World J. Surg. 28, 321–326, 2004
DOI: 10.1007/s00268-003-7397-6

WORLD
Journal of
SURGERY

Aging and Wound Healing

Ankush Gosain, M.D. Luisa A. DiPietro, D.D.S., Ph.D.

Burn and Shock Trauma Institute, Department of Surgery, Loyola University Medical Center, 2160 South First Avenue, 60153 Maywood, Illinois, USA

Published Online: February 17, 2004

Abstract. Impaired wound healing in the elderly presents a major clinical and economic problem. With the aging population growing in both number and percentage, the importance of understanding the mechanisms underlying age-related impairments in healing is increased. Normal skin exhibits its characteristic changes with age that have implications for wound healing. Additionally, the process of wound healing is altered in aged individuals. Although historically healing in the aged was considered defective, there is now consensus that healing in the elderly is delayed but the final result is qualitatively similar to that in young subjects.

The elderly, those older than 65 years of age, are the fastest growing segment of the American population [1]. At present, at 12.4% (35 million) of the total United States population, it is estimated that this group will comprise 20% (53 million) of the population by the year 2030 [2]. Moreover, trauma is the fifth leading cause of death for persons in the U.S. over the age of 65 [3]. Thus, it is clear that an understanding of the effect that aging plays on wound healing is of vital importance.

The first clinical description of impaired wound healing with age was recorded in the medical literature almost one hundred years ago [4]. Since that time, investigators have studied aspects of aging and wound healing ranging from the cellular level to the clinical level. In animal models of wound repair in the aged, there is a 20% to 60% delay in the rate of healing as compared to young animals [5]. The consensus is that the effect of aging on wound repair is primarily a temporal delay and not an actual impairment in the quality of healing [6].

Most literature on wound healing focuses on acute cutaneous wounds and will be the focus of this review. The principles of delayed and impaired wound healing that will be discussed are generally applicable to any organ system. The scant literature present on the healing of chronic wounds will be discussed. This review will summarize the changes seen in normal skin with aging. The effects of aging on the individual phases of healing will be examined. The effect of extrinsic influences (e.g., ultraviolet radiation, medical comorbidities) on wound healing will be detailed. Finally, recent clinical trials aimed at improving wound healing will be discussed.

Correspondence to: L.A. DiPietro, D.D.S., Ph.D.

Changes in Aging Skin

The German physician Rudolf Virchow first described the skin as a protective barrier for the internal viscera almost a century and a half ago [7]. It is now known that skin serves to protect against the entry of microorganisms, regulate water loss, protect against ultraviolet (UV) radiation, assist in thermoregulation, and as a component of the immune system [8]. The changes seen in aged skin are a combination of effects from intrinsic and extrinsic aging. Intrinsic aging is defined as the changes in skin that occur in sun-protected areas, independent of environmental insults. Extrinsic aging is comprised of the cumulative changes of long-standing environmental exposure, most notably to UV radiation from sunlight [9]. The sum effect of intrinsic and extrinsic aging is a progressive loss of function, increased vulnerability to the environment, and decreased homeostatic capability [10]. Clinically, aged skin is characterized by atrophy, drying, roughness, alterations in pigmentation, sagging, wrinkling, and the presence of benign and malignant tumors.

The epidermis, consisting mainly of squamous epithelial cells (keratinocytes), functions as a barrier against water entry. The thickness of the epidermis remains fairly constant with age [11], but there is a flattening of the dermal-epidermal junction, giving the appearance of atrophy [12]. In addition, the time for keratinocytes to migrate from the basal layer to the skin surface, a key process in repair, increases by 50% in aged individuals [13].

The dermis, comprised of fibroblasts and the extracellular matrix (ECM) (types I and III collagen, elastin, and glycosaminoglycans), is divided into the superficial papillary dermis and the deep reticular dermis. The papillary dermis forms ridges that maintain contact with the epidermis. With age, there is a flattening of these rete ridges, resulting in decreased surface contact between the dermis and epidermis [14]. This predisposes to separation of the dermal-epidermal junction with laterally applied tension [12]. The cellular content of the dermis, consisting of fibroblasts, mast cells, and macrophages, is decreased with age. Importantly, there is an age-related decrease in the number and function of antigen-presenting cells (e.g., Langerhans cells, mast cells) in aged skin [15]. The protein content of the dermis, primarily collagen, is decreased with age as well. This is the result of both decreased production and increased degradation [16]. The quality of the collagen that remains is altered, with fewer organized, rope-like bundles and a greater

