Tissue Repair and Regeneration Process Regulation

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Biao Cheng, Min Xuan, Xiaobing Fu, Hongwei Liu, and Chunmeng Shi

8.1 Systemic Factors Affecting Tissue Repair and Regeneration

As the bio-psycho-social medical model concept is widely accepted, the impact of these factors on various diseases is increasingly being valued by clinicians. Trauma itself is a process in which external harmful stimuli affect the overall function through local effects. Wound healing, especially in the repair of large wounds, requires the mobilization of a whole body of psycho-neuro-immuno-endocrine-based regulatory mechanisms to respond to injury stimuli. As far as systemic factors are concerned, the health of the overall function plays an important role in wound healing, and factors such as the surrounding environment can also interfere with the outcome of the repair, which should be the focus of attention.

8.1.1 Psychological Factors

Trauma itself is a serious psychological and physiological stress. In addition, patients lack reasonable understanding of treatment and prognosis. Therefore, traumatic patients generally have negative psychological states such as anxiety, fear, and depression, while anxiety has a signal function; it sends a dangerous signal to the individual. When anxiety occurs, the human autonomic nervous system is activated, the cardiovascular system is strengthened, and the secretion of the adrenal glands is increased. It is characterized by rapid heartbeat, feeling cold or fever, shortness of breath, accompanied by experiences of nervousness, worry, fear, etc. Negative psychological state can impair the body's immune system function, which indirectly affects wound healing. On the contrary, a positive state of mind can promote the normal immune response of the human body. The neuro-endocrine axis of the body maintains a virtuous cycle.

The effects of psychology on wound healing include stress, as well as coping style, rich emotions, complex environment, and social support. British medical researchers found that patient's immune system function will be rapidly enhanced after the patient's depressed emotional vent; this method is economical and effective. British medical researchers asked 18 of the 36 patients to be clinically tested to write down their most unpleasant experiences, and 18 others wrote daily chores. It was found that the group of patients who wrote their inner feelings healed faster. It is confirmed that the venting and adjustment of emotions have a direct impact on wound healing.

The rapid development of cell molecular biology has enabled people to have an unprecedented understanding of life phenomenon and a certain understanding of the biological basis of psychological stress. Psychological stressors regulate the function and fate of various target cells through a neuro-immuno- endocrine network, affecting many physiological and pathological processes including wound healing. In short, under psychological stress, changes in all organs of the body (including plasticity changes in central nervous system function) are guided and based on changes in neuro-endocrine system [1].

8.1.1.1 Psychological Stress and Nerve: the Role of the Endocrine System in Skin Healing

Under stress, intricate neuroendocrine changes include those in the renin angiotensin system (RAS) and hypothalamic-pituitary-adrenal (HPA) axis activation, commonly known as the stress system. Adrenal catecholamines act on the HPA axis to maintain energy balance, RAS redistributes blood

B. Cheng · M. Xuan
General Hospital of Southern Theater Command of Chinese People's Liberation Army, Guangzhou, China

X. Fu (✉)
The Chinese People's Liberation Army General Hospital, Beijing, China

H. Liu
The First Affiliated Hospital of Jinan University, Guangzhou, China

C. Shi
Army Medical University, Chongqing, China

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flow to ensure blood supply to vital organs, nerve stimulation and fear, sadness, anxiety, contradiction, nervous changes from the high-level cortex, visual, taste and body, and the humoral signals such as hormones and cytokines activate the stress system and induce a series of behavioral and physiological responses. In the process of stress regulation, there are multiple levels of action sites in the central and peripheral stress systems. In addition to the HPA axis and the blue nucleus/norepinephrine-parasympathetic sympathetic system, there are other stress sites and mechanisms in the body, such as central dopaminergic neurons and hippocampus, and peripheral reproductive hormone axis, growth hormone axis, thyroid axis, and metabolic reactions. They play an important role in cognitive integration and neurohormonal and neurochemical effects in stress response. In order to adapt to psychological stress, neutrophils release substance P (SP) and activate mast cells or other inflammatory cells together with other inflammatory mediators from the sensory nerve to participate in the inflammatory response. The corticosteroid-releasing factor (CRF) and SP initiate a systemic stress response by activating neuroendocrine pathways such as the sympathetic nervous system, hypothalamic–pituitary axis, and the renin–angiotensin system. They release stress hormones (such as catecholamine, cortisol, growth hormones, glucagon, and renin). The skin and its attachments are important target organs for major stress mediators (such as corticotropic releasing hormone, ACTH, cortisol, catecholamine, prolactin, substance P, and nerve growth factor) and potential stress response immunoregulators. More skin exposure to a variety of exogenous and endogenous stressors than other organs provides an ideal clinical model for studying peripheral and systemic responses to stress, including psychological stress.

Brain–skin contact and local neuroimmune endocrine loops are both the pathophysiological basis of skin function and related changes, and the triggering factor for stress triggering and aggravation. To study the effects of psychological stress on wound healing, Detillion et al. performed adrenalectomy to remove endogenous cortisol to observe changes in psychological stress in solitary wound healing. It was found that positive social interaction participated in the activity change of HPA axis in rodents, which can promote wound healing. Ebrecht surveyed 24 non-smokers to determine the patient's sensory anxiety, healthy behaviors, and personal factors, as well as salivary cortisol levels at 2 weeks before and after biopsy. The results showed that the healing rate was negatively correlated with the perceived stress scale (PSS) and the general health questionnaire (GHQ).

Activation of the stress system leads to adaptive behavioral changes and physical changes. In addition to being closely related to the nervous and endocrine systems, the most basic stress hormones (glucocorticoids and catecholamine) can affect major immune functions and T helper (Th1) response to Th2 selectivity. To a certain extent, they may enhance the production of pro-inflammatory cytokines and activate the adrenocorticotropic hormone–mast cell–histamine axis, thereby feeding back the stress system and enhancing or reducing the immune response.

8.1.1.2 The Role of Psychological Stress and the Immune System in Skin Wound Healing

Psychoneuroimmunology (PNI) is a discipline that has studied immune and endocrine, central, and peripheral nervous systems for nearly 40 years. Neurotransmitters, hormones, and neuropeptides have been shown to regulate immune cells and communicate with neural tissue by secreting large amounts of cytokines. A key role of the central and peripheral nerves is to maintain cell-mediated (Th1) and humoral (Th2) immune responses, and psycho-neuroimmunology becomes the pathophysiological basis for understanding the link between the immune systems. Stress-induced immune disorders are sufficient to cause health-related consequences. PNI is the basis for a close correlation between the spirit/brain and the immune system.

The relationship between the central nervous system and the immune system is mainly accomplished by chemical messengers secreted by nerve cells, endocrine cells, and immune cells. Psychological stressors can damage this network. Early investigations have found that mental stress affects the body's immune function, and stress plays a non-negligible role in the immune system. Emotional anger is associated with corticosteroid secretion, immune function, and non-adaptive changes in surgical recovery. Assuming the relationship between extroverted and introverted emotional anger or non-anger control and delayed healing, the results do show that human emotional anger is closely related to wound healing. In acute stress, endogenous stress hormones enhance the skin's immunity by increasing lymphocyte trafficking and cytokine gene expression at the site of antigenic invasion. In acute wounds, when women are in high stress, there are two key cytokines IL-1 and IL-8 in the wound area, indicating that the production of pro-inflammatory factors in the local microenvironment of wound healing is affected by psychological stress.

The regulation of the immune system by psychological stress is complex, so the effect on wound healing also shows different outcomes. As Weinman's research suggests, the openness of traumatic experience can lead to upregulation of the immune function, which is the key to promoting wound healing. In order to confirm that stress increases wound infection, Rojas found that the skin wounds had viable bacteria through quantitative observation. It was found that inhibition stress (RST) delayed the healing ability by 30%, and the conditional pathogen increased compared with the control group, and was statistically significant ($P < 0.05$). Further

studies have found that RST-induced glucocorticoids play an important role in the mechanism of bacterial clearance. If treated with the glucocorticoid receptor antagonist RU486, pathogenic bacteria will be reduced ($P < 0.05$). Therefore, stress-damaged bacterial clearance during wound healing leads to a marked increase in the incidence of conditioned pathogen infections; psychological stress delays wound healing, reduces immune/inflammatory responses, and also influences bacterial clearance. In short, the complex effects of psychological stress on the immune response present a variety of patterns of response to wound healing.

The skin's nerve and the central nervous system (including psychology), the endocrine axis, and the immune system are a complete stress system. To fully understand the various physiological and pathological functions of the skin, including cell growth, immunity, inflammation, and healing, it is necessary to have a deeper understanding of the dense sensory neural network, the complex central nervous system (including psychology), the release of the multi-layered surface, as well as active specific receptors expressed on the target cells of a large number of skin.

8.1.1.3 Related Signal Pathways Involved in Psychological Stress and Cytokines in Wound Healing

Psychological stress changes the cytokines around and in the brain, but physiological evidence is still lacking. At present, it is necessary to study the role of acute and chronic psychological stress factors in the cytokine network in humans, explore the pathophysiological significance of cytokines in the process of psychological stress, and investigate whether cytokines play a synergistic or antagonistic role in complex networks. The balance between pro-inflammatory cytokines (such as IL-1b, IL-6, TNF-α) and anti-inflammatory cytokines (such as IL-1 receptor antagonists, IL-4, IL-10, TGF-β) reflects neurological and mental strength, neuroimmunity, and neuroinflammatory reactions. Maes suggested that psychological stress as an immobilization can lead to activation of the inflammatory response system. Moreover, stress-induced cytokine secretion can induce and maintain depressive symptoms. Psychological stress (such as sleep deprivation) will reduce skin function recovery and increase the activity of IL-1β, TNF-α, and NK cells. Therefore, the effects of psychological stress on cytokines also affect skin function and wound healing. Wound healing is an orderly, complex physiological process involving inflammation, proliferation, and shaping, which are effected by different cells, cytokines, and matrix metalloproteinases. Cytokines are involved in inflammation and immune responses, as well as cell differentiation and proliferation. Therefore, changes in wound healing caused by the effects of psychological stress on cytokines have also become the focus of research. It has been shown that in animals, sustained stress induces changes in the kinetics of epidermal proinflammatory cytokines, such as IL-1α and IL-1β, as well as the expression of growth factors in the early stages of wound healing. The inflammatory response mediated is often considered to be a predisposing factor for scar formation. It is currently one of the strategies for the treatment and prevention of keloids by inhibiting the IL-6 receptor and its downstream effectors or molecules.

Environmental stress-induced biological and psychological changes are often accomplished by a number of signaling proteins, particularly in the HPA axis. This system also potentially acts on reactive oxygen species (ROS) and acts on cytokines, and finally controls DNA regulation to methylate the promoter regions of these genes. This also suggests that environmental stressors have mechanisms that induce long- term biological changes. In addition, two pathways for stress signaling, the JNK/SAPK and p38 pathways, were studied. The core molecules of these two pathways are c-Jun N-terminal kinase (JNK) and p38 kinase, which are also called stress-activated protein kinase 1/2 (SAPK1/2) and p38, both belonging to mitogen-activated protein kinase (MAPK). The amygdala is thought to be the key to mediating stress-induced changes in hippocampal function. Yang demonstrated that stress immediately causes phosphorylation of extracellular signal-regulated kinase (ERK) in the CA1 region of the hippocampus and central amygdala (CEA) and basolateral amygdala (BLA), indicating that the signal path is activated.

The team of Academician Xiaobing Fu has made some explorations in this regard. First, the animal model of psychological stress was observed for wound healing. C57BL/6 mice aged 6–8 weeks were used (provided by SPF Animal Laboratory, Institute of Neuroscience, Fourth Military Medical University). They were taken into lab 1 week before the experiment to adapt to the new breeding environment. The stress mice model was prepared according to the restraint stress model produced by Padgett et al. in 1998. After establishing this stress model, the serum cortisol level in mice was four times higher than that in the control group. At 20:00 h every night, the mice were introduced into a well-ventilated 50 mL centrifugal tube (four rows of appropriate holes were placed on the wall of the centrifuge tube, a hole was made at the tip of the centrifuge tube, and a hole in the middle of the centrifugal tube cover so that the mouse's tail reached out). Activities restriction,fasting and water cessation was done during period. After the mouse enters the 50 mL centrifuge tube, it cannot be turned over and turned around, it is not movable. After 12 h of restraint, the mice were taken out at 8:00 h the next morning. After three times of restraint, the model of full-thickness skin defect in the back of the mouse was made. (A special puncher was used on both sides of the back spine of the mouse, 0.8 mm in diameter, 0.64 mm^2 in area, and two round full-thickness skin wounds were cut), and continue

to bind five times after the wound was made. At the same time, the control group and the stress group were fasted, water-free, and wounded in the same period of time, so that the experimental group and the control group had the same conditions except for the constraint factor. In a certain period after trauma, the re-epithelialization rate of the wound in the stress group was higher than that in the unstressed group. Reverse transcription PCR was used to detect the expression of ACE, AT1, and AT2 mRNA in skin tissue around the wound (Figs. 8.1, 8.2, and 8.3).

Compared with normal controls, the expression levels of ACE, AT1, and AT2 mRNA increased after infection, and the expression levels of ACE and AT1 were down-regulated and then increased in the stress group, while AT2 was not down-regulated and raised immediately after wounding. The peaks of the three mRNA expressions in the stress group and the unstressed group differed at time points. From the relative expression level, the expression level of AT2 was lower than that of ACE and AT1 in normal control tissues, but the up-regulation of AT2 expression was highest after trauma.

Images acquired with the Olympus Model FV-1000 laser scanning confocal microscope and image acquisition system (Figs. 8.4, 8.5, and 8.6).

It can be seen from the figure that in the skin of the normal control group, ACE is slightly expressed in the basal layer of the epidermis and the connective tissue of the dermis. At2 is expressed in the basal layer of the epidermis and in the hair follicles and sweat glands. At1 is not expressed.

In Figs. 8.4, 8.5, and 8.6, C represents the unstressed group, S represents the stress group, d represents the post-traumatic days, and M is the abbreviation of merge. Red is ACE, blue is AT1, and green is AT2. Compared with normal tissues, the expression of ACE, AT1, and AT2 was enhanced after trauma, both in the stress group and in the unstressed group. ACE is mainly expressed in dermal connective tissue. The expression of AT1 is the weakest and is relatively strong outside the hair follicle. AT2 is expressed in the epidermal base and hair follicles and sweat glands. There is almost no overlap in the expression of ACE and AT2. This study showed that the wound healing rate of mice under moderate stress was significantly higher than that of the unstressed group.

