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5 Tissue Repair and Regeneration Disorders: Repair and Regeneration of Chronic Refractory Wounds

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5.1 Epidemiological Characteristics of Chronic Refractory Wounds in Chinese Human Body

Chronic refractory wounds (commonly known as ulcers) can be formed by many reasons. The International Society for Wound Healing defines it as a wound that fails to achieve anatomical and functional integrity and secondary healing through a normal, orderly, and timely repair process. Clinically, it mostly refers to those who fail to heal and have no tendency to heal after more than one month of treatment. It depends on the wound size, the cause, the general health of the individual, and other factors. It mostly occurs in patients with severe chronic and acute injuries such as diabetes mellitus, trauma, varicose veins, angiosclerosis, paraplegia, and long-term bedridden. It has the characteristics of complicated pathogenesis, long course of disease, many disciplines involved, difficult treatment, and high treatment costs.

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5.1.1 The Pathogenetic Characteristics of Chronic Refractory Wounds on the Body Surface

In 1998, Academician Xiaobing Fu completed the first epidemiological study on chronic refractory wounds on the body surface in China. Through the investigation of more than 30,000 inpatient surgical patients in 15 hospitals in different regions, it was found that patients with chronic refractory wounds on the surface accounted for 1.5–3%, mainly due to traumatic infection (67.5%), pressure ulcer (9.2%), venous ulcer (6.5%), diabetic ulcer (4.9%), and other factors (11.9%). In terms of the affected population, the chronic refractory wounds caused by trauma are mainly young and middle-aged people aged 20–50 years old, while those caused by diabetes, pressure ulcers, and venous ulcers are mainly elderly people over 60 years old [1].

A cross-sectional, retrospective epidemiological study completed in 2009 found that patients with chronic refractory wounds accounted for 1.7‰ of all hospitalized patients, and complications of age-related diseases such as diabetes mellitus and pressure ulcer have become the main causes of chronic refractory wounds on the body surface (Fig. 5.1) indicating that the characteristics of chronic refractory wounds in Chinese human body have been consistent with those in western developed countries. In this study, more than 1/3 of patients with chronic refractory wounds were caused by diabetes, especially in the 40–60 and 60–80 age groups, accounting for 29.4% and 49.0%, respectively. This is highly correlated with the rapid growth of diabetes in our country. Since 1980, China has conducted five surveys on the prevalence of diabetes in 1980, 1994, 2002, 2007, 2008, and 2010 and found that the prevalence of diabetes has experienced a catastrophic development from 0.67% → 2.28% → 2.60% → 9.70%, rising to 11.6% in 2010. In 2013, the top three countries with diabetes in the 20–79 age group were China, India, and the United States, with 98.4 million, 65.1 million, and 24.4 million, respectively. It is expected that the rankings of these three

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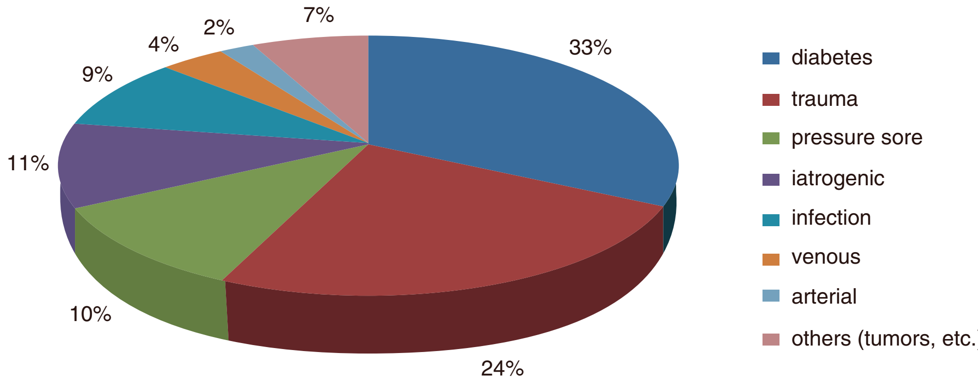

Fig. 5.1 Causes of wound formation (2009)

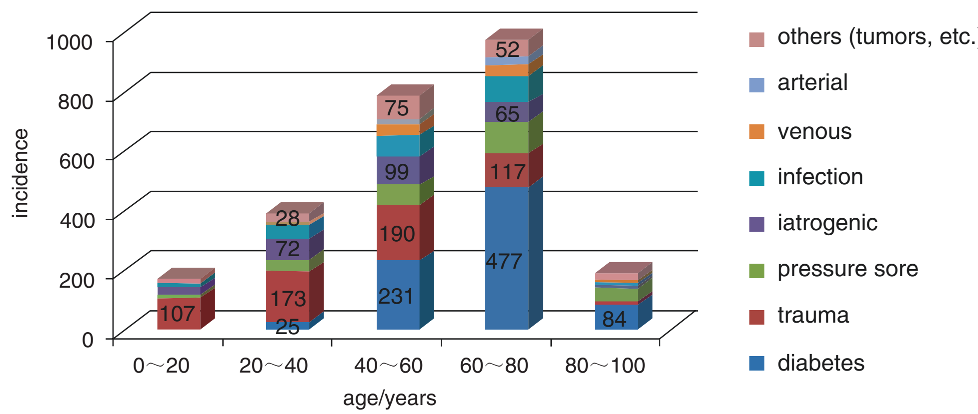

Fig. 5.2 Age distribution of chronic refractory wounds on the surface (2009)

countries will remain unchanged in 2035, with the number of patients increasing to 142.7 million, 109 million, and 29.7 million, respectively [2]. According to the China Health Statistics Yearbook in the past 5 years, the incidence of trauma due to various reasons has not changed significantly in 10 years [3]. From this we can understand that diabetes has replaced trauma as the primary cause of chronic refractory wounds on the surface.

Our study also found a significant change in the age distribution of patients with chronic refractory wounds on the surface. The average age of patients 10 years ago was 36 years old, which is a whole difference of 22 years compared with the average age of 58 years old. Older patients now occupy a significantly larger proportion, with the highest age of onset being 40–60 years old and 60–80 years old (Fig. 5.2). Retired people become the main patient group. A US-wide survey of pressure ulcers found that 73% of these patients occurred in the elderly over 65 years old, and the characteristics of the disease in China are consistent with those reported in developed countries. The incidence of chronic refractory wounds on the surface shows an aging trend, and these changes are related to the recent aging of China's population.

Regarding diabetic foot, a multicenter prospective study in 2003 showed that the majority of patients with foot disease in China were elderly, with low education and low income, and many had complicated complications of macrovascular and microvascular disease. Neuropathic ulcers are more common in patients with foot ulcers. The foot disease patients in the northern region are young, have a long course of diabetes, and have a short course of foot disease. The common factor affecting the severity of diabetic foot in the north and south is ABI. Recent studies have found that the annual incidence of new-onset ulcers in diabetic patients in China is 8.1%, the annual incidence of new-onset ulcers in diabetic foot ulcers is 31.6%. Independent risk factors leading to the occurrence of foot ulcers include kidney disease, insulin levels, and a decrease in HDL levels [1, 4, 5].

The most serious consequences of diabetic foot disease, as well as the greatest psychological and life effects on patients, are the problem of amputation. The investigation of amputation rate in 39-grade 3A hospitals nationwide in 2009 found that diabetes amputation (toe) accounted for 28.2% of all amputations (toes), accounting for 39.5% of nontraumatic amputations; in 2010, we found that amputation (toe) of diabetic foot ulcers accounted for 19.03% of total amputation (toe), including 2.14% for large amputations and 16.88% for small amputations, and the annual incidence of amputation was 5.1%. The independent risk factors of large amputations in patients with diabetic foot ulcers included the increase of

WBC and the previous history of foot ulcers, independent risk factors of small amputations included prolonged course of diabetes, elevated WBC, foot ulcer infection, foot deformity, history of revascularization surgery, and decreased postprandial blood glucose levels [6–9].

Pressure sores, as another type of chronic refractory wounds that are highly correlated with the elderly, bring heavy burden on families and society. The occurrence of pressure sores will prolong the length of hospital stay and increase the mortality rate of the disease, so the nursing staff must strengthen the prevention knowledge of pressure sores. A survey of 39,951 patients in 12 hospitals nationwide completed in 2012 found that the prevalence of pressure sores was 1.577%, and the prevalence of hospital-acquired pressure sores was 0.628%. Excluding reversible stage I pressure sores, the prevalence rate was 1.121%. The prevalence rate of pressure sores was slightly higher in military hospitals (1.72%) than in local hospitals (1.498%), higher in tertiary hospitals (1.694%) than in secondary hospitals (1.114%), and higher in traditional Chinese medicine hospitals (1.684%) than in western hospitals (1.55%). The prevalence rate of pressure sores in different hospitals was 1.114–1.72%. Among them, only 46.517% of hospitalized patients with Braden score ≤ 16 used decompression mattresses, 75.149% could turn over regularly, and nearly 1/3 of the patients did not use any decompression devices. In 76 patients with Braden score > 16 still occurred pressure sores, accounting for 12.063% of patients with pressure sores, and only 35.526% of these patients used decompression mattresses, and 56.579% could turn over regularly. It is believed that the cause of pressure sores may still be related to the lack of decompression mattresses and unscheduled turn over.

Iatrogenic injury refers to injury caused by the operation of medical personnel and not related to the primary disease during the medical procedure. The distribution of iatrogenic wounds involves all departments of the hospital, including surgery, internal medicine, and other specialties. Because both subcutaneous and deep artificial implants can cause wounds, so there are more surgical occurrences (including brain surgery using titanium mesh instead of skull implantation after exposure, brain pacemaker exposure, cardiovascular pacemaker exposure, artificial blood vessel exposure, oral and maxillofacial surgery/head and neck surgery implant exposure), especially in orthopedics and plastic surgery, due to the extensive use of biological materials (internal fixation equipment after limb fractures, prostheses after joint replacement, various plastic surgery materials), resulting in a higher incidence of iatrogenic wounds. This is mainly due to the continuous emergence of various biological materials, the expanding indications of surgery, and the increasing number of operations for elderly patients as their life expectancy increases.

5.1.2 The Pathogenic Microbiological Characteristics of Chronic Refractory Wounds on the Body Surface

Through a study of 1,488,201 cases, we screened 2513 patients with chronic refractory wounds that met the criteria and organized the contents of the bacterial culture in their medical records. It was found that 1853 cases were not cultured, 660 records could be analyzed, 144 records showed negative culture results, 4 records could not be classified, and up to 77.8% of patients in the records were treated with intravenous antibiotics. This suggests that in the clinic the bacterial culture rate of the wound and the use of antibiotics should be emphasized and strengthened. Analysis of 660 positive results showed that 36 species (347 strains) of Gram-negative bacilli, 17 species (265 strains) of Gram-positive cocci, 5 species (7 strains) of Gram-positive bacilli, and 1 specie (4 strains) of Gram-negative cocci, 7 species (42 strains) of fungi, a total of 66 sortable positive records (665 strains). *Staphylococcus aureus* (without methicillin-resistant *Staphylococcus aureus*) is the most common pathogen, followed by *Pseudomonas aeruginosa, Escherichia coli*, and coagulase-negative *Staphylococci*. Gram-positive bacilli and Gram-negative cocci are rare. *Candida albicans* is the most common fungus (Figs. 5.3 and 5.4). In this study, 473 cases with 1 pathogenic microorganism, 55 cases with 2 species, 12 cases with 3 species, 5 cases with 4 species, and 2 cases with 5 species were sequentially detected. Monopathogenic bacterial infection is the most common.

5.1.3 Health Economics Characteristics of Chronic Refractory Wounds on the Surface

Through comparative studies, it was found that although the proportion of self-pay was significantly reduced (58.9%, 42.3%) in 10 years, the individual treatment burden of patients was reduced, compared with 8.9 days (2007) and 8.6 days (2008) published in the National Statistical Bulletin on Health Development, the average hospital stay for chronic refractory wounds in the study was 21 days (P50; P25, P75: 12, 40), increasing the hospital stay for up to 13 days. Males with chronic refractory wounds due to diabetes had the longest hospital stay (P50 = 31 days; P25, P75: 19, 52.3) ($P < 0.01$). The average cost per patient was 12227.0 yuan ($1798.1) (P50; P25, P75: 6801.7, 26794.4) (Table 5.1), compared with the average medical expenses of 4123 yuan (P50 = 1600 yuan) for urban and rural residents, including 7606 yuan (P50 = 3375 yuan) in urban area and 2649 yuan (P50 = 1100 yuan) in rural area, the average indirect cost per hospitalization (mainly including transportation, escort, etc.) was 360 yuan (including 514 yuan in urban area and 294

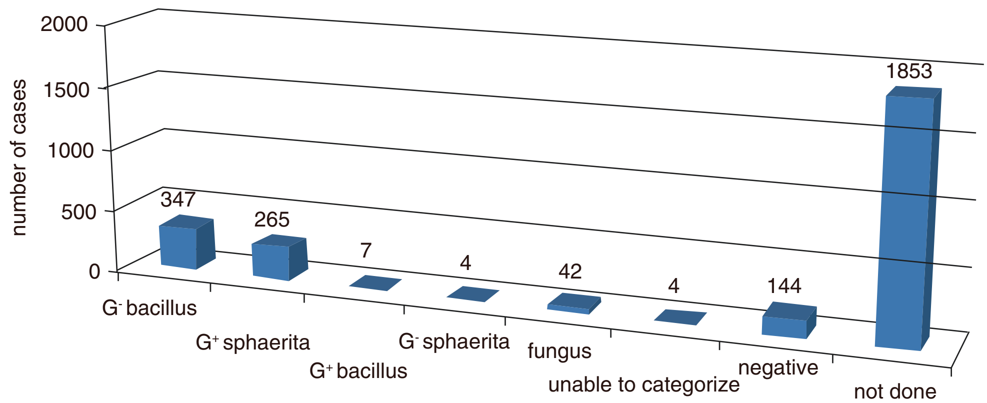

Fig. 5.3 Classification of pathogenic microbiological examination results of chronic refractory wounds on the surface

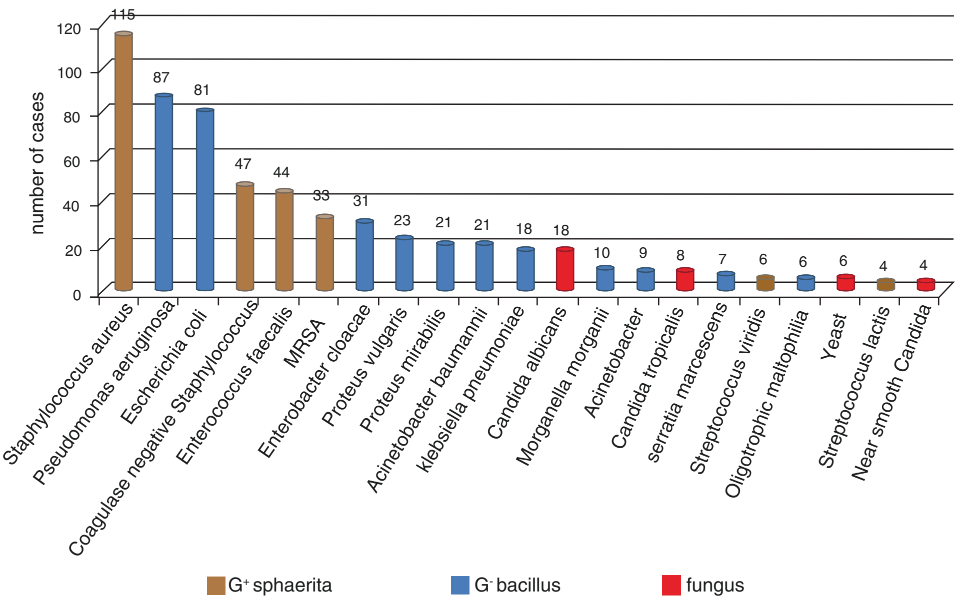

Fig. 5.4 Top 20 pathogenic microorganisms in positive test results of chronic refractory wounds on the surface

yuan in rural area), as well as the national per capita health cost was 854.4 yuan/person (2007) and 984 yuan/person (2008), it can be seen that the chronic refractory wounds caused a heavy health economic burden. In addition, the analysis of the distribution of medical expenses found that the cost of nursing is only 4%, and the cost of medicines accounts for 38% of the total cost. Uneven and unreasonable distribution of medical expenses may be related to relevant national policies.

Through the above research, it can be found that with the rapid development of China's society and economy, the aggravation of population aging, changes in lifestyle patterns and the accompanying changes in disease spectrum, diabetes mellitus has become the primary cause of chronic refractory wounds in China. The treatment of these wounds is particularly difficult due to the advanced age, infectious factors and basic diseases such as diabetes. The country needs to develop an overall plan for early prevention, early detection and early treatment of chronic wounds, and further improve the universal health care system to effectively reduce the incidence of chronic refractory wounds on the surface.

5.2 Study on the Mechanism of Local Skin Damage on Refractory Diabetic Wounds

Skin tissue wound healing refers to a series of pathophysiological processes in which local tissues are repaired by proliferation or regeneration after traumatic or other disease processes because skin tissue defects to form wounds [10]. Wound healing is regulated by the body's genes, making wound healing events have their own laws. The regulatory capacity of organisms can withstand the adverse effects of systemic or local factors through related mechanisms. Once the systemic disease or local pathological changes exceed the body's ability to regulate, it may lead to delayed wound healing or nonhealing, thus forming "out of control" wound repair.

Table 5.1 Health economics characteristics of different causes of chronic refractory wounds

Pathogeny	Diabetes	Trauma	Oppression	Iatrogenic	Infectivity	Venous	Arterial	Other	Total
Observed indicator	**Male**				**Median (P25,P75)**				
Average length of stay (d)	31 (19,52.3)	30 (17,52)	29.5 (19,55.5)	21 (12,35)	24 (15.5,41.5)	15 (9,26)	23 (11,33.5)	19 (10.3,29.5)	23 (13,42)
Average hospitalization fee (yuan)	17181.67 (9064.8,34219.1)	13689.1 (7671.4,33067.3)	17755.1 (8892.7,41064.8)	10444 (5234.4,18962.4)	10139.2 (5517.2,16087.4)	8694.1 (5391.3,11901.5)	18052.1 (9830,41138.3)	11837.9 (6374.3,22863.3)	13308.2 (7136.4,29870.1)
Mean healing time (d)	76 (45,163)	80 (45,176)	106 (53,383)	113 (60,259)	88.5 (47,234.8)	112 (53,601.5)	96 (62.5,156)	80 (53.5,389)	81 (46,208.8)
Observed indicator	**Female**				**Median (P25,P75)**				
Average length of stay (d)	27.5 (17.8,50)	27.5 (16,48.3)	18 (9.8,30)	19 (12,29)	23 (12,44)	18 (12,24)	23 (11,37)	17 (9,23)	20 (12,35)
Average hospitalization fee (yuan)	14068.6 (8391,26699)	13870.6 (6757.3,25191.6)	9839.7 (4975,20804.2)	8575 (4931.3,17223.9)	8411.1 (5330.9,13633.6)	9938.9 (6912.8,13875.1)	11602.9 (9168,20286.3)	10987.2 (5395.2,23139.3)	10942.6 (6052.4,22078.1)
Mean healing time (d)	117 (69,195)	71 (43,160)	76 (40,186.5)	132.5 (61.8,329.8)	70 (44,111)	205 (67,493.5)	67 (52.5,79.3)	220.5 (87.5,464.5)	89 (46,208)

Among all the related factors leading to the "out of control" wound repair, diabetes combined with refractory wounds is one of the most important areas for attention.