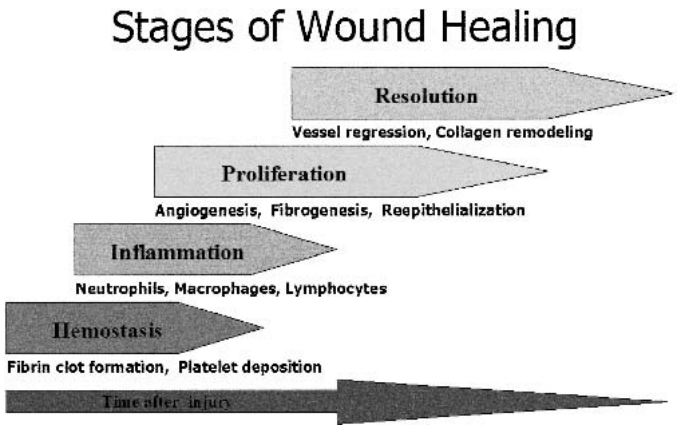

Fig. 1. The process of wound healing is divided into four phases for ease of discussion.

degree of disorganization seen. The quantity of elastin, a determinant of skin elasticity, is fairly constant with age. However, like collagen, elastin in the aged dermis displays a disordered morphology, resulting in decreased elasticity of the skin [17].

The microcirculation of the dermis is decreased with age, and this affects its ability to adapt to injury and changes in temperature. There is a marked reduction in cutaneous blood flow in aged as compared to younger humans [18]. Along with these changes in blood flow, dermal lymphatic drainage decreases with age, diminishing the ability to clear the wound of pathogens and also inhibiting wound contraction [19]. Age-related alterations in the dermal appendages result in decreased secretions from sweat and sebaceous glands, slowed hair growth, and diminished perception of pain and pressure [20, 21].

Most of the changes witnessed in aged skin are the result of long-standing sun exposure (UV light). Excessive exposure to sunlight results in sunburn and immune suppression in the acute setting and skin cancer and photoaging with chronic exposure [22]. As in intrinsically aged skin, the dermis is the site ofmost of the changes in photoaged skin [23]. Activation of matrix metalloproteinases (MMPs) by UV radiation results in disorganized collagen fibrils and the accumulation of abnormal elastin-containing materials [9].

Age-Related Changes in Phases of Healing

Although the process of wound healing is a continuum, it is classically separated into a series of overlapping phases for the ease of discussion (Fig. 1). During hemostasis a fibrin clot is formed at the site of endothelial injury and platelets aggregate. Platelets adhere to the injured endothelium and release chemokines, thereby attracting the cellular components of the inflammatory phase [24]. The inflammatory phase of wound healing is characterized by the presence of neutrophils, macrophages, and lymphocytes [25]. The inflammatory cells then serve to release proinflammatory cytokines (e.g., transforming growth factor [TGF]-α and interleukins) and growth factors (e.g., fibroblast growth factor [FGF] and vascular endothelial growth factor [VEGF]), ingest foreign materials, increase vascular permeability, and promote fibroblast activity [26, 27]. The proliferative phase begins several days after the initial injury. In this phase, capillary growth and granulation tissue formation occur. Cellular proliferation and abundant collagen synthesis by fibroblasts lead to re-epithelialization and construction of a preliminary dermis. The final phase of wound healing, resolution, is a long process of tissue remodeling and increasing wound strength. During this phase, type I collagen synthesis and turnover continues, and fibroblasts differentiate into myofibroblasts, allowing further wound contraction. These four phases (hemostasis, inflammation, proliferation, and resolution) have been studied in detail and exhibit characteristic changes with aging. Decreased levels of growth factors, diminished cell proliferation and migration, and diminished extracellular matrix secretion have been demonstrated.

Alterations in Hemostasis and Inflammation

With endothelial injury, collagen is exposed, promoting the adherence of platelets to the injured endothelium. Platelet adherence to the endothelium is enhanced in aged subjects [28, 29]. Additionally, the release of alpha-granules, which contain TGF-β, TGF-α, and platelet-derived growth factor (PDGF), by platelets increases with age [30].