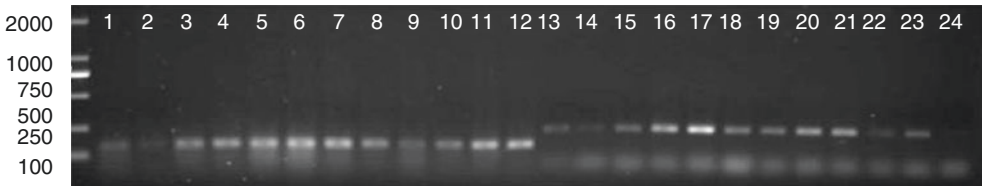

Fig. 8.1 ACE mRNA expression

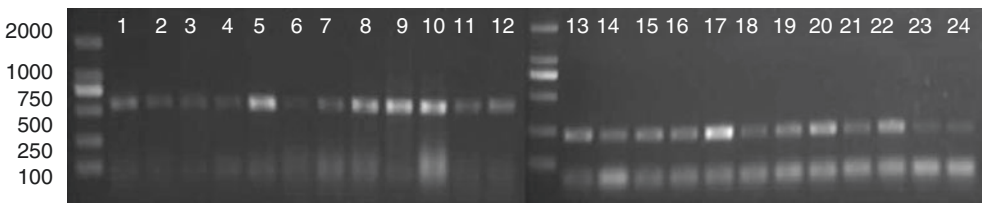

Fig. 8.2 AT1 mRNA expression

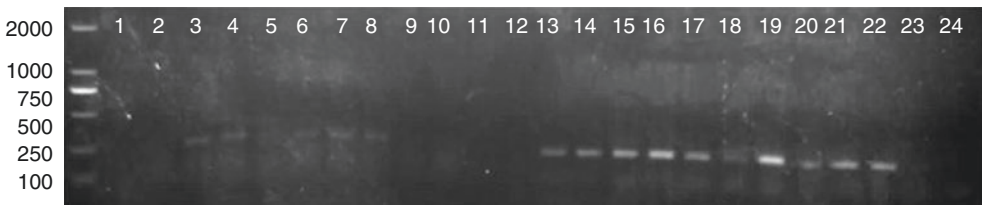

Fig. 8.3 AT2 mRNA expression

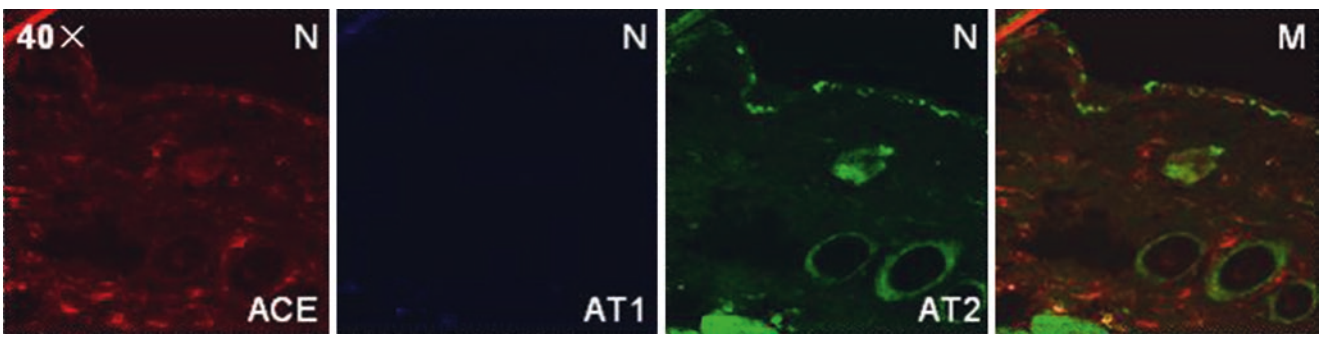

Fig. 8.4 Expression of ACE, AT1, and AT2 in normal skin tissue

Tips that moderate stress may accelerate wound healing. The amount of ACE, AT1, and AT2 in the local tissue RAS system during wound healing was significantly different between the stress group and the unstressed group. In short, stress affects the speed and quality of wound healing. Adjusting stress levels can help improve tissue repair. Activation of the local angiotensin system in skin tissue may be one of the stress response pathways. Reactive changes in Ang I, Ang II, and ACE in systemic and local skin tissues may occur during stress response.

In addition, important members of the Xiaobing Fu academic team also used music therapy to psychologically intervene in 62 surgical patients, 55 of whom were satisfied [2]. After the music-assisted intervention, the tension was improved, the pain was relieved, and the clinical score was based on the original score. Declined 28 people were reduced from VAS > 8 points to 6–8 points; 27 people were reduced from high (6–8 points) to good (3–5 points). Another five people felt relieved, but not obvious (no change in VAS score). Only two people reported no change; the patients were male and aged <30 years. Observing the effect of music therapy on the patient's heart rate, playing light music for the patient during the operation, the patient's heart rate decreased significantly, and there was significant statistical difference before the music treatment ($P < 0.05$). The patient's systolic blood pressure also decreased. The change in diastolic blood pressure was more pronounced and there was a statistical difference ($P < 0.05$). All patients underwent orthopedic suture removal 5–7 days after surgery, and the wounds healed well without a significant difference. It shows that patients with plastic surgery can have anxiety psychological stress before surgery, and the proportion is higher than other surgical patients. Increased responsiveness of systemic Ang I, Ang II, and ACE may occur during stress response. The blood in the preoperative and postoperative period is mainly the change of ACE content in the important components of the angiotensin system. The use of music therapy during surgery can improve the patient's anxiety state. Although there is no significant change in the time of surgical wound healing, the pain of surgery has been significantly improved.

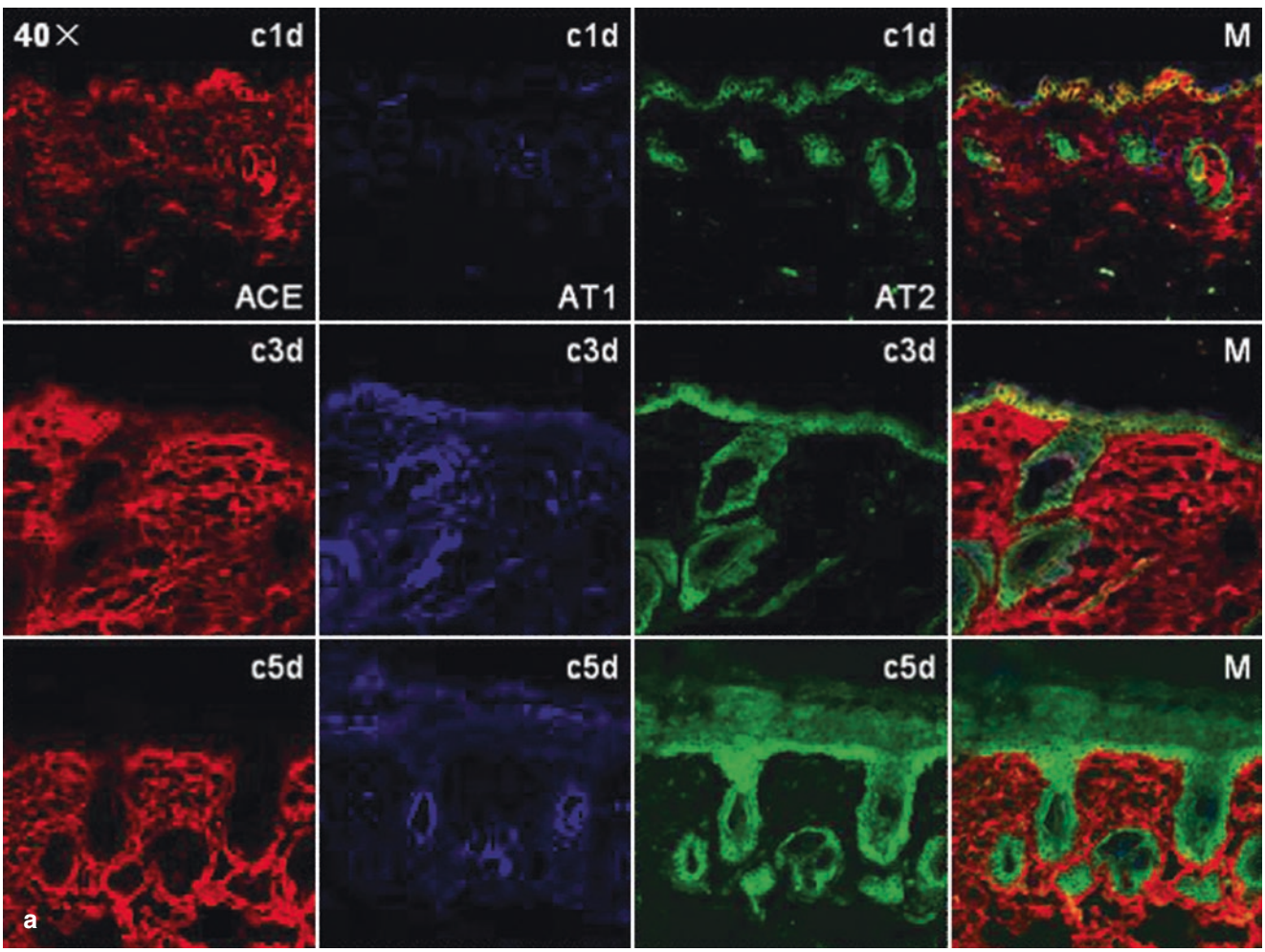

Fig. 8.5 Expression of control group ACE, AT1, and AT2

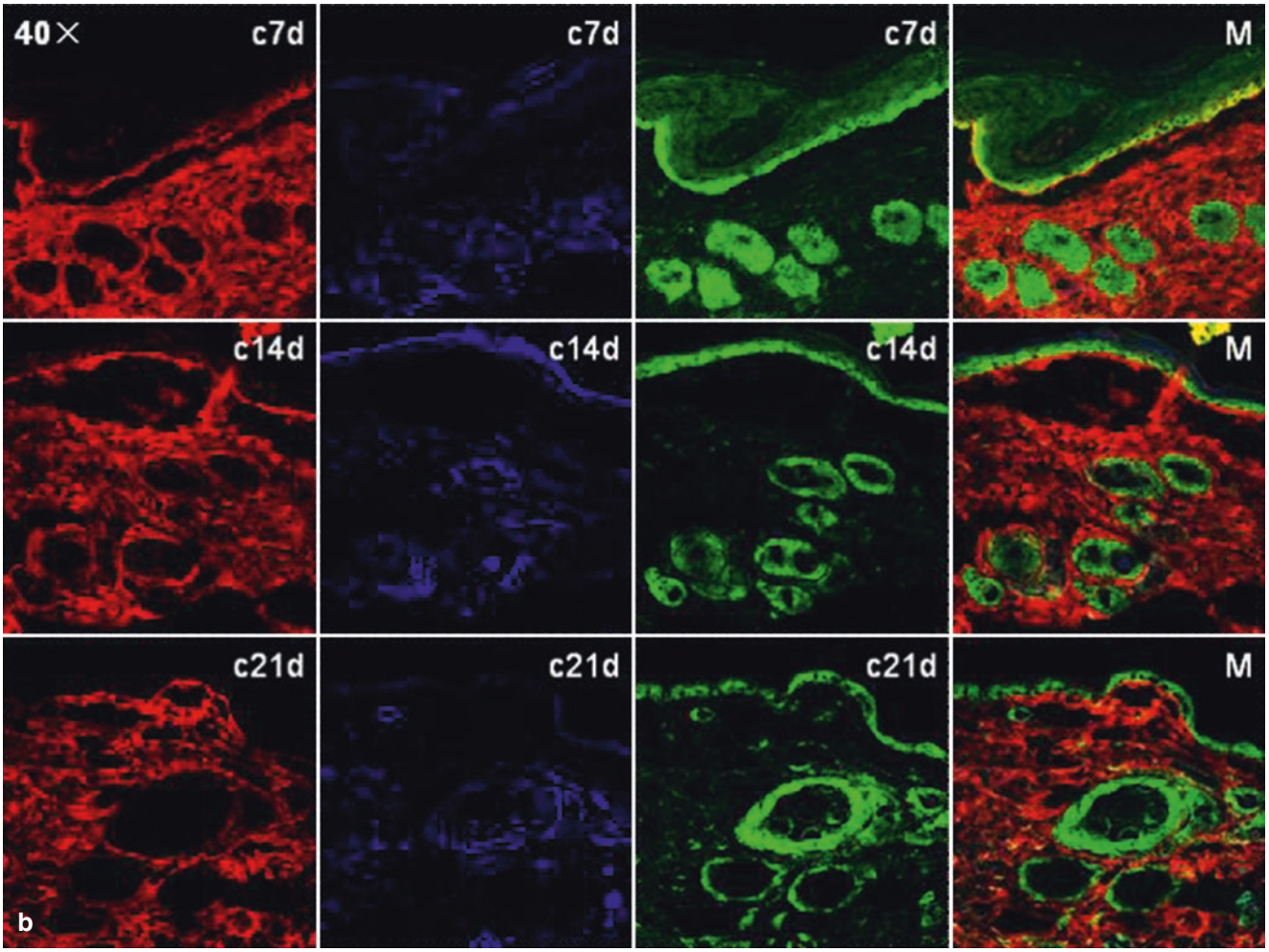

Fig. 8.5 (continued)

