Wound Healing

85 Impaired Wound Repair and Delayed Angiogenesis

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Introduction

Initially, skin was thought of as a simple barrier to protect the internal organs from the outside world, but now it is understood to be incredibly more complex than that. Skin serves multiple functions including the regulation of water loss, thermoregulation, protection from ultraviolet (UV) radiation and entry of microorganisms, and is an integral part of the immune system [1]. Skin ages via two processes: intrinsic aging and extrinsic aging. Intrinsic aging is seen in sun-protected areas of skin and is subject to the same generalized aging conditions as any other cell or organ system. Extrinsic aging occurs in sun-exposed areas and is the cumulative effect of intrinsic aging plus the environmental exposure to the aging process. The biggest extrinsic factor affecting skin aging is UV radiation encountered from sun exposure, which is also termed photoaging [2]. As skin ages, it becomes progressively atrophied, dry, and rough, with alterations in pigmentation, decreased turgor, and increased wrinkling. This leads to a progressive loss of function, leaving the aged skin with a decreased ability to regulate homeostasis and more vulnerable to the environment [3].

Traumatic injuries are the fifth leading cause of death for persons over the age of 65 in the USA, and it is estimated that there will be well over 50 million people over the age of 65 by the year 2030 [4, 5]. Understanding the impact of aging on skin wound healing will be vital in dealing with this growing population in the future. Wound healing is a complex process involving multiple concurrent stages, dozens of cell types, and hundreds of mediators. As research on wound repair progresses, there is an increased understanding of how each of these components changes over time within an individual wound, between different wound conditions, and from wounds of differently aged individuals. Many studies dating back almost a century show that wounds from older individuals do not heal as well as those from younger individuals [6]. More recent studies have provided information on how aging affects the individual components of wound healing including the inflammatory response, deposition of the wound matrix, and angiogenesis [7–9]. In short, the age of an individual has as profound an effect on the process of wound healing as nearly any other identifiable condition or disease may have.

Age-Related Changes in the Components of Skin

Before addressing the issue of how aging affects the wound-healing process, the changes in the milieu in which this wound-healing process occurs must be examined. Skin is a multilayered organ, with each layer's constituents optimized for its function. The outermost layer is the epidermis and is largely composed of squamous epithelial cells called keratinocytes and a smaller population of pigment-producing cells called melanocytes. The epidermis functions as a barrier against moisture loss and water entry. With age, the thickness of the epidermis does not change although the density of melanocytes decreases and the dermal–epidermal junction becomes flattened, giving the appearance of atrophy and cellular heterogeneity [10].

The dermis is composed of multiple cell types, structures, and fibers. Surrounding the hair follicles, sweat glands, and other intradermal glands are various fibers collectively referred to as the extracellular matrix (ECM). The ECM is composed of types I and III collagen, elastin, and glycosaminoglycans. The dermis is divided into two layers, the superficial papillary dermis and the deep reticular dermis. The papillary dermis maintains contact with the epidermis through the formation of papillary ridges. It is these ridges that become flattened with age, resulting in decreased surface contact and the previously mentioned appearance of atrophy, as well as decreased resistance to shear forces with lateral tension in the elderly skin [10, 11]. There is also a decrease in the cellular component of the dermis including fibroblasts, mast cells, macrophages, and other immunologically important cells including Langerhans' cells in the epidermis and dendritic cells in the dermis [9, 10].

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The primary structural proteins in the dermis are collagen, fibronectin, and elastic fibers. Interestingly, while there is a decrease in both the number and diameter of elastin fibers in the papillary dermis with age, in the reticular dermis the opposite is true with an *increase* in the number and diameter of the elastin fibers [10]. Fibronectin has several functions including regulation of inflammation, cell adhesion, and migration, and is closely associated with fibroblast production of collagen [12]. In isolated cell culture, some studies show an increase in fibronectin synthesis with age, but other in vivo studies show an age-related decrease in fibronectin expression with reduced levels of collagen [12, 13]. Collagen, the major protein found in the dermis, may be reduced in quantity with aging skin, but there still exists some controversy on this point, as some studies show a decrease with age, others describe an increase in collagen with age, and yet others show no change in collagen content with age [12, 14–16]. While there is controversy on the quantity, there is certainly a change in the quality of the collagen in aged skin. Collagen in young skin is typically described as rope-like bundles of dense collagen I fibers, arranged in a lattice or basket weave pattern. In aged skin, the collagen is coarser, with individual bundles being primarily straight, loosely woven fibers, and with an increase in density of the collagen network [10–12].

