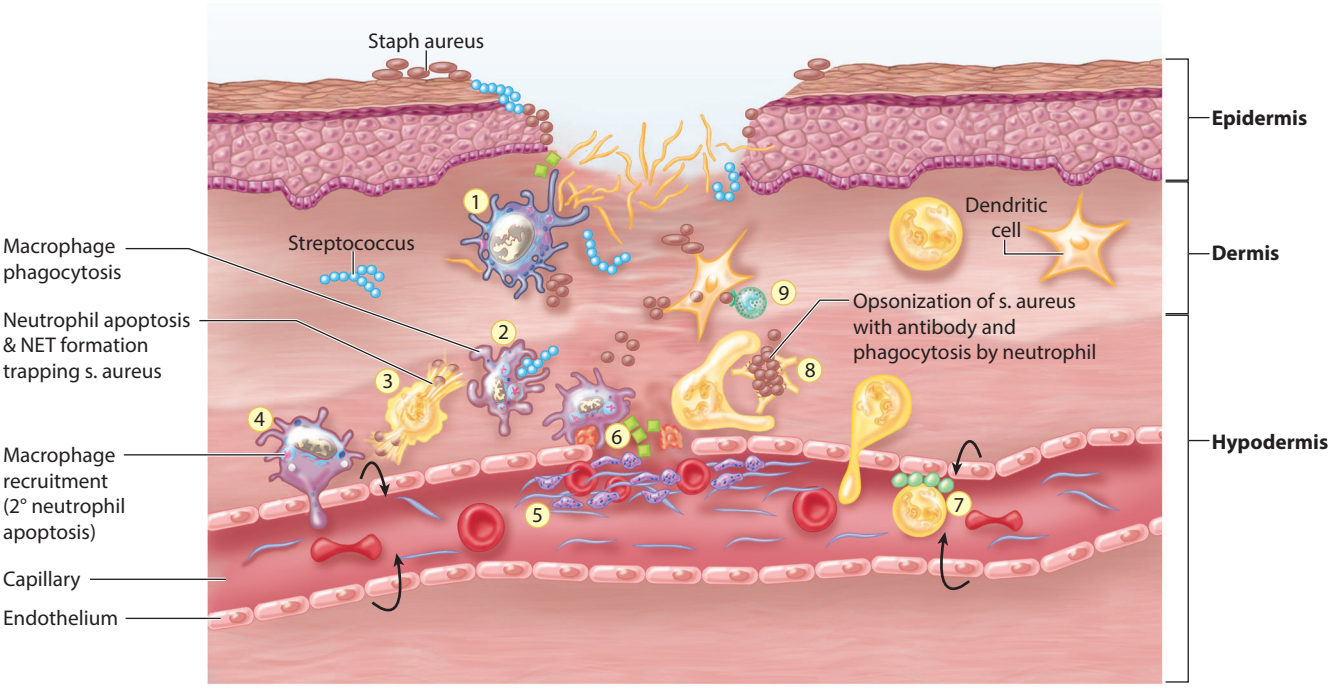
FIGURE 2-11B Inflammation: killing the pathogens



B

Wound in the inflammatory phase when high levels of phagocytic cells are present to break down the necrotic tissue and attack the pathogens. Slough, a by-product of the autolytic process, is visible at the edges and in the right side of the wound.