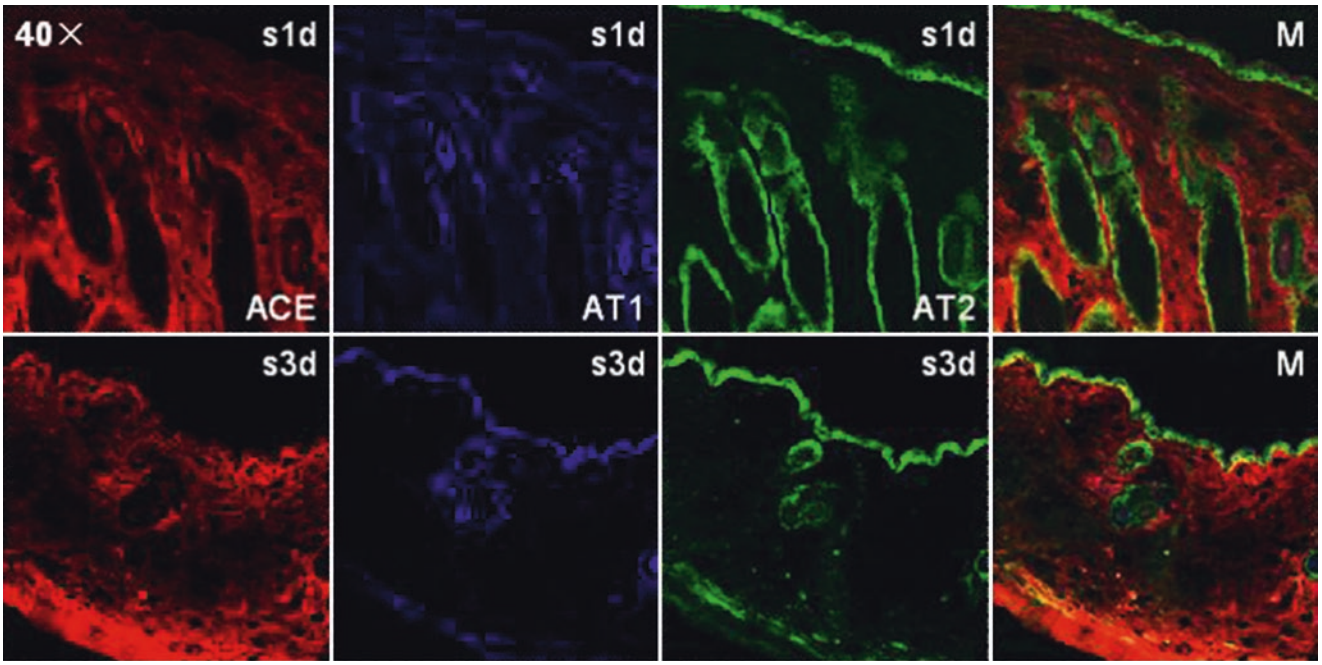

Fig. 8.6 Expression of ACE, AT1, and AT2 in stress group

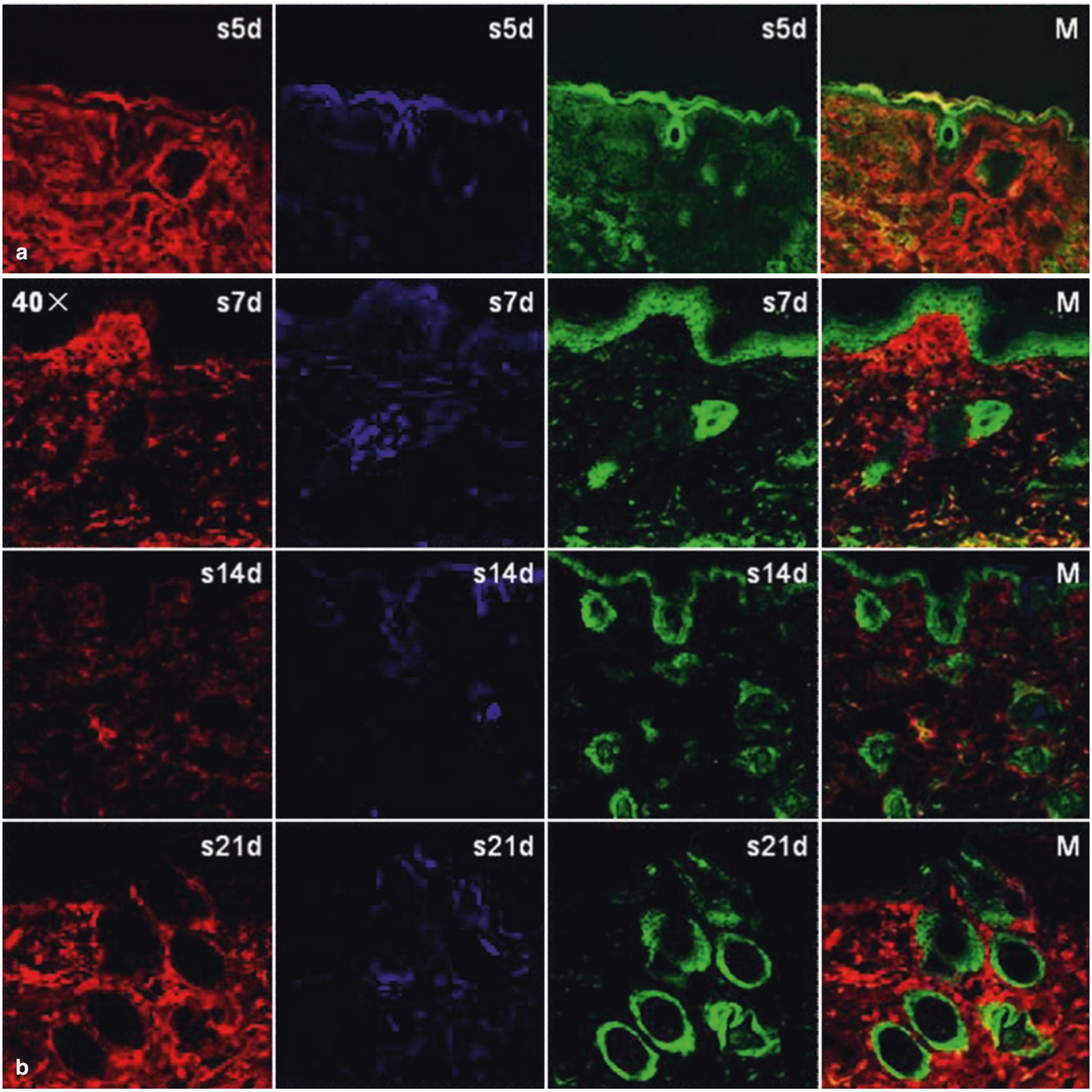

Fig. 8.6 (continued)

The whole study suggests that the psychological stress system may have some connection with the systemic and local RAS system, that is, when the skin is damaged, the systemic and local RAS are activated, and the tissue local ACE, Ang II, and other components regulate the biological behavior of repairing cells and the producing of biologically active target proteins (neuropeptides, heat shock proteins, etc.) and changes on the phenotype of repair cells, thereby affecting cell proliferation and apoptosis, angiogenesis, and collagen synthesis activities, and ultimately regulating skin damage repair process. This study used music-assisted interventions to explore whether music can help alleviate stress during surgery. It will provide new ideas and means for improving the healing outcome of tissue fibrosis, repairing difficult wounds, and treating scars.

Psychological stress has made some progress in the study of the effects of wound healing. Psychological stress involves a complex network of the central nervous system, endocrine system, and immune system. When psychological stress changes, breaking the equilibrium state of this network will

lead to the changes in body physiology and pathology, especially in skin damage repair activities, and also cause the changes in tissue healing speed and outcome. However, there are many unknowns about the detailed mechanisms of how the "psychological" becomes a "physical" physiological response, especially the changes in the number of neuroendocrine substances under stress, and the transmission pathways of stress signals. In addition, we need to study the relationship between the regularity of these substances and the levels at which the regulation of stress are mutually activated or mutually inhibited to ensure the continuity and effectiveness of the body's regulation, further research is needed. Understanding the psychological, neurological, immune, and endocrine regulation of the skin helps to provide new strategies for accelerating the speed of skin tissue repair and improving the quality of skin healing.

8.1.2 Age

Age is the main systemic factor that affects wound healing. As elderly patients grow with age, the body's ability to regulate is reduced, body water is reduced, and various metabolic rates slow down. After being stimulated by external damage, the stress response is weak, the immune system response is poor, and cell movement, proliferation, and maturation are significantly slowed down, which can affect wound healing. Especially in the early stage of injury, the inflammatory response is weak, the secretion of various cytokines is insufficient, the regeneration of new aging is delayed, the synthesis of collagen fibers is reduced, and the skin becomes dry, resulting in slow wound contraction. Children and young people have stronger metabolism, cell proliferation, collagen synthesis, and epithelial regeneration time are shorter than the elderly, as well so that wound healing is faster.

8.1.2.1 Biological Structure and Related Functional Characteristics of Aging Skin

The biological structure and related functional characteristics of aging skin are shown in Table 8.1 [3].

1. Epidermis
   As the outermost layer of the skin, as the age increases, the size of the keratinocytes increases, the shape is irregular, the number decreases, the volume of the cells increases, and the inter-cell desmosomes gradually disappear. The nipple and corresponding protrusion of the skin are flattened, and the connection with the dermis is loose, resulting in a decrease in the skin barrier function, a decrease in hydration ability, and a dry skin. At the same time, due to aging, there are fewer basal cells or germinal cells with mitosis, and thus the production of correspond-

Table 8.1 Major structural and functional changes in aging skin and related clinical manifestations

Skin composition	Primary function	Changes after aging	Functional change	Clinical symptoms
Epidermis	Barrier between the body and the environment	Cuticle thinning, renewal of keratinocytes is slow, the lipid components of the stratum corneum are reduced, the number of melanocytes is reduced, the acidification is weakened, and the PH value changes	The integrality of the skin barrier is weakened, the recovery of the barrier after injury slows down, the wound healing slows down, the stratum corneum hickens, and the photosensitivity increases	Dry skin, increased sensitivity to bacterial, viral and fungal infections, increased incidence ofcontact dermatitis and irritant dermatitis, slow wound healing/chronic wounds
Dermis	To provide elasticity and tension	Thinned, the proliferation of fibroblast was decreased, the ability of collagen I and III synthesis was decreased, the collagen fiber and elastic fiber were decreased	Reduced elasticity, reduced resistance to all kinds of damage and friction	Wrinkles, delayed wound healing, blisters, ulcers
Subcutaneous fat	Support, insulation	Decrease	Reduced support for the dermis and blood vessels	The sensitivity to various damages increased and the heat regulation function weakened
Blood vessel	To provide nourishment	The structure of blood vessels atrophied, the density of blood vessels decreased and the blood vessels became thinner	Reduced blood supply, reduced nutrition, reduced response to changes in stress	Contusion, purpura, reduced heat regulation
Sweat gland	Sebum secretion	Decreased sebum production and secretion	Reduced sebum on the skin surface	Dry skin, hyperplasia of sebaceous glands
Hair follicle	Hair production	Less hair density, less melanin production	Less hair density, less melanin production	Hair removal
Nerve ending	Sense	Diminish	Reduced sensitivity to changes in pressure and heat	Increased risk of skin damage

ing stratum corneum cells may be reduced. Under normal conditions, the epidermis forms cells from the basal layer and gradually proliferates, divides, and moves upward until the outermost layer of the epidermis, and the cells gradually form keratin, and the cell dynamic process from the basal layer to the stratum corneum is keratinized. With age, the ability of epidermal cells to divide and proliferate is reduced, the rate of cell renewal slows down, and the connection between the epidermis and the dermis is more toward the direction of shear. These are the causes of epidermal flattening.

2. Leather
   There are fibroblasts, various types of fibers, matrix, and accessory structures such as sweat glands, hair follicles, and sebaceous glands in the dermis. With the increase of age, the number of fibroblasts decreases, the subcellular level shows less cytoplasm, the lipid brown particles increase, and the cell viability decreases. The number of elastic fibers and collagen fibers is reduced and the arrangement is disordered. The synthesis capacity of the extracellular matrix is reduced, the activity of collagenase is increased, and the decomposition of collagen is increased, resulting in a thinning of the dermis. There is elastic tissue degeneration, mainly characterized by thickening, curling, and kinking of elastic fibers, forming amorphous, granular materials, disordered or aggregated, deposited in dermal tissue. It is generally believed to be caused by the degradation of normal elastic fibers and the abnormal expression of elastic fiber components such as elastin.
3. Blood Vessels and Nerves
   As the age increases, small blood vessels begin to degenerate, capillary vasospasm gradually disappears, and the number of capillaries decreases. The neurological structure undergoes degeneration, the pain sensitivity value decreases, the stress ability to various stimuli decreases, and the tissue regeneration ability becomes weak.
4. Skin Appendages
   The accessory organs of the skin mainly include the sweat glands, sebaceous glands, and hair. Due to the decrease in the number of skin blood vessels in the elderly, the number of viable glands is reduced, the function of the secreted cells is disordered, and even fibrosis occurs, the amount of secretion is decreased, and the response to various types of stimulation is lowered. The skin is an organ that is very sensitive to sex hormones. Therefore, sweat glands, sebaceous glands, and hair are regulated by sex hormones. As the age increases, the hormone secretion capacity decreases and the hormone level decreases, resulting in enlarged pores, whitening, reduction, and thinning of hair. Sebum production and sebaceous gland morphology cannot maintain parallel changes and balances. Human skin is smooth and sebum plays an important role. The skin evaporation ability of the elderly is mostly lost, and the heat dissipation function is obviously degraded.
5. Other
   The number of mast cells, Langerhans cells, and melanocytes in the dermis gradually decreases with age. The reduction in the number and function of such cells in the skin tissue of the elderly can cause a decrease in the mediator effect of immune cells, which affects the healing process of the wound. The number of melanocytes in aging skin decreases. The melanocytes are larger in volume and the dendrites are increased, which may also cause neuroimmune disorders and affect the wound healing.

   The skin barrier function also changes. The stratum corneum of the outer layer of the skin serves as a barrier between the body and the external environment. In the case of aging, the lipid component is reduced, resulting in a weakened barrier function and a dry skin. With aging, the pH of the skin changes. The weakening of the skin barrier function causes various external stimuli and antigens to easily enter the skin, and the incidence of skin diseases such as allergies and contact dermatitis increases.

8.1.2.2 The Impact of Aging on the Healing Process

In general, the purpose of wound repair is to restore damaged tissue and ensure its original integrity. Much of the damage is caused by changes in gene expression, and when aging occurs, c-Fos expression decreases at the transcriptional initiation level. In addition, transcription factors such as NK-κB, AP-1, and Sp-1 were reduced, and several growth-related genes decreased with the activity of E2F transcription family members. At the same time, hormone levels in tissues, mitogen growth factors, and receptor changes in these factors are also responsible for delayed healing of wounds in elderly patients.

1. Infiltration of Inflammatory Cells
   After trauma, the infiltration ability of inflammatory cells (including monocytes, macrophages, and B lymphocytes) around the wound in elderly patients is reduced; these changes will cause delays in healing. In addition, the number of dermal cells in the elderly patients decreased, resulting in decreased release of histamine and decreased migration of capillary endothelial cells, which also affected the rate of healing.
2. Repair Cell Proliferation and Differentiation
   Experiments have shown that the ability of old rat mesenchymal cells to transform into myofibroblasts decreased after injury, which affected the closure time of the wound. As age increases, the number and proliferation activity of fibroblasts decrease, affecting their ability to produce and

regulate collagen, which is the key factor leading to the above performance.

3. Synthesis and Deposition of the Matrix
   An important step in wound healing is the filling of the wound and the filling of the defect. The collagen in the elderly patients changes in both quantity and quality, resulting in poor filling and slowing of contraction. At the same time, the display of collagen in the elderly patients is disordered, and the decrease in the diameter and number of elastic fibers causes the tension to become smaller, which not only causes the wound healing to be hindered, but also makes the wound after healing easy to crack.
4. Epithelialization
   Animal experiments have shown that the expression of matrix metalloproteinase-1, 9 and matrix metalloproteinase inhibitor-1 in aged animals is significantly lower than that of young animals, resulting in changes in the biological behavior of keratinocytes. It causes delayed reepithelization during wound healing.

8.1.2.3 The Impact of Other Changes in Aging on Healing

Wound healing is a complex and orderly process. In normal healing conditions, the recovery of the skin barrier is dependent on keratinocyte migration of the skin, contraction of fibroblasts, and reconstruction of the extracellular matrix. In the elderly, wound healing is impaired, characterized by slowing of keratinocyte proliferation, decreased rate of epithelialization, decreased proliferation of fibroblasts, slowing of granulation tissue formation, and excessive inflammatory response. The nervous, endocrine, and immune systems are widely distributed in the body. The nervous system has synaptic-mediated structural continuity, and its branch ends govern various tissues and organs. Taking the skin as an example, there is a difference in the innervation of epidermal keratinocytes. In addition to keratinocytes, dendritic cells with special effects in the epidermis, Langerhans cells, melanocytes, Merkel cells, and fibroblasts in the dermis are closely related to nerve fibers. Broadly speaking, the endocrine and immune systems can be regarded as the efferent part of the nervous system reflex arc. Stress can directly or indirectly affect the functional status of the three major systems. The mode of action between the systems is not only direct or indirect, but also simultaneous and sequential. The nature of interaction between systems can be enhanced, weakened, modified, allowed, or coordinated by frequency conversion, time change, variable force, etc. The attribute of the interaction between systems, there are physiological and pathological points, is the process of mutual transformation of quality and quantity. The neural, immune, and endocrine networks more closely encompass the interactions and multiple connections between the three systems. As the age continues to grow, the internal environment of the elderly may be on the edge of a relatively "unbalanced" state or "unbalanced", manifested by decreased stress, nervous system disorders, decreased hormone levels, low immune function, the ability of the body to repair and maintain its own stability is also reduced. How to adjust the body's nervous system, hormone secretion level and immunity to the state of young people, and make the metabolism of cells (especially adult stem cells) re-energize to reverse or delay the process of aging, has become a new direction of current geriatric research [4].

