Study on the effect of Shixiang plaster on the expression of CD31, serum FN, and VEGF in a rat model with chronic wounds

Ji Fei1, Ling-Li Wang2, Man Liu1, Peng Liu1, Jing-Hua Ruan1, Kai-Wei Zhang1*1

1College of Chinese Orthopedics and Traumatology, Guizhou University of Traditional Chinese Medicine, Guiyang 550001, China. 2Pharmaceutic Preparation Centre, The First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, Guiyang 550001, China.

*These authors contributed equally to this work and are co-first authors for this paper.

Correspondence to: Kai-Wei Zhang, College of Chinese Orthopedics and Traumatology, Guizhou University of Traditional Chinese Medicine, No. 50, Shidong Road, Guiyang 550001, China. E-mail: drhosi@sina.com.

Abstract

Background: Chronic wounds pose a significant surgical challenge, often requiring traditional treatments with limited efficacy. This study explores the promising impact of Shixiang plaster, a classic Chinese ointment, on wound healing. We investigated the cluster of differentiation 31 (CD31) expression, serum fibronectin (FN), and vascular endothelial growth factor (VEGF) levels in SPF rats with induced wounds to elucidate the mechanism behind Shixiang plaster’s effectiveness. We investigated the effect and explored the role of Shixiang plaster on the expression of CD31, serum FN, and VEGF in chronic wounds.

Methods: The study involved 36 SPF rats divided into model, rb-bFGF, and Shixiang plaster groups. Penicillin was injected into the rats before modelling for 3 days to prevent infection. The skin was excised 2 cm below the horizontal line of the inferior border of the shoulder bone in the middle of the rat column up to the deep fascial layer and inoculated with a certain concentration of Staphylococcus aureus; the wound was covered aseptically for 3 days. The trauma area of the rats was observed at 3, 7, and 14 days, respectively. Histopathology was observed using haematoxylin eosin and Mason staining. CD31 expression was detected using immunohistochemistry staining. FN and VEGF expression was detected using serum ELISA. Statistical analyses were carried out by the method of SPSS.

Results: Regarding wound morphology, at 3 days, the recovery area of the Shixiang plaster group was larger than that of the other two groups, at 7 days, the wound healing rate of the Shixiang plaster group was significantly higher, and at 14 days, the wounds of the Shixiang plaster group had been mostly healed, with a healing rate of 98.3%. Haematoxylin eosin staining revealed a large amount of granulation tissue at 3 days in the Shixiang plaster group, and the epidermal scales disappeared at 14 days, with thinner epidermal thickness at 1 lesion and a large reduction in inflammatory cell infiltration. Masson staining showed that at 3, 7, and 14 days, blue staining was the most abundant and deeper in the Shixiang plaster group, with richer collagen and a compact tissue matrix. Immunohistochemical testing showed strong positive expression of CD31 in the Shixiang plaster group, with abundant neovascularisation and large official lumens extending towards the surface of the wound. Statistically significant elevated expression of FN at 7 and 14 days was determined by ELISA in the Shixiang plaster group, and VEGF expression was significantly increased at 7 days, but expression had been expressed at a low level at 14 days. Conclusion: Shixiang plaster exhibits remarkable efficacy in healing chronic wounds. The proposed mechanism involves FN’s promotion of angiogenesis and cell proliferation, VEGF’s impact on angiogenesis and inflammation, and CD31’s regulatory role in inhibiting inflammation while promoting angiogenesis.

Keywords: Shixiang plaster; chronic wounds; CD31; FN; VEGF
**Highlights**

It has been clinically and scientifically validated that Shixiang plaster promotes wound healing and reduces healing time. However, the underlying mechanism is unclear. We show that the mechanism of Shixiang plaster’s action on chronic wounds may be related to the expression of serum fibronectin (FN), vascular endothelial growth factor (VEGF), and cluster of differentiation 31 (CD31).