Conflicting conclusions have been drawn about the age-related alterations in the inflammatory phase of healing. Although some aspects of healing are depressed in this stage, others are enhanced. Decreased amounts of nitric oxide, a vasoactive mediator, are secreted by aged endothelial cells [31]. Accordingly, there is decreased capillary permeability at the site of injury, and the diapedesis of neutrophils is decreased. In contrast, leukocytes display an age-related increase in secretion of and response to many inflammatory mediators [32–34]. The infiltration of macrophages and B-lymphocytes into wounds is delayed in models of wound healing in middle-aged and elderly mice [6]. The arrival of T-lymphocytes to the wound bed is also delayed in aged animals, but the final level is increased as compared to young animals [15]. In aged animals, lymphocytes display a decreased proliferative response, a decreased number of naïve cells, and an increased number of memory cells [35–37]. Changes in macrophage function have been suggested to be critical to age-related repair defects. Young animals treated with an anti-macrophage serum prior to wounding heal at rates comparable to those of older animals [38]. Additionally, wound repair can be accelerated in aged mice by the intraperitoneal injection of macrophages harvested from young mice [39]. Accelerated wound repair was not seen with the injection of macrophages from old mice. Wound macrophages from aged animals display a decreased percentage of macrophages that are phagocytic, as well as a decreased phagocytic ability [15]. Production of growth factors by macrophages declines with age as well [40]. It can be concluded that there is an age-related decline in macrophage function.

Alterations in Proliferation

Keratinocytes, fibroblasts, and vascular endothelial cells display a reduced proliferative response in aged animals [41, 42]. Re-epithelialization, collagen synthesis, and angiogenesis all exhibit an age-related delay [40]. There is a general decrease in the number and size of dermal fibroblasts with age [43]. Aged fibroblasts have also been shown to exhibit a diminished response to growth factors and diminished replicative capacity [43, 44]. These changes result in an age-related delay in wound closure in animal models as well as in human wounds [45–47]. Whole wound studies have shown decreased rates of epithelialization and contraction in older animals [48, 49] and humans [50–52].

As the epidermis requires nutrition to migrate and proliferate, the process of angiogenesis is believed to be important for optimal wound repair [53]. Conflicting data are present in the literature, with the majority of studies indicating a decrease in angiogenesis with age [40, 54], and a few showing an increase [6, 55]. Excisional and subcutaneous implant models have been used to show that wound capillary ingrowth is delayed in aged animals [56, 57]. Reduced levels of the angiogenic factors FGF, VEGF, and TGF-β have been implicated as partially responsible for this delay [31, 40]. Indeed, replacement of these factors is able to reverse the delay [58].

The rate of collagen production, as measured by hydroxyproline content, is decreased with aging [59]. The decreased deposition of connective tissue has been shown to be primarily a deficit of type I collagen [42, 60]. Recent work has shown that while the deposition of collagen is delayed, the final collagen content in mature wounds does not differ in young and aged animals [40].

In laying down the new collagen framework, the existing ECM must be degraded, a process mediated by the matrix metalloproteinases (MMPs). The invasion of endothelial cells requisite for angiogenesis requires MMP activity as well [61]. Three types of MMPs—collagenases, stromelysins, and gelatinases—are secreted by keratinocytes and fibroblasts and can be found during wound healing [62]. The activity of MMPs is balanced by their naturally occurring inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Studies have shown that chronic non-healing wounds demonstrate an elevated level of MMP expression [63]. Additionally, delayed healing in the aged has been shown to result from an overexpression of MMPs, an underexpression of TIMPs, or both [64–67]. Recent studies have shown that changes in MMP and TIMP expression with age are not global but rather are tissue and cell specific [41].

Alterations in Resolution

Young animals display a greater level of collagenase activity than older animals, allowing greater turnover and remodeling of newly formed collagen [68]. The hyperproliferative wound-healing disorders, hypertrophic scars and keloids, are rarely seen in aged wounds. Some evidence suggests that this may be due to decreased levels of circulating TGF-β [69]. Blockage of TGF-β, a molecule known to enhance collagen deposition, is able to prevent excessive scar formation in adults [70].