So far, it has been considered that the occurrence of diabetic foot is based on diabetic vascular neuropathological changes [11], which are generally manifested as delayed wound healing and ulcer formation with insufficient blood supply and infection. Related molecular biology studies show that:

(a) The migration of macrophages is inhibited, directly or indirectly impairing the chemotaxis of neutrophils and other tissue cells.
(b) Abnormal expression of a variety of growth factors, these growth factors are involved in cell proliferation, chemotaxis, synthesis, secretion, and other activities [12].
(c) Neuropeptide regulation pathway disorder, which inhibits cell proliferation, chemotaxis, and growth factor synthesis related to wound healing [13].
(d) The imbalance between matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) leads to new extracellular matrix (ECM) deposition and neovascular reconstruction disorders, which may be due to abnormal expression of growth factors.
(e) Abnormal cell biological behaviors: the number and bactericidal ability of inflammatory cells decreased, the chemotactic ability of monocytes decreased; fibroblast proliferation ability, response to growth factors, and collagen synthesis decreased significantly.
(f) The formation of glycation end products (AGEs) leads to an increase in oxygen free radicals, triggering an imbalance of oxidative stress.
(g) Inhibition of capillary regeneration in wound.

Recent studies have noted that vascular lesions in diabetic lower extremity ulcers are characterized by stenosis caused by thickening of the vascular basement membrane. After ulceration, vascular repair penetrates from residual diseased capillaries to the ulcer, mainly due to insufficient blood supply leading to the regeneration of capillaries in the wounds. Vascular regeneration after exogenous trauma includes revascularization, in addition to the above mechanisms. The possible reason for this difference is that the occurrence of diabetic lower extremity ulcers is due to the numerous biological behavioral abnormalities already existing in the diabetic skin, which leads to the pathological process with peripheral neuropathy and vascular lesions as the main clinical features and gradually develops into spontaneous ulcers. Although the repair process after the ulcer contains all the elements of wound healing, it is essentially a continuation of the same pathological process, which is related to several characteristic differences associated with the initiation of wound healing after the integrity has not been damaged in the case of external trauma. It is based on such differences that they differ in many aspects related to healing. Therefore, the study of the "out of control" mechanism of diabetic wound healing includes not only the occurrence of lower extremity ulcers but also the difficulty of wound healing caused by exogenous trauma.

Whether it is a spontaneous ulcer of diabetes or a wound caused by exogenous trauma, it has the same clinical feature—difficult to heal. Although diabetic vasculoneuropathy is considered to be the mechanism for diabetes refractory to healing, this understanding does not explain many biological behavioral abnormalities in diabetic skin and wound healing, and the treatments based on this understanding has only achieved unsatisfactory results in a large number of human experiments and clinical practice. The reason is that diabetic vasculoneuropathy may be only the pathological outcome of diabetic complications. Therefore, it is necessary to understand whether the various biological behavioral abnormalities in the healing process of diabetic wounds have a common "switch" by understanding the characteristics of diabetic skin and its posttraumatic healing, so as to clarify the initiating factors of the occurrence of refractory in mechanism and provide a more effective starting point for clinical treatment.

5.2.1 The Characteristics of Diabetic Skin

Diabetic wound healing is a noninvasive—invasive—repairing biological process. Therefore, whether there are abnormal histological, cytological, and molecular biological behaviors of diabetic skin before trauma is obviously important for the "out of control" of the whole wound repair process.

Studies shown that the wound healing in diabetic rats is inextricably linked to the increase of blood glucose and the high concentration of sugar in local tissues. Diabetic wound healing is associated with metabolic changes in diabetes, and local high glucose and AGEs accumulation are important features of biochemical changes in diabetic skin [14].

AGE Reader (DiagnOptics, Groningen, Netherlands) can simply and noninvasively detect the skin of the forearm and automatically obtain the skin autofluorescence (SAF) value. In clinical practice, the SAF value is used to reflect the accumulation of AGEs in human tissues. Studies have shown that the accumulation of type I collagen AGEs in the skin of patients with diabetic foot ulcers is significantly increased, and the accumulation of AGEs is correlated with SAF. SAF test can be used as a simple, rapid, and noninvasive method to screen chronic vascular complications of diabetes. Compare to glycosylated hemoglobin test, SAF can more accurately reflect the actual time of diabetic patients with glucose metabolism disorders and oxidative stress. Therefore, SAF test may be used as a simple method to assess local skin tissue and overall metabolic control status.

Diabetic skin usually has a thin appearance. At the histological level, the thickness of the epidermis and dermis are obviously thinned. In addition, in animal experiments, it was found that the layers of keratinocytes in diabetic skin were not clear, some epidermis lacks stratified arrangement, and the number of spinous cells was significantly reduced. The collagen in dermis of the diabetic is thin and disorderly arranged, and some collagens can be denatured and broken. The focal infiltration of chronic inflammatory cells can be seen in the collagen degeneration area [15].

In addition to histological changes, the content and properties of collagen in diabetic skin tissue also showed abnormal changes, and the hydroxyproline content and collagen solubility of skin tissue decreased significantly.

Tissue repair cells include epidermal keratinocytes, fibroblasts, and vascular endothelial cells. Immunohistochemical examination showed that the number of apoptotic cells in the skin of diabetic patients increased significantly.

A large number of reports have reported abnormal expression of growth factors in diabetic skin. It is generally believed that growth factors with healing-promoting effects in diabetic skin tend to be downregulated, but many experimental studies provide contradictory evidence. In the diabetic rat model, the expression of FGF-2 was also not decreased, and the expression of FGFR was higher than that of normal skin. However, the immunofluorescence double labeling technique showed that FGF-2 was coexpressed with AGEs at the same site [16, 17], thus it is reasonable to presume that there is a lack of growth factors with normal functional activity in diabetic skin.

Inflammatory cells usually play a role in entering the periphery of the wound after wound formation [18].

However, in the skin of noninvasive diabetes, focal infiltration of inflammatory cells in the region of collagen degeneration can be observed, and the increase in myeloperoxidase (MPO) content suggests the number of neutrophils in diabetic skin tissue increased significantly, and the content of malondialdehyde (MDA), which indirectly reflected the oxidative damage of cell membrane, was significantly higher than normal, aMMP-2 level and aMMP-2/TIMP-2 ratio were significantly higher than normal. Combined with the positive expression of Vimentin antigen, it reflects the excessive infiltration of inflammatory cells and a certain degree of tissue damage in the skin tissue under the pathological condition of diabetes.

The above characteristics of diabetic skin tissue indicate that there are histological and cellular biological changes without being subjected to exogenous trauma. This series of diabetic skin tissue behaviors involves various aspects related to wound healing, meaning that diabetic skin has a different starting point from normal wound and will inevitably affect the healing process after trauma.

5.2.2 The Characteristics of Wound Healing in Diabetes

Diabetic wounds showed pathological features of chronic prolonged inflammatory response and tissue repair delay [19].

Fibroblasts are one of the major repair cells [20]. The number of local fibroblasts in diabetic skin burn wounds was significantly reduced, and collagen deposition was also significantly reduced.

Vascular endothelial cells are involved in the initiation and development of inflammatory responses. The functions of chemotaxis, activation, migration, proliferation, and differentiation play an important role in the formation of new blood vessels. There is a neovascularization disorder in the healing process of diabetic wounds [21, 22], which is characterized by a decrease in the number of functional neovascularizations. The mechanism depends not only on the proliferation of vascular endothelial cells but also on the assembly of new blood vessels, which is an important link leading to the inhibition of vascular remodeling [14].

An important sign of wound healing is the reepithelialization of the wound. The proliferative activity of epidermal keratinocytes during re-epithelialization is one of the most important repairing actions in wound healing. Normal and orderly proliferation regulation is a necessary guarantee for the smooth healing of wounds. Studies shown that the decreased expression and activity of cell cycle regulators in epidermal keratinocytes is one of the mechanisms leading to delayed re-epithelialization and refractory wound healing under diabetic pathological conditions.

The expression of growth factors in diabetic wound healing showed different degrees of change. In the mechanism of diabetic wound healing, there is not only a change in the amount of growth factors, but also the deficiency of growth factors with normal functional activity caused by glycosylation of growth factors is an important condition of the growth factor. The local application of the exogenous growth factor FGF-2 can promote collagen neogenesis, improve repair cell function, and increase the expression of FGF-2 mRNA in diabetic wound tissue, which supports this inference.

The inflammatory response is an important stage in wound healing [23]. After the normal skin trauma, the acute inflammatory cells accumulate to the wound edge under the chemotaxis of inflammatory mediators to exercise their mission, forming a relatively clear inflammatory reaction zone in histology. After the diabetic skin trauma, a large number of inflammatory cells are diffusely infiltrated, and persist in the wound healing process. Furthermore, diabetic wounds often stagnate in abnormal inflammatory states, suggesting that there are not only abnormal inflammatory reactions in diabetic wounds but also obstacles in the transition from the inflammatory phase to the subsequent phase.

It was confirmed that the repair process of diabetic full-thickness injury showed delayed inflammatory phase, unsmooth transition in inflammatory phase-repair period and delayed repair period, that is, the wound showed an imbalance of inflammation/repair (Fig. 5.5) [24]. So what are the characteristics of macrophages that are closely related to them in this process?

Macrophage infiltration and polarization transformation were observed in the full-thickness skin defect repair model of diabetic rats. It was found that macrophage infiltration in diabetic wounds was "slow-in and slow-out." The number of macrophage infiltration in the diabetic group was less than that in the normal group on the first and third days after trauma (Figs. 5.6 and 5.7). On the third day after injury, the

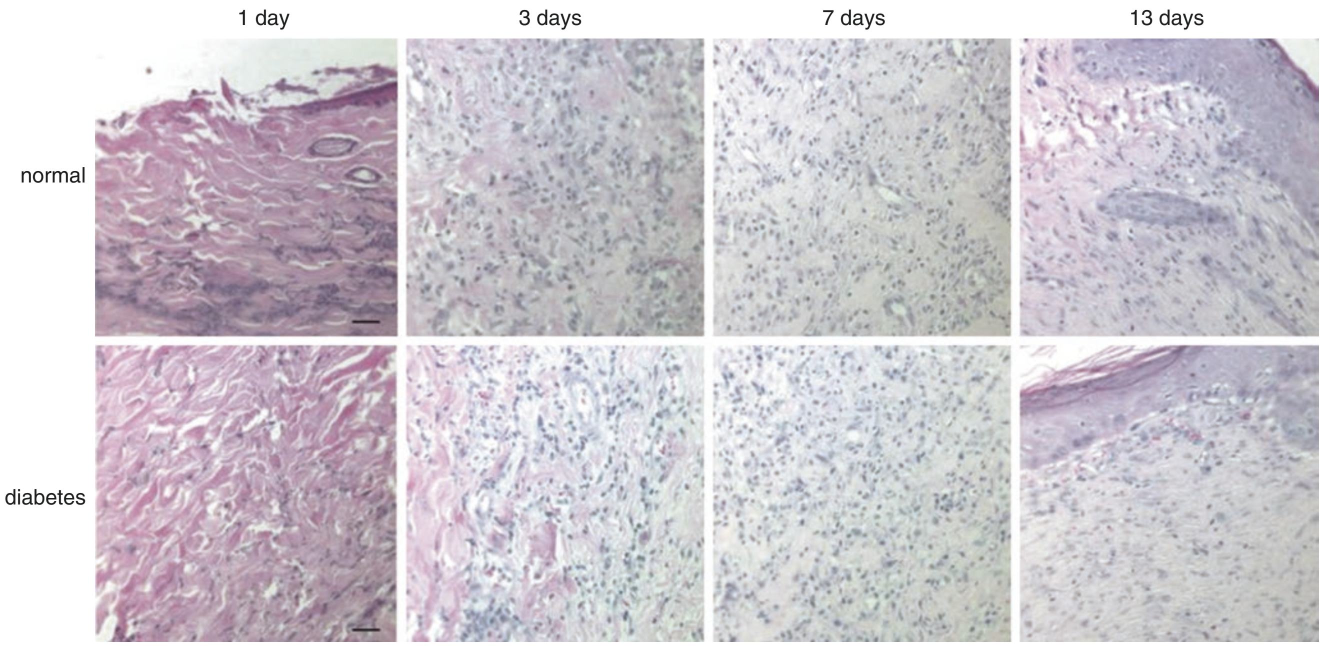

Fig. 5.5 HE staining shows the histological morphology of the wounds at each time point in both groups. (Scale 20 μm)

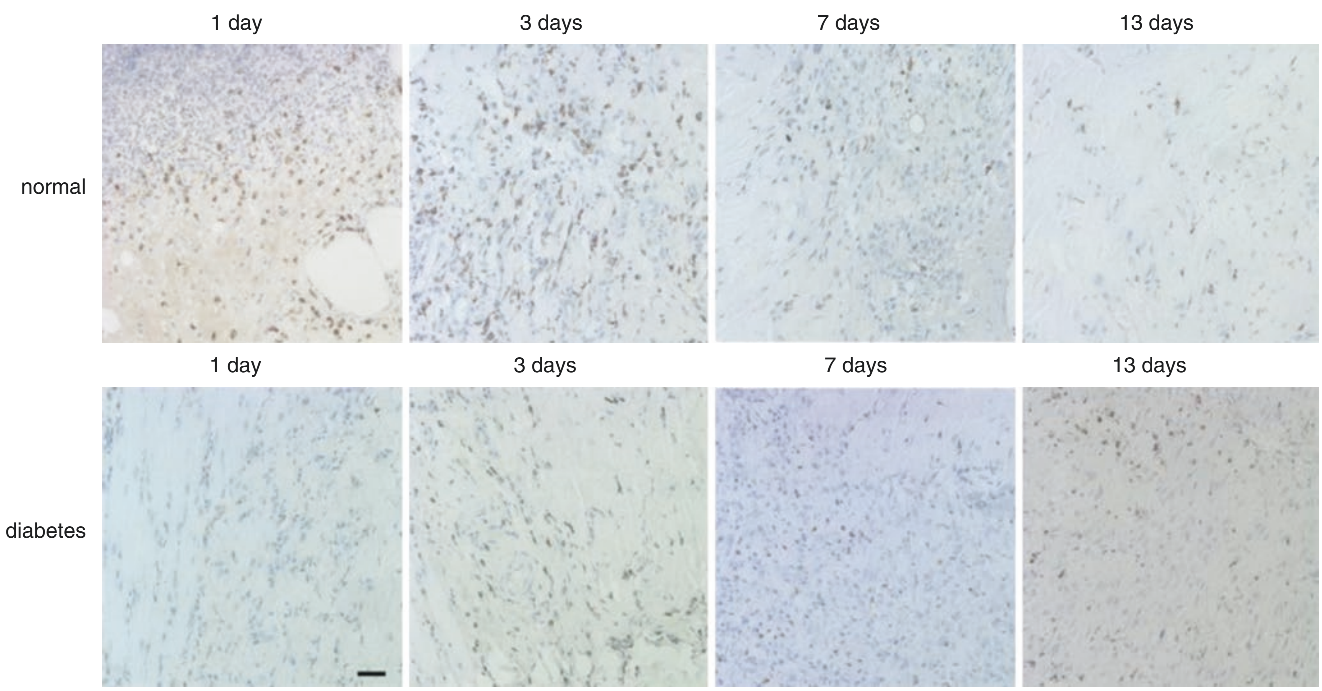

Fig. 5.6 Immunohistochemistry showed a difference in macrophage infiltration and regression between the two groups (scale 20 μm)

number of macrophages in the diabetic group was not only insufficient, but no typical phagocytosis was seen (Fig. 5.8).

On the seventh day after injury, there were still more M1 macrophages infiltrated in the diabetic wound, and the number of M2 macrophages was far less than that of the normal group. It is suggested that the wounds of diabetic mice show an imbalance of inflammation/repair accompanied by abnormal macrophage infiltration and polarization.

The exploration of the tissue characteristics and healing characteristics of diabetes skin has revealed the regularity of the diabetic wound healing, that is, the healing factors such as tissue cells, extracellular matrix, and growth factors interact and influence each other at various stages of healing through their own behavioral abnormalities, forming a "out of control" network for diabetic wound healing. It is worth noting that the diabetic skin is always accompanied by the presence of a high-glucose environment, the accumulation of AGEs, or the associated glycosylation effects, whether in the noninvasive skin tissue or in the wound repair process. This raises the question of how the product of glucose metabolism play a role in the "out of control" network of diabetic wound healing.