Dermal appendages including hair follicles and sweat glands are affected by the aging process as well. Hair follicle numbers are diminished with age, but their structure is largely unchanged save for a small decrease in the number of surrounding melanocytes [17]. Sebaceous glands, which produce a waxy substance that coats hair shafts and reduces water evaporation, also decline with age [18]. Sweat glands are also reduced in number and function with age [19].

The microvascular blood flow to skin is decreased with aging as well. There is as much as a 40% reduction in cutaneous blood flow at 70 years of age compared to the skin of a 20-year-old individual [20]. There is also thinning of blood vessel walls and basement membranes, with decreased numbers of perivascular cells, which may promote extravasation of plasma into the interstitial spaces [21]. Lymphatic drainage in the elderly is also reduced, leading to greater edema and increasing the likelihood for ulcers [22].

In short, aging skin has decreased potential for replication and migration at baseline. There is a decrease in the ECM components and its architecture, resulting in decreased tensile strength. There is a reduction in dermal skin appendages including sweat glands and hair follicles [17, 18]. There is also a reduction in the nutrient supply in the form of decreased microcirculation. Lastly, there is a greater tendency toward fluid accumulation due to increased permeability of the vasculature coupled with a decrease in lymphatic drainage. *Table 85.1* below lists the age-related changes in human skin.

Normal Wound Healing

Generally, wound healing is thought of as occurring in three or four overlapping phases (*Fig. 85.1*) [23]. The first phase, which is not always included, is hemostasis. After a wound has occurred, the body will attempt to stop bleeding by constricting vasculature, depositing platelets, and activating the clotting cascade. Endothelial cells normally line blood vessels, shielding platelets, and clotting factors from exposure to underlying collagen and basement membrane, and secrete inhibitors of platelet aggregation and clotting factors [24]. Once exposed to these normally hidden tissues, platelets are activated and aggregate via a combination of factors including ADP, von Willebrands factor (VWF), collagen, and thromboxane [25]. Following activation, they secrete cytosolic proteins and alpha-granules, which contain numerous mediators of clotting and inflammation including transforming growth factor (TGF)-β, TGF-α, platelet derived growth factor (PDGF), CD-40 ligand, and P-selectin [12, 25]. This leads to the release of fibrin, intracellular granules, and exposure of normally covered extracellular domains, all of which act as potent stimulators for inflammatory cells.

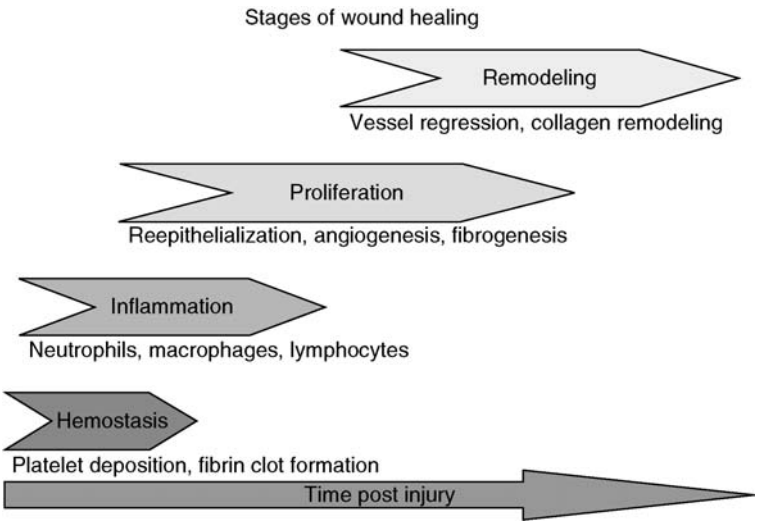
Table 85.1

Summary of the changes occurring in human skin with age

Clinical	Histological
Atrophy	Flattening of the dermal–epidermal junction
Drying	↑ Turnover time
Roughness	↓ Fibroblasts, mast cells, and macrophages
Alterations in pigmentation	↓ Collagen content
Sagging	Disorganized collagen and elastin
Wrinkling	↓ Microcirculation
Benign and malignant tumors	↓ Skin appendages
	↓ Lymphatic drainage