Most studies of the resolution phase of healing focus on wound strength as a measure of collagen content and cross-linking. Measurement of bursting strength is a classic method of studying wound strength [47, 54]. Studies of both intestinal anastomoses [71] and cutaneous wounds [72] have shown that older animals gain wound strength at a slower rate and have decreased strength as compared to younger animals. These findings have been shown to extend to human subjects; incisional wounds in patients over the age of 70 displayed lower tensile strength than those in patients younger than age 70 [73]. Laparotomy wound dehiscence is also more likely in patients over the age of 60 [74, 75]. The delay in wound closure also results in an increased incidence of infection and medical complications [74]. Unfortunately, these studies did not consider comorbidities common in the older population (e.g., diabetes, vascular disease).

Extrinsic Influences

With advancing age, concurrent medical disease and other factors that adversely effect wound healing become more common. Paramount to ensuring optimal wound healing is aggressive control of diabetes and vascular disease, which can impair granulation tissue formation. Corticosteroids, which inhibit lymphocyte function and collagen synthesis, should be withdrawn whenever possible [76, 77]. Alternatively, the deleterious wound healing effects of corticosteroids can be reversed with systemic administration of vitamin A [78, 79]. Poor nutritional status contributes to a delayed inflammatory response and delayed synthesis of matrix proteins and should be optimized [80]. Additionally, cigarette smoking impairs granulation tissue formation by the action of nicotine on the vascular system, and should be stopped [81].

Therapeutic Options

Although the studies on age-related alterations in wound healing discussed above have focused on acute wounds, clinical studies tend to focus on the treatment of chronic wounds (e.g., pressure ulcers, vascular insufficiency ulcers). Many studies over the last decade have examined the role of systemically or topically applied growth factors in wound healing. TGF-β1, basic FGF (bFGF), PDGF, and others have been shown to be of benefit in various animal models [31, 82, 83]. TGF-β1 applied to dermal wounds accelerated the rate of wound closure in aged rats [42]. It is difficult to know the mechanism by which the improved healing was effected, as TGF-β1 has multiple functional effects in the wound – attracting fibroblasts, enhancing collagen deposition, decreasing MMP-1 synthesis, and increasing TIMP-1 expression. Other studies have focused on the ability of topical estrogen to accelerate healing [84, 85]. Once again, the mechanism of this improvement is unclear, as the topical estrogen may be acting by increasing TGF-β1 expression [84]. Ultimately, results from studies in humans have been largely disappointing, and no clear recommendations on the use of growth factors can be made.

Due to overall disappointing results with the transition of a treatment option from animal studies to human studies, new models of aged wounds are being developed. Investigators have postulated that the primary defect in chronic wounds is local tissue hypoxia from scarring, fibrin cuffing, edema, increased venous pressure, and microvascular disease [86, 87]. Previously developed models have attempted to reproduce these conditions by the administration of glucocorticoids, exposure to ionizing radiation, or decreasing blood supply to the wound [88–90]. Recently, a model has been developed that uses rabbits aged 60 months to represent humans in the 7th and 8th decades of life [91]. Additionally, by interrupting the major arterial circulation as well as the dermal microcirculation to the wound, the setting of wound ischemia is created. In this model there is a profound impairment of granulation tissue formation and re-epithelialization. Additionally, this is the first animal wound-healing model in which treatment with TGF-β1 fails to promote healing. Further work has shown that this result is due to reduced expression of receptors for TGF-β as well as reduced activation of intracellular signaling pathways in response to TGF-β [92].

Other studies have examined the use of skin substitutes, electrical fields, vacuum devices, and supplemental oxygen, among other techniques in wound healing. Although no study has shown universal benefit, subgroups of patients have been shown to benefit with

Table 1. Summary of the changes that take place in human skin with age.

Clinical	Histologic
Atrophy	Flattening of the dermal–epidermal junction
Drying	Increased turnover time
Roughness	Decreased fibroblasts, mast cells, and macrophages
Alterations in pigmentation	Decreased collagen content
Sagging	Disorganized collagen and elastin
Wrinkling	Impaired microcirculation
Benign and malignant tumors	

Table 2. Summary of the age-related changes in the individual phases of healing.

Hemostasis	Proliferation
Enhanced platelet aggregation	Delayed re-epithelialization
Increased release of alpha-granules	Delayed angiogenesis
	Delayed collagen deposition
Inflammation	**Resolution**
Decreased vascular permeability	Reduced collagen turnover and remodeling
Increased secretion of inflammatory mediators	Delayed wound strength
Delayed infiltration of macrophages and lymphocytes	Decreased wound strength
Impaired macrophage function	
Decreased secretion of growth factors	

each of these techniques. Therapeutic approaches that may hold promise for the future of wound healing include gene therapy and the use of stem cells [93].