**Medical history of objective**

Shixiang plaster is a traditional clinical formula used to treat chronic wounds. It was developed based on the theory of "promoting pus discharge and removing necrotic tissue," which originates from Wu Qian’s "Yizong Jinjian" (1742 C.E.). "Necrosis refers to damaged tissue. Various texts state: if necrotic tissue is not removed, new tissue will not regenerate... Indeed, medication for removing necrotic tissue is essential in surgery." Modern clinical research has shown that Shixiang plaster has significant effects on chronic wounds, including shortening healing time and alleviating pain.

**Background**

Chronic wounds (CW) are wounds that are unable to achieve anatomical and functional integrity through a normal, orderly, and timely repair process under the influence of various internal and external factors, and instead enter a pathological inflammatory reaction [1]. The duration of the disease often exceeds one month, and the proportion of Chinese surgical inpatients with CW ranges from 1.5% to 3.0%. Currently, the cause of CW in China has changed from trauma to chronic disease, which often reduces the patients’ working ability and quality of life. The incidence of CW is characterized by an older age group, complex pathogenesis, difficulty in treatment, long treatment period and high disability rate, which brings great economic and psychological burden upon the patients and their families [2]. CW are the result of a variety of complications such as diabetes, pressure sores, bed rest, or venous insufficiency. Immune dysfunction is an important factor in the pathogenesis of chronic non-healing wounds. Wound repair includes regeneration, repair, and reconstruction of the affected area [3]. Normal wounds heal in an orderly manner according to the processes of inflammation, hyperplasia, and remodelling [4]. However, CW may stagnate at a certain stage in the healing process for various reasons and may not continue to develop, resulting in delayed or non-healing of the wound and even invasion of the surrounding normal tissues [5]. Current research suggests that CW are mostly caused by tissue ischemia, hypoxia, infection, and a decrease in the number of local growth factors, leading to a relative balance between the body’s immunity and bacterial virulence so that the healing process is stagnant in the inflammatory phase and cannot continue to progress [6, 7].

Recently, research on chronic nonhealing wounds has become increasingly profound, especially in the field of plastic surgery. Studies on chronic nonhealing wounds have mainly focused on cellular and molecular research. Several treatments are commonly employed to manage CW, including traditional approaches such as debridement, anti-infection measures, and flap grafting [8]. Simultaneously, emerging therapies including negative pressure wound therapy, stem cell therapy, and cell growth factor therapy have gained attention [9]. However, these methods have drawbacks such as inconsistent and costly outcomes, cumbersome procedures, and techniques necessitating operating theatre interventions, placing a substantial burden on both physicians and patients [10]. An established remedy in wound treatment is Shixiang plaster, a classic topical ointment recognized for its efficacy in promoting wound healing, reducing healing duration, and alleviating pain [11]. CD31, also known as platelet-endothelial cell adhesion molecule-1/cluster of differentiation 31 (PECAM-1/CD31), has a molecular weight of 130 kDa and is a member of the immunoglobulin superfamily that is crucial in eliminating ageing neutrophils from the body [12]. CD31 is present on platelets, neutrophils, monocytes, specific T-cells, and endothelial cell tight junctions, and likely participates in leukocyte migration, angiogenesis, and integrin activation during wound repair [13-15]. FN, an essential scaffolding protein that regulates cellular activity, plays a pivotal role in maintaining tissue structure and directing extracellular matrix (ECM) composition [16]. During wound repair, FN contributes to comprehensive structural and functional restoration by repairing not only the epidermis but also the dermis, hair follicles, sweat glands, nerves, and blood vessels [17]. VEGF, also known as the vascular permeability factor, is a highly specific pro-vascular endothelial cell growth factor that increases vascular permeability, alters the ECM, and promotes vascular endothelial cell migration, proliferation, and angiogenesis [18, 19]. Therefore, CD31, FN, and VEGF may be key factors in studying the effects of Shixiang plaster on chronic non-healing wounds. In this study, we established a rat model of CW to observe the effects of Shixiang plaster on CD31, serum fibronectin, and vascular endothelial growth factor in rat wounds. This study aimed to explore the potential mechanisms underlying the effectiveness of Shixiang plaster in treating CW.