↑, increased; ↓, decreased

Figure 85.1
The four overlapping phases of wound healing



The second phase of wound healing, the inflammatory phase, occurs from the time of injury through 1 week. This phase is dominated by inflammatory cells beginning with neutrophils which fight invading pathogens and degrade damaged tissues. Macrophages follow, removing debris and apoptotic cells, and coordinating the interaction of other cell types. Many studies have examined the inflammatory phase of wound healing in great detail. Early studies characterizing the inflammatory cell invasion show the sequential infiltration of neutrophils, macrophages, and lymphocytes in the healing wound [26]. Subsequent studies reveal that although the first leukocyte to arrive in the wound is the neutrophil, it is not a necessary component for wound healing to occur [27]. Unlike neutrophils, the macrophage is a critical mediator of tissue repair [28]. Macrophages are recruited to wounds within a few days of injury by various chemoattractants, including chemokines [29, 30]. Macrophages perform dual functions in the wound. Not only do they engulf and phagocytose wound debris, providing a clean bed for migrating proliferative cells to lay down new matrix and blood vessels; they also produce some of the angiogenic and fibrogenic growth factors that recruit and promote the cells involved in the growth phase of repair [31–33]. Finally, as neutrophil and macrophage populations decline in the wound, T lymphocytes become the dominant leukocyte in the later stages of inflammation [34].

It should be noted that the overall role of inflammation in wound healing is not completely understood. Inflammation is not seen as necessary and in some cases may actually be detrimental in some forms of wound healing. Fetal wound healing, which is typically scarless through the first two trimesters, has a significant reduction in neutrophils and macrophages in both number and function [35]. Additionally, when inflammation is induced in fetal wounds the skin heals with scars [36]. Intestinal healing in the fetus also occurs in the absence of inflammation but still forms scars [37]. Inflammation has been shown to be entirely dispensable in some systems, such as the fetus, calling into question the true function of each cellular element.

The proliferative phase is dominated by the replacement of missing tissues and occurs from shortly after injury through 3 weeks. Keratinocytes, fibroblasts, and endothelial cells proliferate and migrate in the wound laying down new collagen, ECM, blood vessels, and epithelial covering. The final stage of wound healing, remodeling, begins at 2 weeks. During remodeling, the excessive blood vessels initially laid down to support inflammation now regress toward normal vascular density, immature collagen is resorbed and replaced with mature collagen, and tissue is continually modified to approximate normal structure.

Age-Related Alterations in Hemostasis

As stated previously, following injury, collagen is exposed and platelets adhere to the newly exposed collagen.

During aging, platelet adherence to collagen is enhanced which may be one mechanism that facilitates activation of platelets in the elderly [38]. There is also an increase in the platelet release of alpha-granules containing TGF-α, TGF-β, and PDGF with age [39]. Not only are platelets affected by aging, but the clotting cascade is also altered by the aging process. Some of the changes in coagulation that are documented include elevated serum concentrations of activated clotting factors, fibrin breakdown products including d-dimers, and inhibitors of clot destroying enzymes like plasminogen activator inhibitor-1. There are also complimentary decreases in the activity of coagulation inhibitors such as antithrombin III and activated protein C [13, 40–42]. With this tendency toward increased coagulation comes an increase in some of the inflammatory mediators, but also a decrease in the some of the stimulants for chemotaxis [13, 43, 44]. This presents a mixed composition for inflammation, with some aspects increased and others decreased. These changes are summarized in *Table 85.2* below.