Summary

Through a combination of intrinsic and extrinsic aging, human skin undergoes a multitude of changes that can potentially affect the process of wound healing (Table 1). Each of the phases of healing demonstrates characteristic age-related changes as well (Table 2). When taken as a whole, it is reasonable to conclude that there are global differences affecting wound healing between young and aged individuals. It is therefore unlikely that a single therapeutic approach will serve to abrogate all of these changes. Future work should focus on therapies applicable to certain subpopulations of patients (e.g., those with venous or arterial insufficiency, immunosuppressed) that commonly develop chronic wounds. Additionally, more detailed model systems need to be developed to test potential therapeutic agents before they progress to clinical trials.

Résumé. Un défaut de cicatrisation chez les personnes âgées représente un problème clinique et économique majeur. Avec l'augmentation de la population âgée en nombre absolu et en pourcentage, on a besoin de comprendre les mécanismes derrière ces défauts de cicatrisation en rapport avec l'âge. La peau normale présente des caractéristiques qui changent avec l'âge ce qui a des implications dans la cicatrisation des plaies. De plus, le processus de cicatrisation chez le vieillard est altéré. Alors qu'autrefois, on considérait que la cicatrisation était défectueuse chez le vieillard, le consensus général aujourd'hui est que la cicatrisation n'est que retardée: le résultat final est qualitativement similaire à celle du sujet plus jeune.

Resumen. La defectuosa cicatrización de heridas que se observa en los ancianos constituye un problema clínico y económico mayor. Con el crecimiento de la población de edad avanzada tanto en número como en porcentaje, se incrementa el interés en lograr una mejor comprensión de los mecanismos que determinan este defectuoso proceso relacionado con la edad. La piel normal preseneta cambios característicos relacionados con el avance de la edad que tienen implicaciones en cuanto a la cicatrización. Además, el proceso de cicatrización se ve alterado en las personas de edad avanzada. Aunque históricamente se ha considerado que la cicatrización es defectuosa en el anciano, ahora hay consenso en que la cicatrización está retardada, pero que el resultado final es cualitativamente similar al de personas más jóvenes.

Acknowledgments.

This work was supported by grants GM50875 and GM55238 from the National Institutes of Health (NIH). A.G. is supported by the NIH T32-GM08750 Training Grant in Trauma and Burn Research.

References

1. U.S. Census Bureau. Census 2000. U.S. Census Bureau, Washington, DC, 2000
2. U.S. Census Bureau. Population Projections Program, Population Division, U.S. Census Bureau, Washington, DC, 2000
3. McMahon DJ, Schwab CW, Kauder D. Comorbidity and the elderly trauma patient. World J. Surg. 1996;20:1113–1119
4. DuNouy PL. The relation between the age of the patient, the area of the wound, and the index of cicatrisation. J. Exp. Med. 1916;24:461–470
5. Quirinia A, Viidik A. The influence of age on the healing of normal and ischemic incisional skin wounds. Mech. Ageing Dev. 1991;58:221–232
6. Ashcroft GS, Horan MA, Ferguson MW. Aging is associated with reduced deposition of specific extracellular matrix components, an upregulation of angiogenesis, and an altered inflammatory response in a murine incisional wound healing model. J. Invest. Dermatol. 1997;108: 430–437
7. Virchow R, Cellular Pathology, London, John Churchill, 1860;
8. Harrist TJ, et al. The skin. In Rubin E, Farber JL, editors, Pathology Philadelphia, Lippincott-Raven, 1999;1236–1299
9. Fisher GJ, et al. Pathophysiology of premature skin aging induced by ultraviolet light. N. Engl. J. Med. 1997;337:1419–1428
10. Gilchrest BA, Garmyn M, Yaar M. Aging and photoaging affect gene expression in cultured human keratinocytes. Arch. Dermatol. 1994;130: 82–86
11. Whitton JT, Everall JD. The thickness of the epidermis. Br. J. Dermatol. 1973;89:467–476
12. Kurban RS, Bhawan J. Histologic changes in skin associated with aging. J. Dermatol. Surg. Oncol. 1990;16:908–914
13. Gilchrest BA, Murphy GF, Soter NA. Effect of chronologic aging and ultraviolet irradiation on Langerhans cells in human epidermis. J. Invest. Dermatol. 1982;79:85–88
14. Montagna W, Carlisle K. Structural changes in aging human skin. J. Invest. Dermatol. 1979;73:47–53
15. Swift ME, et al. Age-related alterations in the inflammatory response to dermal injury. J. Invest. Dermatol. 2001;117:1027–1035
16. Bernstein EF, et al. Long-term sun exposure alters the collagen of the papillary dermis. Comparison of sun-protected and photoaged skin by northern analysis, immunohistochemical staining, and confocal laser scanning microscopy. J. Am. Acad. Dermatol. 1996;34:209–218
17. Lavker RM, Zheng PS, Dong G. Aged skin: a study by light, transmission electron, and scanning electron microscopy. J. Invest. Dermatol. 1987;88:44S–51S
18. Tsuchida Y. The effect of aging and arteriosclerosis on human skin blood flow. J. Dermatol. Sci. 1993;5:175–181
19. Gniadecka M, Serup J, Sondergaard J. Age-related diurnal changes of dermal oedema: evaluation by high-frequency ultrasound. Br. J. Dermatol. 1994;131:849–855
20. Montagna W, Carlisle K. Structural changes in ageing skin. Br. J. Dermatol. 1990;122:61–70
21. Pochi PE, Strauss JS, Downing DT. Age-related changes in sebaceous gland activity. J. Invest. Dermatol. 1979;73:108–111