1. Changes in the Nervous System of the Skin
   The skin contains a dense network of sensory nerves, and the intact nervous system is essential for wound healing. Experiments have confirmed that primary afferent sensory nerves as nociceptors are extremely important for initiating inflammatory responses and successful tissue repair. With the onset of aging, this system partially loses the function of releasing factors, leading to delayed healing of wounds. At the same time, many physiological functions of the skin (such as metabolism, immunity, etc.) are also inseparable from nerve innervation. In addition, the neuropeptide released from the sensory nerves in the skin not only satisfies the requirements of innervation in the skin, but also is an important substance that directly regulates the function of keratinocytes, Langerhans cells, mast cells, microvascular endothelial cells, and infiltrating immune cells. In particular, tachykinin, substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide and somatostatin on the proliferation of cells, the production of cytokines, etc. have a regulatory effect. With increasing age, the release of sensory neuropeptides is reduced, and the effects on various types of cells in the skin are weakened.
2. The Effect of Hormonal Changes on the Skin
   Sex hormones are another important factor in wound healing. Providing repaired patients with direct or indirect hormone replacement therapy can increase cell proliferation activity and cytokine production, affect the post-injury inflammatory response, increase matrix deposition, and promote epithelial healing in elderly patients. With age, changes in estrogen and androgen levels after menopause directly affect the phenotype of endothelial cells and fibroblasts, and play a role in wound repair by regulating their adhesion and proliferation behavior. In addition, studies have found that inflammatory response changes in male patients are significantly different from female patients, resulting in delayed healing of acute wounds. Sex hormones play an important role in the body's local and humoral immunity, which may be the main mechanism affecting healing.
3. Changes in the Immune Function of the Skin
   Compared with young people, the immune function of the skin of the elderly has changed significantly. Mast cells, monocytes, and neutrophils increase, and the num-

ber of t lymphocytes also increases with skin aging, but their response to external antigens is low. In contrast, the number of Langerhans cells and the ability to migrate are reduced. A decrease in skin immune function and barrier function leads to an increased incidence of bacterial, viral, and fungal infections in the elderly.

4. Skin Blood Vessels and Blood Supply Changes
   With age, there is a certain regular change in the degradation and disorder of small blood vessels. Many capillaries and veins are missing. The nipples in the skin tissue disappear, the capillary sputum gradually disappears, and the number of capillaries is inevitably reduced. According to relevant research, the number of blood vessels in the skin of the elderly is reduced by at least 30%. As skin aging worsens, the number of vascular reductions is more prevalent and more severe. The skin damaged by light has only a few swelling and distortion of blood vessels. The blood flow of elderly patients is reduced by 35%, which leads to the failure of local perfusion, which cannot meet the oxygen and nutrients needed for wound healing, and is also the main reason for poor wound healing.
5. Changes in the Secretion Capacity of Growth Factors in the Skin
   The characteristics of aging are mainly manifested by the decrease of the overall function of the cell, and this relative effect is regulated by growth factors. The application of transforming growth factor-β1 (TGF-β1) in local and system can enhance the healing of wounds and regulate the functions of several types of repair-related cells, such as matrix production and angiogenesis. TGF-β1 plays a role in regulating cell differentiation and extracellular matrix formation in the late stage of healing. Vascular Endothelial Growth Factor (VEGF) is a polypeptide that is also a potential, special mitogen that promotes endothelial cell proliferation, migration, and endothelial surface retraction (allowing vascular penetration) and angiogenesis in living organisms and affects wound healing and tissue reshaping. Animal experiments have found that the expression level of growth factors in aged animals is lower than that of younger animals.
6. Skin Barrier Function and Dryness
   Skin barrier function and dryness refers to a series of symptoms such as tightness, desquamation, and itching caused by moisture or lipid deficiency. It is the most common skin problem in the elderly. The self-repairing ability of dry skin is weakened, and the healing rate of skin wounds (such as pressure sores, scratches, etc.) is slowed down and the skin is intact.

The main members of the team of Xiaobing's Fu academicians collected medical records of hospitalized patients with plastic surgery in a plastic surgery department in a southern hospital from July 2011 to December 2013 [5]. 120 cases of refractory wounds were screened for 2 months of non-healing of skin tissue defects. The causes of wound formation, age, location of wounds, and length of hospital stay were retrospectively investigated. 2136 inpatients were counted, including 120 patients with refractory wounds, accounting for 5.6%. The main cause of wound formation was metabolic disease (43.3%), followed by traumatic infection (20%) and tumor (20%) ($\chi^2 = 62.917$, $P < 0.01$). The high-incidence age of patients with wound healing was 40–60 years old, followed by 60–80 years old ($\chi^2 = 29.562$, $P < 0.01$).

The most difficult sites for refractory wounds were limbs (61.6%), and the most common were the feet (38.3%) ($\chi^2 = 17.546$, $P = 0.002$). Middle-aged and elderly patients with metabolic diseases become the main group of refractory wounds. Unhealthy wounds often occur in the limbs, which seriously affects the patient's actions, causing delays in hospitalization, and bringing great burdens to family members and society.

Later, the team of Academician Xiaobing Fu conducted a more in-depth study on the differential expression and correlation of autophagy in the healing process of acute wound healing in rats of different age groups [6]. Autophagy, a system involved in intracellular degradation during aging, has been shown to be closely related to the body's aging. Skin tissue, as part of the organism's organic whole, may also be associated with autophagy. The role of autophagy in age-related changes in human skin is still unclear. The changes of autophagy-related genes LC3, Beclin-1, and p62 expression during acute wound healing in rats of different age groups were studied to observe the difference of autophagy activity between the old and young rats in the normal skin and in the process of wound healing.

SPF grade SD rats (provided by Guangdong Experimental Animal Center) were selected, in which young rats were 3 months old, $n = 16$, and weighed 200–300 g. The older rat group were 18 months old, $n = 16$, weight 600–800 g. All of the above were half male and female, and the mixed feed was kept in a single cage.

They were exposed to natural light and the room temperature was controlled at around 25 °C. After 3 days of routine feeding in rats, the hair in the back area was removed with an electric razor and hair removal cream, and 10% chloral hydrate was anesthetized at a dose of 3 mL/kg. After anesthesia was successful, a skin puncher was used at each 1.5 cm on each side of the spine in the middle of the back of the rat, a circular full-thickness skin resection wound of 1.2 cm in diameter was made, the whole skin and superficial fascia were cut, and then wetted with physiological saline to wipe the wound blood, and the wound covered with aseptic dressing, and the above treatment was carried out in a single cage. The wounds of the 0th, 4th,

7th, 14th, and 21st days were taken from the two groups. All the specimens were collected under aseptic conditions. The wounds and surrounding skin specimens were cut from the outer edge of the new epithelium at 2–3 mm. Part of the specimen was fixed in a 10% neutral formalin solution and a portion was stored in a –80 °C refrigerator. The specimens were placed in a 10% neutral formalin solution for 48 h and then routinely dehydrated, embedded in paraffin, serially sectioned, and sliced 5 μm thick. Under light microscopy, in normal skin, the younger group of rats had thicker epidermis, and the subcutaneous tissue and dermis were not clearly defined. In the elderly group, the thickness of the epidermis was thinned, and the connection between the epidermis and the dermis became flat and relaxed. The amount of collagen fibers and elastic fibers in the dermis is reduced, irregularly arranged, and often broken into pieces. At 4 days, the dermis of the young group showed obvious expansion of capillaries, a large number of neutrophils and macrophages infiltrated, and a large amount of granulation tissue was formed; the neutrophil infiltration and the number of dermal capillaries in the elderly group were less than those in the young group. At 7 days, the young group showed that the wound was basically filled with granulation tissue and the inflammatory cells were reduced. The wounds in the old group were not filled with granulation tissue, and there were still a large number of inflammatory cells infiltrating. At 21 days, the young group's wounds healed basically, the granulation tissue was fibrotic, and the scar tissue was transformed. Masson trichrome staining showed that the number of collagen fibers increased compared with the old group. After 21 days, the old group was completely or nearly healed. At this time, a small amount of inflammatory cell infiltration was observed. The newly accumulated dermal collagen accumulated loosely, the number was small, the arrangement was irregular, the fragments were diffuse, and the regular accumulation of collagen was less (Figs. 8.7, 8.8, and 8.9).

Under electron microscopy, the autophagic cells can be seen in the damaged organelles, such as the swelling and degeneration of mitochondria, surrounded by a vacuole-like bilayer membrane-like structure, or a bilayer membrane surrounding the mitochondria to form auto phagosomes, also showing a remnant that ultimately cannot be degraded by autophagy lysosome, etc. In this experiment, in the keratinocytes and fibroblasts of normal skin of young and old rats, autophagic vacuoles of cytoplasmic bilayer membrane or multilayer membrane were observed, and the size was different, organelle residues such as mitochondria, endoplasmic reticulum, ribosomes can be seen, the number of autophagic vacuoles in the elderly group was higher than in the younger group (Fig. 8.10).

LC3 and Beclin-1 were expressed in the epidermis and dermis of normal skin of young and old rats. After trauma, positive expression was also observed in the epidermis, dermis, hair follicles, new granulation tissue, vascular endothelial cells, macrophages, and fibroblasts at the

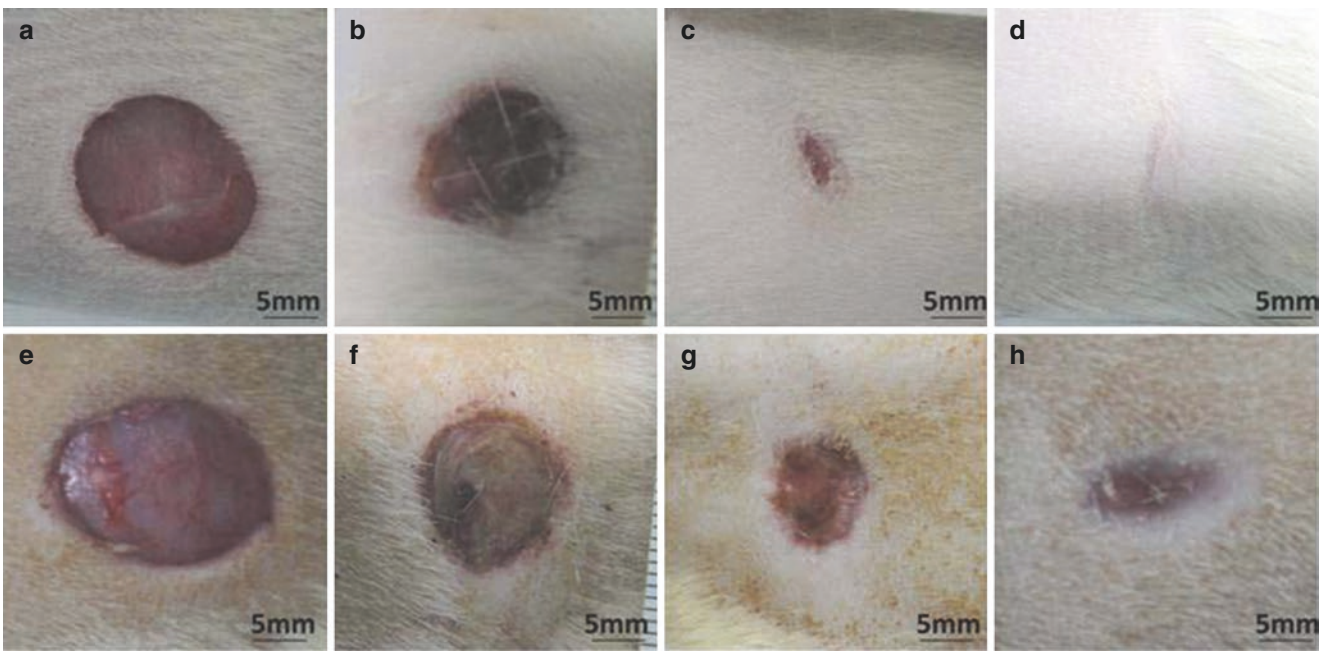

Fig. 8.7 Wound healing in rats of different age groups

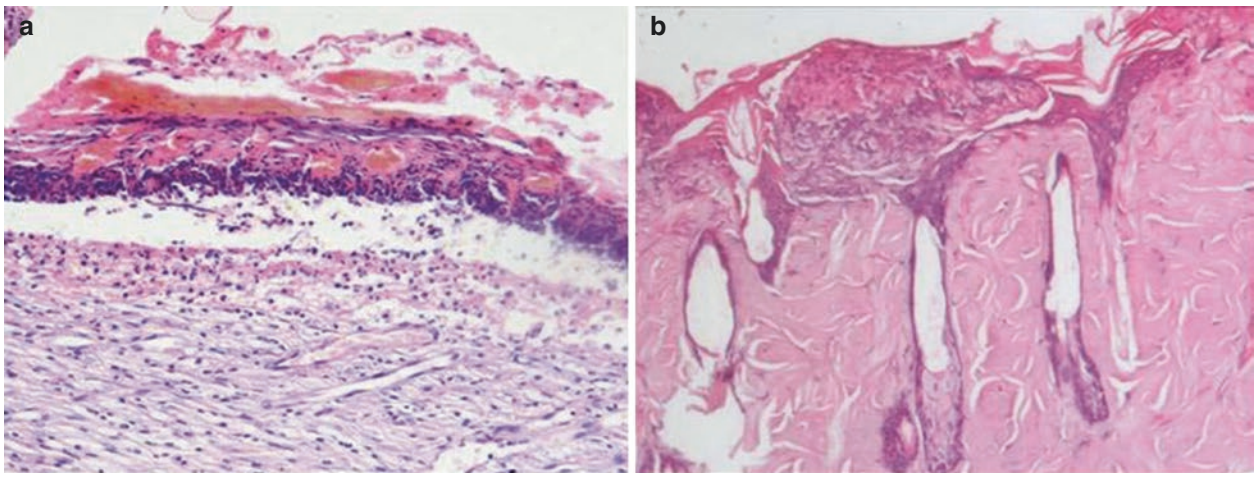

Fig. 8.8 4 Days' skin wounds of rats of different age groups (HE staining, ×100)