Age-Related Alterations in the Inflammatory Phase

There are many conflicting studies regarding changes seen during the inflammatory phase of wound healing in the aged skin. There are reports of acute inflammatory reactions being slower and less intense in aging skin, equivalent in young and old skin, and faster and more intense in older skin [45–47]. Complicating matters, some aspects of wound healing appear to be enhanced while others are markedly decreased.

There is plenty of evidence to suggest that neutrophil functions are impaired by aging, but their role in wound healing is controversial [48]. Neutrophils isolated from elderly humans have a decreased respiratory burst, diminished capability to phagocytose, and diminished chemotactic ability [49–51]. However, studies demonstrate no difference in wound debridement, cellularity, or connective tissue formation in the wounds of control and neutropenic animals [27]. Although neutrophils may play a role as a first line of defense against bacterial invasion, they do not appear to play a role in the proliferative phase of repair. More recent studies confirm that the role of neutrophils in uncontaminated wounds is probably minimal or even perhaps detrimental [52, 53]. The macrophage however, seems to be required for wound healing to occur normally and age-related changes in this cell type are likely to be important to healing outcomes [28].

In one series of experiments, the role of macrophages in young, middle aged, and elderly mice was deciphered. Middle aged and elderly mice showed a delay in wound closure compared to young mice. When young mice received intraperitoneal (ip) injections of rabbit antimacrophage serum, their healing was delayed, similar to that of untreated aged mice. Wound repair was accelerated in aged mice that received ip injections of macrophages harvested from young mice but not from old mice [54, 55]. There are many possible explanations for these effects including decreased numbers, diminished chemotaxis, or impaired cytokine production in the elderly macrophages. Whether macrophage populations are diminished with age is uncertain. Some studies suggest that hematopoetic stem cells do have a limited life span; there is a marked hypocellularity in the bone marrow of elderly humans; and CD68 positive cells (markers of macrophage populations) are decreased with age, but others demonstrate the opposite with increased macrophage population in the bone marrow and similar numbers and composition of macrophages in young and old mice [56–60]. Macrophage invasion into wounds is decreased in middle aged and elderly skin in some studies but not in others [8, 9].

The ability of macrophages to phagocytose is diminished with age. In one study, macrophages obtained from elderly mice ingested fewer particles than macrophages obtained from young mice [9]. Many other studies corroborate this general understanding that macrophage phagocytosis is impaired in aging skin [9, 61, 62]. The mechanisms to explain this decrease in macrophage function are not clear however. Glucose utilization in macrophages is decreased with age; several cell surface receptors required for macrophage activation and recognition are decreased including MHC class II. However, the Fcγ receptor, which is critical for macrophage phagocytosis, does not show a decrease in number or function [9, 12, 63]. Several of the signal transduction pathways in macrophages obtained from aged individuals including

Table 85.2

Summary of age-related changes in hemostasis

↑ Platelet adherence to collagen	↓ Inhibitors of coagulation
↑ Platelet aggregation	↑ Inhibitors of clot lysis inhibitors
↑ Release of alpha-granules	↑ Concentrations of active clotting factors
↑ Concentrations of active clotting factors	

↑, increased; ↓, decreased

the MAP kinases ERK, p38, and JNK are deleteriously affected and may partially explain the discrepancy [64].

The ability of the macrophage to coordinate the arrival and interaction of other cells participating in the wound repair process is also diminished with age. The production of interleukin(IL)-1 and IL-6 is decreased in the aging macrophage, along with the production of VEGF, and TNF-α [12, 15, 64, 65]. As is the case with much of the above information, conflicting reports showing increases in each of these cytokines are reported in the literature [12, 58, 64]. Despite the conflicting data, the following can be said of the aging macrophage with a fair degree of validity: phagocytic activity, cytokine (including TNF-α, VEGF, and FGF-2) and chemokine (including MIP-1α and CCL5) production, infiltration, and antigen presentation are all decreased [15, 58, 62, 63, 66]. As these are all vital components of the wound healing process, the age-related functional deficits displayed by macrophages probably contribute significantly to the healing impairment seen in aging skin. Many of these deficits involve cytokines that have influence beyond the inflammatory stage of wound healing and extend into the proliferative phase as well. These deficits during the proliferative phase lead to identifiable changes in wound healing and are summarized in *Table 85.3*.