FIGURE 2-12 Inflammatory phase: wound debridement

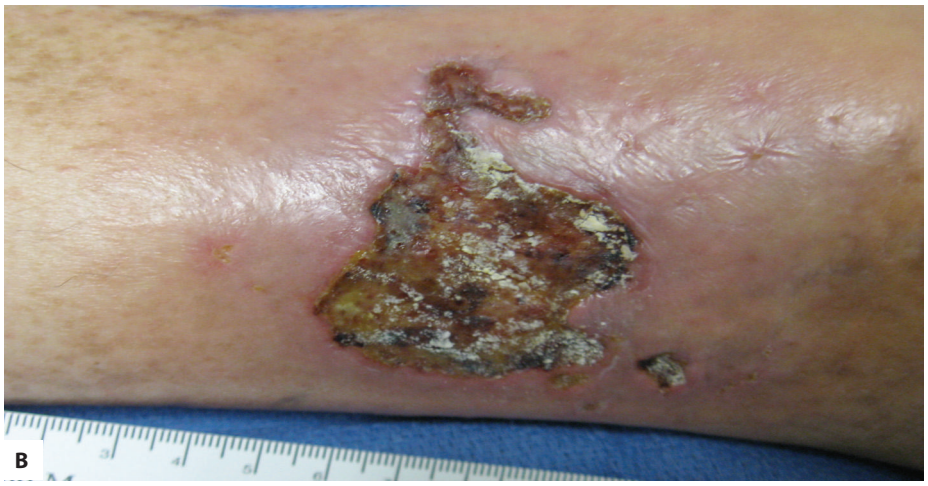


Key	Cell	Macrophage	NK	Platelets	RBC	PMN	Fibroblast	Endothelial
	State	Upregulated M1	Upregulated	Baseline	Baseline	Upregulated	Upregulated	Upregulate

A

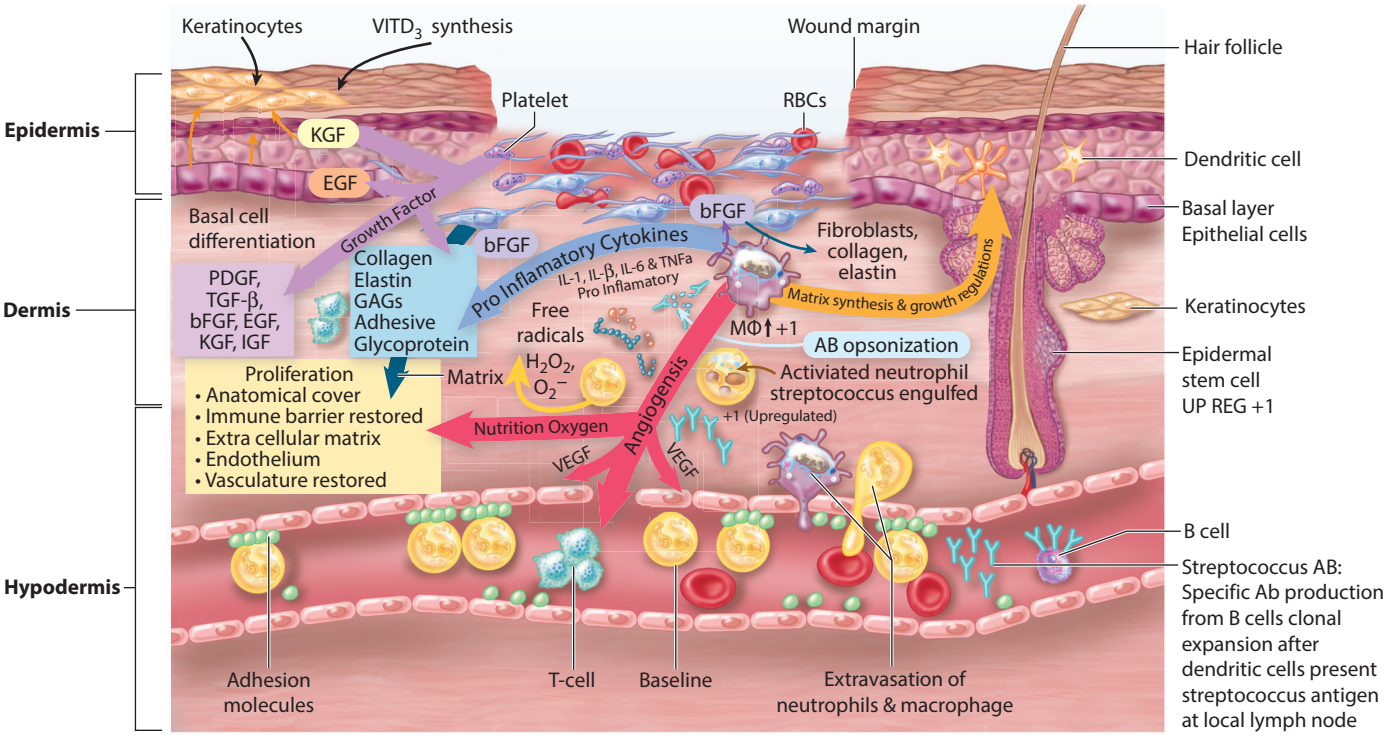
A. During the wound debridement phase of inflammation, macrophages are activated and begin phagocytosis of cellular debris while releasing enzymes to liquefy the ECM.