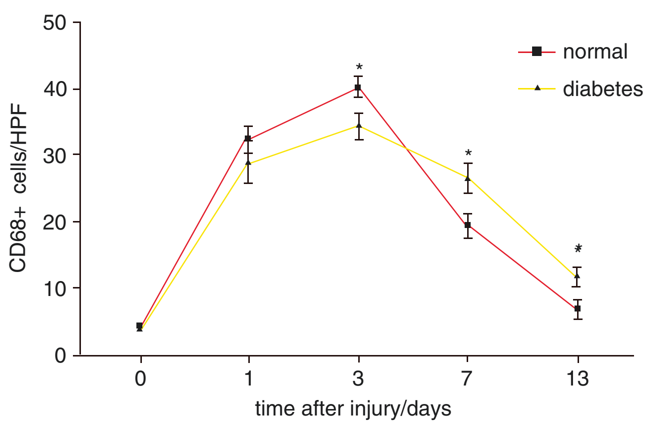

Fig. 5.7 Macrophage infiltration in the dorsal wound of diabetic rats was "slow-in and slow-out"

5.2.3 The Relationship Between Metabolic Disorders and Healing Factors

Diabetes is characterized by persistent pathological hyperglycemia, which triggers the activation of abnormal metabolic pathways, leading to the disorder of the internal environment on which cells depend to survive, causing pathological changes in the function and structure of cells, tissues, and organs. It is an important factor causing the occurrence and development of diabetic complications. Among them, nonenzymatic glycosylation induced by long-term hyperglycemia is one of the main metabolic remodeling activities, and its biochemical outcome is local high glucose and AGEs accumulation. Numerous studies have confirmed that the characteristic of short-term diabetes is mainly increased sugar content in skin tissue, while the characteristics of long-term diabetes are increased sugar content in the skin tissue and a large accumulation of AGEs. AGEs have a wide range of biological activities. Diabetic skin tissue is a predisposing site for glycosylation due to the presence of a large number of long half-life tissue components. At the same time, long-term high glucose environment makes tis-

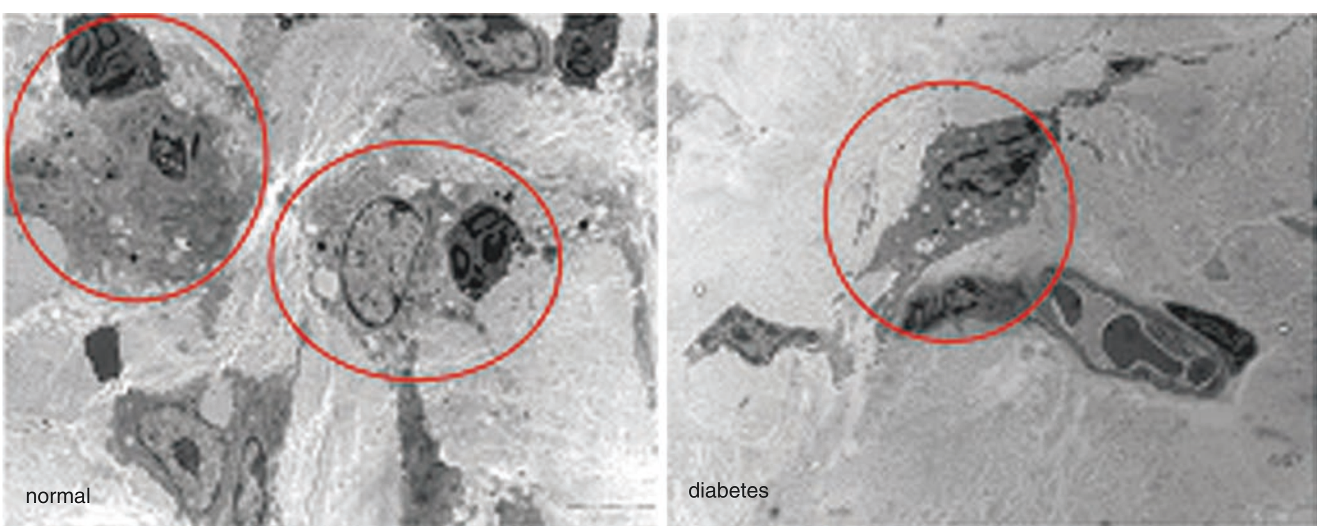

Fig. 5.8 Phagocytosis of neutrophils by macrophages observed under electron microscopy on the third day after trauma (×3400)
In a mouse model of full-thickness skin defect repair, on the third day after trauma, typical phagocytosis of neutrophils neutrophils (segmented cells) by macrophages (including vesicle cells) was observed, while only a small number of macrophage infiltration was observed in the diabetic group, and no typical phagocytosis of neutrophils by macrophages was observed

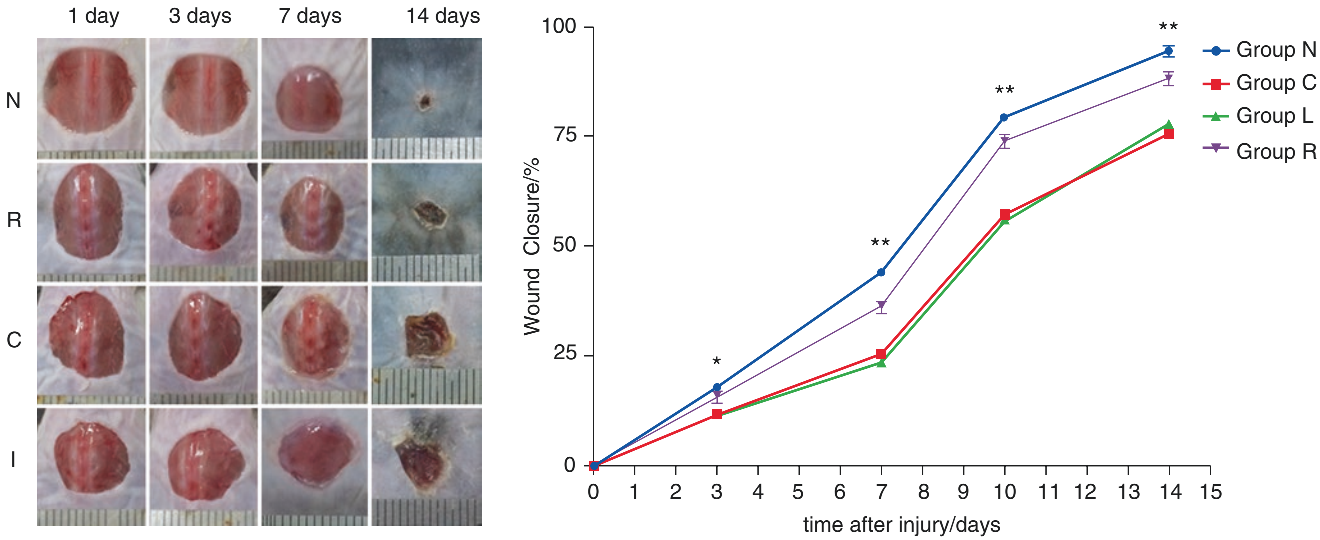

Fig. 5.9 Diabetic rat model of full-thickness skin defect and trauma The healing speed of RAGE antibody intervention group (R Group) was faster than that of diabetic control group, but it was lower than that of normal mice (N Group). On the third day after injury, the percentage of wound healing in R Group was significantly higher than that in normal saline control group with diabetes (C Group). There was significant difference between the two groups ($P < 0.05$), which was more obvious on the seventh and tenth day after injury. There was no significant difference between the IgG control group (I Group) and the C Group. Comparison of wound healing rate in each group: $^*P < 0.05$

sue cells involved in wound healing easily express glycosylation product receptor (RAGE), thereby constructing a structural pathway for the pathological effect of glycosylation products (Fig. 5.9).

In vitro experiments showed that cultured endothelial cells and fibroblasts have strong adherence ability and good cell viability. After high glucose intervention, the cell growth speed is accelerated, the cell volume is enlarged, and the metabolism is vigorous. After 48 h of AGE-BSA intervention, the number of endothelial cells decreased, parts of the cell membranes were intact, and cytoplasm appeared foaming. Under the intervention of AGE-BSA, the volume of endothelial cells and fibroblasts became smaller, the cell membrane shrunk, the division phase decreased, the cell adhesion ability decreased, the proliferation of fibroblasts and endothelial cells was inhibited, and apoptosis increased, angiogenesis of microvascular endothelial cells was inhibited (Fig.5.10).

By establishing a glycosylated extracellular matrix model to better mimic the environment of diabetes, it was further confirmed that glycosylation of extracellular matrix secreted by fibroblasts could inhibit fibroblast adhesion and proliferation and lead to increased apoptosis cells. The expression of RAGE, the receptor of AGEs, was increased in diabetic skin tissue. Glycosylated extracellular matrix upregulates p53 and p21 gene expression in human fibroblasts by RAGE mediation, affecting the orderly operation of cell cycle and promoting apoptosis by regulating the expression of Bcl-2 family proteins.

Study on the differences between high glucose and AGEs on the behavior of repair cells showed that AGEs could inhibit the proliferation of repair cells in 48 h. The adverse effects of high glucose and AGEs on the biological behavior of repair cells were time-effect relationship and dose-effect relationship, and the damage of AGEs was greater than that of high glucose. In addition, the study also found that fibroblasts were more tolerant to high glucose and AGEs damage than endothelial cells, and endothelial cells were more tolerant than keratinocytes, indicating that pathological damage of diabetes has different degrees of pathological effects on different tissue repair cells.

In vitro experiments also confirmed that with the increase of high glucose and AGEs concentration, the apoptotic rate of neutrophils decreased, and the release of neutrophil elastase and reactive oxygen species increased. While macrophages stimulated by high glucose, the expression of iNOS protein was enhanced and the expression of Arg-1 protein was inhibited, and the high glucose environment impaired the pro-vascularization ability of activated macrophages. At the same time, AGEs intervention inhibited the phagocytosis and inflammation-promoting ability of macrophages and enhanced their pro-inflammatory ability, all of which were dependent on RAGE pathway.

Pathological hyperglycemia persists in diabetic patients, and the formation of AGEs is accompanied by accumulation of various active intermediate metabolites. High glucose can spontaneously oxidize to form AGEs in an open aerobic environment, during which glyoxal (GO) and H_2O_2

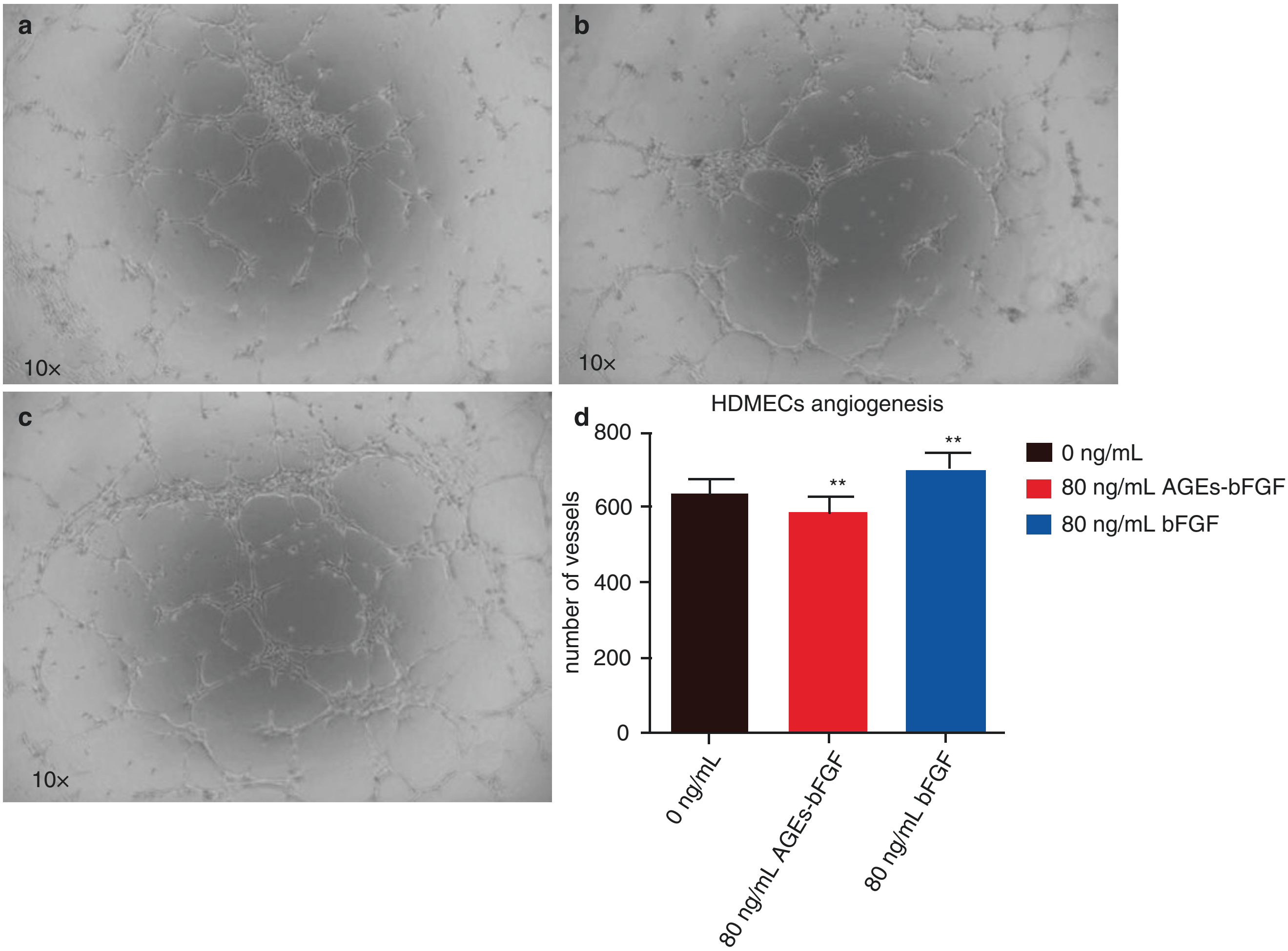

Fig. 5.10 Angiogenesis ability of human dermal microvascular endothelial cells under different intervention conditions (inverted microscope ×10)
The results of angiogenesis experiments showed that 80 ng/mL bFGF significantly promoted HDMEC angiogenesis (637.25 ± 15.97 vs. 701.00 ± 14.73, $P < 0.01$, A, C, D), while 80 ng/mL AGEs-bFGF significantly inhibited angiogenesis of skin endothelial cells (637.25 ± 15.97 vs. 586.00 ± 10.82, $P < 0.01$, A, B, D). Compared with the 0 ng/mL group: $^{**}P < 0.01$. A—normal endothelial cell culture medium; B—80 ng/mL AGEs-bFGF; C—80 ng/mL bFGF; D—comparison of cell angiogenesis ability of human dermal microvascular endothelial cells under different intervention conditions.

can be produced. AGEs can increase the release of neutrophil reactive oxygen species (ROS) in a dose-dependent manner. GO can promote the glycosylation of proteins and has a toxic effect on repair cells. The production of reactive oxygen species accelerates the formation of AGEs, affects the viability of repair cells, and promotes the production of ROS and MDA, thus forming a vicious circle between oxidative stress and AGEs independent of the high-glucose environment, affecting each other, causing each other, resulting in a series of chain reactions and amplification effects.

The local accumulation of high glucose and AGEs as the direct products of metabolic remodeling in diabetes initially mediated the biological abnormalities in diabetic skin by altering the skin microenvironment. These phenotypically different biological abnormalities are an integral and interrelated group of syndromes due to the common initiating factors of metabolic abnormalities in nature, namely, "Underlying Disorder" of diabetic skin. At the same time, local metabolite accumulation is one of the important sources of environmental stimuli, acts as a "switch" for the "out of control" network of diabetic wound healing during the whole process of wound repair. The presentation and confirmation of this series of concepts provides a powerful basis for the ultimate understanding of the nature of "out of control" in the healing of diabetic wounds.

5.2.4 The Exploration of Intervention Methods for Diabetes Complicated with Refractory Wounds

5.2.4.1 Overview of Mechanisms and Interventions for Diabetic Refractory Wounds at Home and Abroad

A lot of researches have been carried out on the related mechanisms of diabetic refractory wounds at home and abroad, especially the occurrence and development of diabetic foot, and some achievements have been made [25].

So far, it has been considered that diabetic vascular neuropathological changes are an important cause of diabetic refractory wound. With the deepening of research, people also noticed the relationship between biochemical changes of diabetes and diabetic complications. It is believed that in the mechanism of high glucose-mediated diabetes complications damage formation, four metabolic remodeling constitute the main pathways of metabolic abnormalities: polyol pathway, formation of AGEs, protein kinase C (PKC) pathway, and hexosamine pathway. These metabolic remodeling can affect the development of diabetic complications through the excessive production of peroxide.

On this basis, people have explored relevant interventions. These interventions can be broadly divided into two categories. One is for the main causes of vascular neuropathy, including antiinfective treatment, improving blood supply and neurotrophy, growth factors application, reducing local mechanical load, and diabetic foot care. The second type of means is to achieve the purpose of promoting healing by intervention of certain drugs on biochemical abnormalities of diabetes and subsequent pathways. Studies have shown that aminoguanidine can selectively inhibit the activity of nitric oxide synthase (iNOS) by blocking AGEs and advanced lipoxidation end products (ALE), and promote the healing of diabetic refractory wounds [26].

In summary, the research to date has greatly promoted the research progress of related mechanisms through the description of the pathophysiological and molecular biological abnormalities of diabetic wounds. However, it is worth noting that the exploration of interventions based on this is still unsatisfactory.

5.2.4.2 Establishment of Intervention Means

In view of the experience and deficiencies in the previous researches on the mechanism of diabetic refractory wounds, we believe that the following factors must be considered in the establishment of interventions:

(a) The establishment of interventions must be based on systematic mechanism research.

Previous mechanism studies involved various factors including cells, extracellular matrix, growth factors, etc., in the process of wound repair, and many pathophysiological changes in diabetic skin were also observed. At the same time, the possible pathways of metabolic remodeling caused by abnormal glucose metabolism on the occurrence of diabetic complications were explored. However, an obvious drawback of the above studies is the lack of systematic organic correlation among the various elements.

Diabetic skin tissue already has histological and cellular biological changes in the absence of exogenous trauma. At the same time, local high glucose and AGEs accumulation, as the direct product of metabolic remodeling in diabetes, initially mediates the biological abnormalities of diabetic skin by changing the skin microenvironment. The different biological abnormalities on these appearances are a collective and interrelated group of syndromes, namely, “Underlying Disorder” of diabetic skin, due to the common initiating factors of metabolic abnormalities in nature. Based on this understanding, the interventions we have chosen can reverse or reduce the “Underlying Disorder” of diabetic skin, thus achieving the goal of improving the wound healing.

(b) Interventions should give priority to upstream events in the pathogenesis.

Although diabetic vascular neuropathy is considered to be a mechanism of diabetes refractory to healing, this understanding does not fully explain many biological behavioral abnormalities in diabetic skin and wound healing. The reason is that diabetic vascular neuropathy may be only the pathological outcome of diabetic complications. Through the analysis of the “Underlying Disorder” of diabetic skin and the characteristics of posttraumatic healing, we summarized the nature of the diabetic refractory wounds, that is, the diabetic refractory wound is a pathological evolution process based on diabetic metabolic disorders and mediated by subsequent events of metabolic abnormalities [27, 28]. Compared with diabetic skin blood vascular neuropathy, the accumulation of metabolites in local tissues is an upstream event in the development of this disease. The microenvironmental change caused by the increase of glucose content and accumulation of metabolites in skin tissue caused by diabetic metabolic disorders, namely, “cutaneous environmental disorders,” is one of the initiating factors leading to the diabetic refractory wounds. Therefore, for the prevention and treatment of diabetes complicated with refractory wounds, it is undoubtedly the focus should be placed on the initial stage of “cutaneous environmental disorders,” that is, through the intervention of the upstream link related to wound healing, to stop the occurrence or development of its follow-up effects, so as to provide means for effective and feasible prevention and treatment strategies, and achieve an ideal preventive and therapeutic effect on diabetes complicated with refractory wounds.

(c) Interventions should try to avoid strategies that are specific to a particular aspect of the wound healing process.

The exploration of the skin tissue characteristics and healing characteristics of diabetes has revealed the regularity of the diabetic refractory wound healing, that is, the healing elements such as tissue cells, extracellular matrix, and growth factors interact and influence each other through their respective behavioral abnormalities at various stages of healing, forming a network of "out of control" for diabetic wound healing. Therefore, a single intervention, or an intervention strategy for a single process, is often difficult to achieve a better healing effect.

Based on the above principles, we have selected arginine and aminoguanidine as interventions to improve and prevent "Underlying Disorder" and refractory wounds of diabetic skin tissue on the basis of a large number of experimental studies.

5.2.4.3 The Main Role of Arginine and Aminoguanidine in the Mechanism of Diabetes Complicated with Refractory Wounds

The Effect of Arginine on the "Underlying Disorder" of Diabetic Skin Tissue

Arginine is a conditionally essential amino acid, which can be synthesized by the body to meet the metabolic needs under normal circumstances. Arginine not only has the effect of lowering blood glucose and promoting insulin secretion but also promotes the repair of diabetic refractory wounds by accelerating collagen synthesis and reconstituting the nitric oxide pathway to restore the normal proliferation of cells.

The content of glucose in the skin tissue was significantly decreased after the application of arginine, indicating that arginine can effectively correct the increase of local tissue glucose content caused by glucose metabolism disorder, which may reduce the pathophysiological changes of local tissues. Arginine can reduce hyperglycemia in diabetic rats through the polyamine formation pathway, and arginine can also lower blood glucose by stimulating insulin release or increasing tissue sensitivity to insulin.