Age-Related Changes During the Proliferative Phase

Many age-related alterations in wound healing are described. Clinical and laboratory studies describe delays in re-epithelialization, decreases in collagen synthesis, and organization in wounds of aged humans and rats [67–70]. Additional studies establish decreases in wound-breaking strength and increases in wound disruption [71, 72]. Results from excisional wound studies on mice continue to confirm prior reports describing age-related delays in re-epithelialization [15, 69]. In one study (*Fig. 85.2*), aged mice show a significant delay in terms of time to complete closure, as well as the portion of the wound bed re-epithelialized [15]. This study correlates with other studies showing delayed epithelialization in aged humans and mice [12, 69, 73].

Many studies show that the proliferative capacity of keratinocytes decreases with age [74, 75]. Others show that there is a decline in the rate of normal keratinocyte turnover in aged skin [76]. Additional studies describe a decrease in keratinocyte migration in aged skin as well. Hypoxia is a potent stimulus for keratinocytes from young persons to migrate, but the opposite effect is seen in keratinocytes from aged individuals [77]. This decrease in hypoxia associated keratinocyte migration in aged skin is partially related to a decrease in MMP production. MMP-1 and MMP-9, which are both associated with keratinocyte migration in wounds, are upregulated in young keratinocytes but downregulated in aged keratinocytes [77, 78]. Combining these results with prior studies, there is an implication that there is a baseline decrease in the proliferation and migration of keratinocytes in aged skin. Once an injury occurs, the normal keratinocyte response to proliferate and to migrate across the wound is impaired. This impairment results in a delay of wound closure, increasing the chance for infection or chronic wound development. The mechanism behind this impairment is still not fully understood, but may be partially related to decreases in keratinocyte proliferation capacity,

Table 85.3

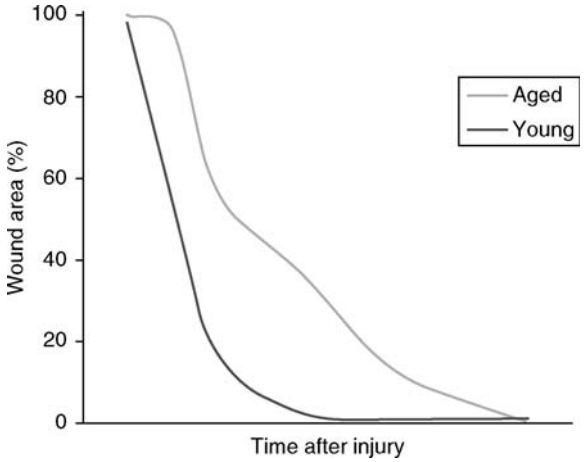
Summary of age-related changes during the inflammatory phase of healing

↓ Macrophage function	↓ Neutrophil function
↑ Secretion of inflammatory mediators	↓ Vascular permeability
↓ Secretion of growth factors	↓ Infiltration of macrophages and lymphocytes

↑, increased; ↓, decreased

Figure 85.2

Representation of the time course of excisional wound re-epithelialization in young and aged mice. Young mice have smaller wound areas across all healing times and reach complete closure before aged mice (Swift et al. [15])



and migration capacity, and may be mediated through age-related decreases in specific cytokine production.

Not only are keratinocytes impaired by aging, but the major proliferative cell in the dermis, the fibroblast, displays significant age-related impairments as well. It is generally accepted that there is a decrease in number, size, and proliferation of fibroblasts in skin with age [12, 61, 79]. Some studies show a decrease in the in vitro life span of fibroblasts from aged human donors [80]. Others document a decrease in migration and proliferation of explanted rat fibroblasts with age [81]. Many studies evaluating the migratory deficiencies of aged fibroblasts demonstrate a decline in motility for fibroblasts independent of chemotactic stimulus and a decline in fibronectin associated migration [12, 82, 83]. These functional declines are due to multiple factors. Injection of senescent fibroblast mRNA into young fibroblasts impairs their ability to synthesize DNA [84]. Synthetic machinery is also decreased, with aged fibroblasts tending to have poorly developed endoplasmic reticulum [85]. Additionally, a great number of studies examine the decreased responsiveness of fibroblasts to a wide variety of cell signaling molecules and cytokines.