1. Activated macrophage phagocytosis of cellular debris and release of enzymes.
2. Macrophage phagocytosis of *Streptococcus*.
3. Apoptosis of neutrophils, formation of neutrophil NETs, trapping *Staphylococcus aureus* in preparation for phagocytosis by macrophages.
4. Macrophage recruitment, secondary to neutrophil apoptosis.
5. Platelet aggregation and entrapment in fibrin.
6. Macrophage phagocytosis of cellular debris and release of cytokines.
7. Adhesion of neutrophils to endothelium in preparation for exocytosis.
8. Opsonization of *Staphylococcus aureus* with antibody and phagocytosis by neutrophils.



B. The necrotic tissue, termed *eschar*, on the wound surface will be attacked from the lower side by the macrophages and other phagocytic cells.

FIGURE 2-13A **Inflammatory phase: neo-angiogenesis**



A

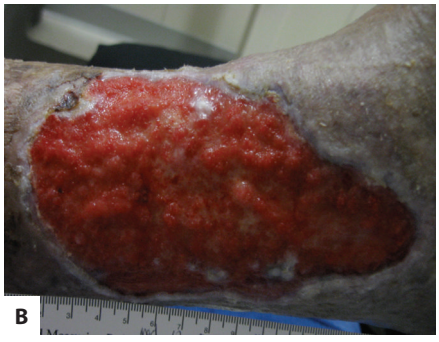
Key

Cell	Macrophage	NK	Platelets	RBC	PMN	Fibroblast	Endothelial	Epithelial
State	Upregulated M1	Upregulated	Baseline	Baseline	Baseline	Upregulated	Upregulated	Upregulated

In preparation for proliferation, angiogenesis begins to (1) support new tissue formation and (2) facilitate the removal of debris and waste products as a result of the destruction of cells and tissue.

1. Growth factors are released from the platelets, including PDGF, TGF-β, bFGF, EGF, KGF, and IGF.
2. ECM matrix formation begins accelerating with the formation and release of collagen, elastin, GAGs, and adhesive glycoproteins.
3. Pro-inflammatory cytokines from the macrophages and other sources (including IL-1, IL1β, IL-6 and TNF-α) are being down-regulated and have a diminishing effect.
4. Neutrophils release free radicals (including H_2O_2, O_2^-, and OH^-), which are destructive to bacteria in the wound milieu.
5. Clonal expansion of specific B cells begins after the dendritic cells present *Streptococcus* antigen in local lymph nodes.
6. Antibodies released by B cells opsonize specific target cells and provide a further signal for neutrophil engulfment.
7. Macrophages release VEGF to stimulate endothelium progenitor cell recruitment and differentiation as well as endothelial mitosis.
8. The foundation of proliferation is accomplished including the following actions: covering of the anatomical structures, restoration of immune barrier, construction of ECM, fabrication of endothelium, and restoration of circulation with vascular reconstruction.