Current studies have confirmed that arginine mainly affects cell physiology through the conversion of arginine to polyamines and nitric oxide (NO), while polyamines and NO regulate and stimulate cell proliferation. Arginine is a precursor for the synthesis of NO. NO can activate guanylate cyclase in cells and participate in a variety of intracellular metabolic processes [29]. Arginine is converted to putrescine and polyamine by ornithine. Polyamines are closely related to cell growth, which can stimulate DNA and RNA biosynthesis. Putrescine also promotes cell proliferation. In diabetic skin tissue, the NO content is insufficient due to the decrease of iNOS activity [30]. This lack of NO may be an important cause of tissue repair cell proliferation disorders. The supplement of exogenous arginine can significantly increase the activity of iNOS and increase the level of local NO, so as to achieve the purpose of reconstructing cell proliferation behavior [31, 32].

In view of the above pharmacodynamic effects of the arginine, prophylactic application of arginine to diabetic patients can improve the "Underlying Disorder" phenomenon of diabetic skin, thereby reducing the occurrence of diabetic skin tissue complications.

The Prevention and Treatment of Aminoguanidine on Refractory Wounds

Aminoguanidine is a hydrazine compound with a nucleophilic effect. It can competitively bind to the early glycosylation product to form inactive substitutes that cannot cause protein cross-linking, thereby inhibiting the formation of AGEs and ameliorating the biological behavioral abnormalities of healing factors such as tissue repair cells, extracellular matrix, and growth factors mediated by AGEs.

Our study shows that after prophylactic application of aminoguanidine, the content of AGEs in diabetic skin tissue is significantly reduced, the collagen solubility of skin is significantly increased, and the wound healing rate is significantly increased. In addition, aminoguanidine significantly inhibits the vicious circle between AGEs and oxidative stress, and improves the toxic effects of H_2O_2 on fibroblasts.

5.2.4.4 Prospects for the Intervention of Diabetes Complicated with Wound Healing

Diabetes is one of the most common diseases in daily life. With the development of the economy and the improvement of living standards, the incidence is increasing year by year.

Diabetes combined with refractory wound healing is one of the important complications of diabetes.

In our country, in stark contrast to the increasing incidence of diabetes with refractory wound healing, there is a lack of awareness and means of prevention and treatment. Patients with diabetes complicated with refractory wounds and high-risk groups have neither specialist treatment nor effective health education. At the same time, some western developed countries attach great importance to the prevention and treatment of diabetic refractory wounds, and have begun to form the principles of diagnosis, treatment, prevention and care, and conducted a large number of researches on relevant interventions. In this field, China is clearly in a backward state.

Therefore, relying on systematic research accumulation of the mechanism of diabetes wound healing and interventions with independent intellectual property rights, in-depth study on the prevention and treatment mechanism and optimal application of arginine and aminoguanidine for diabetes combined with refractory wound, and finally complete the pre-clinical experiments of arginine and aminoguanidine, thus laying the foundation for the establishment of standardized and systematic diagnosis and treatment of diabetic refractory wounds, which not only has academic value but also has broad market prospects.

5.3 Establishment of Innovative Treatment Methods for Refractory Wounds

For a long time, people have been looking for ways or drugs to shorten the healing time of wounds while improving the quality of wound healing to make perfect healing. For representative diabetic foot and radiation ulcers, we have created four innovative key treatment technologies based on routine surgery, wound negative pressure therapy, modern moisturizing functional dressings, etc., which significantly improved the cure rate:

(a) Surgery plus photon therapy.
(b) Modified cytokine therapy.
(c) A new comprehensive treatment technology system based on scaffold materials and cell therapy.
(d) For the first time in the world, the “4G” system has been used to realize the same standard in different levels of medical institutions for the treatment of complex wounds, which has played an important role in improving the cure rate of refractory wounds.

5.3.1 Surgery Plus Photon Therapy

He-Ne laser can accelerate local blood flow velocity and increase capillary permeability, thereby improving inflammation local microcirculation, affecting enzyme activity, strengthening intracellular ribonucleic acid, protein and glycogen synthesis, enhancing the activity of phagocytes, enhancing their phagocytic ability, improving the immune function of tissue cells, improving the body’s immunity and metabolic level, and improving the body’s nutritional status. In addition, it also promotes fibroblasts proliferation, neovascularization, collagen synthesis and granulation tissue growth, and helps control infection. Semiconductor gallium, aluminum, and arsenic lasers can expand microvessels, promote blood circulation, increase oxygen carrying capacity of red blood cells, improve tissue utilization of oxygen, activate red blood cell surface enzyme system, increase ATP production and DNA and RNA synthesis, promote material metabolism and energy metabolism, and improve the immunity of the individual, thereby facilitating the repair and regeneration of the damaged tissue.

In addition, electrical stimulation can promote blood vessel formation and enhance the migration of epidermal cells. The mechanism is mainly magnetic field nonthermal biological effects. The electromagnetic effect generated by the applied magnetic field is transmitted to the target tissue through a rich neural network. As a result, the metabolic activity of the cell is strengthened, including the biological activity of macrophages, granulocytes, and lymphocytes, which plays a more active role in the antiinfective process. Increased red blood cell metabolic activity improves hemoglobin oxygen carrying capacity, increases oxygen supply, and improves internal respiratory function. The cell activity of the lesion tissue is enhanced, on the one hand; it can promote the absorption of inflammatory exudate, on the other hand; it can accelerate the repair of damaged tissue and inhibit the adhesion of scar. The generation of microcurrent can have a series of effects on the bioelectrical activity in the body, such as strengthening the activity of Na^+, K^+, Cl^-, changing the membrane potential, enhancing the permeability of cell membrane, and promoting the exchange of substances inside and outside the cell membrane. Therefore, the magnetic field has the effects of sedation, analgesia, and alleviation of inflammatory response, which may be associated with the increased activity of cholinesterase, monoamine oxidase, histamine, and kininase.

Short-wave has the function of promoting blood circulation and promoting the absorption of inflammatory substances, at the same time, it can increase the function of phagocytic cells, and the action site is deep, which has a good effect on the control of wound inflammation. At present, there is a kind of warm-up therapy (TM) on the foreign market. The device uses advanced medical foam dressing to retain the thermal temperature of the wound surface as the greenhouse effect, and the wound temperature rises to 38 °C. Because it is not directly contact with the wound, there are no adverse reactions, and good results have been achieved for the treatment of venous ulcers and pressure sores.

In response to radiation ulcers, we innovatively expand the debridement domain, excise more tissues outside the wound margin, reduce and optimize the ineffective necrosis-clearance-repair process based on the characteristics that the necrosis scope of this type of ulcers exceeds the naked eye observation domain. In addition, innovative photon therapy technology is used to accelerate the healing speed of chronic refractory wounds represented by diabetic foot, improve the quality of healing, and achieve remarkable results. Related studies have shown that high-power red photons can promote the synthesis of adenosine triphosphate (ATP); enhance the body's antioxidant capacity; promote the synthesis of DNA, RNA, and protein; promote the material exchange and metabolism of cells; increase glycogen utilization; increase cell respiration; improve blood circulation; reduce wound exudate and promote exudate recovery; promote cytokines production and accelerate cell division; accelerate the regeneration of damaged nerves; promote the growth of granulation tissue and skin, wound healing and ulcer healing; accelerate division of bone cells, promote the healing of callus; increase the phagocytosis of white blood cells and enhance the body's resistance; reduces the 5-hydroxytryptamine (5-HT) content in the inflammation site and acts as an analgesic effect. At present, relevant products in China have been applied in clinical practice and achieved good therapeutic effects.

5.3.2 Modified Cytokine Therapy

Growth factors not only directly participate in the inflammatory response of the wound but also affect a series of biological processes such as the transformation of tissue repair cell cycle. After exogenous application of growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF), the wounded "inactivated" macrophages are activated and release growth factors such as TGF, TNF and FGF. Exogenously applied growth factors plus endogenously released growth factors can act directly on tissue repair cells, thereby initiating the repair process. In the early 1990s, scholars abroad used the autologous platelet-derived growth factor to treat chronic wounds including diabetic ulcers, bedsores, ulcers caused by arteriovenous diseases of the lower extremities, and all of them achieved unexpected results.

The therapeutic effect of transgenic growth factor therapy on wound healing has received more and more attention in recent years. At the beginning of the twentieth century, a multicenter, large-sample clinical study completed in China showed that the recombinant bovine basic fibroblast growth factor (bFGF) had 2.5 days, 4 days, 5 days, and 3.5 days earlier healing promoting effect on shallow II degree, deep II degree burn, granulation wound and donor site than the control in the same period, and no adverse reactions occurred. So far, the domestic pharmaceutical administration has officially approved the recombinant bovine basic fibroblast growth factor, and recombinant human epidermal growth factor (EGF), which are widely used in clinical treatment of various acute and chronic wounds. Modified cytokines are currently widely used in ophthalmology, otolaryngology, gynecology, etc., due to their good repair effects and are also used to repair damaged myocardium and islet cells.

5.3.3 A New Comprehensive Treatment Technology System Based on Scaffold Materials and Cell Therapy

In the course of clinical practice, aiming at diabetic foot wounds, radioactive refractory wounds, and other causes, such as wound healing or refractory wounds caused mainly by repairing cell damage or dermal scaffold loss, we have established a "one-stop" approach to promote tissue repair by supplementing repair cells and providing scaffold materials, which plays an important role in promoting repair and reducing the amputation rate.

In the past half century, with the deepening of people's understanding of the mechanism of wound repair and tissue regeneration, especially Dr. Winter's research on the wet environment and wound healing effect and related mechanisms in the 1960s, people gradually realized that the dressing should also be endowed with functions such as promoting or accelerating wound healing and improving repair quality.

Tissue engineering is the comprehensive application of cell biology and engineering principles. In the laboratory, a part of human tissue cells are cultured and expanded in vitro, then these cultured cells are planted and adsorbed on a biomaterial scaffold, and then transplanted to the needed parts of the human body to repair tissue defects, replace part or all of the functions of the tissues and organs, or as an extracorporeal device, temporarily replace some of the functions of the organs to achieve the purpose of improving the quality of life and prolonging life. At present, the tissue engineering products that can be applied in the local treatment of trauma wounds mainly include tissue engineering artificial skin, tissue engineering cartilage (bone), tissue engineering tendon, and tissue engineering peripheral nerve. Now the most researched and most likely to be applied clinically are various types of stem cells. Stem cell application plays two main roles in wound repair and tissue regeneration. On the one hand, pluripotent stem cells transform into related tissue repair cells to promote repair and regeneration under the action of local microenvironment in the injury site. On the other hand, stem cells exert autocrine and paracrine effects on wounds, secrete a large number of growth factors related to tissue repair and regeneration, and participate in the process of repair and regeneration. At present, there have been reports that local application of mesenchymal stem cells has a positive effect on angiogenesis of diabetic foot and regeneration of skin sweat glands after severe burns.

Under the guidance of this idea, we combine a variety of derived stem cells (umbilical cord, bone marrow, etc.) with scaffold materials (chitosan, hydrocolloid, hydrogel, etc.), which have a good affinity with tissue cells, and then through 3D printing and other technologies form a three-dimensional structure with a variety of biologically active factors, which plays an important role in promoting repair and reducing amputation rate.

5.3.4 Utilizing the "4G" System to Improve the Cure Rate of Refractory Wounds

For the first time in the world, the "4G" system has been used to achieve the treatment of complex wounds with the same standard in different levels of medical institutions, which has played an important role in improving the overall cure rate of refractory wounds.

The community is the basic unit for the distribution of urban residents in China. By working with community health institutions, we extend the medical services for wound repair to the community, provide a convenient medical environment, and have better operability to meet the medical requirements of most patients with wound diseases. Taking the wound repair department as the core and the community medical care as the network, the layout of the wound repair medical service constructed is obviously adapted to the occurrence law of wound diseases and is represented as the overall pattern of "small wards and big outpatient service." In order to realize this pattern, we have realized the treatment of complex wounds with the same standard in the different levels of medical institutions for the first time in the world by using the "4G" system. This system enables the wound treatment experts of large hospitals to truly observe the wounds while away from the grassroots or community health institutions, thereby directly participating in the wound treatment process, which not only facilitates patients' medical treatment but also ensures the quality of medical care and plays an important role in improving the cure rate of refractory wounds. The specific operation of the wound repair department and the community medical linkage mechanism involves the coordination and interaction of the wound repair department, the health administrative department, and the community health institution. To this end, based on the experience accumulated in the initial operation phase of the linkage mechanism, through repeated communication with the health administrative departments and community health institutions, we have determined the policy direction of institutional guarantees and mechanism incentives, which laid a good foundation for mobilizing the subjective initiative of all parties. We have been specially commented by international peers and highly praised the topic of "Looking to the East." [33–38]

5.4 Theory and Practice of Wound Healing Center Construction

With the rapid development of the social economy, the human disease spectrum has undergone significant changes. In the past, some major infectious diseases that seriously endangered the physical and mental health of the people have been eliminated, but the wounds caused by trauma and various diseases have become one of the diseases with the highest incidence in society today. Wound formation involves a variety of factors including trauma, systemic (local) diseases, and aging population, involving a wide range of people. Wound treatment is not only the basis of all trauma treatments but also the key to preventing late complications and promoting the early recovery of trauma patients. Whether in Europe and the United States or in China, wound epidemiological studies have shown that due to socio-economic development, the main factors leading to wound formation have been transformed from traditional trauma-infective factors to complications caused by various chronic diseases and senile diseases, such as ulcers and bedsores caused by diabetic foot and varicose veins of the lower extremities. In the past 20 years, the increasing number of various wounds and the complexity of various causes of wound formation have led to an increasing demand for wound treatment [39]. Multicenter epidemiological survey of chronic refractory wounds shows that the age group with high incidence of chronic wounds in China is 40–59 years old (3 1%) and 60–80 years old (38%), which not only includes the young and middle-aged people but also covers the ageing population who requires a large amount of medical care. The changes in the spectrum of these diseases are closely related to economic development and harmonious development of society. The development of high technology has provided many new treatments and methods for wound healing and has made wound treatment more complicated and diversified. Establishing a scientific and rational diagnosis and treatment model is a guarantee to promote the development of new technologies for wound healing and to benefit trauma patients. In traditional treatment systems, it will be very difficult to continue to treat complex wounds by a single surgery (such as orthopedics, burns, or general surgery) or a subject in the internal medicine (such as diabetes and endocrinology). The establishment of a comprehensive wound center (specialist), which consists of personnel in surgery, anesthesia, internal medicine and nursing, makes it necessary to obtain systematic treatment of complex wounds caused by multiple factors, and is the key to improving the cure rate. This model, like the previous treatment of severe diseases, is based on the integration of multiple disciplines according to the critical illness treatment needs. Wound centers and intensive care units are innovative treatment models that follow the development of medical technology [40].

After Professor Xiaobing Fu and other experts systematically proposed the idea of constructing a standardized and exemplary wound center in 2005, China began to practice in related fields [41]. In the early stage, Professor Han Chunmao, from the Second Affiliated Hospital of Zhejiang University, started the practice of establishing a wound therapy specialty in a medical unit based on the burns department and using the academic and technical advantages of the burn department in treating wounds. Later, some medical units carried out the construction of wound treatment specialist for a single disease, such as Professor Xu Zhangrong from the 306 Hospital of the People's Liberation Army, using the technical advantages of diabetes specialists in the treatment of diabetes and introducing surgical personnel, established the Diabetes Foot Treatment Specialist in the 306 Hospital of the People's Liberation Army, which achieved remarkable results in the treatment of diabetic foot. In addition, the outpatient departments of some hospitals have formed a specialist dressing room for wound treatment through the strengthening of the dressing room technology and equipment. According to the survey, there are more than 200 wound centers (specialties). In 2014, we conducted a survey of the composition of wound healing centers or specialists named after Wound Healing Centers or Wound Care Units in 69 third grade A hospitals in China. Among the 69 wound centers, 46% developed from the expansion of the original burn surgery function, 32% developed from the strengthening of the original outpatient dressing room, and 12% were set up in the endocrinology department or diabetes center, the other 10% were branch departments established by departments such as vascular surgery, orthopedics, hand and foot surgery, and plastic surgery. The characteristics of these wound centers are as follows: the treatment team is under the doctor's guidance (leadership) and participated by the nurses, which has changed the situation in which there were only nurses but no doctors in the past; most of the doctors and nurses involved in the wound treatment have carried out wound treatment specialist training or participated in related study classes in the early stage, and systematically understand the basic theories and basic skills of wound treatment; because doctors and nurses participate in wound treatment together, the scope and disease types of wound treatment specialists are significantly expanded; because of the formation of specialty, many new treatment techniques and methods can be used centrally, improving the utilization rate of treatment equipment and treatment technology; the cure rate, average hospitalization days, bed occupancy rate, and drug proportion of chronic refractory wounds are greatly improved. Great results have been achieved in both health economic and social benefits.

In view of the characteristics of "small wards and big outpatient service" for chronic refractory wounds [1], we combined the current characteristics and operation modes of Chinese medical treatment, changed traditional treatment methods, and innovatively established a new mode of bidirectional linkage and referral between complex refractory wound treatment specialists and community medical institutions. The new model has initially solved the problem of patients with chronic refractory wounds that have difficulty in seeing a doctor, expensive medical treatment and long hospital stay, and are highly recognized by international colleagues. According to incomplete statistics, by the end of October 2014, we had advocated the establishment of more than 50 wound centers in China. The cure rate of these centers for refractory wounds increased from 54% before the establishment of the centers to 93%, and the average hospitalization days decreased from 47 days to 26 days, and the proportion of drugs decreased from 17% to 14%.

The specialized training of doctors and nurses and the development and rapid transformation of innovative treatment technology are the key to ensuring the connotation development of the construction of wound specialty centers and improving the success rate of treatment. In March 2010, led by Academician Xiaobing Fu of the Chinese Academy of Engineering, the China Organizational Rehabilitation Society applied to the World Diabetes Foundation (WDF) and the Access to Healthcare (AtH) for the project—"National Chronic Wound Care Training Program for Doctors and Nurses Dealing with Patients with Diabetic Mellitus" and received a total of $550,000 support from the two foundations [42]. The project is composed of well-known domestic professors and scholars in China, who are responsible for the preparation, development, implementation and management of educational courses, compiling teaching materials, and teaching as a main lecturer in the training course. In 2011, the People's Military Medical Publishing House officially published the book *Treatment of Diabetic Foot and Related Chronic Refractory Wounds*, which was widely welcomed and concerned by trainees as a project training textbook and a reference book for understanding the basic knowledge in this field [43]. In 2013, the book was further revised and updated on the basis of the first edition and the *Treatment of Diabetic Foot and Related Chronic Refractory Wounds* (second Edition) was reprinted, which further enriched and improved the textbook content. It has been expanded to 10 people based on the original 6-person project expert group. By the end of the project in 2015, more than 60 classes have been opened in more than 20 cities across the country, with more than 8600 participants, highly recognized by international counterparts. From the perspective of project development, the project has the following characteristics:

(a) The project is highly targeted.
(b) The training content keeps pace with the times.
(c) The teaching standard of the project is unified.
(d) The project has strong teaching staff.
(e) The project has good practicability.
(f) The training form is pragmatic. Recently, we have followed up the learning effects of some trainees, especially the theoretical knowledge and practical skills, which proved to be good.

