Age-Related Aspects of Cutaneous Wound Healing: A Mini-Review

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Key Words

Aging · Cutaneous wound healing · Repair process · Collagen synthesis

Abstract

As the aging population in developed countries is growing in both numbers and percentage, the medical, social, and economic burdens posed by nonhealing wounds are increasing. Hence, it is all the more important to understand the mechanisms underlying age-related impairments in wound healing. The purpose of this article is to give a concise overview of (1) normal wound healing, (2) alterations in aging skin that have an impact on wound repair, (3) alterations in the repair process of aged skin, and (4) general factors associated with old age that might impair wound healing, with a focus on the literature of the last 10 years.

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Introduction

With the dramatic increase in the size of the elderly population over 65 years of age, the increased risk of age-related pathologies, including chronic wounds, and the associated clinical and socioeconomic burdens, the investigation of aging effects on wound healing becomes a priority topic.

Cutaneous wound healing occurs via a complex and dynamic series of overlapping phases involving numerous cell types, tissues, cytokines, chemokines, growth factors and proteolytic enzymes. The cellular activities, including migration, proliferation, phagocytosis, and synthesis of extracellular matrix (ECM) proteins are tightly regulated, and the precise chronology of the wound-healing phases encompassing hemostasis, inflammation, proliferation, and tissue remodeling, is crucial for optimal repair [1]. Aberrancies, prolongations or interruptions in these processes can delay wound healing or even cause nonhealing chronic wounds. Since the first report on the relation between age and wound repair in 1916 [2], age-related aspects of wound healing have been studied on clinical, cellular, and molecular level, in vitro and in vivo, in humans and in animal models. But many of these studies are difficult to interpret for several reasons, e.g. a loose definition of aging in many animal models and for cellular aging, a lack of control for comorbidities, and last but not least, failure to define the health status using the SENIEUR protocol. However, it seems that wound healing in healthy old people is delayed, not defective, and that age-related alterations might predispose the elderly to effects of various factors that impair wound healing, and underlie chronic wounds. As the knowledge of the molecular mechanisms involved in normal wound healing is fundamental to the understanding of age-related factors that alter events of wound repair,

Fig. 1. Phases of normal cutaneous wound healing. Wound healing starts within seconds after injury with hemostasis, followed by the distinct, but overlapping, phases of inflammation, proliferation and tissue remodeling. The final phase can last for more than 1 year.

and might cause pathologic conditions, this review will give a short overview of normal wound healing, and highlight alterations in the repair processes that are caused by normal aging as well as some factors associated with old age that adversely affect wound healing.

Normal Wound Healing

Normal wound healing is an innate immune response to tissue injury with the aim to restore tissue integrity and the barrier function of the skin [recently reviewed in ref. 1]. It starts within seconds after the injury with hemostasis, followed by three phases, i.e. inflammation, proliferation and tissue remodeling (fig. 1). To stop hemorrhage, the injured microvessels constrict, the coagulation cascade becomes activated, platelets aggregate, and a fibrin clot, which plugs the wound and serves as a provisional matrix, is formed. The release of numerous cytokines, chemokines, and growth factors, including platelet-derived growth factor (PDGF), epidermal growth factor, vascular endothelial cell growth factor (VEGF), and transforming growth factor- β (TGF- β), from the clot and the surrounding tissue promotes the migration of inflammatory cells to the site of injury [1].

The inflammatory phase is characterized by the sequential infiltration of neutrophils, monocytes and lymphocytes. The major function of neutrophils, whose

number peaks at 24 h, is to cleanse the wound by phagocytosing infectious invaders, and devitalized tissue debris. Within 24–48 h, monocytes migrate into the wound, and differentiate into mature macrophages. Macrophages seem to be key regulators in the wound-healing response and exert various important functions: they remove debris and apoptotic cells, including spent neutrophils, help to fight infections, promote and conclude inflammation, and secrete cytokines and growth factors that recruit and activate other cells involved in the repair process. Classically activated pro-inflammatory M_1 macrophages are present during the early inflammatory phase while alternatively activated anti-inflammatory M_2 macrophages, which promote tissue regeneration by stimulating keratinocytes, angiogenesis and ECM synthesis, prevail at later stages of the repair process. The role of lymphocytes, which are the last inflammatory cells to enter the wound (>3 days after injury), has not been clearly defined. However, T cells might influence the wound-healing process by direct cell-cell interactions with macrophages, platelets, keratinocytes, and fibroblasts, and/or via the release of cytokines known to play a role in tissue remodeling [3].