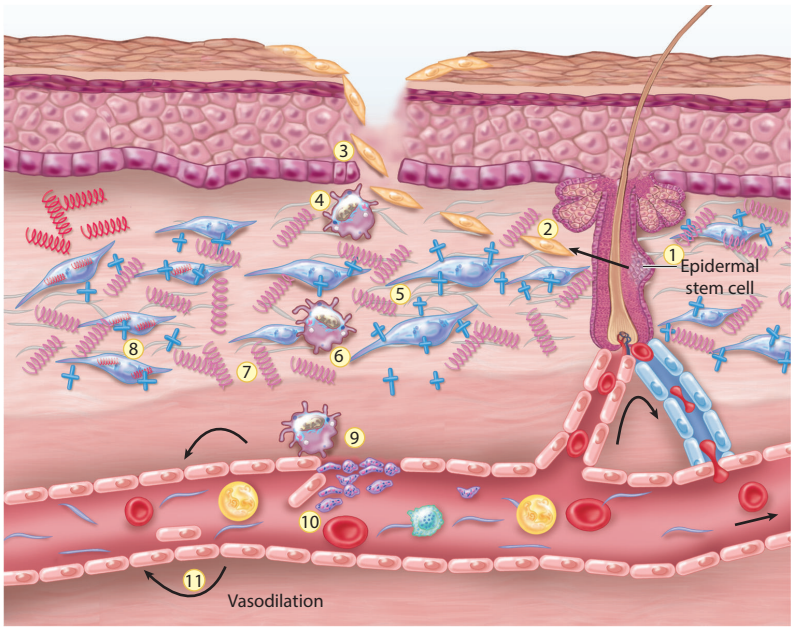
FIGURE 2-13B **Neo-genesis**



B

Healthy extracellular matrix supports the growth of new capillaries as seen in this lateral ankle wound. The capillaries give the surface of the wound a bumpy, granular appearance, thus the nomenclature "granulation."

FIGURE 2-14A **Proliferation**

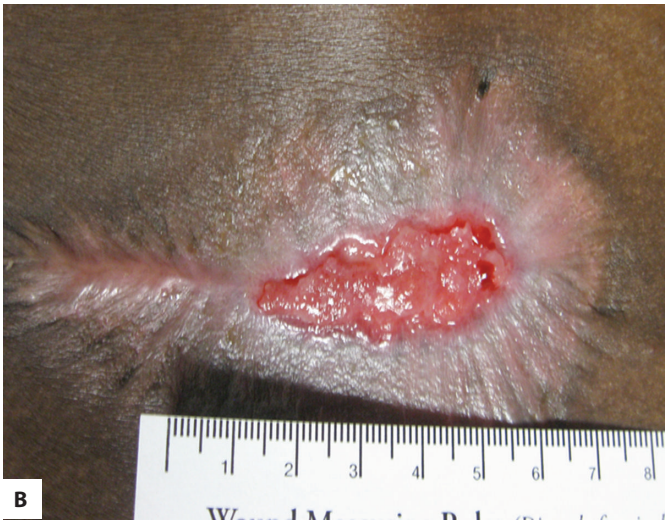


Key	Cell	Macrophage	NK	Platelets	RBC	PMN	Fibroblast	Endothelial	Epithelial
A	State	Upregulated M2 Anti-Inflammatory	Baseline	Upregulated	Baseline	Baseline	Upregulated-change to Myofibroblast	Upregulated	Upregulated

Proliferation is a complicated and intricate process composed of the following:

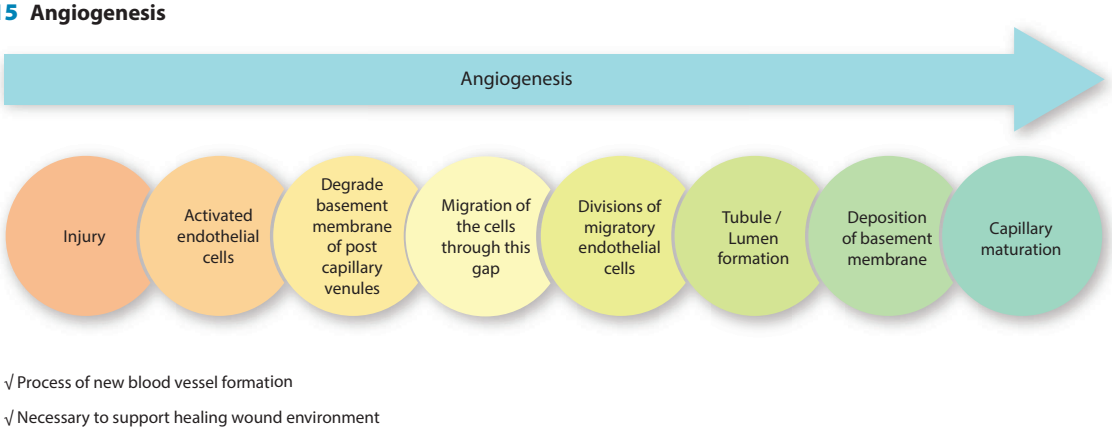
1. Epidermal stem cell activation.
2. Keratinocyte migration from epidermal stem cells.
3. Increased mitosis of basal epithelial cells.
4. Macrophage scavenging cellular debris, and collagen for remodeling while simultaneously releasing enzymes.
5. Laminin attaches to collagen and fibroblast cells via adhesins.
6. Macrophage continues to phagocytose fibrocytes and collagen to facilitate migration.
7. Elastin (pink) and collagen (silver) are important components in the restoration of function.
8. Fibroblast differentiates into myofibroblast evidenced by the presence of α contractile fibers.
9. Macrophage phagocytosis of the old clot (old fibrin, platelets and RBCs).
10. Endothelial progenitor cells are attracted by VEGF and other factors.
11. Vasodilation facilitates the process of tissue construction.