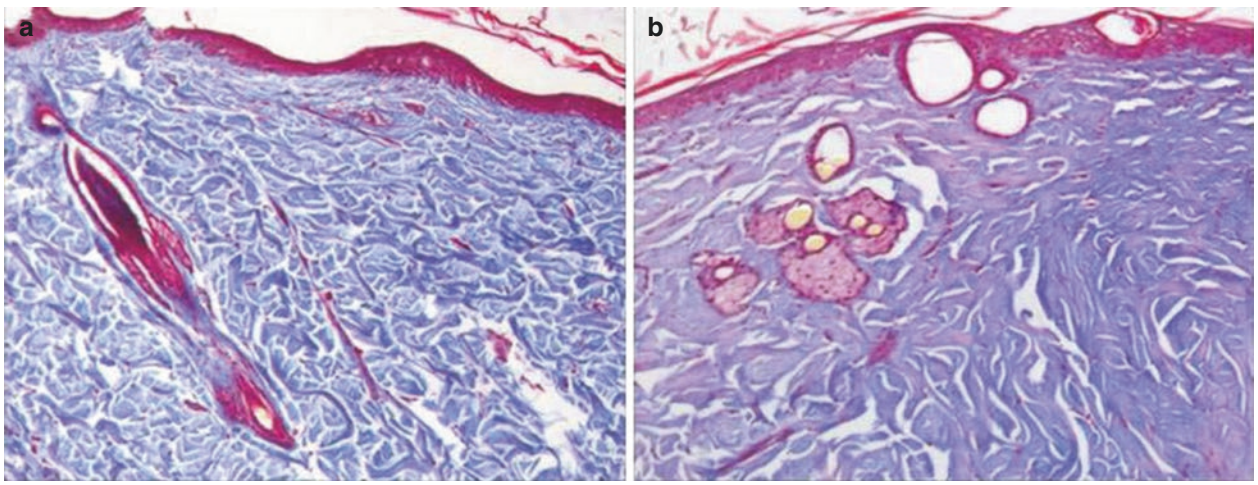

Fig. 8.9 21-Day wound skin in rats of different age groups (Masson trichrome staining, ×100)

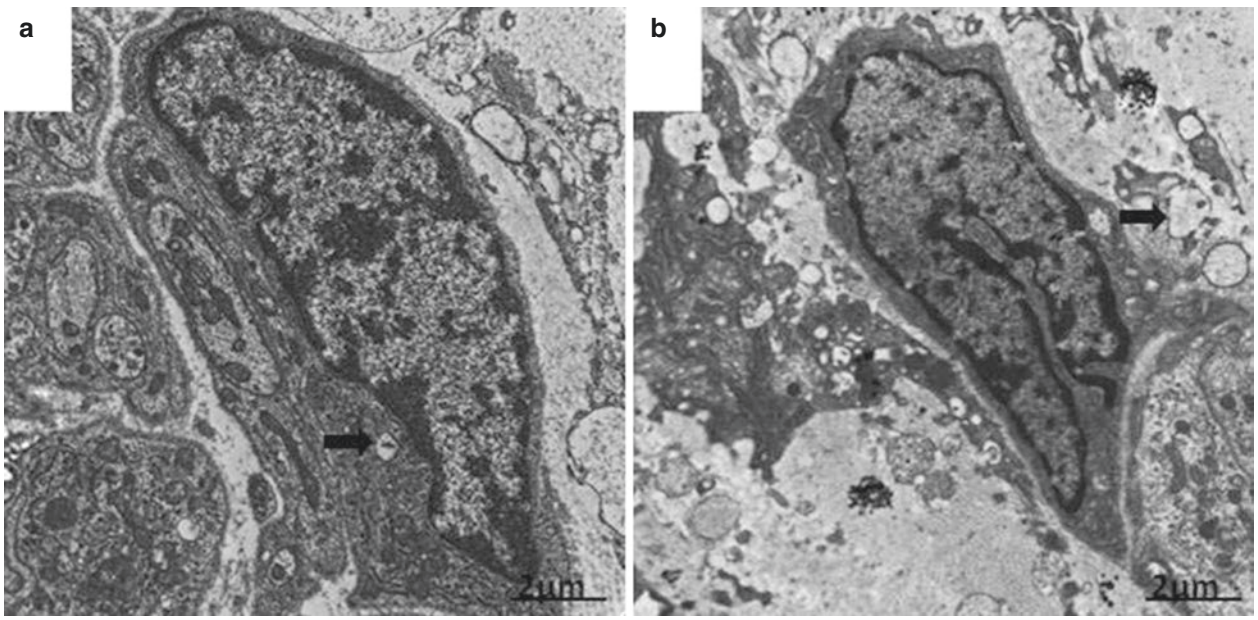

Fig. 8.10 Autophagosome morphology and structure of normal skin fibroblasts in different age groups (electron microscopy, ×30,000)

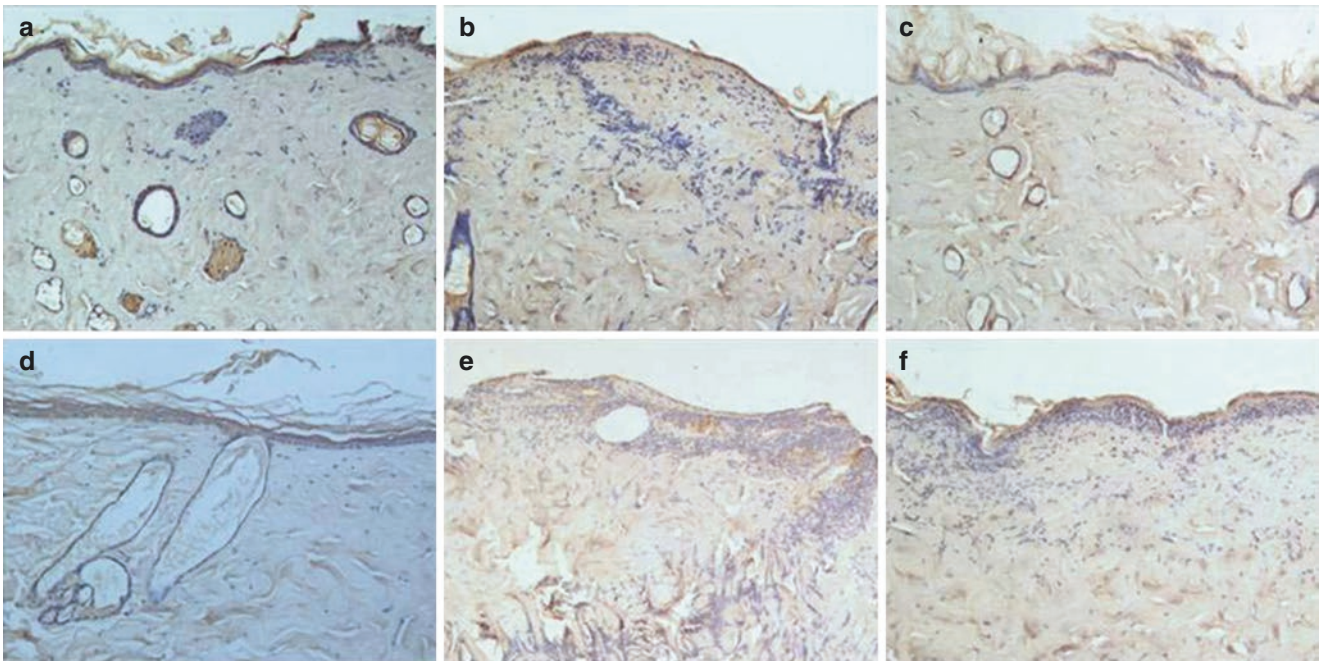

Fig. 8.11 Expression of LC3 in skin wounds of rats of different age groups (Envison method, ×100)

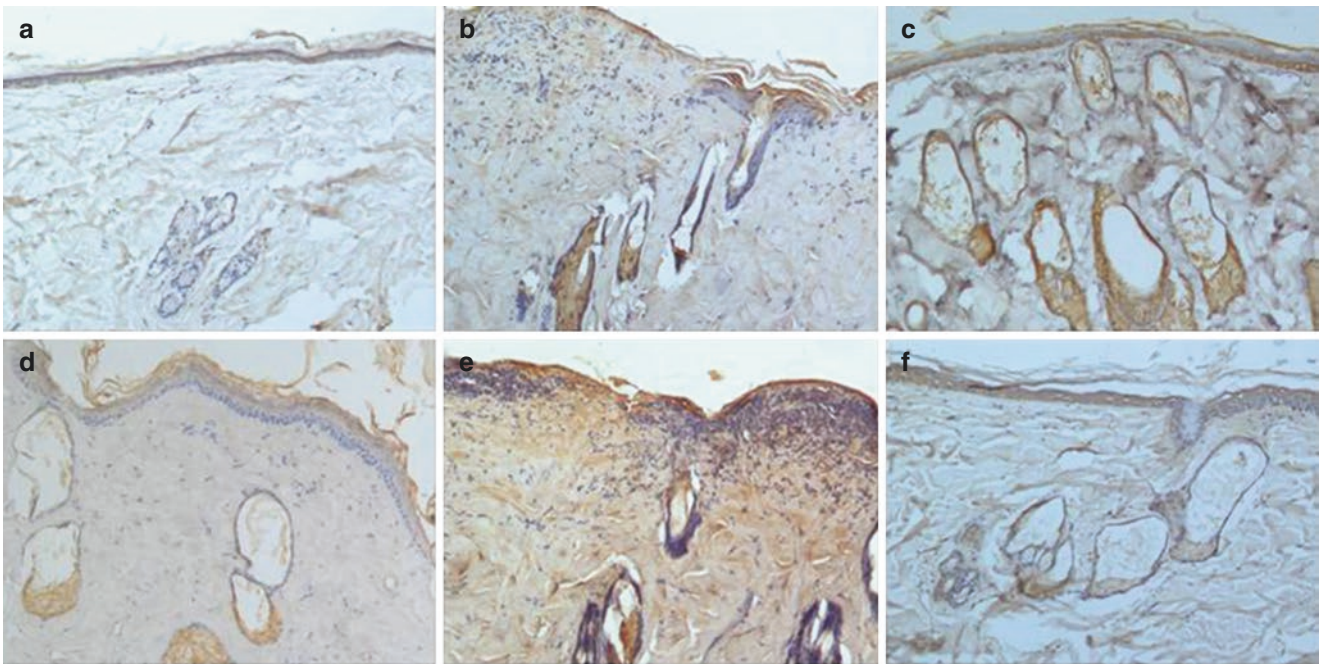

Fig. 8.12 Beclin-1 expression in skin wounds of rats of different age groups (Envison method, ×100)

wound margin. The expression of two proteins in the pre-traumatic group was higher than that in the young group. The expression of LC3 and Beclin-1 protein increased with time. At 4 days, 7 days, 14 days, and 21 days, the expression of LC3 was more in the young group, while Beclin-1 was more expressed in the old group (Figs. 8.11 and 8.12).

In terms of individual effects, there was a significant difference in the expression of lc3 in the young rats group at 4 days, 7 days, 14 days, and 21 days (F = 65.283, P < 0.001), 4 days < 7 days < 14 days < 21 days; similarly, there was a significant difference in expression between the four phase points in the aged group (F = 35.236, P < 0.001), of which 4 days < 7 days < 14 d ays < 21 days; in 14 days, the expression of lc3 in the young murine group was significantly higher than that in the old rats (P < 0.05). There was no significant difference in the expression of the two groups at the other three time

points. In terms of main effect, there were significant differences between the age group and the time ($P < 0.001$); however, there was no interaction between the two factors ($P = 0.072$), indicating that the two had no effect on the expression of lc3 (Table 8.2).

The expression of Beclin-1 mRNA in skin wounds of rats of different age groups: in the young rats, the expression of Beclin-1 was significantly increased in 4, 7, 14, and 21 days ($F = 166.748$, $P < 0.001$), 4 days < 7 days < 14 days < 21 days; similarly, the expression of four time points in the aged rats group was also significantly increased ($F = 38.875$, $P < 0.001$), 4 days < 14 d ays < 7 days < 21 days; at each time point, the expression of Beclin-1 in young rats was significantly lower than that in the old rats ($P < 0.05$). In terms of main effect, there were significant differences between age group and time ($P < 0.001$); and there were interactions between the two factors ($P < 0.001$), indicating that both of them affected the expression of Beclin-1 (Table 8.3).

The changes of mRNA expression of p62 in skin wounds of rats of different age groups showed that there was a significant difference in p62 expression loss in 4, 7, 14, and 21 days in young rats ($F = 12.842$, $P = 0.002$). Similarly, there was a significant difference in expression reduction at four time points in the aged group ($F = 4.257$, $P = 0.045$), and the mRNA reduction in both age groups was 4 days < 7 days < 14 days < 21 days. There was no significant difference in the expression of young rats and aged rats at 4 days and 7 days ($P > 0.05$). At 14 days and 21 days, the decrease in p62 expression in the young mouse group was significantly lower than that in the old mouse group ($P < 0.05$). In terms of main effect, there were significant differences between the group and time factors ($P < 0.001$); and there was an interaction between the two factors ($P = 0.047$), indicating that the two affected the expression of p62 (Table 8.4).

In different age groups, the expression of LC3- II/I and Beclin-1 protein increased gradually at 4, 7, 14, and 21 days after injury, while in p62 protein the expression gradually decreased ($P < 0.05$). The protein level expression of LC3-II/I, Beclin-1, and p62 was consistent with gene level expression (Figs. 8.13 and 8.14).