22. Montagna W, Kirchner S, Carlisle K. Histology of sun-damaged human skin. J. Am. Acad. Dermatol. 1989;21:907–918
23. Smith JG, et al. Alterations in human dermal connective tissue with age and chronic sun damage. J. Invest. Dermatol. 1962;39:347–350
24. DiPietro LA, et al. MIP-1alpha as a critical macrophage chemoattractant in murine wound repair. J. Clin. Invest. 1998;101:1693–1698
25. Ross R, Benditt EP. Wound healing and collagen formation: fine structure in experimental scurvy. J. Cell Biol. 1962;12:533–551
26. Polverini PJ, et al. Activated macrophages induce vascular proliferation. Nature 1977;269:804–806
27. Hunt TK, et al. Studies on inflammation and wound healing: angiogenesis and collagen synthesis stimulated in vivo by resident and activated wound macrophages. Surgery 1984;96:48–54
28. Grigorova-Borsos AM, et al. Aging and diabetes increase the aggregating potency of rat skin collagen towards normal platelets. Thromb. Haemost. 1988;60:75–78
29. Silverman EM, Silverman AG. Granulocyte adherence in the elderly. Am. J. Clin. Pathol. 1977;67:49–52
30. Yonezawa Y, Kondo H, Nomaguchi TA. Age-related changes in serotonin content and its release reaction of rat platelets. Mech. Ageing Dev. 1989;47:65–75
31. Rivard A, et al. Age-dependent impairment of angiogenesis. Circulation 1999;99:111–120
32. Doria G, Frasca D. Regulation of cytokine production in aging mice. Ann. N. Y. Acad. Sci. 1994;741:299–304
33. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu. Rev. Med. 2000;51: 245–270
34. Mascarucci P, et al. Age-related changes in cytokine production by leukocytes in rhesus monkeys. Aging (Milano). 2001;13:85–94
35. Ginaldi L, et al. Changes in the expression of surface receptors on lymphocyte subsets in the elderly: quantitative flow cytometric analysis. Am. J. Hematol. 2001;67:63–72
36. Bruunsgaard H, et al. Proliferative responses of blood mononuclear cells (BMNC) in a cohort of elderly humans: role of lymphocyte phenotype and cytokine production. Clin. Exp. Immunol. 2000;119:433–440
37. Plackett TP, et al. Aging enhances lymphocyte cytokine defects after injury. FASEB J. 2003;17:688–689
38. Cohen BJ, Danon D, Roth GS. Wound repair in mice as influenced by age and antimacrophage serum. J. Gerontol. 1987;42:295–301
39. Danon D, Kowatch MA, Roth GS. Promotion of wound repair in old mice by local injection of macrophages. Proc. Natl. Acad. Sci. U. S. A. 1989;86:2018–2020
40. Swift ME, Kleinman HK, DiPietro LA. Impaired wound repair and delayed angiogenesis in aged mice. Lab. Invest. 1999;79:1479–1487
41. Reed MJ, Ferara NS, Vernon RB. Impaired migration, integrin function, and actin cytoskeletal organization in dermal fibroblasts from a subset of aged human donors. Mech. Ageing Dev. 2001;122:1203–1220
42. Puolakkainen PA, et al. The enhancement in wound healing by transforming growth factor-beta 1 (TGF-beta 1) depends on the topical delivery system. J. Surg. Res. 1995;58:321–329
43. Plisko A, Gilchrest BA. Growth factor responsiveness of cultured human fibroblasts declines with age. J. Gerontol. 1983;38:513–518
44. West MD. The cellular and molecular biology of skin aging. Arch. Dermatol. 1994;130:87–95
45. Bruce SA, Deamond SF. Longitudinal study of in vivo wound repair and in vitro cellular senescence of dermal fibroblasts. Exp. Gerontol. 1991;26:17–27
46. Grove GL, Kligman AM. Age-associated changes in human epidermal cell renewal. J. Gerontol. 1983;38:137–142
47. Butcher EO, Klingsberg J. Age, gonadectomy and wound healing in palatal mucosa of the rat. Oral Surg 1963;16:482–492
48. Billingham RE, Russell PS. Studies on wound healing with special reference to the phenomenon of contraction in experimental wounds in rabbits' skin. Ann. Surg. 1956;144:961–980
49. Cuthbertson AM. Contraction of full thickness skin wounds in the rat. Surg. Gynecol. Obstet. 1959;108:421–432
50. Fatah MF, Ward CM. The morbidity of split-skin graft donor sites in the elderly: the case for mesh-grafting the donor site. Br. J. Plast. Surg. 1984;37:184–190
51. Holt DR, et al. Effect of age on wound healing in healthy human beings. Surgery 1992;112:293–297