Human fibroblasts have an age-associated decrease in mitogenic response to epidermal growth factor (EGF), insulin, dexamethsone, and transferrin [86]. They also show decreased responsiveness to FGF-7, also known as keratinocyte growth factor (KGF) [87]. Fibroblasts also require higher concentrations of PDGF to stimulate proliferation in aged individuals [12]. TGF-β1 responses are also found to be diminished in fibroblasts derived from aged individuals [88]. These studies suggest that fibroblasts may become desensitized to these stimuli with age. For some of these decreased responses, possible mechanisms have been elucidated. For instance, one study shows striking differences in EGF receptor (EGFR) number, affinity, and rate of EGF/EGFR internalization in early-passage dermal fibroblasts derived from newborn versus young adult versus old adult donors, and another shows a decline in insulin receptor numbers in aged fibroblasts [89]. TGF-β receptor types I and II are decreased in hypoxic, but not normoxic conditions in aged fibroblasts from human donors, and downstream phosphorylation is also decreased [88]. Some of these receptors are not only important for proliferation and migration of fibroblasts, but are also involved in the production of fibroblast derived cytokines and fibrogenesis in fibroblasts.

Fibroblast growth factor receptor (FGFR) expression is downregulated with age and the production of many FGFs are also found to be decreased. Some of these decreases include FGF-2, FGF-7, VEGF, and TGF-β1 [15, 90]. This reduction in synthetic ability of fibroblasts is not limited to cytokines alone, as declines in fibronectin and collagen production are also described. As stated previously, conflicting studies exist regarding whether there is a change in collagen production with aging [12, 14–16]. Several studies show no age-related changes in collagen production [12, 15, 69, 91]. Studies that show an age associated increase in collagen production include increased production in cultures of fibroblasts from rats and pigs serially cultured to mimic aging and decreased type I collagen mRNA production from TGF-β stimulated human fibroblasts. Despite this conflicting information, many studies demonstrate a clear decrease in collagen production with age. Some of the studies showing this decrease in production include an increase in the ratio of immature type III collagen (with a decrease in the proportion of mature type I collagen) with age; reduced collagen production after age 30 in humans; delayed collagen content (but ultimately similar final levels) in aged mice; and reduced mRNA production of type I collagen with age [8, 15, 92, 93]. *Tables 85.4* and *85.5* describe the age-related changes seen during the proliferative and remodeling phases of wound healing.

Age-Related Changes During Remodeling

During the remodeling phase of healing, both collagen degradation and synthesis occur along with the

Table 85.4

Summary of age-related changes seen during the proliferative phase of healing

↓ Collagen deposition	↓ Proliferation of keratinocytes, fibroblasts
↓ Migration of keratinocytes, fibroblasts	→ Re-epithelialization
↓ Receptor numbers and response	

↑, increased; ↓, decreased; →, delayed

Table 85.5

Summary of age-related changes seen during wound remodeling

→ Wound strength	↑ Collagen degradation
↓ TIMP	↓ Wound strength
↓ Lysyl oxidase (LOX) crosslinking	