Many modern advanced technologies and products can be well transformed and applied in the wound treatment specialty, so that the technical requirements of wound treatment can be met to the greatest extent [44]. The emergence of modern advanced dressings has developed the traditional dressings into a new function of actively promoting wound repair by isolating the wound and preventing re-contamination [45]. The research, development, and application of a class of new drug, genetic engineering growth factors, has made people take a solid step toward the dream of intervention and regulation of wound healing. The application of red light in American aerospace technology has shown significant effects on chronic wounds. Negative pressure therapy, hyperbaric oxygen therapy, traditional medicine, etc. have also played an important role in wound healing.

The innovative wound management model is the foundation for the orderly development of wound centers (specialties). Establishing a two-way linkage mechanism between wound treatment specialists (centers) in large hospitals and community health institutions is an important means to solve the problem of treating patients with wounds and reducing medical expenses. This single-disease two-way linkage mechanism is collaboration between the doctor in a community health institution after specialized training by wound therapists and the wound treatment specialist in a large hospital in the treatment of complex and refractory wounds. The usual technical guidance of community health institutions can be guaranteed through the established “Wi-Fi-networking+” technology. In 2011, we established the first professional wound treatment specialist in Shanghai Ninth People’s Hospital. In the first year of operation, 125 patients were treated, and all of them had wounds that failed to heal using traditional conventional methods. Among them, 121 cases were cured, with a cure rate as high as 96.8%. Especially in 2015, the newly built wound treatment specialist of Shanghai Ninth People’s Hospital successfully cured the chronic wounds caused by the Japanese artillery shrapnel 68 years ago and the wounds caused by electric shock 52 years ago, which caused good responses in the academic circles. The practice of the Shanghai Ninth People’s Hospital shows that with this innovative model, the cure rate for patients who need wound treatment has increased from 60% in the past to around 94%, and the cost of each treatment has dropped from around 150 yuan to 30–40 yuan. Especially, the treatment of patients in community medical institutions at home has saved a lot of time and effort. For the hospital, the average hospitalization day has dropped from more than 20 days to 14 days, and the proportion of drugs is only 16%, which achieve significant social and economic benefits. The adoption of this innovative model has greatly promoted the orderly flow of patients, reduced the average hospitalization days, and significantly reduced medical expenses.

The Chinese Medical Association Trauma Surgeons Branch has started to promote the “1239” three-year action plan for the construction of China’s wound center (wound repair specialist) to form the basic framework for the development of wound repair discipline, and standardize and enhance the professional level of wound repair in China. According to the plan, we will achieve corresponding results in the following aspects:

(a) Establish an expert platform representing the national level.
(b) Two wound repair training institutions with good teaching and clinical practice conditions have been built and incorporated into the training system of the Chinese Medical Association.
(c) Build three distinctive wound treatment specialists that can represent the national level and form a demonstration unit for the construction of wound repair specialists in China.
(d) According to the regional distribution, nine regional wound repair specialists will be established nationwide within three years.

At present, great achievements have been made at the academic and technical levels. In 2016, the first *Chinese Guideline for Diagnosis and Treatment of Skin Wounds* (2015 Edition) was published.

It is believed that under the joint efforts of medical workers at all levels of the country dedicated to the prevention and treatment of wounds, the wound center will surely usher in a leap forward.

5.5 Chronic Ulcer

5.5.1 Introduction

Chronic ulcer is a general term for wound associated with the wounded site and the host which does not be healed normally in the desired time or for a long time [46]. Its definition has not yet been uniformly given. Clinically, it is customary to treat wounds that have not been healed by regular treatment for one month and have no obvious healing tendency as chronic ulcers. Chronic ulcer on the surface is a difficult problem in surgical clinics. Due to the different reasons for its formation, the time of prolongation, the depth of ulceration, and the location of the disease, the treatment methods and prognosis are not the same [47].

5.5.1.1 The Classification of Ulcers

There is no uniform classification standard for ulcers at present. The main classification criteria are as follows:

1. According to the concept of traditional wounds.
   Divided into surgery, burns, daily injuries, bedsores, infectious ulcers.
2. According to the time of ulcer prolongation and degree of contamination Divided into clean, contaminated, infected, and ulcer wounds.

3. Using skin continuity as a measure and according to anatomical depth.
   Divided into superficial, half-thickness, full-thickness, and deep tissue ulcers below the skin.
4. European RYB classification.
   The open wounds of stage 2 or delayed healing are divided into red, yellow, black, and mixed wounds according to the tissue morphology.
5. According to the cause of ulcer formation.
   (a) Traumatic ulcer: with a clear history of trauma, such as car accidents, gunshot wounds, crushing or thermal burns; it can form residual ulcer wound in the late stage, deep to the skin layers or even the muscles, tendons, joints and bone tissue; extensive unstable scars formed after a burn can be broken due to local tension or infection without healing persistently. Much longer duration, and it will cause cancer and form Marjolin's ulcer [48] (Fig. 5.11).
   (b) Autoimmune ulcer (drug induced ulcer): The patient is in a high immune state, which leads to the ulcer gradually expanding and deepening, lasting for several years or even decades.
   (c) Tuberculous ulcer: A special infection combined with sinus, which is caused by *Mycobacterium tuberculosis*.
   (d) Pressure ulcer: It is an ulcer formed by ischemic necrosis of the skin or subcutaneous tissue due to local compression. It occurs in the bony protrusion of elderly patients in bed (Fig. 5.12). Clinical manifestations are generally divided into the following three phases. The first phase: erythema phase. The second phase: vesicular phase. The third phase: ulcer phase. The prevention of bedsore generally has the following aspects. Avoid local compression for a long time. Skin care: keep the skin clean and dry. Strengthen functional exercise: relieve the pressure of local tissue constantly. Preventive cushions and devices. Behavioral and psychological education.
   (e) Cancerous ulcer (malignant ulcer): refers to the primary or secondary cancerous ulcer on the body surface (Fig. 5.13).
   (f) Radiation ulcers: it is often complicated in the treatment of radiotherapy accompanied by chronic inflammation and fibrotic changes in the deep and surrounding tissues of the ulcer. More frequently, it is also accompanied by intractable pain and other sequelae (Fig. 5.14).
   The clinical manifestations are as follows: varying ulcer size and depth; extensive fibroplasia; and degeneration around ulcer.
   (g) Vascular ulcer: lower extremity ulcers caused by varicose veins and vasculitis of lower limbs are much common in the distal leg and ankle [49] (Fig. 5.15), with dark base, rough skin around the wound obvious pigmentation.
   (h) Diabetic ulcer: one of the serious complications of diabetic patients (Fig. 5.16). Most diabetic patients with foot ulcers are associated with neurological diseases.

Fig. 5.11 Marjolin's ulcer

Fig. 5.12 Pressure ulcer

Fig. 5.13 Cancerous ulcer

Fig. 5.14 Radiation ulcer

Fig. 5.15 Vascular ulcer

(i) Infectious ulcer: ulcers that cannot heal due to repeated wound infections (Fig. 5.17).

The etiological classification can guide the treatment of the cause and highlight the individual characteristics of different ulcers. However, although the causes of various ulcers are different, once the ulcers are formed, the local pathophysiology, and healing mechanism are roughly the same [50].

Fig. 5.16 Diabetic foot

Fig. 5.17 Infectious ulcer

5.5.2 The Healing of Chronic Ulcers

The essence of chronic ulcer healing is the common result of the dynamic interactive response of the body to damage stimulation, orderly and controllable proliferation and differentiation of tissue cells. Due to the pathophysiological state of chronic ulcers, it is determined that microscopically involved complex interactions and responses among various cells, extracellular matrix, soluble mediators, and cytokines, and macroscopically experienced three different but overlapping stages:

(a) Inflammation stage.
(b) Proliferation stage.
(c) Maturation and reconstruction stage.

5.5.2.1 Inflammation Stage

Monocytes in the blood circulation are concentrated by the chemokines at the wound and differentiate into macrophages. Macrophages migrate into the wound 48–96 h after trauma. The anti-infective effects of macrophages include phagocytosis and the production of active free radicals such as nitrous oxide, oxygen, and superoxide. Once the infiltration of macrophages is blocked, the wound healing process will be severely impaired.

5.5.2.2 Proliferation Stage

Fibroblasts and endothelial cells are the main cells growing at this stage. The new collagen-rich matrix produced by fibroblasts replaces the original fibrin clot and plays an important role in the formation of granulation tissue.

Angiogenesis activated during healing and repair is a fundamental biological mechanism. This process is characterized by invasion, migration and proliferation of endothelial cells.

5.5.2.3 Maturation and Reconstruction Stage

The main feature of the maturation stage is the deposition of collagen on the wound surface. The rate, quality, and total amount of matrix deposition determine the strength of the scar.

5.5.3 The Treatment of Chronic Ulcers

5.5.3.1 Etiology Treatment

For chronic ulcers caused by diabetes, the first thing is to control blood glucose level. Fasting blood glucose is generally required to be stable below 10 mmol/L stablely. For vascular factors, local blood circulation and oxygen supply should be improved, including bed rest, elevation of the affected limb, and application of improved microcirculation drugs [51]. For pressure ulcers, relieve local pressure and pay attention to changing position. For radioactive ulcers, local radiation exposure should be stopped.

5.5.3.2 Conservative Therapy

It is the basis and premise of surgical treatment. The focus is on symptomatic treatment, such as systemic nutritional metabolism support, preparation of a local wound bed [52]. Physiotherapy such as ultrashort wave, infrared irradiation is adopted to improve local blood circulation. Cleaning the wound local dressing, timely removing necrotic tissue and secretions, and carrying out bacterial culture of secretion and drug sensitivity test is also the part of conservative therapy.

5.5.3.3 Debridement

Surgical intervention is used to treat ulcer wounds to achieve the transformation from contaminated (yellow, black, or a mixed) wounds to relatively clean (red) wounds, from unprepared healing to ready healing wounds [53].

5.5.3.4 Application of Various Flaps and Myocutaneous Flaps

The principle is to completely remove the necrotic tissue, together with the scar tissue around and at the base. If the bone is damaged, the necrotic bone tissue should be removed together. The remaining cavity is preferably covered with an adjacent flap or myocutaneous flap [54]. It is easy to recur after skin grafting because of weak skin friction resistance, so it is not the first choice for repairing bedsores. Unless the condition is serious, in order to prevent the loss of protein and temporarily relieve pain, it can be considered to temporarily close the wound before the flap is transferred [55, 56].

At present, the use of various flaps and musculocutaneous flaps to repair wounds is still a very effective means [57, 58]. The flaps and myocutaneous flaps have rich blood supply and good anti-infective ability, as well as the advantages in pressure resistance and friction resistance. The repair of wounds generally uses island-shaped myocutaneous flaps, axial flaps, arbitrary flaps, or other tissue flaps with abundant blood supply and abundant tissue. These tissue flaps have rich blood supply, strong anti-infective ability, and can improve local blood circulation, improve the biological effects of growth factors, help to remove residual necrotic tissue, and promote wound healing.

It should be selected according to the cause, location, wound condition, patient occupation, and age [59, 60]:

(a) For the head wound, because the scalp is thick and the blood supply is rich, in the case of no bone exposure, as long as the tissue above the skull periosteum can be preserved, it can be treated with full thickness skin; if the skull is exposed, the wound can be repaired with a local flap or a trapezius myocutaneous flap.
(b) For face and neck wounds, the skin flaps can be selected include local flaps, thoracic triangle flaps, trapezius myocutaneous flaps, pectoralis major myocutaneous flaps, and sternocleidomastoid myocutaneous flaps.
(c) For the front chest wound, the selected flaps include the ipsilateral latissimus dorsi musculocutaneous flap, the contralateral pectoralis major myocutaneous flap, and the rectus abdominis myocutaneous flap.
(d) For abdominal, thoracodorsal wounds, local flap is generally used for repair. If the defect is large, two flaps can be used in parallel for repair (Fig. 5.18).

Wound Medication

Scientific and proper use of wound medication can greatly accelerate the healing of ulcers [61]. Cell aging, tissue ischemia, and bacterial colonization are the main factors for

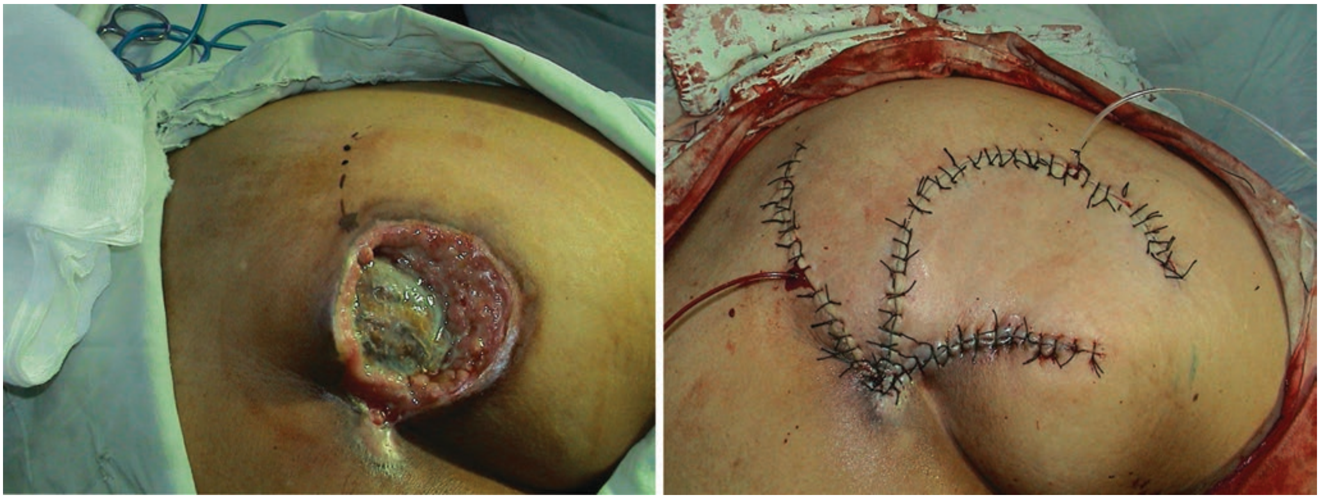

Fig. 5.18 Repair of sacrococcygeal wound with local flap

ulcer formation and prolonged unhealing. The compatibility of local drugs should focus on the control of infection, promoting tissue regeneration and improving local ischemia, follow the stage law of ulcer repair, and make the overall consideration of many factors such as systemic and local conditions, effectiveness and adverse reactions, compatibility, and dosage forms [62].

Vacuum Sealing Technology

It is a new therapeutic technology for repairing and covering soft tissue wounds by using medical polymer composite composed of polyvinyl alcohol-absorbent gelatin sponge. The principle is that this material acts as an intermediary between the drainage tube and the drainage surface, making the drainage from the point to the surface, changing the open wound into a relatively closed wound to prevent pollution and secondary infection; under the action of negative pressure, the blood flow of the wound increases, stimulating the growth of granulation tissue, and having the effect of compression hemostasis [63].

Hyperbaric Oxygen Therapy

By increasing oxygen diffusion, the blood oxygen content and the oxygen partial pressure of the wound are increased, the oxygen supply of the lesion tissue is corrected, and the bactericidal ability of the white blood cells is enhanced. The hyperbaric oxygen can reduce the whole blood viscosity, plasma viscosity and platelet aggregation rate, can increase red blood cell deformability, and improve microcirculation. The hyperbaric oxygen can promote the establishment of collateral circulation, improve capillary permeability, effectively prevent extravasation of plasma water, and reduce wound edema, thereby improving the microenvironment of wound healing [64, 65].

Bioengineered Skin Products

With the rapid development of materials science and life sciences, more and more bioengineered skin or skin substitutes have been used to treat acute and chronic wounds including burns [66–68].

Bioengineered skin may work by delivering living cells that are suitable for the environment. There is evidence that some viable "skins" release growth factors and cytokines, but fail to fully explain the mechanisms by which they work.

Gene Therapy

This technology can now be used to introduce specific genes into wounds using a variety of physical or biological vectors, including viruses. To date, most of the research on wound healing gene therapy has been limited to laboratory and animal models, but some gene therapy that promotes human wound healing still makes us look forward to.

Stem Cell Therapy

It is speculated that pluripotent hematopoietic stem cells are more effective than the introduction of specific genes. Because it can differentiate into various cell phenotypes, including fibroblasts, endothelial cells, keratinocytes, etc., which are the key cells in the healing process. There have been many reports of successful clinical applications [69–73]. However, stem cell research itself is still controversial. The main problem is that although the results in the laboratory are very encouraging, the results in clinical practice are not satisfactory.

Platelet-Rich Plasma Therapy

Platelet-rich plasma (PRP) usually is defined as plasma with higher platelet concentration than healthy whole blood. It can also refer to plasma rich in platelets, high platelet concentrates, and autologous platelet gels [74]. After injecting it into the tissue defect, it can make up for the defect of too little growth factor in the wound, and start the repair mechanism faster, induce tissue regeneration.

Antioxidant Treatment
One way to correct abnormalities in wound healing in diabetic patients is to reduce the amount of free radical products. Raxofelast, an antiallergic drug, is a protective membrane antioxidant that improves wound healing in diabetic rats by stimulating angiogenesis.

5.5.4 Tuberculous Wounds

5.5.4.1 Overview

Tuberculosis (TB) is infected by *Mycobacterium tuberculosis* (MT). In recent years, tuberculosis has been classified as a major infectious disease by the World Health Organization. In China, the tuberculosis epidemic is equally serious as well [75]. The fifth national tuberculosis epidemiological sampling survey report shows that the annual incidence of tuberculosis in China is about 1.3 million, accounting for about 14% of the total number of tuberculosis cases worldwide, ranking second in the world. According to relevant study, about 100,000 patients in China suffer from subcutaneous and skin soft tissue damage caused by tuberculosis bacilli each year, and some tuberculous wounds are formed [76, 77]. In recent years, the incidence of TB in patients with diabetes mellitus (DM) ranks first in the world [78]. It is predicted that the incidence of tuberculous wounds will increase further.

Because tuberculous wounds have the characteristics of insidious onset, slow progress, and high rate of misdiagnosis and missed diagnosis, sinus-type wounds have been formed in most patients when they visit the hospital [79]. Most of the sinus-type tuberculous wounds are sporadic and scattered in clinics, which leads to low attention in the academic circles. There are few reports at home and abroad [80, 81]. At present, medical diagnosis and treatment of such diseases is mainly based on internal medicine in China, and there are few studies on tuberculous wounds.

5.5.4.2 Epidemiological Characteristics of Tuberculous Wounds

So far, the epidemiological data of tuberculous wounds in China have rarely been officially reported [82]. The epidemiological data has positive guiding significance for the government to formulate prevention and control policies and to specifically improve the level of clinical tuberculous wounds diagnosis and treatment. Recently, we reviewed the clinical data of hospitalized patients with extra-pulmonary tuberculous wounds in a hospital from January 2010 to December 2012, systematically analyzed the clinical epidemiological characteristics and rules, in order to fill the gap in this aspect of China, and broaden the theoretical and practical areas of wound healing field, so as to provide reliable epidemiological data for improving the quality of tuberculous wound healing and enhancing overall prevention and control measures for tuberculosis. Of the 5863 extra-pulmonary tuberculosis patients, 235 had tuberculous wounds, with an incidence of 4.01%. The age distribution of the patients ranged from 1 to 87 years (median 35 years), with an average age of 6.9 ± 17.9 years. Among them, 163 (69.4%) were rural patients and 72 (30.6%) were urban patients (Fig. 5.19). Among the causes, the peripheral lymph node tuberculosis was the first (112 cases, 47.7%), followed by bone and joint tuberculosis (95 cases, 40.4%). The main site of the disease was neck, 99 cases (88.4%). There were 173 patients (73.6%) without systemic symptoms. Patients with erythrocyte sedimentation rate greater than 20 mm/h accounted for 76.1%, and the positive rate of *Mycobacterium tuberculosis* culture was 43.8%. In the pathological examination, 129 cases (74.6%) were found to be tuberculosis, and 44 cases (25.4%) were negative samples. The average time from the onset of clinical symptoms to the diagnosis of tuberculosis was 4.4 months, which was much higher than that reported in the literature from 9 to 54 days. Among the patients with a diagnosis time of more than 6 months, rural residents accounted for 85.4%.The patient treated with dressing change was 54.9%, but the cure rate was only 4.7%; the cure rate of surgical treatment was 89.8%. To a certain extent, this reflects the epidemiological data and epidemic status of local areas in China during a certain period.