52. Orentreich N, Salmanowitz VJ. Levels of biological functions with aging. Trans. N. Y. Acad. Sci. 1969;2:992–1012
53. Strigini L, Ryan T. Wound healing in elderly human skin. Clin. Dermatol. 1996;14:197–206
54. Holm-Pedersen P, Viidik A. Tensile properties and morphology of healing wounds in young and old rats. Scand. J. Plast. Reconstr. Surg. 1972;6:24–35
55. Passaniti A, et al. A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. Lab. Invest. 1992;67:519–528
56. Pili R, et al. Altered angiogenesis underlying age-dependent changes in tumor growth. J. Natl. Cancer Inst. 1994;86:1303–1314
57. Yamaura H, Matsuzawa T. Decrease in capillary growth during aging. Exp. Gerontol. 1980;15:145–150
58. Arthur WT, et al. Growth factors reverse the impaired sprouting of microvessels from aged mice. Microvasc. Res. 1998;55:260–270
59. Viljanto J. A sponge implantation method for testing connective tissue regeneration in surgical patients. Acta Chir. Scand. 1969;135:297–300
60. Beck LS, et al. One systemic administration of transforming growth factor-beta 1 reverses age- or glucocorticoid-impaired wound healing. J. Clin. Invest. 1993;92:2841–2849
61. Reed MJ, et al. A deficit in collagenase activity contributes to impaired migration of aged microvascular endothelial cells. J. Cell. Biochem. 2000;77:116–126
62. Salo T, et al. Expression of matrix metalloproteinase-2 and -9 during early human wound healing. Lab. Invest. 1994;70:176–182
63. Wysocki AB, Staiano-Coico L, Grinnell F. Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. J. Invest. Dermatol. 1993;101:64–68
64. Zeng G, Millis AJ. Expression of 72-kDa gelatinase and TIMP-2 in early and late passage human fibroblasts. Exp. Cell Res. 1994;213:148–155
65. Millis AJ, et al. Metalloproteinase and TIMP-1 gene expression during replicative senescence. Exp. Gerontol. 1992;27:425–428
66. Ashcroft GS, et al. Age-related differences in the temporal and spatial regulation of matrix metalloproteinases (MMPs) in normal skin and acute cutaneous wounds of healthy humans. Cell Tissue Res. 1997;290: 581–591
67. Ashcroft GS, et al. Human ageing impairs injury-induced in vivo expression of tissue inhibitor of matrix metalloproteinases (TIMP)-1 and -2 proteins and mRNA. J. Pathol. 1997;183:169–176
68. Platt D, Ruhl W. An age-dependent determination of lysosomal enzyme activities, as well as the measurements on the incorporation of 14-C-proline and 14-C-glucosamine in a subcutaneously implanted polyether sponge. Gerontologia 1972;18:96–112
69. Younai S, et al. Modulation of collagen synthesis by transforming growth factor-beta in keloid and hypertrophic scar fibroblasts. Ann. Plast. Surg. 1994;33:148–151
70. Shah M, Foreman DM, Ferguson MW. Control of scarring in adult wounds by neutralising antibody to transforming growth factor beta. Lancet 1992;339:213–214
71. Howes EL, Harvey SC. The age factor in the velocity of the growth of fibroblasts in the healing wound. J. Exp. Med. 1932;55:557–590
72. Sussman MD. Aging of connective tissue: physical properties of healing wounds in young and old rats. Am. J. Physiol. 1973;224:1167–1171
73. Sandbloom PH, Petersen P, Muren A. Determination of the tensile strength of the healing wound as a clinical test. Acta Chir. Scand. 1953; 105:252–257
74. Halasz NA. Dehiscence of laparotomy wounds. Am. J. Surg. 1968;116: 210–214
75. Mendoza CB, Postlethwait RW, Johnson WD. Veterans Administration cooperative study of surgery for duodenal ulcer. II. Incidence of wound disruption following operation. Arch. Surg. 1970;101:396–398
76. Capewell S, et al. Purpura and dermal thinning associated with high dose inhaled corticosteroids. BMJ 1990;300:1548–1551
77. Pollack SV. Systemic drugs and nutritional aspects of wound healing. Clin. Dermatol. 1984;2:68–80
78. Neifeld JP, Lee HM, Hutcher NE. Lack of effect of vitamin A on corticosteroid-induced immunosuppression. J. Surg. Res. 1975;19:225–228
79. Stephens FO, et al. Effect of cortisone and vitamin A on wound infection. Am. J. Surg. 1971;121:569–571