FIGURE 2-14B **Proliferation**



In addition to the visible bright-red granulation tissue in the wound, the results of myofibroblasts can be seen around the edges where the wound bed has contracted and re-epithelialized. The top edge is rolled with senescent cells at the edge of the wound bed, a condition termed *epibole*.

FIGURE 2-15 Angiogenesis



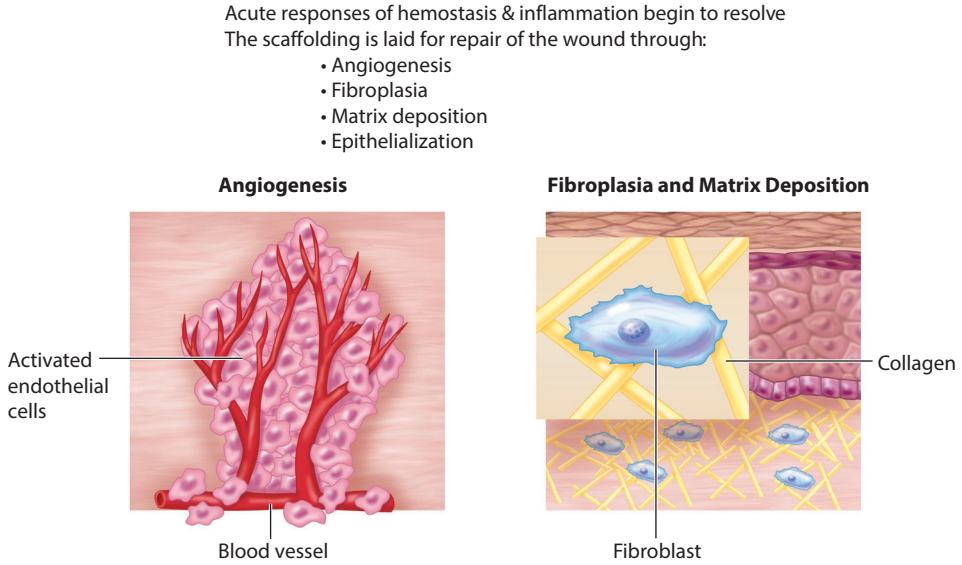
Angiogenesis is the process of new blood vessel formation. The flowchart demonstrates that angiogenesis begins immediately after injury and proceeds through a very orderly sequence, stimulating the extravasation and migration of cells through gaps in the endothelium and progressing through the formation of endothelial cells. The formation of tubules and lumen provides the basis for basement membrane deposition and capillary maturation. Angiogenesis ensures nutrition availability and removal of waste products throughout the healing process.