Through the detection of autophagy-associated marker proteins LC3, Beclin-1, and p62 during wound healing, it

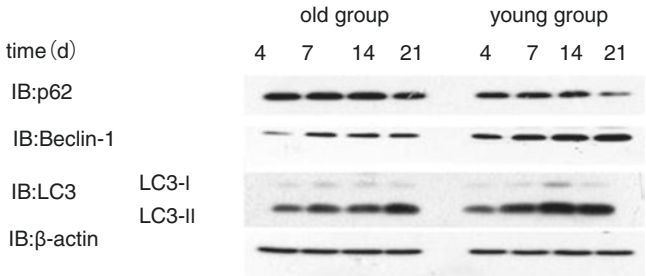

Fig. 8.13 Protein expression of LC3, Beclin-1, and p62 in different age groups (Western blot)

Table 8.2 Comparison of mRNA expression changes of LC3 in skin wounds of rats of different age groups

Group	*n*	4 Days	7 Days	14 Days	21 Days	Total	*F*	*P*
Young	3	0.40 ± 0.08	2.32 ± 0.19	5.29 ± 0.34	7.12 ± 1.22	3.78 ± 2.77	65.283	<0.001
Old	3	0.77 ± 0.30	1.85 ± 0.39	3.55 ± 0.34	7.76 ± 1.69	3.48 ± 2.88	35.236	<0.001
Total	–	0.59 ± 0.28	2.09 ± 0.37	4.42 ± 1.00	7.44 ± 1.36	3.63 ± 2.77	0.897	0.358
t	–	2.091	1.871	6.273	0.526	88.096	2.827	
P	–	0.105	0.135	0.003	0.627	<0.001	0.072	

Table 8.3 Comparison of mRNA expression changes of Beclin-1 in skin wounds of rats of different age groups

Group	*n*	4 Days	7 Days	14 Days	21 Days	Total	*F*	*P*
Young	3	0.05 ± 0.03	5.75 ± 0.82	4.81 ± 0.38	9.25 ± 0.47	4.97 ± 3.46	166.748	<0.001
Old	3	1.39 ± 0.12	20.08 ± 1.56	27.25 ± 7.47	34.03 ± 1.69	20.69 ± 13.16	38.875	<0.001
Total	–	0.72 ± 0.74	12.92 ± 7.92	16.03 ± 13.17	21.64 ± 13.62	12.83 ± 12.37	60.399	<0.001
t	–	19.297	14.075	5.196	24.425	190.845	21.618	
P	–	<0.001	<0.001	0.007	<0.001	<0.001	<0.001	

Table 8.4 Comparison of mRNA expression changes of p62 in skin wounds of rats of different age groups

Group	*n*	4 Days	7 Days	14 Days	21 Days	Total	*F*	*P*
Young	3	–3.03 ± 2.31	–6.38 ± 0.95	–8.31 ± 0.98	–9.92 ± 1.02	–6.91 ± 2.95	12.842	0.002
Old	3	–2.58 ± 0.47	–3.39 ± 0.65	–4.62 ± 0.87	–5.32 ± 1.36	–4.20 ± 1.34	4.257	0.045
Total	–	2.15 ± 0.30	3.63 ± 0.53	5.54 ± 2.17	2.69 ± 1.41	3.50 ± 1.81	29.317	<0.001
t	–	0.334	2.639	4.876	4.692	16.905	3.320#	
P	–	0.755	0.058	0.008	0.009	<0.001	0.047	

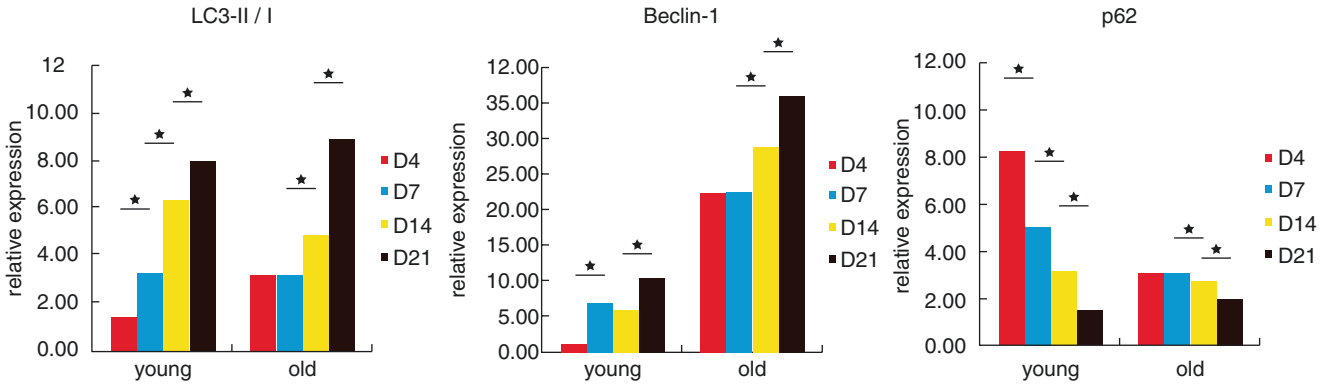

Fig. 8.14 Protein expression of LC3, Beclin-1, and p62 in wounds of different age groups (*$P < 0.05$)

was found that the autophagy ability of skin cells in young and aged rats was enhanced, but young skin could maintain cell homeostasis and keep autophagy within the normal range, thereby promoting wound repair. Older skin may be obstructed by its own autophagy system, resulting in excessive autophagy that is incompatible with itself, causing irreversible damage to the cells and delaying the healing process.

8.1.3 Nutrition

Nutritional factors play an important role in wound healing. Effective energy support can provide sufficient energy and nutrients for the body to repair wounds, reduce the body's own protein degradation, enhance the body's immunity, and promote functional healing of the wound [7]. Energy, carbohydrates, protein, fat, vitamins, and mineral metabolism all affect the healing process of wounds. Under special circumstances (such as wars), due to the limitations of the conditions, nutritional imbalances often occur, and the malnutrition of the body reduces the process of wound healing. It is well known that nutrients and micronutrients are extremely important components in the process of acute and chronic wound repair. After the body is injured, the whole body tissue is in a state of decomposition, and it can last for a long time, which is easy to cause the lack of protein in the body. The entire repair process requires sufficient heat, protein supply, and sufficient vitamins A, B, C and minerals and trace elements; otherwise protein collagen fibers and granulation tissue cannot be formed. In patients with delayed wound healing after surgery, the zinc content in the skin is mostly lower than in patients with good healing. Zinc urinary excretion has been shown to increase in surgical stimulation, trauma, and burns, and zinc supplementation promotes wound healing. In addition, the lack of copper is also associated with poor wound healing.

8.1.3.1 Carbohydrates

Carbohydrates are the main source of energy in the healing process of wounds, and are structurally represented as monomers or multiparts of sugar. When ingested by the human body, the carbohydrate is converted into monosaccharides (mainly glucose) by the action of digestive enzymes. The role of glucose in the healing process of wounds is mainly to provide energy for various cells involved in wound repair, stimulate fiber growth, stimulate collagen production to form new tissue, and produce ATP to meet the energy required for cells to participate in wound repair to accelerate metabolism. Glucose as a source of ATP synthesis also avoids the consumption of amino acids and proteins. The body's response to energy deficiency caused by insufficient carbohydrate intake is increased mortality, decreased human albumin content, decreased muscle tissue ratio (because muscles break down to provide energy), wounds are difficult to heal, and BMI (body mass Index) is too low.

8.1.3.2 Protein

Abnormal protein metabolism and body nutrient disorders may be important ways to prevent wound repair. This view has been increasingly recognized since the discovery of protein detection techniques and metabolic assays. The effect of hypoproteinemia on wound repair is not directly caused by protein deficiency, but is related to the decrease of plasma colloid osmotic pressure and the accompanying tissue edema, which is also the main reason for the delayed repair in the case of war wounds. Other experiments have confirmed that chronic protein-deficient animals are affected by angiogenesis, fibroblast proliferation, collagen synthesis, and deposition.

Experiments have confirmed that the body lacks arginine, which can reduce collagen deposition and poor wound healing. Supplementing 17 g of arginine daily can not only increase the deposition of hydroxyproline, promote wound healing, and improve immune function, but also retain more skin protein under stress for healing of the incision. Arginine

can also promote wound healing and promote insulin and growth hormone secretion through humoral regulation mechanisms. Supplementation with arginine was most effective 3 days before wound healing. Thus, inflammatory cells and fibroblasts are in an activated state. Therefore, patients with pressure ulcers, especially those who are at risk of protein and energy malnutrition or have occurred, need to immediately use sufficient energy and protein for nutritional intervention to meet the additional nutritional needs of patients. In addition to energy and protein, arginine and micronutrients should be supplemented to maintain optimal healing of the incision.

Other experimental studies have found that the methyl group of methionine can synthesize choline to prevent fatty liver, and methionine itself can be converted into cysteine to participate in detoxification and prevent liver poisoning. The ratio of lysine to tryptophan is also important. It is generally believed that the ratio of lysine/tryptophan is preferably (6–7):1 to improve protein utilization. Lysine is also the most important amino acid in the synthesis of proteins. It has been suggested that threonine, serine, tryptophan, tyrosine, histidine, glutamic acid, glycine, alanine, proline, and branched chain amino acids should be considered when supplementing amino acids.

Glutamate is one of the most abundant amino acids in the plasma membrane component and is a major source of energy metabolism in rapidly proliferating cells such as fibroblasts, lymphocytes, epithelial cells, and macrophages. Oral supplementation with glutamate can increase wound tension and levels of mature collagen.

8.1.3.3 Vitamins

1. Vitamin C

   Vitamin C (also known as ascorbic acid) is an acidic polyhydroxy compound with six carbon atoms. It is abundant in a variety of fresh vegetables and fruits. The two enol hydroxyl groups of the 2- and 3-position carbon atoms in the molecule are easily dissociated, releasing H+ and being oxidized to dehydrogenating vitamin C. Vitamin C and dehydrogenated vitamin C form a reversible redox system in the human body, which plays an important role in biological redox and cellular respiration. Vitamin C is involved in amino acid metabolism and synthesis of neurotransmitters, collagen, and tissue interstitial cells; it can reduce capillary permeability, accelerate blood coagulation, stimulate coagulation function, promote iron absorption in the intestine; promote blood lipid decline, promote wound healing, increase resistance to infection, participate in the detoxification process, have anti-histamine, and prevent the formation of carcinogens.

   As early as 1941, foreign cooks studied the effects of vitamin C on wound healing. After more than half a century of unremitting efforts by doctors and scholars, the role of vitamin C in wound healing was affirmed. Hydrogen peroxide used in the treatment of infectious wounds has a strong bactericidal effect, but it also increases the production of oxygen free radicals. No matter what kind of disinfectant, such as hydrogen peroxide, sulfur, bleaching disinfectant, etc., poisoning will interrupt the normal function of the cells. Vitamin C is a scavenger of free radicals. It is also involved in the synthesis of tissue interstitial collagen and improves the permeability of capillaries, so it promotes the formation of fresh tissue and reduces exudation.

   Although vitamin C has an anti-infective effect, it is not very strong and therefore cannot be used as a main drug at the peak of infection, especially in the case of infectious ulcers.

   In the absence of vitamin C, wound healing stops at the stage of fibrosis, and the number of fibroblasts in the wound is normal, but not enough collagen. In severe scurvy patients, not only can new wounds not heal, but old healing scars can also rupture, as the ongoing collagen solubilization far exceeds the synthesis of new collagen. Vitamin C wet compress is beneficial to the formation of skin cells on the wound surface, promotes the growth of granulation tissue, and accelerates the healing of wounds. However, the study also found that the presence and intake of vitamin C at normal levels is usually ineffective. When patients have vitamin C deficiency or trauma, they are supplemented with vitamin C of 100–200 mg per day; when more complex trauma occurs, including stage III, IV, or severe traumatic pressure ulcers, supplementing with vitamin C of 1000–2000 mg per day can promote wound healing.
2. Other Vitamins
   (a) Vitamin A: It is an essential substance for maintaining epithelial growth. At the time of injury, the demand for vitamin A increases. Moreover, vitamin A has a positive effect in the inflammatory phase of wound healing. Vitamin A can partially reverse the adverse effects of long-term steroid treatment in patients with wound healing. If vitamin A is lacking, the wound will heal slowly.
   (b) B vitamins: B vitamins are important for wound healing and are coenzymes involved in energy metabolism. The lack of vitamin B_2 delays the formation of epithelia during wound repair, decreases total collagen content, and reduces the rate of wound healing. When the B vitamins are deficient, intramolecular diplomacy, collagen maturation is impaired, and the tensile force of the incision is decreased. Vitamin B_6 is mainly a coenzyme involved in the synthesis and decomposition of amino acids. In the absence of it, the effect on wound healing is similar to vitamin B_2 deficiency.

(c) Vitamin D: The Matsumoto study showed that 1,25-$(OH)_2$-Vit D_3 induced differentiation of keratinocytes, and inhibited the growth of the cells. Hosomi found that the differentiation of keratinocytes by 1,25-$(OH)_2$- Vit D_3 was accomplished by vitamin D receptor, either in vitro or in vivo. PDGF plays an important role in wound healing. It stimulates the proliferation of fibroblasts, muscle cells, promotes the synthesis of collagen and extracellular matrix, and can recruit fibroblasts, monocytes, and neutrophils. The keratinocytes in the epidermis do not have a receptor for PDGF. Studies have shown that 1,25-$(OH)_2$-Vit D_3 can up-regulate PDGF, and TGF-α is also affected.

(d) Vitamin E: It can promote the proliferation of capillaries, that is, micro vessels, improve the surrounding circulation, and its anti-oxidation effect has an effect on the metabolism of the body, which can promote the growth of granulation tissue and skin.

8.1.3.4 Trace Elements

The lack of trace elements, such as zinc and copper, is also associated with poor wound healing. Zinc deficiency is associated with poor epithelialization and chronic wound healing. Magnesium, as a cofactor for many enzymes, is involved in the synthesis of proteins and collagen. Copper is a cofactor required for good cross-linking of cytochrome oxidase, cytosolic superoxide dismutase, and collagen. Zinc is a cofactor for RNA synthase and DNA synthetase. Iron is essential for the hydroxylation of proline and lysine, and severe iron deficiency can lead to collagen synthesis disorders.

1. Zinc
   Zinc is involved in collagen synthesis, which is a very important step in the healing process of pressure sore incision, so marginal zinc deficiency is thought to be related to delayed healing of the incision. When zinc is deficient, it affects the healing of pressure sores and other chronic incisions. When the plasma zinc concentration is less than 100 μg/mL, it is directly related to tissue repair and can be supplemented with zinc supplementation therapy.
2. Iron
   Iron is a cofactor required for the hydroxylation of proline and lysine during collagen synthesis. Chronic inflammation and infection caused by pressure sores can aggravate anemia and can be treated by iron supplementation and blood transfusion. After the occurrence of pressure sores, the body's need for certain nutrients, especially zinc and iron, increases.
3. Copper
   Copper is involved in the maturation of collagen and has an effect on wound healing. A copper- containing metalloenzyme (lysyl oxidase) catalyzes the oxidation of collagen lysyl residues to form a hydroxyl lysyl group. These groups pass through the cross-linking of extracellular collagen to increase the strength of the scar.
4. Manganese
   Many enzymes require normal participation in their function, including a lysylgalactose converting enzyme. This enzyme is involved in the glycosylation process of the original collagen fibers. Manganese also affects the production of hyaluronic acid, chondroitin sulfate, heparin, and other mucopolysaccharides, which are important factors in the healing process of the incision.
5. Selenium
   Selenium is an important component of glutathione peroxidase. The enzyme protects cells from oxidative damage by promoting hydrogen peroxide reduction. When selenium is deficient, the activity of the enzyme is reduced, and the healing of the incision can also be affected by changing the function of macrophages and multinucleated cells.

8.1.4 Neuro-Immune-Endocrine System

The neuroendocrine response is the earliest systemic reaction in the body after trauma. It is mainly manifested by the immediate hypothalamic–pituitary–adrenal axis (HPA) and sympathetic adrenal medullary axis, releasing hormones such as glucocorticoids and catecholamines. The heavier the injury, the stronger the neuroendocrine response. The neuroendocrine reaction can also affect the immune system. In the early stage of trauma, moderate neuroendocrine response can enhance the body's immune function, prevent or reduce secondary damage, and thus constitute a neuro-immune-endocrine regulation system, and play a regulatory role in wound healing [8].

The disorder of the neuro-immune-endocrine regulatory network can lead to disturbance of metabolic activity in the body, and the whole repair process enters a pathological stage, which affects the healing time. The effects of neurological, endocrine, and hormonal changes on skin repair and regeneration have recently received great attention.

8.1.4.1 The Influence of Nerve on Wound Healing

1. The Skin Is a Nerve-Dependent Organ
   A large number of studies have shown that the skin is a very sensitive nerve-dependent organ as the largest organ in the human body. The sensory nerves from the dorsal root ganglia pass through the dermis, running parallel at the junction of the true and epidermis, penetrating the basement membrane and reaching the layer of epidermal granules vertically to form a three-dimensional network. Cells in the skin (keratinocytes, microvascular endothelial cells, and fibroblasts) can express various types of neuropeptides. Many physiological functions of the skin

(such as metabolism, immunity, etc.) are inseparable from innervation, such as sweating, immune response, thermoregulation, and DNA repair.