↑, increased; ↓, decreased; →, delayed

maturation of collagen structure, and the dermal architecture moves closer to the original normal structure. In aging, levels of collagen degradation in wounds appear to increase. The enzymes that are most active in collagen degradation are the matrix metalloproteinases (MMPs), a family of proteases that have various functions including, acting as intermediary signaling molecules, but are primarily thought to be active as proteolytic degradation enzymes. The MMPs are secreted by various cells including keratinocytes and fibroblasts, and collectively are known to degrade collagen, gelatin, and other ECM components [78]. Tissue inhibitors of metalloproteinases (TIMPs) are naturally occurring inhibitors of MMPs and their presence helps balance the degree of collagen synthesis and breakdown in wound healing. It should come as no surprise that debate exists on whether MMPs and TIMPs are increased or decreased with age and evidence exists in favor of each [12, 44, 61, 70, 77, 78, 94, 95]. However, the preponderance of evidence currently points toward increased MMP levels and decreased TIMP levels in aging skin. As new research continues to examine this process, this notion will likely evolve. Additionally, alterations in protease balance are likely not to be uniform and depending on the cell types, substrates, and wound conditions being examined, it is quite possible that divergent effects will be seen. It is possible that collagen degradation by MMPs may be enhanced while receptor mediated interactions with MMPs may be reduced. Divergent effects may also occur over early wound time points versus later wound time points as wound healing is incredibly dynamic requiring appropriate increases and decreases in each element at appropriate times.

The end result of the remodeling phase is a durable dermis, one measure of which is the strength of the wound. Studies demonstrate a decrease in tensile strength in older individuals in a variety of settings including intestinal anastomoses, cutaneous wounds, and abdominal incisions [71, 72]. Tensile strength of wounds is not solely dependant on the amount of collagen present, but also relies upon the degree of crosslinking of the collagen fibers and the overall architecture. Collagen can be crosslinked by two mechanisms. In enzymatic crosslinking, collagen is crosslinked by a posttranslational modification via the enzymatic activity of lysyl oxidase (LOX). This enzyme crosslinks collagen in a specific pattern and maintains an association with the collagen fibrils preventing nonspecific crosslinking [96]. Enzymatic crosslinking occurs in normal and wounded skin and contributes to improved collagen architecture and tissue strength. The second method of collagen crosslinking is via a nonspecific chemical modification by crosslink oxidation or nonenzymatic glycosylation [97, 98].

Both enzymatic and nonspecific crosslinking are described to be altered with aging. In regard to enzymatic crosslinking, alterations in LOX activity are described with aging. An age associated decrease in LOX activity in skin from elderly monkeys has been described [99]. LOX mRNA levels were also found to be decreased in skin from aged rats in several studies [100, 101]. Despite this described decrease in LOX activity, increases in collagen insolubility were reported with higher intra and intermolecular crosslinking in older subjects [96, 101, 102]. This apparent disparity between decreased LOX activity and increased collagen crosslinking may actually go hand in hand. As previously stated, while LOX does crosslink collagen, it also protects it from nonspecific crosslinking. Some studies show that the specific LOX derived crosslinks decrease with age and the nonspecific chemical and glycosylation crosslinks increase [96]. This increase in nonspecific collagen crosslinks may explain some of the physical changes described in elderly skin including coarse collagen structure, stiffer ECM, and decreased chemotaxis of inflammatory and proliferative cells such as endothelial cells.

Age-Related Changes in Angiogenesis

While some controversy exists as to whether wound angiogenesis is increased or decreased with age, the preponderance of evidence points toward an overall decrease in angiogenesis in aging skin [8, 13, 103, 104]. Many of the previously described changes in cytokines, proliferation, and structural proteins also affect angiogenesis. Studies on both excisional wounds and subcutaneous implant models in aged animals show a delay in wound capillary ingrowth [105, 106]. This delay may be a function of impaired migration because of the altered collagen crosslinking mentioned above, cellular senescence of endothelial cells, or may be due to decreases in growth factor expression.

Many studies have delineated the perturbations caused by altered levels of growth factors. VEGF, PDGF, TGF-β1, and FGF have all been found to impact angiogenesis through their influence on endothelial cell functions and age associated impairments of these growth factors can have profound effects on wound angiogenesis [15, 107–109]. Senescent HUVEC cells fail to migrate in response to FGF and the phosphorylation of FGF receptor-1 substrates is impaired [107]. A decrease in capillary density, along with a decrease in the production of the pro-angiogenic stimuli FGF-2 and VEGF has been demonstrated in excisional wounds from aged mice. In addition, the in vivo response to a defined level of pro-angiogenic stimulus, implanted subcutaneously, decreases in aged animals [15]. Other studies demonstrate similar

impairments including decreased perfusion and capillary density with decreased VEGF levels in aged animals and a concomitant increase in subsequent vascular density with recombinant VEGF supplementation [110]. Other studies show a reduction in sprouting of vessels in aged mice with a rise to levels approaching that of young mice when supplemented with IGF-1, VEGF, TGF-β1, or bFGF [111].