Wound Debridement Autolytic wound debridement begins immediately post-injury and includes the use of cells and enzymatic proteins to break down necrotic tissue. Cells typically involved in debridement include neutrophils, macrophages, and mast cells.[36,65] Enzymatically active proteins, including proteinases and collagenases, degrade the damaged tissue and ECM, thus providing a migratory path to the site of injury for the key cells needed to complete the healing process.[17,19]

PDGF, released by platelets, is chemotactic for monocytes, causing the monocytes to leave adjacent blood vessels and transform into wound-activated macrophages (WAMs) when they reach the interstitial tissue. WAMs continue the process of debris removal, and more importantly, begin the signaling that will orchestrate the remaining transitions and events central to healing (**FIGURE 2-28**).[27,61] Macrophage numbers remain high for approximately 3–4 days, releasing various tissue growth factors, cytokines, interleukin-1 (IL-1), tumor necrosis factor (TNF), and PDGF.[64] In addition to the phagocytic function, the macrophages provide a unifying script for the multiplication of endothelial cells and the sprouting of new blood vessels, both of paramount importance for continued cell migration and proliferation.[3,5,66–68]

The controlled liquefaction of the ECM continues during the debridement process until all of the damaged cells

FIGURE 2-16 Proliferation



Proliferation is composed of four broad processes: angiogenesis, fibroplasia, matrix deposition, and epithelialization.

are removed and cells needed for repair are in place. The rate of autolytic debridement will decline as debris is cleared, new cells proliferate, and healing ensues. The pro-inflammatory cytokines and their effects are presented in **FIGURES 2-2** and **2-3**.

Angiogenesis (Early) Just as the re-epithelialization process begins a few hours after wounding, so too does neo-angiogenesis.[69] Keratinocytes from the wound edges migrate beneath the fibrin clot and over the wound. Activated fibroblasts migrate to the site of injury in response to TGF-β1,[11,61,70] and, in combination

TABLE 2-3 Principal Sources and Primary Activities of Interleukins and Cytokines

Interleukins	Principal Source	Primary Activity	Comments
IL-1α and IL-1β	Epithelial cells, fibroblasts, platelets, macrophages, and other antigen presenting cells (APCs)	Costimulation of APCs and T cells, inflammation and host fever, hematopoiesis	Acute phase response
IL-2	Activated Th1 cells and NK cells	Proliferation of B cells and activated T cells, NK cell function Regulate WBCs	
IL-4	Th2 and mast cells, basophils	B-cell proliferation, eosinophil and mast cell growth and function, IgE and class II MHC expression on B cells, inhibition of monokine production Th0 differentiated to Th2 cells, Th2 cells produce ↑ IL-4 Promotes macrophage 0 to differentiate to M2 macrophage; M2 macrophages are considered repair macrophages and coupled with the secretion of IL-10, TGF-β resulting in decreased inflammation and diminution of pathological inflammation	
IL-6	Activated Th2 cells, APCs, adipocytes, macrophages, hepatocytes, PMNs, and fibroblasts	Acute phase response, B-cell proliferation, thrombopoiesis. IL-6 works synergistically with IL-1β and TNF on T cells ↑ Production of neutrophils in bone marrow	Both pro- and anti-inflammatory Also considered a myokine—produced in response to repetitive muscle contraction
IL-8	Macrophages, epithelial, endothelial, fibroblasts, and other somatic cells	Chemoattractant for neutrophils and T cells Induces phagocytosis. IL-8 can be secreted by any cell with toll-like receptors that are involved in the innate immune response. Usually, it is the macrophage that "see" the invader first Promotes angiogenesis	Capable of crossing blood–brain barrier
IL-10	Activated Th2 cells, CD8+, T and B cells, macrophages, monocytes, and mast cells	Inhibits cytokine production, promotes B-cell proliferation, survival and antibody production, suppresses cellular immunity and mast cell growth Down-regulation of MHC Class II receptor expression	Anti-inflammatory Also known as human cytokine inhibitory factor Inhibition of TNF-α, IL-1 and IL-6 production and inhibition of PMN activation
IL-12	B cells, T cells, macrophages, dendritic cells	Proliferation of NK cells ↑ Cytotoxic activity of NK cells Th0 to Th1 INF-γ production, promotes cell-mediated immune functions Antiangiogenesis via ↑ production of INF-γ	Two different protein chains, which form three distinct dimers: AA, AB, BB
IL-13	Th2 cells, B cells, macrophages	Stimulates growth and proliferation of B cells, inhibits production of macrophage inflammatory cytokines Induces MMPs Induces IgE secretion from activated B cells	
IL-18	Macrophages	Increases NK cell activity, induces production of INF-γ Induces cell-mediated immunity Stimulates NK cells and T cells to release INF-γ	Pro-inflammatory Also known as INF-γ-inducing factor