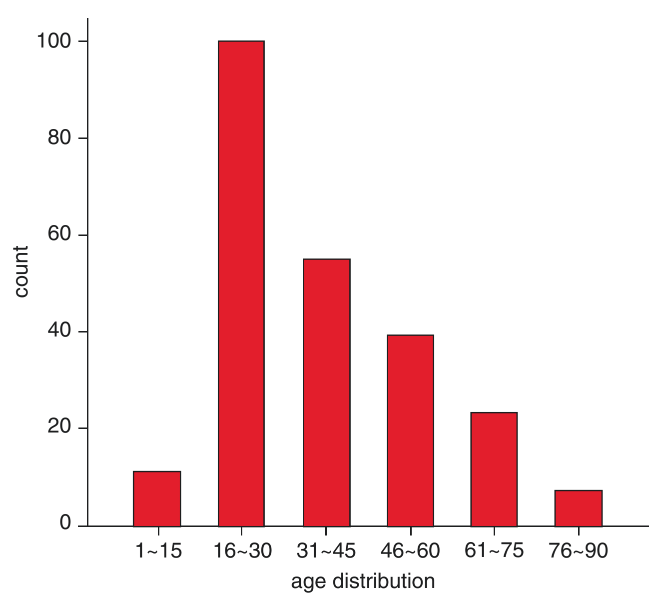

Fig. 5.19 Age distribution of patients

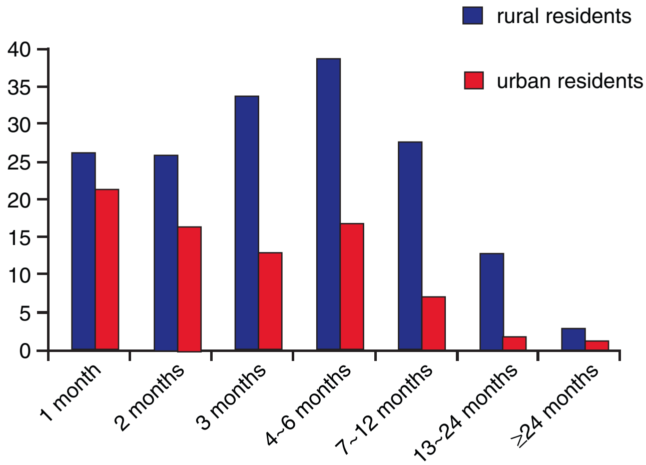

Fig. 5.20 Time distribution of tuberculous wounds diagnosis

The average time from the onset of clinical symptoms to the diagnosis of tuberculosis was 4.4 months (median 3 months), and the time distribution ranged from 1 to 32 months. Among them, the majority of patients were diagnosed within the first 3 months (Fig. 5.20). Among patients with a diagnosis time of more than 6 months, the rural residents have a relatively high composition.

5.5.4.3 The Concept of Tuberculous Wounds

At present, there is no standard concept for wounds caused by *Mycobacterium tuberculosis* in the international and domestic medical community. Based on the initial cause of wound formation, combined with the final clinical features, we proposed the concept of tuberculous wounds, which is caused by *Mycobacterium tuberculosis* invading the local tissues of the body, leading to necrosis of the affected site or adjacent skin and subcutaneous soft tissue, eventually leading to skin ulceration. Tuberculous wounds are a big concept, referring to the wounds caused by *M. tuberculosis*. The common clinical tuberculous wounds are caused by the spread of lymphoid tuberculosis and bone tuberculosis lesions to surrounding tissues and skin. Skin tuberculosis refers to the invasion of skin by *M. tuberculosis*. Once a wound is formed, it is also a category of tuberculous wounds.

5.5.4.4 Clinical Manifestations of Tuberculous Wounds

Because tuberculous wounds are caused by *Mycobacterium tuberculosis* infection, the wounds have their unique clinical manifestations:

(a) A small mouth and a large bottom, and the skin ulcers are generally small, but the invasion of subcutaneous tissue is large and the invaded level is deep (Figs. 5.21 and 5.22).
(b) Easy to invade bone. For example, the chest wall tuberculosis wounds often involve the sternum, and the wounds near the joints are often accompanied by bone and joint tuberculosis.

Fig. 5.21 Neck tuberculosis wound

Fig. 5.22 Seen during surgery

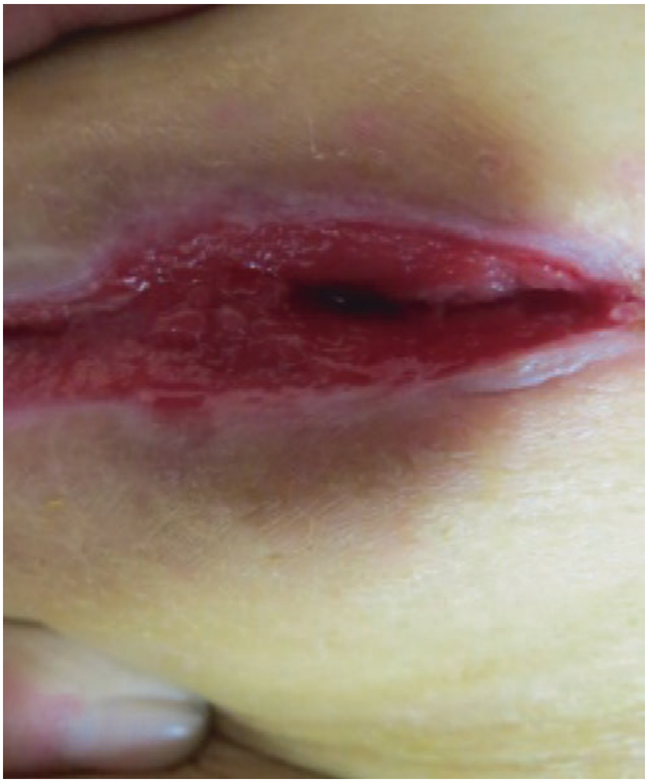

Fig. 5.23 Waist wound

Fig. 5.24 Yellow-green necrotic purulent secretions

(c) Often multiple sinuses are formed, the trajectory is tortuous into a mouse hole (Fig. 5.23), reaching deep into the muscles and even the bone surface.
(d) The affected tissue is necrotic to caseous necrosis, may be accompanied by yellow-green purulent secretions and no obvious foul odor (Fig. 5.24).
(e) Most of the patients have deep and clear primary lesions. Due to the positional factors, the wound site is often lower than the original lesion.

Most patients have no systemic symptoms, and clinical symptoms are different, which may lead to misdiagnosis and missed diagnosis. Most of the wounds are single, while the number of multiple wounds is relatively small. The skin around the sores is red and swollen, with tenderness.

5.5.4.5 Diagnosis of Tuberculous Wounds

The diagnosis of tuberculous wounds needs to be combined with the clinical features of the wound (caseous necrosis, yellow-green purulent secretions, and mouse-hole-like sinus), imaging features, and ultrasound and laboratory tests. However, due to the atypical symptoms in the early stage of tuberculous wounds, it is easy to be confused with inflammatory granulomatosis, tumors, chronic inflammation, and other diseases, and it is difficult to specifically exclude other diseases in imaging, which brings certain difficulties to the diagnosis of tuberculous wounds, so the rate of early misdiagnosis is extremely high.

Tuberculous wounds are caused by *Mycobacterium tuberculosis* infection. Because of its unique strains and immune response, laboratory tests have a higher diagnostic value. Our small-scale epidemiological survey found that PPD test had the highest positive rate in laboratory tests, which was of great significance for diagnosing *M. tuberculosis* infection. However, the PPD test can also be positive after previous infection with *M. tuberculosis* or BCG vaccination. At present, there is no reliable standard to distinguish the natural tuberculosis infection and the reaction after BCG vaccination, so it cannot be used as a diagnosis standard. The diagnosis of tuberculous wounds requires the discovery of M. tuberculosis in wound secretions, but it has the disadvantages of long culture time, high price, high requirements for laboratory and inspectors, and low positive rate. Although the positive rate of biopsy histopathology is higher than that of tuberculosis cultivation, it can be the gold standard for the diagnosis of tuberculous wounds; many patients still have negative results. Therefore, even if the pathological examination is negative, the possibility of tuberculosis cannot be ruled out.

5.5.4.6 Treatment of Tuberculous Wounds

Preoperative Evaluation

Ideal preoperative analysis of indicators for assessing wound conditions should include accurate positioning, assessment of wound area, depth, presence and shape of the subcutaneous sinus, nature of the wound contents, anatomical levels invaded by the wound tissue, and anatomical relationship with adjacent bones and soft tissue.

The sinus tract formed by tuberculous wounds has the remarkable characteristics of small mouth and large bottom, and mouse hole, the appearance and the actual situation in the deep are very different, so it is deceptive. Most patients think it is a superficial small wound, and the surgery should be simple. Inexperienced doctors also tend to think that the lesion is not large and ignore the preoperative assessment, which leads to surgery failure. Conventional methods are susceptible to uneven wound edges, patient position, wound site, and tissue swelling. It is not possible to accurately assess deep wounds with cystic segmentation and musculoskeletal involvement.

In recent years, the authors research team has attempted to use magnetic resonance imaging and 3D reconstruction software to achieve a 3D reconstruction of tuberculous wounds with sinus, attempting to achieve a panoramic and accurate display of its deep morphology and adjacent anatomical relationships, and initially achieved satisfactory results (Figs. 5.25, 5.26, and 5.27), which greatly facilitates the formulation of individualized surgical plans and the notification of the condition [83]. The technology is mainly divided into three parts: image acquisition, image segmentation reconstruction, and visualization. The acquired magnetic resonance images are imported into the medical 3D reconstruction software, and the signal recognition threshold selection tool is used to segment the inflamed tissue, sinus and normal muscle, then 3D reconstruction is performed, and the organization is displayed in different colors. The reconstructed 3D image can clearly show the deep stereoscopic shape of tuberculous wounds, including the anatomical adjacent relationship between inflammatory tissue and normal tissue, the sinus movement and depth, and can be

Fig. 5.25 Chest wall tuberculosis wound

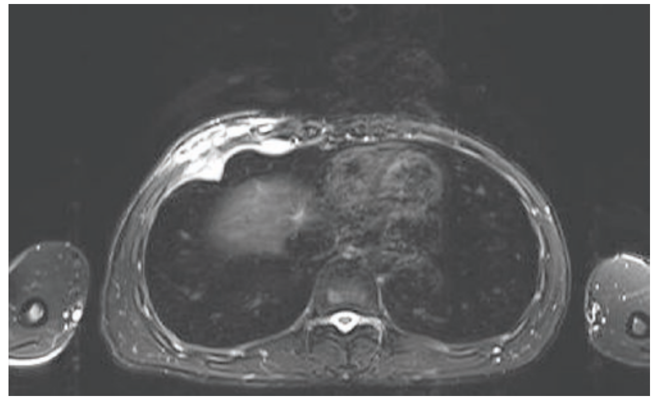

Fig. 5.26 Nuclear magnetic resonance scanning

Fig. 5.27 3D reconstruction

rotated, translated, scaled to observe the internal morphology of the wound from different perspectives. The images obtained are detailed and intuitive, which facilitates the communication among the members of the surgical team and the formulation of surgical plans, so as to reduce the damage to the surrounding normal tissues and reduce the risk of surgery. At the same time, the 3D reconstruction image is intuitive and easy to understand, and also makes the communication between doctors and patients more concise and smooth.

Treatment Plan and Efficacy

In the early stage of the disease, when the local tissue necrosis range is small, after a long period of time, the treatment has a certain effect. However, if the medical history is long, the primary lesion is large, the local necrotic tissue is wide, and the depth of involvement is deep, it is difficult to work by traditional treatments alone. Not only the course of the disease is prolonged but also the condition tends to gradually worsen. Surgical intervention (lesion clearance+wound coverage) is essential (Figs. 5.28, 5.29, 5.30, and 5.31).

Surgery is one of the important means of treating refractory wounds [84]. In surgical debridement, only the complete removal of necrotic tissue can achieve the final complete healing of the ulcer, otherwise the remaining lesions will become the cause of recurrent ulcer. The wounds covers generally require muscle flaps, musculocutaneous flaps, or free flaps. Of course, in order to achieve a complete wound healing, preoperative and postoperative standard anti-tuberculosis

Fig. 5.28 Chest wall tuberculosis wound

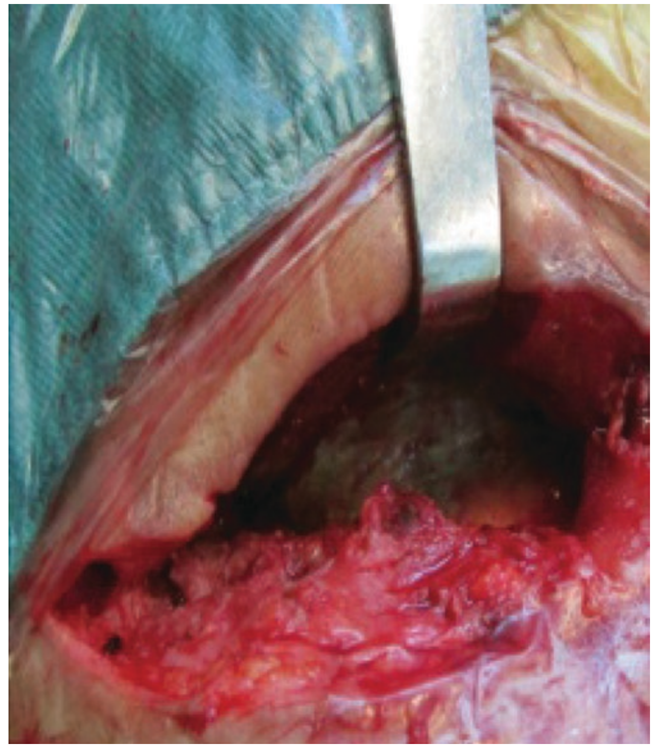

Fig. 5.29 Postoperative debridement

Fig. 5.30 Flap transfer

Fig. 5.31 Wound healing

chemotherapy is also necessary. Recently, we have adopted a new treatment strategy of "lesion clearance+VSD + flap" and achieved good clinical results [85].

5.5.4.7 Research Trends and Prospects of Tuberculous Wounds

Most of these diseases are mainly diagnosed and treated by internal medicine in China, and no systematic study on tuberculous wounds has been reported. Analysis of the reasons, first, compared with other common types of wounds, the incidence of tuberculous wounds is lower and people pay less attention to them; second, the development is slower, the diagnosis cannot be confirmed in time; third, the replication of animal models is very difficult, and the corresponding basic research is difficult to carry out; four, the protective conditions of the research environment are very high. Therefore, research on tuberculous wounds has been almost blank for many years.

The establishment of animal models of tuberculous wounds is the necessary basis for studying the causes, rules; occurrence and development mechanisms of tuberculous wounds. First, experimental animals should be susceptible to mycobacteria, have a similar immune system to humans, and can form pathological changes similar to human tuberculous wounds. Secondly, the choice of animal models of tuberculous wounds should also consider laboratory biosafety issues. Based on the above requirements, animals that meet the requirements are very limited.

At present, the research on tuberculous wounds is still in its infancy, and the animal model of tuberculous wounds is still not mature and stable enough, and there may still be a certain gap from the clinical practice. Although there are still some problems in the animal model of tuberculous wounds, as far as the actual situation is concerned, the research potential of the molecular mechanism related to tuberculosis wounds in the zebrafish model infected by *Mycobacterium marinum* is not to be underestimated. Genes and transcriptional expression pathways associated with tuberculous wounds can be found, and the target genes and target proteins associated with tuberculous wound treatment can be found, making biological target therapy for tuberculous wounds possible.

The future development trend should be the application of high-frequency ultrasound, CT scan, and magnetic resonance imaging without non-invasive real-time imaging (3D reconstruction accurately shows the location, size, shape, internal structure of the wound, and its relationship with surrounding structures), and in the basic research, the occurrence and development mechanism of tuberculous wounds should be systematically in-depth studied.

5.5.5 The Conclusion

Chronic ulcers are a class of diseases with high prevalence, high treatment costs, variable clinical conditions, and poor prognosis, which are often treated by disparate treatment systems. Therefore, it is not advisable to simply evaluate the quality of wound treatment without considering the patient's treatment results. Similarly, it is extremely difficult to evaluate the quality of the clinician's treatment.

Therefore, when making treatment strategies, such a thinking mode should be followed:

(a) Diagnosis and risk assessment.
(b) Active treatment, including stabilizing the ulcer, promoting healing early, and treating complications that can increase the risk of chronic ulcers.
(c) Improve quality of life and reduce pain when ulcers have unconditionally healed.
(d) Make a long-term targeted treatment and nursing plan to slow the development of ulcers and prevent recurrence.

Of course, China's research in the field of chronic ulcers, still exists a large gap between China and foreign countries. Mainly reflecting in the following aspects:

(a) Conceptual backwardness. Chronic ulcers are not deemed to be life-threatening and to belong to natural physiological processes.

(b) Insufficient capital investment. The deficiency of scientific research funds will inevitably lead to the shortage of human resources and the difficulty in forming a scale of the project, and it is unlikely to make a breakthrough at a certain point.
(c) Poor use of funds. Since there is no overall plan, the limited funds are scattered, resulting in low-level duplication.
(d) Academic institutions are not fully equipped.

Therefore, we should strive to do the following works.

(a) Conduct universal education on wound healing, making its importance recognized by most people, which is the basis of other kinds of works.
(b) Improve organizational structure and straighten out relationships. A strong organization is the core of the development of the discipline, which can grasp the development direction and overall planning of the discipline.
(c) Break the traditional concept and take a new road of "medical and commercial cooperation, common development." At present, China's economy is still underdeveloped, and science and technology funds are not sufficient. It is not enough to rely solely on the various research funds. We must find our own way and "medical and commercial cooperation, common development" is a practical way. It has been implemented in foreign countries for many years and proved to be beneficial to both medicine and business.
(d) Hold regular academic meetings on wound healing to learn from each other in order to strengthen academic exchanges. At the same time, top talents should be selected to participate in appropriate international conferences, so as to timely understand the international basic research trends and clinical treatment levels, and track the international advanced level.
(e) Grasp the relationship between basic research and clinical research. Basic research to a great extent reflects the level of science and technology in a country. Once it lags behind, it is difficult to catch up in a short period of time. Therefore, we must pay attention to basic research work. But only focusing on basic research is not enough, because our ultimate goal is to solve clinical practical problems, so it is critical to correctly grasp the relationship between the two. Contemporarily, the situation in China is that there is a serious disconnect between basic and clinical research results, many of which cannot be transformed into clinical services. Only by combining the two closely, accelerating the development and application of new products, can wound healing research really go on the track of healthy development.