Soon after injury, almost in parallel with inflammation, re-epithelialization by proliferation and migration of keratinocytes is initiated. Epidermal stem cells, which reside in the hair follicle bulge and basal layer of the epidermis, also contribute to re-epithelialization of the wound [1]. The proliferative phase, which is characterized by the replacement of the provisional fibrin matrix with granulation tissue, begins within 72 h of injury and lasts for about 14 days. Fibroblasts proliferate in response to growth factors, such as TGF- β 1, PDGF, and fibroblast growth factor, and synthesize ECM components, e.g. type III and type I collagen, glycosaminoglycans and proteoglycans. Concurrently, angiogenesis is induced by hypoxia. The newly formed blood vessels invade the neomatrix, and build a dense network of capillaries to supply the cells in the wound bed with oxygen and nutrients. Once the granulation tissue is formed, some fibroblasts differentiate into myofibroblasts. Myofibroblasts express α -smooth muscle actin, and are key players in wound contraction and ECM remodeling.

At around day 8, the wound-healing process enters the final remodeling phase, which can persist for 1 year or even longer, and is essential for the restoration of full tissue functionality. During remodeling, ECM components are constantly degraded and newly synthesized in order to approximate normal tissue architecture. The fraction of collagen type III decreases over time, and is replaced by the stronger collagen type I. With the formation of

Table 1. Age-related changes in skin and their potential influence on wounds

Morphological alterations	Functional impairment	Potential effect on wounds
Decreased proliferation of keratinocytes	Decreased desquamation	Delayed re-epithelialization
Increased keratinocyte migration time	Decreased desquamation	Delayed re-epithelialization
Decrease in macrophages	Reduced phagocytic activity	Delayed wound healing
Decrease in fibroblasts	Reduced collagen production	Delayed collagen deposition and remodeling
Atrophy of the epidermis	Increased fragility	Increased susceptibility to mechanical injury
Reduction in nerve endings	Reduced sensation of pain	Increased risk of injury
Flattening of the dermal-epidermal junction	Decrease in surface contact	Increased vulnerability to shearing forces
Reduced, disorganized microcirculation	Reduced vascular response	Development of ischemic ulcers
Decrease in Langerhans cells	Diminished immune function	Development of chronic wounds

larger collagen bundles with more intermolecular cross-links, the tensile strength increases, but does not reach more than 80% of unwounded skin. In the remodeling phase, the microvascular density also returns to normal by regression of newly formed capillaries [1].

Alterations in Aging Skin

Skin has several important functions: as barrier to protect against the entry of microorganisms, physical and chemical insults, in thermoregulation, regulation of water loss, as a sensor, and as part of the immune system. With increasing age, these skin functions deteriorate due to morphological and structural changes, influenced by intrinsic factors, e.g. the genetic make-up and changes in hormone levels, and extrinsic factors such as sun exposure and tobacco smoking [reviewed in ref. 4]. Age-related epidermal changes include a decrease in the number of Langerhans cells and melanocytes, and flattening of the dermal-epidermal junction. In addition, keratinocyte proliferation is reduced, and the turnover time, i.e. the number of days for keratinocytes to migrate from the basal layer to the skin surface, is increased by 50%. The dermis of the aged skin displays fewer fibroblasts, macrophages and mast cells, reduced vascularity, and a loss in ECM components such as collagen and glycosaminoglycan. Besides the imbalance of collagen production and degradation, the quality of the remaining collagen is also altered, showing fewer rope-like bundles and a higher degree of disorganization. The morphology of elastin is also disordered, resulting in decreased skin elasticity. Additional age-related changes are diminished sensation to light touch and pressure, reduced sebum secretion, and a decreased capacity to produce vitamin D₃. Morphologi-

cal and functional alterations and their potential consequences for wound healing are summarized in table 1. Alterations in aging skin not only have an impact on wound healing, but also make the skin more susceptible to injury. For example, the reduction of nerve endings reduces pain sensation, thereby increasing the risk of injury, and increased fragility due to epidermal atrophy makes the skin more vulnerable to mechanical forces. Immunosenescence, e.g. reflected by a decrease in Langerhans cells, and increased fibroblast senescence are involved in the development of chronic wounds. Furthermore, microvascular disturbances might lead to the development of ischemic ulcers (table 1).

Impact of Aging on Wound Healing

Although wound healing in healthy elderly people (>65 years of age) is not impaired per se, age-related changes are obvious in all phases of wound repair [5]. Disruption of any step in one of the wound-healing phases leads to a delay in healing by 20–60% [6]. A summary of age-related alterations is given in table 2.