(*Continued*)

TABLE 2-3 Principal Sources and Primary Activities of Interleukins and Cytokines (*Continued*)

Interleukins	Principal Source	Primary Activity	Comments
INTERFERONS			
INF-α, INF-β, INF-γ	Macrophages, neutrophils	Antiviral effects, induction of class I MHC on all somatic cells, activation of NK cells, and macrophages	
INF-γ	Activated Th1 and NK cells, cytotoxic T cells	Induces expression of class I MHC on all somatic cells, induces class II MHC on APCs and somatic cells, activates macrophages, neutrophils, NK cells, promotes cell-mediated immunity, antiviral effects Activates inducible NO synthesis ↑ Production of IgG2g, IgG3 from activated plasma B cells ↑ MHC I and ↑ MHC II expression by APCs Promotes adhesion binding for leukocyte migration Retards collagen synthesis and cross-linking; stimulates collagenase activity	Also called macrophage activating factor Critical for both innate and adaptive immunity Antiviral INF-γ binds to glycosaminoglycan heparin sulfate at the cell surface, binding in general inhibits biological activity
ADIPOCYTOKINES			
C-reactive protein	Hepatocytes, adipocytes Synthesized by the liver in response to factors released by macrophage and adipocytes (eg, IL-6)	CRP is a ligand binding protein (calcium dependent), which facilitates the interaction between complement and both foreign and damaged host cells Enhances phagocytosis by macrophage Modulates endothelial cell functions by inducing the expression of adhesion/"sticky" molecules (ICAM-1, VCAM-1) Attenuates nitric oxide production by down-regulating NOS expression CRP's level of expression is regulated by IL-6	First pattern recognition receptor (PRR) to be identified. Acute phase protein. Physiological role is to bind phosphocholine expressed on the surface of dead or dying cells and some types of bacteria in order to activate the complement system via C1Q complex; therefore, phagocytosis is enhanced. Opsonic-mediated phagocytosis helps amplify the early innate immune response
PROSTAGLANDIIN	Leukocytes and macrophage	Either constriction or dilation of vascular smooth muscle Acts on platelets, endothelium, and mast cells Causes aggregation or disaggregation of platelets Regulates inflammatory mediation Controls cell growth Acts on thermoregulatory center of hypothalamus to produce fever Enzymatic pathway to convert the intermediate arachidonic acid to prostaglandin is found in active WBCs and macrophage	Prostaglandins are potent but have a short half-life before being activated or excreted. Therefore, send only autocrine (acting on the same cell from which it is synthesized) or paracrine (local adjacent cells)

with macrophages, form granulation tissue.[16,69] Both macrophages and fibroblasts produce vascular endothelial growth factor (VEGF), and fibroblasts produce connective tissue growth factor (CTGF),[71] which results in their proliferation via an autocrine loop.[12] The formation of new vasculature requires both extracellular matrix and basement membrane degradation followed by migration, mitosis, and maturation of endothelial cells. Both FGF and VEGF are thought to be central in modulating angiogenesis.[10,28,72–75] As a critical component of healing, new vessel formation will both (1) supply the oxygen and nutrients required and (2) remove the waste products of autolysis. **FIGURE 2-29** provides a broad overview of the key events involved in angiogenesis and **FIGURE 2-30** illustrates a well-vascularized granulating wound bed.

3. Proliferation

The proliferation phase of healing consists of four subphases: angiogenesis, fibroplasia, matrix deposition, and re-epithelialization.[60,69] **FIGURE 2-16** illustrates a broad overview of the events occurring in the proliferation phase, which is characterized by the formation of granulation tissue. This tissue consists of a rich capillary bed, fibroblasts, macrophages, and a loose arrangement of collagen, fibronectin, and hyaluronan.

Angiogenesis is mandatory to supply necessary nutrients to and remove waste products from the wounded tissue. Angiogenesis, discussed as part of both the inflammatory and proliferative phases, begins immediately after injury and is necessary to ensure endothelial cell migration that results in capillary sprouting. Injured endothelial cells, adhering red