80. Thomas DR. The role of nutrition in prevention and healing of pressure ulcers. Clin. Geriatr. Med. 1997;13:497–511
81. Ueng SW, et al. Effect of intermittent cigarette smoke inhalation on tibial lengthening: experimental study on rabbits. J. Trauma 1997;42: 231–238
82. Broadley KN, et al. Monospecific antibodies implicate basic fibroblast growth factor in normal wound repair. Lab. Invest. 1989;61:571–575
83. Mustoe TA, et al. Accelerated healing of incisional wounds in rats induced by transforming growth factor-beta. Science 1987;237:1333–1336
84. Ashcroft GS, et al. Estrogen accelerates cutaneous wound healing associated with an increase in TGF-beta1 levels. Nat. Med. 1997;3:1209–1215
85. Ashcroft GS, Mills SJ. Androgen receptor-mediated inhibition of cutaneous wound healing. J. Clin. Invest. 2002;110:615–624
86. Falanga V. Growth factors and chronic wounds: the need to understand the microenvironment. J. Dermatol. 1992;19:667–672
87. Falanga V. Chronic wounds: pathophysiologic and experimental considerations. J. Invest. Dermatol. 1993;100:721–725
88. Wu L, Mustoe TA. Effect of ischemia on growth factor enhancement of incisional wound healing. Surgery 1995;117:570–576
89. Pierce GF, et al. Transforming growth factor beta reverses the glucocorticoid-induced wound-healing deficit in rats: possible regulation in macrophages by platelet-derived growth factor. Proc. Natl. Acad. Sci. U. S. A. 1989;86:2229–2233
90. Mustoe TA, et al. Reversal of impaired wound healing in irradiated rats by platelet-derived growth factor-BB. Am. J. Surg. 1989;158:345–350
91. Wu L, et al. Transforming growth factor-beta1 fails to stimulate wound healing and impairs its signal transduction in an aged ischemic ulcer model: importance of oxygen and age. Am. J. Pathol. 1999;154:301–309
92. Mogford JE, et al. Effect of age and hypoxia on TGFbeta1 receptor expression and signal transduction in human dermal fibroblasts: impact on cell migration. J. Cell. Physiol. 2002;190:259–265
93. Reed MJ, Koike T, Puolakkainen P. Wound repair in aging. A review. Meth. Mol. Med. 2003;78:217–237