**Materials and methods**

**Experimental material**

**Drug on trial.** Shixiang plaster is composed of medicinal ingredients such as Olibanum, Myrrha, calcined bone, Halloysitum Rubrum, Gardeniae Fructus, and Borneolum Syntheticum. This combination of ingredients can help contract wounds, promote granulation tissue formation, and expel pus. However, there is a concern that the medicinal properties may be too strong and could damage the skin. Therefore, sesame oil and beeswax are added as solvents to reduce specific skin irritation while keeping the wound moist, thus promoting granulation tissue growth and epithelial tissue regeneration. Additionally, they cover the surface of the wound and enhance overall transdermal absorption owing to their liposolubility. Preparation of Shixiang plaster: The Shixiang plaster was prepared by dissolving Calamine (500 g), Olibanum (150 g), Myrrha (150 g), Halloysitum Rubrum (150 g), Gardeniae Fructus (150 g), calcined bone (150 g), and Borneolum Syntheticum (150 g) in 2 L of heated sesame oil to form a paste. The paste was mixed with 200 g of Cera Flava to synthesise the Shixiang plaster. The procedure was performed in the Chinese Medicine Preparation Room of Guizhou Provincial Hospital of Traditional Chinese Medicine. Recombinant bovine basic fibroblast growth factor (rb-bFGF) solution was purchased from Zhuhai Yisheng Biopharmaceutical Co., Ltd. (Zhuhai, China).

**Laboratory animals.** Thirty-six SPF-grade male SD rats weighing 250–300 g were housed in individual cages reared in separate cages at the Yunnan Huashu Biotechnology Laboratory (Experimental Unit Use Licence No. SYXK (Dian) K2020-0006). The treatment of experimental animals in this study adhered rigorously to the Guiding Opinions on the Proper Treatment of Experimental Animals promulgated by the Ministry of Science and Technology in 2006. The animal study protocols were approved by the Animal Ethics Committee of the Guizhou University of Traditional Chinese Medicine (Certificate No. BST-RAT-20230806-01).

**Main reagents and instruments.** Shixiang plaster was prepared by the First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine; rb-bFGF (Approval No.: S10980077), the main ingredient of which is recombinant bovine basic fibroblast growth factor, purchased from Zhuhai Yisheng Bio-Pharmaceutical Co., Surgical Instruments (Sound Medical Devices Co., Ltd., Zhuhai, China). We also used electothermal constant temperature incubator (303-2, Shaoxing Isolai Instrument Co., Ltd., Shaoxing, China), slicer (LEICARM2135, LEICA, Wetzlar, Germany), scanner (SQS-12P, Shenzhen Shengqiang Science and Technology Co., Ltd., Shenzhen, China).
Modelling. Normal, neoplastic, and granulation tissue specimens were taken from the back of rats in the model group, rb-bFGF group, and Shixiang plaster group. Certain tissue specimens were fixed with 4% paraformaldehyde and made into paraffin wax blocks for preservation, and some were stored in a refrigerator at − 80 °C.

Observational indicators and methods

Observation of wound healing and wound healing rate. At 3, 7, and 14 days after modelling, the local wounds of the rats were photographed, the wounds were observed in general, and the wound area was counted using ImageJ software, according to Equation (1): Wound Healing Rate = [(Original Wound Area − Unhealed Area at the Time Point)/Original Wound Area] × 100%

Histopathological changes observed by haematoxylin eosin (HE) and Masson staining. Tissue specimens were taken from the modelling areas at 3, 7, and 14 days and fixed in 4% paraformaldehyde at 4 °C overnight, dehydrated in ethanol gradient (2 days 70% ethanol, 2 days 96% ethanol, 2 days 100% ethanol), degressed in acetone for 8 h, and then paraffin embedded. Next, were taken and stained with HE and Masson trichrome staining.