Hemostasis and Inflammation

Hemostasis, the initial step in wound healing, is characterized by fibrin clot formation, platelet activation, and subsequent release of mediators stimulating the influx of inflammatory cells. With increasing age, adherence of platelets to the injured endothelium, and release of PDGF, TGF- β and TGF- α from α -granules seem to be enhanced [5].

In response to proinflammatory mediators released from platelets and resident mast cells, circulating neutrophils are recruited to the wound within a few hours after

Table 2. Age-related alterations in wound healing

Hemostasis	Inflammation	Proliferation	Remodeling
Increased platelet aggregation Increased platelet degranulation	Altered adhesion molecule profile Early increase in neutrophils Delayed monocyte infiltration Increase in mature macrophages Impaired macrophage function: – reduced phagocytic capacity – increased secretion of pro-inflammatory mediators – decreased VEGF production Delayed T cell infiltration	Reduced response to hypoxia: – diminished HIF-1 α signaling – decreased SDF-1 expression Delayed angiogenesis Delayed collagen deposition: – reduced fibroblast proliferation – reduced fibroblast migration – reduced responsiveness to TGF- β_1 Delayed re-epithelialization: – reduced keratinocyte proliferation – reduced keratinocyte migration	Reduced collagen turnover Increased fibroblast senescence Accelerated maturation Improved scarring

the injury. Concurrently, monocytes begin to enter the wound and differentiate into mature tissue macrophages. Cell infiltration is regulated by adhesion molecule expression, which is altered in old individuals, and might affect the early inflammatory wound-healing response. Indeed, an early increased neutrophil response, and a delayed monocyte influx with increased numbers of mature macrophages have been shown in aged compared with young controls [7]. Whereas neutrophils are not essential for successful wound healing, macrophages play a crucial role. Thus, it has been shown that selective depletion of macrophages leads to delayed wound closure with diminished granulation tissue formation, decreased angiogenesis, decreased collagen and growth factor synthesis, and reduced numbers of myofibroblasts [8]. A recent study by Lucas et al. [9] on time-dependent restriction of macrophage depletion during distinct phases of wound healing provided evidence that macrophages exert different functions during the diverse phases of repair. Recruitment of macrophages during the inflammatory phase controlled granulation tissue formation, angiogenesis and epithelialization. Macrophage depletion in the proliferative phase resulted in hemorrhage and curbed tissue maturation. Furthermore, macrophage recruitment during the inflammatory phase promoted alternative activation. There is some evidence that phagocytosis of neutrophils might cause a switch from the classically activated M₁ phenotype to the alternatively activated M₂ phenotype. Intriguingly, the phagocytic activity of wound macrophages from aged mice with delayed repair is significantly reduced compared with young animals [10]. This might also account for the increased production of proinflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis fac-

tor- α , and reduced VEGF secretion [5, 11]. In addition to these alterations, T cell infiltration is also delayed but with an ultimately higher peak in the aged [7, 10]. The impact of these mechanisms on wound healing remains elusive and needs further investigations.

Proliferation

The mid-phase of wound healing is characterized by re-epithelialization, granulation tissue formation and angiogenesis involving keratinocytes, fibroblasts, and endothelial cells. All these cells display age-related changes, including diminished proliferation and migration, reduced response to growth factors, and decreased cytokine secretion, resulting in delayed wound closure, and decreased angiogenesis and ECM deposition [5]. There is strong evidence suggesting that some of these alterations can be attributed to a diminished response to hypoxia with reduced hypoxia inducible factor (HIF)-1 α signaling and subsequently decreased stromal-derived factor-1 expression [12]. Due to vascular disruption, the microenvironment of the early wound is hypoxic. In young individuals, temporary hypoxia stimulates wound healing by inducing cytokine and growth factor production, and by promoting cell proliferation, migration and angiogenesis. In elderly individuals, however, the response to hypoxia seems to be impaired. Thus, Xia et al. [13] have shown that the migratory activity of keratinocytes from old donors was significantly depressed under hypoxic conditions whereas it was increased in young keratinocytes. Expression of matrix metalloproteinase (MMP)-1 and MMP-9, which are required for keratinocyte migration, was not induced by hypoxia in keratinocytes from elderly donors, but was upregulated in cells from young controls. Fibroblasts from aged donors also

exhibit a loss of responsiveness to hypoxia that is associated with depressed migratory activity. Moreover, the age-related decreased response to TGF- β_1 is further diminished by hypoxia [14]. Hypoxia is a well-known potent inducer of angiogenesis. Thus, it is not surprising that a reduced response to hypoxia is also involved in the age-related delay of angiogenesis during wound healing. Reduced neovessel density correlates with decreased HIF-1 α dependent stromal-derived factor-1 expression [12]. The reduced VEGF levels found in wounds of aged mice are also consistent with depressed HIF-1 α signaling [11]. It was recently shown that the capacity to stabilize HIF-1 α becomes depressed with aging, and that diminished HIF-1 α signaling impairs mobilization and homing of bone marrow-derived angiogenic cells [15, 16].