5.6 Treatment of Chronic Refractory Wound with Osteomyelitis

5.6.1 Overview

5.6.1.1 The Concept and Mechanism of Osteomyelitis

Osteomyelitis is an infectious inflammatory response of bone caused by microorganisms, which can be limited to a certain part of the bone and can also involve various areas of the bone, such as bone marrow, cortical bone, periosteum, and surrounding soft tissue. In clinical practice, it is often divided into acute osteomyelitis (several days to weeks) and chronic osteomyelitis (several weeks to months or even longer) according to the course of disease. The latter generally develop from the former to the formation of dead bones and become chronic.

The pathogenesis of osteomyelitis is mainly divided into two types: exogenous and hematogenous. Exogenous osteomyelitis is caused by open fractures, surgery or spread of infection in adjacent tissues. Hematogenous osteomyelitis is mainly caused by bacteria reaching a certain bone tissue through the blood circulation from other infection lesions in the body.

Chronic osteomyelitis is a biofilm infection, in which only a very small proportion of the microorganisms are free to swim (plankton), active (culturable), and sensitive to systemic medication. However, the vast majority of pathogens are colonized, elastically adherent (on dead bone, autologous or external grafts), and deeply implanted in microbial adhesion membranes (biofilms). Once the microorganism enters a colonized state, it cannot be cleared by the host's immunity, nor can high concentrations of antibiotics in the blood kill it. Over time, the toxins produced by microorganisms and the alkaloids produced by host cell-related immune side effects accumulate, causing severe local and systemic damage (sepsis, soft tissue loss, and chronic edema). At the body level, when the inflammation blocks the blood vessels, partial ischemic osteonecrosis is secondary. Bone tissue that lacks blood supply will gradually separate from normal bone tissue and gradually become the so-called dead bone. In the vicinity of the infected area, reactive hyperemia occurs with an increase in local osteoclast activity, which further aggravates bone loss. The hallmark of chronic osteomyelitis is that the diseased soft tissue is wrapped with infected dead bone. The infected area of the bone is surrounded by sclerotic and relatively ischemic bone, which is covered with thickened periosteum, scarred muscles, and subcutaneous tissue. Because of the ischemic scar tissue surrounding the lesion, systemic antibiotics are difficult to achieve.

5.6.1.2 The Classification and Diagnosis of Osteomyelitis

Classification

In 1985, Cierny and Mader proposed the Cierny-Mader classification for adult patients with osteomyelitis, becoming the first osteomyelitis classification system that combines the course, classification and the treatment plan [86]. The Cierny-Mader classification classifies the main factors affecting treatment, that is, the extent of osteonecrosis, the overall health of the patient, and the impact of the disease on function, consisting of patient classification and anatomical classification.

Patients with osteomyelitis can be classified into three types according to the physiological indicators of the patient's response. Type A: The patient has a normal response to infection and surgical treatment. The patient has normal immune function and good local blood supply. Type B: The patient's body is damaged and the wound healing ability is reduced, and the B-Local (BL) and B-Systematic (BS) factors damage the immune function and healing ability of the patient. Type C: Patients with mild disability, other illnesses, and after treatment may be worse than untreated.

According to anatomical indexes, osteomyelitis can be classified into four types. Type I: Intramedullary osteomyelitis, characterized by endosteal lesions. Type II: Superficial osteomyelitis, lesions limited to the bone surface, secondary to surface defects. Type III: Localized osteomyelitis, the lesion is a stable and well-defined dead bone and cavity of full-thickness cortex, completely eliminating the lesion will not cause instability. Type IV: Diffuse osteomyelitis, which is mechanically unstable before or after treatment and requires complex reconstruction.

The combination of anatomical and physiological indexes can divide osteomyelitis into 12 clinical stages. For example, type B patients with systemic impairment factors have type III osteomyelitis, which is defined as stage III BS of osteomyelitis. This classification method is very helpful for the choice of treatment options, and it can decide whether to adopt simple method or complex method, radical surgery or palliative treatment, limb saving, or amputation.

Diagnosis

The diagnosis of osteomyelitis should be a combination of clinical manifestations, laboratory tests, and imaging examinations, but there is no non-invasive examination to completely diagnose or exclude osteomyelitis. The gold standard for diagnosis is histological and microbiological examination of dead bone by biopsy. During physical examination, attention should be paid to the integrity of the skin and soft tissue, determine the location of the tender point, check the stability of the bone, and judge the condition of the limb nerves and blood vessels. Laboratory tests are generally not specific and the severity of the infection cannot be determined. The vast majority of patients had elevated ESR and CRP, but only 35% of patients with elevated white blood cell counts.

It can be examined by a variety of imaging methods, but there is no way to clearly confirm or exclude osteomyelitis. The purpose of imaging examination is to help confirm the diagnosis and preoperative preparation.

The gold standard for diagnosing osteomyelitis is bacterial culture and susceptibility testing after biopsy. Biopsy not only confirms the diagnosis but also helps to select sensitive antibiotics. *Staphylococcus* is the most typical pathogen, especially in posttraumatic infections. Anaerobic and Gram-negative bacteria are generally rare, soft tissue, and bone specimens should be submitted for microbiological studies.

5.6.1.3 Overview of the Treatment of Osteomyelitis

Chronic osteomyelitis with chronic refractory wounds must be treated surgically, with the goal of treating infection, reconstructing soft tissue and bone structures. The principles of surgery are as follows.

(a) Thorough debridement: remove all kinds of internal plants; completely remove dead bones, fistula, abscesses, necrosis and bad granulation tissue according to the preoperative evaluation range.
(b) Treatment of ineffective cavities: application of flap transfer or antibiotic bone cement bead filling.
(c) Maintain stability: external fixes, etc.
(d) Closed wounds: in order to prevent recurrence of infection, it must be covered with well-vascularized tissue. At the same time, the treatment of osteomyelitis requires a multifaceted approach, in addition to antibiotic application, surgical debridement and reconstruction, should also consider the patient's accompanying disease and appropriate treatment, such as smoking cessation, blood glucose control.

Classification for Different Patients

Type B patients are those who have underlying disease, and they are affected by the primary disease and respond to stress, trauma, or infection. There is a risk of failure in the treatment of type B patients, because there may be a higher probability of metabolic disorders, impaired immune function, bacterial infections, wound rot, and excessive bleeding. If the risk and/or complications of treatment exceed the benefits of the treatment itself, the patient is defined as a type C patient. Type C patients are not treated with surgery, but simply given palliative symptomatic treatment. During the treatment process, improving patients' basic health condition could bring benefits to type B patients which could eventually make the outcome similar to type A patients. Choosing a safe surgical procedure and avoiding the use of surgical grafts can also reduce the risk of treatment. However, if the treatment does need the application of internal plants, the entire treatment process is best staged, and reconstructed surgically after the application of antibiotics in order to reduce the tissue tension generated by the local inflammatory reaction, remove the remaining pathogenic strains, and protect soft tissue coverage to increase treatment success rate.

Classification for Different Anatomies

Classification for different anatomies (Fig. 5.32). Type I (intramedullary) osteomyelitis is not associated with skin and soft tissue wounds and is not described here.

Type II (superficial) osteomyelitis. This type of osteomyelitis is associated with skin and soft tissue defects, so normal soft tissue coverage must be restored when making a preoperative plan. Clinical physical examinations, vascular ultrasound, and angiography are often used to assess defect conditions and potential surgical procedures for repair surgery. Remove the diseased soft tissue to a vigorous, soft edge and bite off the diseased bone until the "red pepper sign" appears. Thereafter, it is necessary to synthesize vascular perfusion and local tissue conditions to develop a long-term and feasible surgical approach for soft tissue coverage. Reconstruction of type II osteomyelitis is often performed by closed vacuum suction with skin grafting, local transfer flap or free tissue flap transplantation.

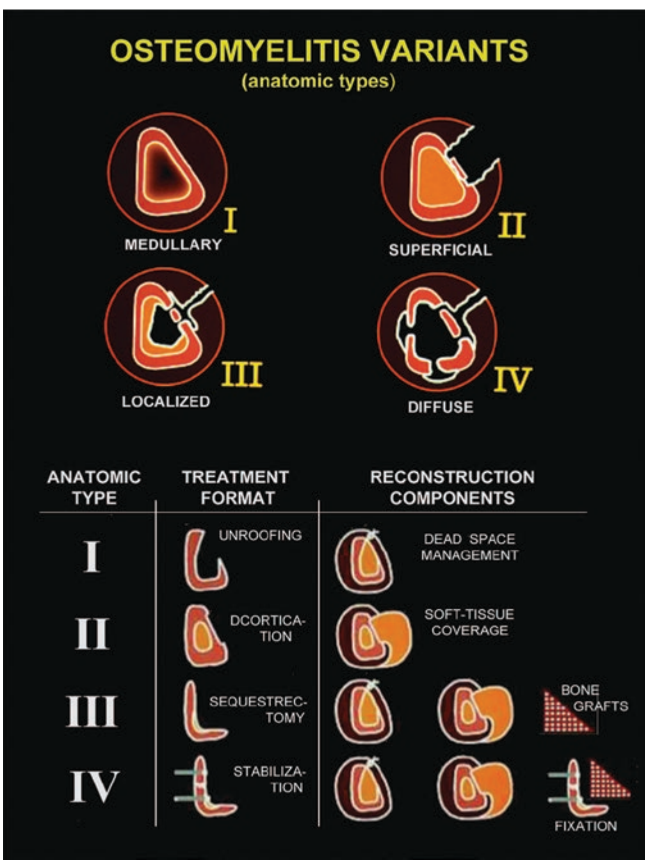

Fig. 5.32 Cierny-Mader classification

(a) Type III (limited) osteomyelitis. Type III osteomyelitis is characterized by both type I and type II osteomyelitis, and there are often severe soft tissue defects after debridement surgery. If debridement removes bone tissue to a large extent that may affect the mechanical stability of the remaining bone, prophylactic stabilization of the affected limb is required to achieve stable results, including the use of bone metastasis, external fixators, or the implantation of antibiotic loads (antibiotic-coated plants or fillers, antibiotic rods, etc.) in situ after debridement surgery. The soft tissue defect is the same as the type II osteomyelitis that has been discussed. If it is necessary to treat the ineffective cavity or reconstruct the mechanical structure after debridement, local antibiotic treatment is often required for a period of time before the reconstruction surgery.
(b) Type IV (diffuse) osteomyelitis. Debridement surgery for type IV osteomyelitis lesions can cause bone instability. Mechanical instability, potential cavities, bone defects, and other serious underlying diseases (type B patients) make the treatment of type IV osteomyelitis most difficult. On the one hand, almost all treatments require staged reconstruction to make later reconstructions as clean and sterile as possible. On the other hand, it can also complete the soft tissue recovery and bone reconstruction in the form of free bone flap with vascular pedicle flap transformation, or open bone metastases, or different combinations of shortening and lengthening of bone, in order to replace the above-mentioned staged surgical plan.

5.6.1.4 The Main Differences and Difficulties Between the Refractory Wounds with Osteomyelitis and Common Conventional Refractory Wounds

(a) Refractory wounds with osteomyelitis are more difficult to accurately assess and diagnose than conventional refractory wounds, and are easily missed diagnose, misdiagnosed, or neglected. The main lesions of refractory wounds with osteomyelitis tend to be deep and may be multilacunar, the wounds that can be observed by the naked eye are often only the tip of the iceberg. Not only the patients often do not care at the beginning, but the inexperienced clinicians may also missed diagnose and misdiagnosed, making the disease unable to be effectively treated and controlled in the early stage.
(b) Refractory wounds with osteomyelitis differ from conventional ones in the presence of dead bone lesions. In addition, the lesions often involve the bone marrow cav-

ity and are often accompanied by the presence of orthopedic implants. Bacteria will form biofilms on the surface of dead bones and implants that cannot be removed by routine flushing and antibiotics. Even if extensive debridement is carried out, as long as the debridement of dead bone or intramedullary cavity is not complete and bacterial biofilm remains, it will soon return to the level before debridement. However, in clinical practice, it is often difficult to confirm the extent of dead bone and bone infection, and it is difficult to remove intramedullary lesions. Expanding debridement for complete removal will not only increase the size of invalid cavity but also further damage the mechanical stability. It is very difficult to give consideration to both "effective and thorough" and "minimally invasive and precise" and to further maintain clean and stable wound after debridement.

(c) Refractory wounds with osteomyelitis often involve the bones and joints stability, resulting in abnormal activities of the soft tissues around the wounds and making the wounds difficult to heal. The maintenance of local stability must be strengthened during the treatment.

5.6.2 The Osteomyelitis-Diagnose-Score (ODS) and Diagnosis and Treatment Process

5.6.2.1 The Osteomyelitis-Diagnose-Score

In 2011, Professor Schmidt et al. [87] in Germany established the osteomyelitis-diagnose-score (ODS) to assign scores to five indicators of "clinical history and risk factors," "clinical manifestations and laboratory test results," "imaging examination results," "microbial culture," and "histopathology." Each index is scored from 0 to 6 points, and the total score is obtained by adding the five scores. When the total score is≥18 points, the diagnosis is grade A, that is, the diagnosis of osteomyelitis is "reliable"; when the total score is in the range of 8–17 points, the diagnosis is grade B, that is, the diagnosis of osteomyelitis is "possible"; when the total score is<8 points, the diagnosis is grade C, that is, the diagnosis of osteomyelitis is "less likely." Among the five indicators, each diagnostic factor can only be rated as 6 points at most. This means that even if a diagnosis score of one indicator is greater than 6 points, its weight in the total score will not be greater than the other 4 diagnostic indicators. The detailed scores of each indicator are shown in Table 5.2.

Table 5.2 Diagnosis and treatment of osteomyelitis

I. Assess patients
II. Preoperative examination
1. Laboratory tests: biochemistry, blood routine, coagulation, urine routine, erythrocyte sedimentation rate, C-reactive protein, calcitonin
2. Diagnostic examination: vascularity index, ultrasound, transcutaneous oxygen partial pressure
3. Imaging examination: X-ray, magnetic resonance, CT, radionuclide scanning
4. Tissue biopsy: culture, histological section, PCR
III. Clinical classification
1. Anatomical classification: medullary cavity, superficial, localized, diffuse
2. Physiological classification: type A patient, type B patient (BL/BS), type C patient
IV. Treatment
1. Limb salvage
2. Amputation
3. Palliative: type C patient, incurable patient
V. Improve the general condition of the patient: treatment of concomitant diseases
VI. First operation
A. One-time surgical reconstruction:
1. Debridement, tissue biopsy, local and systemic antibiotics
2. Dealing with invalid cavities: limb salvage or amputation
(a) Wound: self-healing in the second stage; directly closing or delaying the closure of the wound
(b) Bone: with vascularized bone flap; first stage shortening
(c) Fixation: orthopedic surgery, external fixator
(d) Drug sustained release: antibiotic beads
B. The first step in multi-stage surgery:
1. Debridement, tissue biopsy, systemic antibiotics
2. Replace: replace the instrument, re-rinse, lay the sheet and replace the gloves
3. Temporary fixation: external fixator, antibiotic coated plants
4. Dealing with invalid cavities
(a) Wound: self-healing in the second stage; directly closing or delaying the closure of the wound
(b) Bone: bone removal; with vascularized bone flap
(c) Fixation: orthopedic surgery, external fixator, antibiotic coated plants
(d) Drug sustained release: antibiotic beads, antibiotic filler

Table 5.2 (continued)

VII. Discharge, outpatient follow-up: monitoring wounds, rechecking indicators (erythrocyte sedimentation rate/C-reactive protein); rehabilitation exercise
VIII. Second surgery
A. One-time surgical reconstruction
1. Prophylactic application of antibiotics, removal of internal plants, debridement, tissue biopsy (negative frozen section: no inflammation)
2. Replace: replace the instrument, re-rinse, lay the sheet and replace the gloves
3. Reconstruction
(a) Wound: direct closure
(b) Bone: bone graft; with vascularized bone flap; joint prosthesis
(c) Fixation: orthopedic surgery; external fixation or internal fixation; joint prosthesis
(d) Drug sustained release: antibiotic beads; permanent filler; antibiotic-coated plants
B. The second step of multi-stage surgery
1. Prophylactic application of antibiotics, removal of internal plants, debridement, tissue biopsy (positive frozen section: acute inflammation)
2. Same as VI B (above) or amputation
IX. Discharge and outpatient follow-up: monitoring wounds, rechecking indicators (erythrocyte sedimentation rate / C-reactive protein); rehabilitation exercise
X. Third surgery
A. One-time surgical reconstruction: (negative frozen section: no inflammation) same as VIII A (above)
B. The third step of multistage surgery: (positive frozen section: acute inflammation) same as VI B (above) or amputation
XI. Fourth surgery: biological reconstruction: callus healing, shortening of operation, bone removal, joint resection
XII. Discharge, outpatient follow-up: monitoring wounds, rechecking indicators (erythrocyte sedimentation rate/ C-reactive protein); rehabilitation exercise

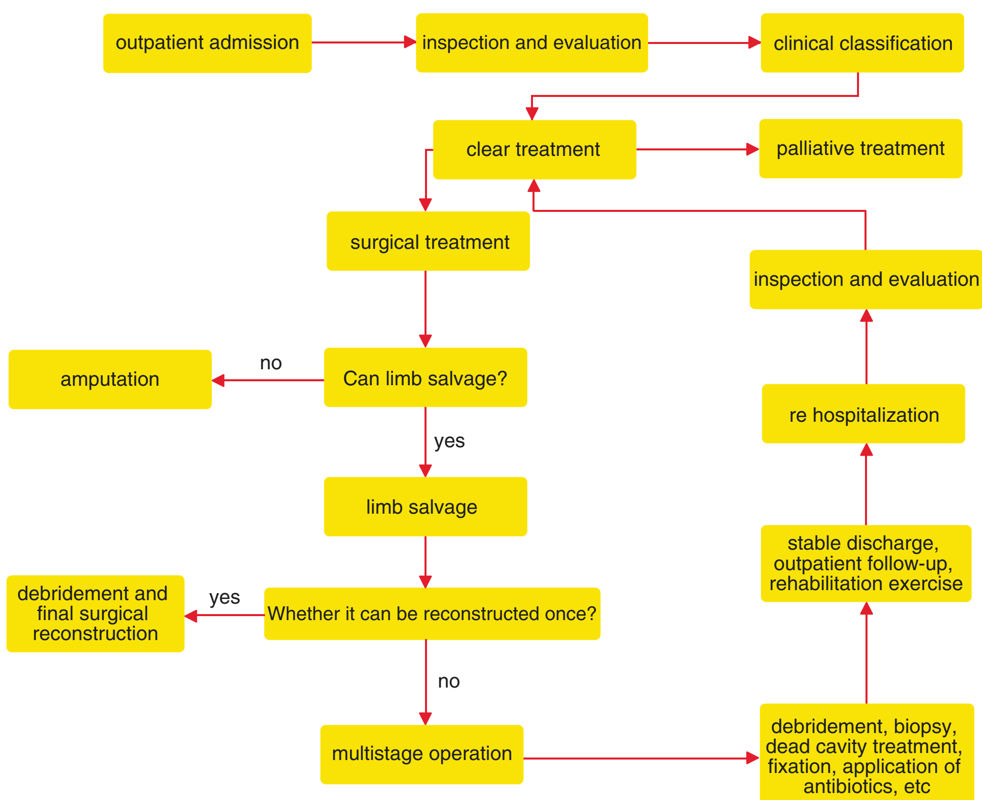

Fig. 5.33 Flowchart of diagnosis and treatment of osteomyelitis

5.6.2.2 The Diagnosis and Treatment Process of Osteomyelitis

The diagnosis and treatment process of osteomyelitis is shown in Table 5.2.