Immunohistochemistry staining to detect CD31 expression. To assess the status of angiogenesis, the 5-µm thick sections were taken and 0.01 M citrate buffer was added for antigen repair. The sections were blocked with 3% H₂O₂ water incubated at room temperature for 20 min. Then, 5% bovine serum albumin V was added and incubated at 37 °C for 30 min for sealing. The rat primary antibody against CD31 was added and incubated overnight, and secondary antibody was added and incubated at 37 °C for 30 min, followed by diamobenzidine tetrahydrochloride chromatography and haematoxylin re-staining. The dehydration, transparency, and sealing of the films were performed for analysis.

Serum ELISA for skin damage repair-related factors. All the reagents, samples, and standards were prepared according to the ELISA kit. Next, 50 µL of standards or samples were added per well and then immediately 50 µL of prepared assay reagent was added. The mixture was shaken, mixed, and incubated at 37 °C for 1 h. The mixture was aspirated and washed 3 times. Then, 100 µL of prepared assay reagent was added and incubated at 37 °C for 30 min. The mixture was aspirated and washed 5 times. To this mixture, 90 µL of substrate solution was added and incubated at 37 °C for 10–20 min. Finally, 50 µL of the stop solution was added. Absorbance was immediately measured at 450 nm.

Statistical methods

SPSS software (version 22.0) was used for statistical analysis. Data were expressed as mean ± standard deviation (± s) and analysed by ANOVA if normal distribution and chi-square were satisfied, or by rank sum test if normal distribution or chi-square were not satisfied. This experiment was tested for normality and analysed using analysis of variance. Statistical significance was set at P < 0.05.

Results of the study

Observation of the gross morphology of the wound

At 3 days, the model group had a smaller area of trauma recovery, and the Shixiang plaster group had a larger area of recovery than the rb-bFGF group. The wound healing rate was significantly higher in the Shixiang plaster and rb-bFGF groups than in the model group. Furthermore, the wound-healing rate in the rb-bFGF group was lower than that in the Shixiang plaster group. At 7 days, large portions of the wounds in the model group did not have scabs, a few wounds in the rb-bFGF group did not have scabs, and all wounds in the Shixiang plaster group had scabs. Compared to the model group, the wound healing rate was significantly higher in both the Shixiang plaster and rb-bFGF groups. Additionally, there was no significant difference in the wound healing rate between the rb-bFGF and Shixiang plaster groups. At 14 days, the wounds in the model group also crusted over but were still redder and swollen; the wounds in the rb-bFGF group were redder than those in the Shixiang plaster group. Most wounds in
the Shixiang plaster group recovered. Compared to the model group, the wound healing rate was significantly higher in both the Shixiang plaster and the rb-bFGF groups. There was no significant difference in the wound healing rate between the rb-bFGF and Shixiang plaster groups. The wound area was measured using NIH ImageJ image analysis software to calculate the wound healing rate. The wound healing rate was calculated using Equation (2), as shown in Figure 1, 2:

\[
\text{Wound Healing} = \left( \frac{\text{Initial Wound Area} - \text{Wound Area}}{\text{Initial Wound Area}} \right) \times 100\%
\]

The rats were weighed before each photo session, and changes in their body weights are shown in Figure 2.

**HE staining and observation of wound tissue**

The model group exhibited several inflammatory cells in the wound on day 3, the infiltration of inflammatory cells in the skin tissue was reduced at 7 and 14 days. The epidermal cells covered the wound tissue at day 14 with poor continuity. In the Shixiang plaster group, several inflammatory cells appeared in the wounds tissue at 3 days, and a large number of granulation tissues were formed. At 14 days, epidermal scales mostly disappeared from the skin tissues, the thickness of the epidermis at the skin lesions was markedly thinner than that of the model group, and the infiltration of inflammatory cells significantly reduced, and the wounds surface was recovered better. The thickness of the epidermis at the skin lesions at 14 days was even thinner. In the rb-bFGF group, the epidermis at the skin lesions was thicker at 3 days, and the infiltration of inflammatory cells was slightly reduced compared to that of the model group. At 7 days and 14 days, the epidermal scales basically disappeared from the skin tissues, and the thickness of the epidermis at the skin lesions was thinner than that of the model group, the infiltration of inflammatory cells was reduced, and epidermal cells covered the traumatic tissues in a continuous and good way, as shown in Figure 3.