An increase in anti-angiogenic factors, such as members of the thrombospondin (TSP) family, is found in wounds of aged animals, and may provide a second layer of inhibition on endothelial functions beyond the simple deficiency of stimulatory growth factors. TSP is known to inhibit neovascularization through a variety of mechanisms including limiting vessel density, inducing apoptosis in endothelial cells, and decreasing the response of endothelial cells to various stimulatory signals including VEGF [112]. In one study, TSP-2 expression was increased in fibroblasts from the wounds of aged mice; TSP knockout mice have increased angiogenesis compared to wild-type counterparts [7]. Several studies describe an increase in TSP levels with age [7, 13, 113].

SPARC (also termed osteonectin), is a multifunctional glycoprotein that modulates cellular-ECM interaction, inhibits cellular proliferation, and regulates the activity of growth factors. SPARC can bind directly to VEGF, inhibit VEGF-receptor interaction, and prevent VEGF-induced phosphorylation of VEGF receptor-1 [114]. SPARC has been shown to inhibit the proliferative and migratory effects of FGF and VEGF on endothelial cells [115, 116]. SPARC expression increases with age in several models both in vivo and in vitro [116, 117]. Specifically, SPARC increases with age in human periodontal ligament cells' in murine wounds using a sponge implant model, and in fibroblasts and endothelial cells obtained from the skin of young and old human donors. This age-related increase in SPARC could contribute to the decrease in VEGF and angiogenesis that is observed in aging. The regulation of SPARC itself is not completely understood, although some studies show that TGF-β1 and PDGF increase the expression of SPARC, while bFGF decreases the expression of SPARC [118, 119]. While there are probably many reasons for the decrease in VEGF expression and endothelial cell migration in aging skin and wounds, the increase in inhibitors of angiogenesis like TSP-1 and SPARC provide additional means of inhibition, beyond mere cellular senescence in wounds from aged individuals.

Regardless of the underlying mechanism, endothelial function and angiogenesis is impaired in aging skin (*Table 85.6*). Whether due to an age-related increase in angiogenic inhibitors; a decrease in growth factors like VEGF, EGF, or FGF, a decrease in endothelial migration and proliferation, or an impairment of downstream receptor signaling, the end result is that angiogenesis in wounds from aged individuals is impaired. A significant decrease in angiogenesis can often negatively impact healing. However, the influence of the age-related angiogenic impairment on the healing capacity of any particular wound probably varies due to individual wound conditions.

Table 85.6

Summary of age-related changes seen in angiogenesis

→ Capillary ingrowth	↓ Migration and proliferation of endothelial cells
↑ Inhibitors of angiogenesis	↓ Angiogenic cytokines
→ and ↓ Vascular density	

↑, increased; ↓, decreased; →, delayed

Conclusion

Even though angiogenesis and wound healing may be delayed in an aged individual, if the person is otherwise healthy, and the wound is in optimal condition, no functional detriment might be observed. The wound will close, no infection will set in, and the individual will be none the wiser that it took a few extra hours to close, or that there were fewer blood vessels, or that the strength of the skin across the wound will take an extra few weeks to achieve maximal strength. The real impact of the impairment in wound healing and angiogenesis is not on the healthy aged individual, but on the aged individual with baseline impairments: a lower extremity laceration on an elderly woman with peripheral vascular disease, or a man with diabetes, for example. For these individuals who already have a baseline deficiency in the maximal potential for wound healing, a slight reduction in angiogenesis or a slight delay in wound closure, cytokines, or growth factor activity could mean the difference between an infection and a clean wound, a chronic ulcer or a healthy scar.

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