Skin cells can perform neuron-like functions such as the expression of neurotransmitters and their receptors. Among the myriad of neurotransmitters and neurohormones, there are currently more than 20 types of skin, most of which are neuropeptides (Table 8.5).

2. The Effect of Nerves on Wound Healing
In the process of wound healing, the wound area will be further increased after the denervation of the wound, and the contracture will be restricted, resulting in a healing disorder; paraplegia and diabetic patients with neurotrophic disorders often lead to difficult wound healing, and even prolonged unhealed wounds; in the early stage, there is often a temporary phenomenon of excessive nerve innervation. There are indications that neurological factors have a regulatory effect on inflammation, neovascularization, granulation hyperplasia, and post-healing shaping stages in wound healing. Neurotrophic factors and neuropeptides (such as SP, CGRP, VIP, SOM, and opioid peptides) act as neuromodulators, neurotransmitters, and neurohormones to effectively regulate the function of skin cells, determine the final biological response of the cell, affecting the healing outcome and regeneration ability.

8.1.4.2 The Impact of Endocrine on Wound Healing

1. The skin Is a Large Endocrine Organ
The skin produces many important endocrine and exocrine substances (Table 8.6). Special mention is made of fat cells, which are capable of secreting leptin (LP), lipoprotein lipase (LPL), resistin, angiotensinogen (AGT), and adiponectin (Adiponectin). Among them, adiponectin, also known as GBP28, enhances insulin sensitivity and stops inflammation. Apolipoprotein E is one of the major apolipoproteins in plasma. It is polymorphic and mainly synthesized and metabolized by the liver. It plays an important role in plasma lipoprotein metabolism, tissue repair, inhibition of platelet aggregation, immune regulation, and inhibition of cell proliferation. It has been determined that leptin is involved in endocrine function, inflammatory response, and has the potential to promote vascular and granulation tissue formation and re- epithelialization, and is a new important factor in the process of wound repair. Adipocytokines are also involved in the regulation of endothelial function as inflammatory factors.

2. The Effect of Endocrine on Wound Healing
The neuroendocrine system of the skin involves the local production of neuroendocrine mediators, which interact with the corresponding specific receptors by paracrine and autocrine.

The renin-angiotensin system (RAS) is one of several hormonal systems that regulate the function of the body, and produces an endocrine effect together with angiotensin II (AT II). In the skin, the local or tissue renin-angiotensin system affects cell proliferation and differentiation. In addition, fat cells can also secrete other peptides and

Table 8.5 Neurotransmitters and their receptors produced by various cells in the skin

Kinds of skin cells	Neurotransmitter and neurohormones	Neurologic receptor
Keratinocyte	NGF, SP, CGRP, VIP, NKA, ACh, DA, AR, NE, β-EP, CA, SOM	NGFR, VIPR, NPYR, 5-HTR, CGRPR, NK-1/2/3R, μ/ζ-opiete-R
Merkel cell	SP, CGRP, MEK, NGF, NKA, SOM, VIP, NPY	NGFR, NK-1R
Langerhans' cells	NGF, SP, CGRP, SOM, VIP, MEK, NKA	NK-1/2R, SOMR, NPYR
Mastocyte	NGF, CA, SP, CGRP, NKA, SOM	NK-1R
Fibroblast	NGF, SP, β-EP	NGFR, NK-1R, SOMR, NPYR, 5-HTR
Adipocyte	–	AR-β1, 2, 3
Microvessel endothelial cell	ACE, Ang, NO, ET, β-EP	NGFR, NK-1/2/3R, NPYR
Sweat gland cell	–	NK-1R, μ-opiete-R
Sebaceous gland cell	–	NPYR, μ-opiete-R

Table 8.6 Hormones and their receptors produced by various cells in the skin

Kinds of skin cells	Hormone	Hormone receptor
Keratinocyte	PTHrP, CRH, ACTH, α-MSH, corticotropin, androgens, atRA, elcosanoid	TSHR, CRH-1R, MC-1R, M-1R, VIPR-2, IGF-1R, GHR, GR, AR, PR, THR, ER-β, RAR, RXR, VDR, PPAR-α/β/γ
Merkel cell	Estrogens	ER
Langerhans' cells	GRP, PACAP, α-MSH, POMC	GRPR, PACAPR I/II/III, MC-1R/5R
Mastocyte	POMC	MC-1R mRNA, non-protein level
Melanocyte	PTHrP, CRH, Ucn, ACTH, α-MSH, epinephrine, IGF-I	TSHR, CRH-1R, MC-1R, 2R, MR, M-1R, 5-HTR, GHR, ER-β, RXR-α, VDR
Fibroblast	ACTH, α-MSH, IGF- I/II, IGFBP-3, Estrogens	PTHR, TSHR, CRH-1R, MC-1R, M-1R, GHR, AR, THR, ER-β/α, RXR-α
Adipocyte	LP, LPL, resistin, AGT, ApoE	IR, GR, GHR, TSHR, Gastrin/CCK-B R, GLP-1R, Ang II-R, VDR, THR, AR, ER, PR, LR, IL-6R, PPAR-γ
Microvessel endothelial cell	CRH, Ucn, ACTH, α-MSH	MC-1R, VIPR-2, RAR-2, GHR, AR, ER-β, RAR, RXR, PPAR-γ
Sweat gland cell	Ucn, androgens	MC-1R/5R, VIPR-2, GHR, AR, PPAR-γ
Sebaceous gland cell	CRH, androgens, estrogens, atRA, calcitiol, eicosanoids	CRH-1R/2R, MC-1R/5R, μ-opiete-R, VIPR-2, GHR, AR, ER-β/α, RAR, RXR, PPAR-α/β/γ

non-peptide factors, which play a role in the axis of angiotensinogen-angiotensin II-prostacyclin, affecting the relaxation and growth of blood vessels.

The effects of hormone levels on downstream cells and tissues, including the cascade and interaction of cytokine signaling pathways, positive and negative regulation of target proteins are gradually attracting attention. Sex hormones maintain organ development, regeneration, and tissue metabolism, including normal skin, epidermal thickness, mitotic capacity, and vascularization, as well as elastin characteristics and collagen tissue content, which are important factors in the process of wound healing. It is currently believed that estrogen can down-regulate tumor necrosis factor-α (TNF-α) by binding to its receptor and affecting gene expression through the action of activator protein-1 (AP-1), increased matrix deposition, stimulated hair follicle keratinocyte proliferation, and enhanced keratinocyte growth factor (KGF) expression, affecting epithelial regeneration. In addition, estrogen affects skin healing and regeneration through inflammatory reactions, matrix deposition, re-epithelialization, and scar maturation. Studies have shown that androgen receptors (AR) expressed in the skin also affect healing by participating in inflammatory responses, cell proliferation, and matrix deposition. In short, the terminal organ of the skin acting as a sex hormone must be affected by it when it is damaged, repaired, and regenerated. Thyroid hormones also have a major impact on wound healing and regeneration.

Androgen, estrogen, corticosteroids, and their physiological and pathological signaling pathways in healing are important. Understanding the response of the cascade of signaling pathways, especially in combination with immunity, is of great importance in understanding the role of wound healing in the healing of wound healing mechanisms.

8.1.4.3 The Impact of Immunity on Wound Healing

1. The Skin Is an Immunoreactive Organ
   The skin is the largest tissue and organ of the human body. Due to its special structure and function, it forms a natural barrier between the body and the external environment. Skin is often seen as a tissue organ that has a unique immune function and is closely related to the systemic immune system. It not only has nonspecific immune defense function, but also participates in the whole process of antigen recognition, immune cell activation, and skin immune response of body-specific immunity (Table 8.7).

Table 8.7 Various cells and related immune responses in the skin

Kinds of skin cells	Immune response
Keratinocyte	Creating a unique microenvironment for antigen uptake and recognition. There are two major features of Keratinocyte in SIS: The expression of MHC-II Antigens, which act as helper cells in T cell mediated immune responses. It produces a lot of cytokines such as IL-1, IL-2, IL-6, GM-CSF, TNF, IFN
Langerhans' cells	Bone marrow-derived dendritic cells are distributed in the basal layer of epidermis and adnexal epithelium, accounting for 3–8% of the total number of epidermal cells. The chemical property and surface markers are similar to those of macrophages. It is generally accepted that the Langerhans' cell, which resides in the epidermis of normal humans, is immature and has its full function only after entering the dermis or draining lymph nodes. It is the main antigen presenting cell of skin and participates in skin immune reaction. It can ingest, process and present Antigen, and control T cell migration. Langerhans' cell T cells secrete important cytokines and participate in immune regulation, immune surveillance, immune tolerance, skin graft rejection, and so on
Mastocyte	Mainly located in the dermis around the papilla vessels, deep dermis rare, almost non-existent in the epidermis. Mast cells have different membrane receptors (such as IgEFcR, which bind to IgE) on their surface. Mast cells produce and release a variety of bioactive mediators, which can be divided into vasoactive substances, chemokines, active enzymes and structural glycoproteins. They participate in delayed type hypersensitivity
Lymphocyte	In normal human skin, a large number of T cells (more than 90%) are localized around the dermal vessels, mainly around the dermal papilla and capillary. Only the T cells in the lymphocyte can be recycled to the skin organs
Dendritic cell	A specific subset of antigen presenting cells that are widely distributed in the human body. In addition to the dendritic cell of the skin, there are melanocytes, Merkel cells, tissue macrophages, unidentified cells, and dermal dendritic cells
Fibroblast	Dermal fibroblasts can synthesize all kinds of proteins required for activation of t lymphocyte subpopulations, which can prolong the survival time of T lymphocyte by binding adhesion molecules CD44, LFA, ICAM-1 to T lymphocyte. Cytokines produced include: IL-1/6/8, IFN-β, monocyte chemoattractant protein, B factor, C3, CSF2, TGF-α/β. The MHC-II Antigen is expressed locally and acts as an antigen presenting cell, activating T lymphocyte
Adipocyte	Synthesize and secrete complement D (Adipsin, which is the first complement component cloned from an Adipocyte line). Secretion of inflammatory cytokines (such as TNF-β, CRP and IL-6). It secretes Leptin, which has immunomodulatory and T lymphocyte effects on monocytes, macrophages, and natural killer cell cells. In addition, it can affect the production of cytokines by immune cells. Adiponectin reduces lipopolysaccharide-induced tumor necrosis factors expression, impairs the phagocytosis of mature macrophages, and inhibits the proliferation and growth of bone marrow mononuclear cell lines, a negative regulator of the hematopoietic- immune system, to help stop the inflammation
Microvessel endothelial cell	In normal skin, lymphocytes are concentrated around the capillary veins, the tiny endothelial cells in the inner walls of blood vessels that promote lymphocyte circulation from the blood to the skin. In addition, it actively participates in the complex reactions between macromolecules and blood cells and extravascular substances, and participates in the immune and inflammatory processes. The activation of endothelial cells was triggered by cytokines, and the adhesion of activated endothelial cells to leukocytes was increased. Endothelial cell activation plays an important role in cellular immune response

In the 1970s, it was suggested that the skin is a primary lymphoid organ similar to the thymus of primary lymphoid tissue. In the 1980s, the concept of skin-associated lymphoid tissue (SALT) was proposed based on the characteristics of epidermal Langerhans cell-presenting antigen, T-cell pro-epithelia, and keratinocytes producing epidermal thymic activating factor. SALT is thought to include four distinct cells: keratinocytes, lymphocytes, Langerhans cells, and endothelial cells. The SALT concept limits skin immunity to the epidermis, which is incomplete. Cells involved in the skin's immune response, such as T cells and monocytes, are mainly distributed in the dermis; cells involved in the skin's immune response include cells other than SALT cell components, such as mast cells, neutrophils, and fibroblasts and a variety of mediators involved in the immune response (such as cytokines, immunoglobulins, etc.). Therefore, in the mid-1980s, Bos proposed the concept of a skin immune system (SIS). SIS consists of two major parts, cells and body fluids. The cell components include keratinocytes, Langerhans cells, tissue cells (dendritic cells and macrophages), T cells, granulocytes, mast cells, endothelial cells, and the like. The body fluid components include antimicrobial peptides, plasmin, arachidonic acid, complement, secretory immunoglobulin IgA (SIgA), cytokines, and the like. In the mid-1990s, the concept of the dermis immune system (DIS) was proposed, which complemented and expanded SIS.

2. The Effect of Immune Response on Wound Healing
   For external damage, the skin not only has mechanical resistance, but also has immune function and can produce an appropriate immune response. In the inflammatory phase of wound healing, the infiltration of lymphocytes and macrophages, and the sources of pro-inflammatory factors are all related to stress. The degree of immunosuppression is directly proportional to the acute inflammatory response. After the rapid release of glucocorticoids and catecholamines into the blood, glucocorticoids can decrease the activity of T cells and mononuclear macrophages, and various immunosuppressions promote the synthesis of cytokines, resulting in insufficient expression of antigens in the immune response. Catecholamine inhibits the proliferation of T cells, the expression of IL-2 receptors, and the formation of immunoglobulins. Factors that cause immunosuppression are various cytokines produced by prostaglandins and inflammatory cells.

8.1.5 Systemic Disease Factors

8.1.5.1 Metabolic Diseases

Hyperglycemia in diabetic patients can inhibit neutrophil function, and the inflammatory response of the wound is weak, which directly leads to the decrease of fibroblast growth and collagen synthesis. The hyaluronic acid of the dermal papilla of the wound surface of such patients is also reduced compared with normal, while the collagenase content is significantly increased; this phenomenon can affect the healing tissue tension strength and collagen aggregation. In addition, due to vascular pathological changes in diabetic patients, blood perfusion is low, with tissue hypoxia, and the risk of wound infection increases. The wounds of uremic patients are not easy to heal. The main mechanism may be systemic malnutrition, low blood volume, and insufficient oxygen supply. In addition, hyperlipidemia can also reduce the synthesis of collagen in fibroblasts. Diabetic patients are prone to traumatic infections. When blood glucose is >200 mg/dL, the function of leukocyte phagocytic bacteria is inhibited. Therefore, blood sugar levels in diabetic patients must be controlled during wound healing.

8.1.5.2 Cardiovascular Diseases

Patients with atherosclerosis have altered vascular function, affecting blood supply to the wound and resistance to local infection. In addition, high blood pressure, high blood lipids, and other factors can affect the wound healing process.

8.1.5.3 Nerve Damage Diseases

For example, ulcers caused by leprosy are not easy to heal and are caused by nerve involvement. The damage of the autonomic nerve changes the local blood supply, and the effect on regeneration is more obvious.

8.2 Local Factors Affecting Tissue Repair and Regeneration

8.2.1 Bacterial Colonization and Infection

Bacterial biofilm is a membranous structure formed by some bacteria attached to the wound surface and formed with the extracellular matrix. It consists of bacteria and its secreted products, extracellular matrix, necrotic tissue and so on. Since it is a membranous structure composed of a plurality of components present at the cellular level, it is often determined by fluorescein staining or the like in the study [9]. The formation of biofilms consists of three stages: attachment of wound microbes, secretion of EPS and formation of colonies, and maturation and spread of colony cells [10].