Flowchart of diagnosis and treatment of osteomyelitis (Fig. 5.33).

5.6.3 The "6R Technology" for the Treatment of Chronic Refractory Wounds with Osteomyelitis

In the long-term treatment of various types of osteomyelitis, we have accumulated a lot of valuable clinical treatment experience and summarized them as "6R technology" for osteomyelitis treatment, namely, low temperature plasma radiofrequency debridement (radiofrequency), reaming, rinsing, monitoring reliable wounds (reliable), revascularization, reconstruction/regeneration.

5.6.3.1 Low-Temperature Plasma Radiofrequency Debridement

For bacteria, biofilms and necrotic lesions that affect wound healing, debridement must be effective and thorough, while at the same time, local blood circulation and metabolic disorders in the lesion area require debridement to be minimally invasive and precise, with no or less damage to normal tissue structure. Therefore, the key to the treatment of chronic refractory wounds is debridement, and the difficulty of debridement lies in the consideration of the two principles of "effective and thorough" and "minimally invasive and precise." There are many debridement techniques in clinical application, including self- debridement, enzyme debridement, biological debridement, sharp surgical debridement, ultrasonic debridement, and water knife debridement, but these methods have their own distinct shortcomings in the treatment of chronic wounds. Even the sharp surgical debridement, which is considered to be the gold standard for wound debridement, cannot be tolerated by old, weak, and critically ill patients because of its large trauma and bleeding during operation, especially for patients with coagulopathy disorders or in the process of anticoagulant therapy.

Low-temperature plasma radiofrequency technology was developed in the 1990s and matured by ArthroCare. It uses the energy generated by the high-frequency plasma motion to excite the electrolyte between the bipolar electrode and the tissue into the ion-vapor layer of the plasma, and uses the high-speed plasma to open the intercellular bond and gradually decompose the cells into carbohydrates, so as to realize the ablation of soft tissue. When the temperature reaches 40–70 °C, the target tissue is ablated accurately and limited by the plasma between the electrodes, which makes the water evaporate, the protein degenerate and necrosis, and the blood vessels and tissues around the tissue shrink, close, and solidify. Plasma ablation technology has the advantages of low temperature during operation and precise ablation, which makes it less damage to surrounding tissues during work, thus reducing postoperative pain and healing time. Moreover, the plasma ablation system offers a variety of tool tip options, which can be used in open surgery or closed surgery such as arthroscopy. It has been widely used in the surgical treatment of departments such as otolaryngology, plastic surgery, joint, and spinal surgery.

At the beginning of the twenty-first century, Axel Kramer et al. [88] and other scholars first proposed the use of low-temperature plasma radiofrequency ablation technology for wound treatment. Since then, the research teams at home and abroad have verified the debridement effects of low-temperature plasma radio frequency through in vitro and in vitro experiments [89]. In 2012, the author's team verified the in vivo sterilization effect of low-temperature plasma radiofrequency technology through animal experiments, and compared plasma debridement with traditional surgical debridement and electric knife debridement, confirming the effect of low-temperature plasma radiofrequency debridement is significantly better than that of surgical debridement and electric knife debridement [90]. After the success of the animal experiment, the author's team applied the low-temperature plasma radiofrequency technology for debridement treatment of clinical chronic osteomyelitis wounds and obtained very satisfactory therapeutic results. It is fully verified that the low-temperature plasma radiofrequency technology can take into account the "effective and thorough" and "minimally invasive and precise" of debridement in the treatment of chronic refractory wounds with osteomyelitis, which is a new technology worthy of vigorously promotion.

5.6.3.2 Reaming

Due to the infection in the medullary cavity of type I (intramedullary), type III (limited), and type IV (diffuse) osteomyelitis, and the medullary cavity in the lesion site of patients is often occluded by the infection lesions or abnormal hyperplasia of bone, so it is necessary to perform a debridement in the medullary cavity and open the closed medullary cavity during the operation. There are various potential risks in the conventional graded reaming, including injury of intramedullary blood supply, fat embolism, and osteonecrosis caused by the thermal effects of cortical bone. In order to reduce the various complications that may occur during the reaming process, Synthes in the United States produced a new "reamer-irrigator-aspirator" (RIA) reaming system in 2003. During the process of reaming, it can maintain continuous saline perfusion and continue suction to reduce the pressure in the medullary cavity. The materials in the medullary cavity are sucked away at the head of the reaming knife, and the continuous and stable infusion of normal saline driven by gravity can ensure the smooth suction channel. Compared with the traditional reaming method, the RIA reaming system has the following advantages:

(a) It can effectively reduce the pressure in the medullary cavity and prevent the fat particles from infecting the bacteria into the systemic circulation system.
(b) It can effectively reduce cortical osteonecrosis caused by high temperature of reaming.

(c) One-time reaming can significantly shorten the operation time and reduce trauma.
(d) The fragments produced by reaming can be collected for microbial bacterial culture, drug sensitivity test and histopathological examination.

5.6.3.3 Rinsing

Flush

Pulsed flushing can spray the flushing liquid according to the set pressure and frequency, which is convenient for flushing the wound attachments away from the tissue. It is a safe and more effective method of wound cleaning than ordinary rinsing. The flushing fluid in the debridement surgery is aseptic isotonic saline, at least 9000 mL. Generally, no other drugs are added to the rinsing liquid, and hydrogen peroxide solution with a volume fraction of 3% can be used when it is identified or suspected to be contaminated or infected with anaerobic bacteria.

Lavage

In the First World War, Carrel and Dakin underwent continuous lavage treatment for the wounded soldiers, and they received amazing results. After continuous practice and improvement, now closed lavage drainage has the advantages of promoting the rapid discharge of necrotic tissues and infections, local high concentration of antibiotics that are conducive to local bactericidal, reducing the cavity of negative pressure suction promote healing, painless patients in the lavage process, and simultaneous lavage of multiple lesions. It has become one of the important means of osteomyelitis treatment.

5.6.3.4 Monitoring Reliable Wounds

In the first stage of repair and reconstruction, the chronic osteomyelitis wounds that have been initially treated by surgery have a recent recurrence caused by poor infection control, which leads to surgical failure. This is often related to the fact that the wound bacterial level cannot be controlled within a certain range.

It is necessary to monitor the stable level of the osteomyelitis wounds. If debridement is doubtful or the level of debridement is not good or the condition is complex, it is necessary to closely monitor the wound and systemic inflammatory reaction in time to ensure the deep wound is stable recently. After no severe inflammatory reaction, the next step is to cover the wound and rebuild the continuity of the bone. Vacuum sealing drainage (VSD) can be used as a temporary cover for osteomyelitis wounds in this process.

In 1992, Dr. Fleischman was the first to use VSD in Germany. In 1994, Professor Juward introduced VSD technology to China, which was gradually widely used in surgical clinics. The mechanism of vacuum sealing drainage is to significantly increase the blood flow of the wound, promote the removal of necrotic tissue and bacteria, accelerate the growth of granulation tissue and cell proliferation and repair, promote capillary neogenesis, promote collagen synthesis in the proliferative phase and contractile fiber synthesis in repairing phase, rapidly enhance leukocyte activity, and phagocytic function reduces the number of bacteria, thus promoting accelerated healing of various wound including osteomyelitis.

In 1960, Canadian doctor L.J. Papineau [91] first proposed the Papineau method for the treatment of osteomyelitis, namely open cancellous bone grafting, which mainly includes:

(a) Complete lesion removal.
(b) Open cancellous bone transplantation.
(c) Continuous repeated washing.

Since then, Cierny has discussed it in more detail, that is, after thorough debridement, one-stage granular cancellous bone grafting is performed in the invalid cavity of the bone defect, after the operation, the dressing is continued until the surface of the grafted bone covered the granulation tissue, and then free skin grafting is performed to cover the wound. With the widespread application of VSD technology in orthopedics, if it is difficult to close the wound after the lesion is completely removed, VSD technology can be combined with the Papineau method to promote the growth of fresh granulation and accelerate wound healing.

The Papineau method combined with VSD technology has the following advantages over the ordinary Papineau method:

(a) The method is simple without changing dressings.
(b) Effective antibiotic flushing is possible.
(c) Granulation grows more quickly and evenly. However, it must also be noted that if ivory-like sclerotic bone or dead bone is found to be exposed without granulation when replacing the VSD, the sclerotic bone or dead bone must be removed again.

Infect necrotic tissues and exudates on chronic refractory wounds with osteomyelitis are often very viscous, making the effect of VSD greatly discounted and the tube easily blocked. Therefore, it is necessary to apply VSD on the basis of thorough debridement and to design each sponge and each tube during the operation. After the operation, saline should be continuously or intermittently applied to lavage multiple tubes of VSD. This not only ensures the patency of the VSD device but also further flushes the local tissue necrotic exudates and metabolites to accelerate wound healing.

5.6.3.5 Revascularization

After extensive and thorough debridement of the lesion, soft tissue transfer techniques are used to fill the invalid cavity and reconstruct local blood supply. There are various methods for soft tissue flap transfer, including the local vascularized pedicled myocutaneous flap, and the free tissue flap with microsurgical anastomosis. For larger skin and soft tissue defects, the choice of flaps is a very challenging task, requiring clinicians to have very rich clinical experience, and should follow the principle of "from the near to the distant, simple, and not complex." First of all, it is necessary to make a comprehensive evaluation according to the specific conditions of the affected limbs. This includes the area of the skin defect, the surrounding tissue condition, the blood supply of the limb (angiography if necessary), and whether or not a bone defect is combined. The appropriate donor sites are selected according to the results of these evaluations. The commonly used flaps include local flaps, sural nerve flaps, medial and lateral head flaps of the gastrocnemius, medial and lateral flaps of the calf, dorsalis pedis flaps, cross leg flaps, thoracic umbilical flaps, and latissimus dorsal myocutaneous flaps. Secondly, larger defects such as upper leg preferred medial and lateral head flaps of the gastrocnemius; lower leg can choose sural nerve flaps, dorsalis pedis flaps, medial and lateral leg flasps, the middle leg has a larger choice, and the above flaps can be used. When the skin defect is very large or combined with tibia defect, the soft tissue condition of the lower leg is very poor, and there is no alternative flaps locally, the cross-leg flap, free thoracic umbilical flap, latissimus dorsi flap, or composite tissue flap of the contralateral fibula should be considered. In addition, when designing skin flaps, consideration should be given to the area of the skin defect to be repaired and the amount of soft tissue required for invalid cavity filling. The preferred flap is the myocutaneous flap or the flap with some fascial tissue. Finally, for the filling of ineffective cavities without skin defects, the muscle flap is preferred because the local muscle flap has complete blood supply and good antiinflammatory effects [92]. The flaps after removal of the epithelium can also be used to fill the invalid cavity with the tissue flaps that preserve the dermis, subcutaneous fat, and deep fascia [93]. It must be borne in mind that before the soft tissue is transplanted; the infected lesion should be thoroughly debrided so that the tissue flap can have a healthy recipient area, which helps to ensure the success of the operation.

5.6.3.6 Structural Reconstruction and Tissue Regeneration

Postoperative infections in orthopedics often require complete removal of the implant at the expense of stability to control infection, while extensive and thorough debridement of osteomyelitis often affects the stability of the limbs, so the external fixation becomes the most commonly used fixation to stabilize and reconstruct the structure. The external fixation has many advantages, such as minimally invasive, easy to operate, and wide indications. It is the most commonly used fixation technique for the treatment of chronic osteomyelitis with severe bone and soft tissue defects.

In the 1950s, Dr. Ilizarov of the Soviet Union designed and applied a ring-shaped external fixator and minimally invasive technique (Ilizarov technique) for the treatment of chronic osteomyelitis with bone defects, nonunion, bone and joint deformity, and obtained good clinical results. And after years of animal experiments and clinical experiments, the stimulating effect of stretch stress on tissue growth and regeneration has been found, that is, the law of stretch regeneration, also known as the law of tension-stress (LTS). LTS believes that a certain tension generated by slow and continuous stretching of living tissue can secondary and maintain the regeneration and active growth of certain tissue structures, which are consistent with fetal tissues and are the same cell division. Based on the biological theory of LTS, Ilizarov has continuously developed and summarized a technology of traction bone regeneration, also known as distraction osteogenesis (DO) technology, that is, continuous distraction can stimulate bone growth and induce proliferative compensatory adaptation of muscles, fascia, nerve, and skin. After distraction, the new bone tissue is mineralized by the original intramembranous osteogenesis process, which can be transformed into normal bone structure under physiological stress stimulation. Both bone transport technology and bone reconstruction technology originate from the basic principles of tractive osteogenesis [94].

The mechanisms of DO technology in the treatment of chronic osteomyelitis are as follows: completely clearing the infected lesions, repairing the tissue defect by distraction osteogenesis, and reconstructing the blood supply and function of the affected limb. A large number of basic researches and clinical observations have demonstrated that sustained stretch stress stimulation within physiological limits can activate and maintain the ability of tissue regeneration. According to DO technology theory, after a suitable stretching stress is given to the bone, the bones and their attached muscles, fascia, blood vessels and nerves grow synchronously, which increases the extent of limb extension to some extent [95]. Slow and continuous stretching will stimulate cell proliferation and biosynthesis, and active tissue metabolism. A large number of histological studies of the Ilizarov method have confirmed that bone formation is intramembranous ossification in homogeneous region. Histology has shown that there is thin capillary growth between the bone columns, and vessels of uniform diameter extend from the surface of the original bone end. Microangiography confirms that the neovascularization is in the same direction as the longitudinal axis of the new bone. After the Ilizarov external fixation is firmly fixed, the osteotomy site is still elastically connected. The slow stretching makes the tissue metabolism abnormally active and stimulates cell proliferation and biosynthesis. A growing zone can be formed in the middle of the extended region, with fibroblast-like forming collagen fibers (aligned with the direction of distraction). Osteoblasts on collagen fibers produce bone-like tissue, which gradually forms bone trabecula bone and gradually ossifies after fixation. Active smooth muscle cells appear in the middle layer of blood vessel wall, and new capillaries have many communicating branches that anastomose with the blood vessels in the soft tissue surrounding the traction area, constituting the blood circulation in the extended area and its surroundings [96].

Ilizarov external fixation technology provides a solid and adjustable fixation system for the treatment of osteomyelitis, which maximizes the protection of soft tissue around the bone and promotes bone tissue to play its potential. The Ilizarov external fixator penetrates the limb and bone tissue through multi-planar fine Kirschner wires, connects ring-shaped fixator, and assembles into three-dimensional structure with 3–4 screws. This design is firmly fixed, which can not only eliminate shear and rotation stress but also play its role of simple stretching stress or compression, and at the same time, it can also exert its periodic axial micro-motion during weight-bearing walking to promote bone healing. It can be adjusted in many directions and has the functions of de-angulation, de-rotation, de-lateral displacement, and axial compression with biomechanical requirements. It has more advantageous than single-arm external fixator [97].

5.6.4 The Application of Antibiotics

5.6.4.1 Systemic Application of Antibiotics

Effective antibiotic use is the basis for the treatment of chronic osteomyelitis [98]. Systemic application of antibiotics to control infection must meet two conditions: effective bactericidal concentration and maintenance for a sufficient period of time. Although there are many expert opinions and scientific evidences on the treatment of pediatric hematogenous osteomyelitis or endophyte-associated osteomyelitis, in the treatment of adult osteomyelitis without grafts, the optimal antibiotic medication is still unclear and there is a lack of treatment guide that are universally accepted worldwide [99].

In the past, experts often recommended intravenous antibiotics for 4–6 weeks before continuing oral antibiotics for 2 weeks to 2 months [100–103]. According to expert opinion, the purpose of prolonging the course of intravenous medication is to increase the blood drug level. But now, new opinions suggest intravenous administration only during the first 2 weeks [104]. Although intravenous antibiotics facilitate drug penetration into the bone, in 2009 a Cochrane Meta-analysis that included eight trials comparing the efficacy of oral versus intravenous administrations in adult chronic osteomyelitis found that there was no statistically significant difference between oral and intravenous administration in disease remission rate at 12-month follow-up, but the incidence of adverse reactions was significantly higher with intravenous administration than with oral administration [105]. In multivariate logistic regression analysis, the effectiveness of intravenous administration for 1 week did not differ from 2 to 3 weeks or more.

After surgical treatment, antibiotics can help treat residual infections and prevent further spread of chronic infections. The choice of antibiotics should be based on the results of bacterial culture at the site of infection. Because different pathogens have different sensitivities to antibiotic treatment, fine needle bone biopsy or bone biopsy must be performed. In addition, treatment-related drug resistance needs to be considered during long-term treatment, and multiple antibiotics should be used in combination. The antibacterial spectrum should cover every pathogen. In addition, the time of intravenous antibiotics should be as short as possible, and replaced with oral antibiotics until the symptoms disappear. Adjustments and decisions should be made based on a combination of cost and efficacy.

Overall, the duration of antibiotic treatment for osteomyelitis adjuvant to surgery is generally limited to 6 weeks [106]. Long-term (6 months or more) intravenous or oral antibiotics did not show any significant improvement in the condition compared to 6 weeks of treatment [107].

5.6.4.2 Local Application of Antibiotics

Because scar formation or vascular damage often occur in the injured area, leading to blood circulation disorders, it is difficult for systemic application of antibiotics to achieve an effective drug concentration in the lesion. Therefore, the application of local antibiotics is very necessary. The local antibiotics should have the following characteristics:

(a) The antibacterial spectrum is broad.
(b) The local drug concentration of the antibacterial drugs is high, it is easily soluble in water, and it needs to have sufficient stability.
(c) The adverse reactions of the drugs are small.
(d) Thermal stability, suitable for manufacturing process. In addition to the above-mentioned application of antibiotics for lavage, antibiotic sustained release system, namely, polymethyl meth acrylate (PMMA) beads rich in antibiotics, are commonly used for local treatment. After debridement, the antibiotic beads are packed into the ineffective cavity can significantly increase the local drug concentration and is widely used in clinical practice.

However, PMMA itself has certain defects, mainly including:

(a) Cannot be absorbed, need second operation to remove.
(b) No osteogenesis.
(c) The heat generated by the polymerization leads to the inactivation of antibiotics. In order to make up for the shortcomings of PMMA, high-dose antibiotic mixtures

of calcium sulfate are now widely used in the treatment of osteomyelitis.

Although calcium sulfate beads can avoid secondary surgery removal compared with traditional antibiotic beads as potential foreign bodies, these biodegradable products also have a number of disadvantages, such as:

(a) Not strong enough to bear the role of load-bearing filler.
(b) The number and types of antibiotics that can be carried are limited.
(c) Side effects during the degradation process can release inflammatory stimuli, increasing the probability of spontaneous fistula formation in the wounds.

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