Tissue Remodeling

In contrast to the earlier phases of wound healing, only few data are available on age-related alterations in the final maturation phase. Studies by Ashcroft et al. [5] have shown upregulation of MMP-2 and MMP-9 in cutaneous wounds of healthy elderly humans until 84 days after injury, and downregulation of tissue inhibitor of metalloproteinase-1 and -2, suggesting that intrinsically aged skin is primed for tissue breakdown. This imbalance between MMPs and their natural inhibitors result in elevated proteolytic activity and subsequently decreased collagen deposition. Furthermore, TGF- β_1 , which is known to increase the net deposition of collagen by stimulating its synthesis and diminishing its degradation, is significantly reduced in wound tissue of old individuals [reviewed in ref. 5]. Late-stage wounds of aged mice showed an age-related delay in collagen remodeling with less organized collagen in old compared with young animals at day 11 after injury [17]. Nevertheless, a recent study analyzing scars from healthy volunteers from 1 month to 12 months after injury revealed a shorter maturation time and better scar quality in old compared with young individuals [18]. Thus, whilst aging adversely affects the speed of wound healing during early phases, it may accelerate maturation and improve scar quality under optimal wound care. However, if wound healing occurs under suboptimal conditions, immunosenescence, i.e. age-related changes in innate and adaptive immune response, delayed re-epithelialization, increased fibroblast senescence and delayed collagen deposition may render the wound more susceptible to secondary insults, such as infection and repeated trauma.

Additional Factors Affecting Wound Healing

Various factors associated with aging or predominantly concerning elderly people additionally affect wound healing, e.g. decline of sex steroid hormones, malnutrition, immobilization, psychological stress, medication and comorbidities such as diabetes, peripheral arterial disease and chronic venous insufficiency.

Aging is accompanied by a decline in systemic and local hormone levels, which is especially rapid and dramatic in postmenopausal women. Estrogens, androgens and their steroid precursor dehydroepiandrosterone have substantial effects on cutaneous wound healing. Whereas androgens are negative regulators of repair, estrogens and dehydroepiandrosterone accelerate wound healing by attenuating inflammation, and promoting ECM deposition. A recent microarray-based study has emphasized the importance of estrogen as a regulator in wound healing, showing that the differences in gene expression between wounds in old and young males are almost solely estrogen regulated [19].

Poor nutritional status and stress also contribute to impaired wound healing. Thus, it has been shown that stress fuels further delay in wound repair by suppressing the production of IL-1 α , and IL-8 at the wound site. Impairment of the early inflammatory response slows down the wound-healing process, increasing the risk of infection and development of chronic ulceration [6]. Calorie restriction, a reliable intervention to prevent chronic diseases and slow down aging, does not benefit wound healing in aged subjects [20]. Adequate nutrition is essential for optimal healing of acute and chronic wounds. Malnutrition, which is fairly frequent in the elderly, contributes to delayed repair and, together with impaired mobility, is a major risk factor for developing poorly healing pressure ulcers [21].

With advancing age, concurrent diseases and medications that negatively affect wound healing, e.g. corticosteroids and chemotherapeutic drugs, become more common. Whereas low-dose topical corticosteroid treatment seems to be beneficial in the treatment of chronic wounds [22], systemic glucocorticoids inhibit wound repair by anti-inflammatory effects, and suppression of fibroblast proliferation and collagen synthesis, possibly via attenuation of HIF-1 α activity [23]. Chemotherapeutic drugs interfere with many pathways that are critical to wound healing: they delay cell migration, impair cell proliferation and reduce angiogenesis and ECM formation. Moreover, they weaken the immune system and thereby increase the risk of infection [24].

Chronic wound healing disorders are a common problem in the elderly population. They are usually not seen in healthy aged individuals, but are rather associated notably with diabetes, peripheral arterial disease and chronic venous insufficiency. The underlying mechanisms leading to chronic nonhealing wounds are manifold and complex. Hence, a discussion of this topic is beyond the scope of this mini-review.

Conclusion

From the current literature we can conclude that cutaneous wound healing in healthy elderly people is delayed, but scar maturation is improved in comparison with

young individuals. Impaired wound healing leading to chronic wounds is primarily associated with comorbidities, which are more prevalent in old age. Nevertheless, age (>60 years) is an independent risk factor for less frequent closure of chronic wounds.

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