It is important to understand the characteristics and mode of action of this membranous structure to reveal the emergence of bacterial resistance and its role in the development of chronic refractory wounds. According to research, the formation and action of biofilms in acute wound bacteria is not obvious. Only 6% of wounds can detect the presence of this biofilm, so bacteria are not the main factor to delay

wound healing. However, when the wound changes from acute to chronic, the biofilm can be detected on more than 60% of the wound surface. When the number of bacteria reaches a certain level, the bacterial biofilm may play a decisive role. Sometimes in the wounds from the acute to chronic early stage of wounds or some single factors, the types of bacteria detected may be relatively simple, but in the wounds with bacterial biofilm formation, a variety of factors and a variety of bacteria are common. As a result of mixed infection, this may be why some single-type bacteria have a higher detection rate (up to 90% or more), while in biofilm-forming wounds, the detection rate is relatively low (about 60%).

How is the biofilm of bacteria formed when chronic refractory wounds occur? It is generally believed that the wound is contaminated during the process from acute to chronic wounds. When the amount of contaminated bacteria is $<10^5$/g, the bacteria are only colonized in the wound. There is no delay in wound healing; however, when the amount of bacteria is $>10^5$/g, especially when there are multiple bacteria at the same time, the bacteria adhere to the wound and multiply on the wound to form a clone, and then embed itself in the necrotic tissue, a multi-layered matrix formed by extracellular matrix, a protective layer is formed, similar to a membrane-like structure. At this time, typical symptoms such as redness, swelling, heat, pain, and low partial pressure of oxygen are observed in the clinic. Bacteria can resist the effects of various treatments. The establishment of this biofilm allows these bacteria to escape the killing effect of antibiotics on them.

When the trauma occurs in a harsh environment, when the bacteria of the wound are transformed from pollution to infection, the exotoxin secreted by the microorganisms during the life and during the destruction, such as *Staphylococcus aureus* α toxin, not only causes the destruction of red blood cells and platelets, but also promotes small blood vessel smooth muscle contraction, paralysis, leading to capillary blood flow blockage and local tissue ischemia and necrosis.

The combined effects of a large number of bacterial exotoxin, endotoxin, and proteolytic enzymes after wound infection, and the biological effects of cytokines and free radical damage caused by their cytotoxic effects result in an increase in the number of tissue edema, hemorrhage, and purulent secretions. The protein is lost by a large amount of wounds and the electrolyte is sharply increased. The protein in the granulation tissue of the purulent wound is hydrolyzed in a large amount, and the bacteria invade the surrounding tissue in a large amount, so that the growth of the granulation tissue is slow or the epithelial formation is seriously affected by the excessive proliferation of the granulation, thereby affecting the speed of wound repair.

The mechanism by which bacterial biofilms delay wound healing may include:

1. Effect on Repairing Cells
   In the early stages of acute wound healing, neutrophils can control the extent of infection by eliminating microorganisms. When bacteria aggregate to form a biofilm, it will produce a resistance to neutrophils and affect the changes of fibroblasts and neutrophil chemotactic factors, resulting in the body's repair ability and immune function blocked, which leads to delayed healing.
2. The Role of Acetyl Homoserine Lactone
   Bacteria regulate the expression of related genes by sensing the density of peripheral cells. If the molecules of acyl homoserine lactones (AHLs) reach a certain threshold, the bacterial density sensing system is activated, and the bacteria continuously secrete the extracellular matrix to bind a single bacterium to form a biofilm, thereby hindering the penetration of the antibacterial drug. When the density of bacteria is reduced, a decrease in AHL secretion can lead to a rapid decrease in biofilm, and supplementation of exogenous AHLs can restore biofilm maturation. As a quorum sensing signal molecule, AHLs are also related to the production of bacterial virulence factors, synthesis or degradation of antibacterial drugs, plasmid binding and other biological functions, and directly affect the gene expression of host cells, which is an important molecule leading to persistent or repeated infection.
3. The Impact of the Formation of Drug Resistance Mechanisms on Wounds
   It is generally believed that bacterial infection of the wound can be eliminated by standardizing the use of antimicrobial treatment. The bacteria in the biofilm state have significantly enhanced drug resistance relative to their planktonic state. The presence of the membrane can weaken the neutrophil chemotaxis, and the inflammatory reaction of the surrounding tissue of the membrane is weakened, causing the bacteria and the organism to be in a long-term symbiotic state. The wound stays in the inflammatory phase for a long time and blocks the healing process of the wound. In addition, extracellular polymers consisting mainly of polysaccharides, proteins, lipids, metal ions, and extracellular DNA, EPS can also affect the physical and chemical properties of biofilms, leading to their resistance to antibacterial agents.

At present, the treatment methods for biofilm mainly include:

(a) Partial debridement, destroying the membrane.
(b) Negative pressure treatment.
(c) Topical drug treatment.
(d) Bioengineering alternative therapy.
(e) Hyperbaric oxygen therapy.

However, there is no better method of radical treatment, and many antigens and wound dressings are ineffective

against biofilm infection. Pay attention to the following aspects in clinical work to reduce the production of biofilm:

(a) Careful and thorough debridement and local dressing change.
(b) Reasonable choice of antibiotic type and route of administration.
(c) Operating specifications for the use of various conduits and synthetic materials.

Microbiota is also known as the microbial region, the microflora. Researchers at the Stowers Medical Institute in the United States have found that there is a clear link between the composition of the microbiome and the host's immune response and the body's ability to heal itself. At present, how the microbial group changes and how the immune system responds to the regeneration and repair process is attracting more and more attention.

The immune response mainly constitutes a barrier to effective tissue regeneration and repair. When a part of the planarian population is infected, lesions appear around the eye and become larger and larger until the entire head is degraded. Under normal circumstances, the planarian can regenerate a new head, but the infection interferes with their ability to regenerate in some way.

In the future, humans may develop small-molecule interference immune pathways to improve tissue repair and regeneration, and successfully use this effective method of simple organisms only for higher organisms (such as humans).

8.2.2 Foreign Bodies

Among the local factors affecting wound healing, it is mainly the effect of foreign matter retention on the wound or wound in the wound, including shrapnel, warhead, and other foreign matter being carried into the body. Usually large foreign bodies can be seen by the naked eye or by X-ray, but foreign objects below the millimeter level are hard to find by the naked eye.

The effect of foreign body on wound healing mainly comes from the following aspects: First, the foreign body itself has a large number of bacteria, which easily cause local wound infection; second, some foreign bodies, such as gunpowder particles, phosphorus particles, lead particles, etc., have certain tissue toxicity, which can cause direct damage to surrounding tissues; third, foreign bodies stimulate surrounding tissues, aggravating the reaction process in the acute inflammatory phase. Therefore, the wounds caused by trauma should be removed as much as possible during debridement. Foreign bodies in deep tissues, if they do not affect physiological functions, do not need to be barely removed, so as not to cause large tissue damage. Sharp foreign bodies immediately adjacent to the nerves and blood vessels should be removed in time. Free large bone fragments should be reset as much as possible during surgery, and bone fragments that are small and lose vitality should also be removed. At the time of surgery, the ligature and the suture are also foreign bodies. The shorter and less the retention, the better, to alleviate the local inflammatory response.

8.2.3 Hematoma and Ineffective Cavity

Both hematoma and ineffective lumens have a tendency to increase infection and will directly or indirectly affect wound healing. A non-contaminating surgical incision should be completely stopped when the incision is closed, and the layered suture does not leave a dead space. For contaminated wounds, the use of ligation should be used to stop bleeding as much as possible. Electrocautery or compression to stop bleeding should be the first choice. A drainage strip should be placed when the incision is closed, and taken out 48–72 h after injury. If a localized hematoma is formed, it will exert pressure on the normal tissue of the wound, affecting the blood supply of the wound edge, delaying the healing of the light, and causing tissue necrosis.

8.2.4 Local Blood Flow Supply

Insufficient local arterial blood supply or venous return disorders can lead to a decline in the supply of oxygen and nutrients, dystrophic granulation tissue, slow growth, and impede healing. Ischemia around the wound has both systemic and local factors. Local factors include both the influence of blood vessels itself and the ischemia caused by extravascular tissue hemorrhage and edema.

Under the action of the injury factor, different degrees of cellular and tissue damage occur locally, and the inflammatory process is initiated. The arterioles are transiently contracted, ranging from a few seconds to several minutes, followed by hemodynamics and rheological changes. The three phases: high mobile phase → low mobile phase → blood flow stagnation phase. If the damage factor is too strong or long-lasting, the low mobile phase is prolonged, plasma extravasation is increased, blood viscosity is increased, and blood flow is stagnant. In addition, leukocytes swim out of the blood vessels, accumulate in large areas in the injured area, phagocytose necrotic tissue and foreign bodies, the oxygen consumption is significantly increased, and the metabolic activity is enhanced, resulting in a relative lack of blood supply in the damaged area. Hemorrhage, edema, increased tension, and compression of blood vessels around the wound are another major cause of tissue ischemia

around the wound. Wound repair must have adequate blood flow, on the one hand, to provide sufficient oxygen and necessary nutrients to the wounded area, and on the other hand, to transport locally produced toxic products, metabolic waste, bacteria, and foreign bodies out of the damaged area.

The mechanical causes of local blood supply shortage are mainly local pressure, friction, and increased shear force, such as wound dressing or suture tightness, pressure ulcer formation. Vascularization caused by inflammation of local blood vessels or narrowing of blood vessel caused by small arteriosclerosis, such as venous leg ulcers and diabetic foot ulcers. In addition, smoking can also lead to dysfunction of the blood circulation system, which is mainly manifested in the following two aspects:

(a) Nicotine acts on the smooth muscle of the small arterial wall, causing the arteriole to contract and the blood flow to slow down.
(b) Inhaled carbon monoxide will compete with hemoglobin, which will reduce the oxygen carrying capacity of the blood and affect the oxygen supply of the wound tissue.

8.3 Other Factors Affecting Tissue Repair and Regeneration

8.3.1 Environmental Factors

Studies have shown that the use of moisturizing dressings to maintain a certain degree of humidity on the local wounds will help to form a local hypoxic environment, thereby stimulating fibroblast growth and capillary sprout formation. In moist, hypoxic, and slightly acidic environments, the dissolution of necrotic tissue is enhanced, and the release of various growth factors closely related to tissue repair is increased without increasing the infection rate and significantly reducing wound pain.

The climatic conditions at high altitudes often cause hypoxia in the body. After local tissue injury, when the local blood circulation of the injured tissue is subjected to certain obstacles, the wound tissue is in a low perfusion state and ischemic and hypoxic, and the repair of the injury reaction is inhibited, delaying the healing time.

Under a high-temperature and high-humidity environment, various bacteria grow and reproduce.

Therefore, it is easy to cause healing difficulties, as described in the relevant chapters.

8.3.2 The Impact of Ionizing Radiation on Healing

Any kind of irradiation (including gamma rays, X-rays, alpha and beta rays, electron beams, etc.) can directly cause skin ulcers that are difficult to heal, and on the other hand can indirectly affect the healing process of the wound. The mechanism is that the radiation damages small blood vessels, inhibits fibroblast proliferation and collagen synthesis and secretion. High doses of radiation can significantly delay healing wounds.

The sensitivity of different tissue cells to ionizing radiation is inconsistent. According to Bergonie and Tribondeau, the sensitivity of cell radiation is directly proportional to the ability of cells to proliferate, and inversely proportional to the degree of cell differentiation. Hematopoietic cells are sensitive to radiation damage, followed by skin cells. The effect of ionizing radiation on wound healing is closely related to the type of radiation, the dose of radiation, the way of irradiation, and the time of irradiation. Generally, the larger the irradiation dose and the longer the irradiation time, the heavier the degree of healing. For localized exposure, the same dose of soft X-rays is more effective in delaying healing than gamma rays and hard X-rays. Most of the radiation is absorbed by the superficial skin, which aggravates skin damage. For systemic radiation injury, the healing of the local wound interacts with the overall condition of the body. According to the existing literature, total body irradiation below 2 Gy has no effect on wound healing; When it comes to more than 4 Gy, wound healing is significantly delayed; and 7 Gy or more after systemic irradiation due to obvious damage of hematopoietic function, if not treated, usually not yet The wound healed and the animal has died.

The pathological process of high-dose ionizing radiation significantly delaying wound healing is mainly manifested as: hematopoietic function is inhibited, inflammatory reaction is weakened, especially inflammatory cells such as macrophages and neutrophils which are locally infiltrated by trauma are significantly reduced, and the wound initiation process is delayed; vascular damage, endothelial cell degeneration, necrosis, bleeding is obvious; granulation tissue formation and maturation are significantly slowed down, fibroblasts number and function are impaired; re-epithelialization process is delayed, healing time is prolonged. In recent years, molecular-level research has further deepened the understanding of the mechanism of ionizing radiation to delay wound healing. Studies have shown that the decrease of hematopoietic cell source and radiation-induced apoptosis is an important cause of the reduction of local inflammatory cell volume in trauma, and the radiation effect. Inhibition of cell proliferation and increased apoptosis are important reasons for the decrease in the number of fibroblasts.

The mechanism by which ionizing radiation delays wound healing is “the regulation of disorders by healing factors that are the key link of cell damage”, in which “cells” include both hematopoietic cells and repair cells, while “damage” includes both quantitative and functional damage. An important principle for the healing of open wound wounds is to increase the number of hematopoietic and repair cells in the wound and promote their function.

8.3.3 The Impact of Drugs on Healing

1. Cytotoxic drugs: These drugs can inhibit the growth, differentiation, and collagen synthesis of fibroblasts. Theoretically, they have the effect of delaying wound healing, but they have not been fully confirmed in clinical practice.
2. Steroids: Because it is the most commonly used anti-inflammatory drug in clinical application, it has a significant inhibitory effect on wound healing. Its main mechanism is to inhibit the inflammatory process and promote protein breakdown. It has been clinically proven that the preoperative or intraoperative steroid use cases have significantly increased complications, and systemic use of vitamin A can antagonize the inhibitory effect of steroids on inflammation. Recently, studies have also shown that mastering the application time and dosage of post-traumatic steroid drugs can sometimes promote wound repair. Other anti-inflammatory drugs have less effect on wound healing, but more than the pharmacological dose of aspirin has the effect of delaying wound healing.
3. In the process of debridement, some doctors added vasoconstrictor drugs and adrenaline to local anesthetics in order to reduce wound bleeding. The further research's purpose is to determine whether this measure aggravate local tissue ischemia and secondary wound hemorrhage, and then influence the healing process.

8.3.4 Other

If the local fixation is poor, the adjacent joint wound is difficult to heal. This may be related to premature activity, which may cause exudation reactions in the inflammatory process, aggravate local swelling, and affect blood supply to the injured area. The new granulation tissue is very fragile, which is prone to damage bleeding, affecting the differentiation of fibroblasts and the formation of scar tissue. Premature activity of the fracture is also prone to bone disconnection and pseudo joint formation.

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