**Masson staining of wound tissue**

Masson staining revealed changes in dermal collagen fibres in the traumatised tissue. Masson's trichrome stain stains collagen fibres blue and is used to evaluate collagen fibre formation in wounds. At 3 days, the blue staining of the wound surface was light in all groups, among which the Shixiang plaster group was the deepest. At 7 days, the wound surface of the Shixiang plaster group and the rb-bFGF group had more blue staining, deep colouring, and the wound surface was rich in collagen fibres and neatly and tightly arranged. At 14 days, the wound surface of the model group had less blue staining, lighter colouring, less collagen deposition, and the tissue matrix was sparse. The blue staining of the Shixiang plaster group was many and darker, with more collagen richness, and the tissue matrix was tight. The blue staining of the rb-bFGF group had more blue staining, and the tightness of the tissue matrix was slightly sparse compared to that of the Shixiang plaster as shown in Figure 4.

---

*Figure 1 Wound healing of rats in each group at different time periods. rb-bFGF, topical recombinant bovine basic fibroblast growth factor.*

*Figure 2 Wound healing rate of rats in each group at different time periods (A) and line graphs of body weight of rats in each group at different time periods (B). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.001. # denotes comparisons with Model group, and # denotes comparisons with Shixiang plaster group. rb-bFGF, topical recombinant bovine basic fibroblast growth factor.*

Submit a manuscript: https://www.tmrjournals.com/trmr
Immunohistochemistry

To observe the neovascularisation of the wounds, immunohistochemical staining with the neovascular endothelial cell marker CD31 was performed on the normal, neoplastic, and granulation areas of the wounds in each group at different time points. There was no significant difference in the CD31 positivity rate between the Shixiang plaster and rb-bFGF groups compared with the model group at 3, 7, and 14 days in the normal skin area. At 3 days, there was no significant difference in the CD31 positivity rate in the granulation and neoplastic skin areas in both the Shixiang plaster and rb-bFGF groups compared to that in the model group. At 7 days, the rate of CD31 positivity in the granulation and neoplastic skin areas was significantly higher in both Shixiang plaster and rb-bFGF groups than in the model group. At 14 days, CD31 positivity was significantly higher in the granulation and neoplastic skin areas of the Shixiang plaster group than in the model group, and there was no significant difference in the rb-bFGF group. CD31 positivity was significantly higher in the granulation and neoplastic skin areas on day 14 in the rb-bFGF group than in the Shixiang plaster group (Figures 5, 6).

Determination of FN and VEGF in rat serum by ELISA

FN expression was elevated in the Shixiang plaster group compared with the model group at 3, 7, and 14 days, with the difference being not statistically significant at day 3 and statistically significant at 7 days and 14 days. FN expression was elevated in the rb-bFGF group.
compared with the model group at 3, 7, and 14 days, with the difference being not statistically significant at 3 and 7 days, and statistically significant at 14 days. VEGF expression was also detected. VEGF was related to skin damage repair, and VEGF expression was the lowest after the administration of Shixiang plaster compared with the model group and the rb-bFGF group at 3 days. At 7 days, VEGF expression increased significantly after the administration of Shixiang plaster compared with the model group and the rb-bFGF group, and there was no significant difference between the two groups. However, the expression of VEGF began to decline in the model group. At 14 days, serum VEGF expression was low in all three groups, with no significant differences between the groups (Figure 7).

---

**Figure 5** Immunostaining of CD31 in different parts of rat twound tissues in different groups at different time periods. Scale bar = 50 µm. rb-bFGF, topical recombinant bovine basic fibroblast growth factor.
Figure 6 CD31 expression in rat trauma tissue in each group at different time periods. *P < 0.05, **P < 0.01. ***P < 0.001, ****P < 0.0001. * denotes comparisons with Model group, # denotes comparisons with Shixiang plaster group. rb-bFGF, topical recombinant bovine basic fibroblast growth factor.

Figure 7 Serum FN expression levels in rats of different groups at different time periods (A) and VEGF content in rats of each group at different time periods (B). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. * denotes comparisons with Model group, # denotes comparisons with Shixiang plaster group. rb-bFGF, topical recombinant bovine basic fibroblast growth factor; FN, fibronectin; VEGF, vascular endothelial growth factor.

Discussion

In Chinese medicine, CW fall under the category of “gangrene”, as highlighted in “The Spiritual Pivot – Carbuncle Gangrene” [21]. This text emphasises the urgency of treating carbuncles and associating red and black colouration with life and death. “The Origin of All Diseases” attributes gangrene to disorders of the five organs, linking it to a positive deficiency and evil reality. Its pathogenesis involves factors such as excessive fat, sweetness, phlegm deficiency, blood stasis (microcirculation disorders), positive qi deficiency (decreased immunity), and congestion of heat and toxins (organism damage caused by metabolic disorders) [22]. Traditional Chinese medicine views the disease as a deficiency, with qi and yin deficiencies (metabolic disorders and weakened immunity) as the standard. This deficiency leads to meridian loss of nourishment, internal organ damage, and weakened yin and yang (multisystem dysfunction), making the body susceptible to external events such as dampness and heat. This imbalance allows these pathogenic factors to invade, leading to heat, rotting of flesh, pus formation, and carbuncle-related complications [23].

Shixiang plaster, a renowned topical remedy used at the First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, has demonstrated significant efficacy in the treatment of various CW for over 40 years. Rigorous quality control and safety assessments conducted in preliminary experiments confirmed its reliability [24]. The formula consists of Galantina, Olibanum, Myrrha, calcined bone, Halloysitum Rubrum, Gardeniae Fructus, Borneol, and other herbs. Olibanum and Myrrha, which are regarded as monarch medicines, are pivotal for activating blood circulation, relieving pain, reducing swelling, and promoting muscle regeneration. They are often used together to treat traumatic diseases and play a crucial role in wound healing. Halloysitum Rubrum, with its astringent and bleeding-stopping properties, combines synergistically with calcined bone to aid in sore convergence and the removal of necrotic tissue. Gardeniae Fructus, Borneol, and Calamina serve as adjuvant medicines, contributing to heat clearance, detoxification, swelling reduction, pain relief, and the prevention of itching. To balance the potential effects of the formula and prevent skin damage, sesame oil and beeswax were used as solvents to maintain wound moisture and reduce irritation. The combined action of these ingredients results in a comprehensive therapeutic effect involving astringency, haemostasis, muscle regeneration, pus extraction, detoxification, and skin moisturization [25].

This study focused on the effects of three different treatments on CW healing in rats. HE staining revealed that, at 3, 7, and 14 days after the administration of the drug, compared with the model group, the Shixiang plaster group showed a reduction in inflammation, formation of denser and orderly granulation tissue, regular keratinised epithelial structures, and formation of new hair follicles at the wound site, suggesting that the Shixiang plaster group performed better in functional regenerative repair of the skin. In contrast, Mason’s trichrome staining showed significantly higher levels of fibrosis in the granulation tissue and neoplastic skin area at 14 days in the rb-bFGF group and the neoplastic skin area at 7 days in the Shixiang plaster group. This suggests that both promote the accumulation of collagen
and muscle fibres in the wound area, thereby contributing to wound healing.

Wound healing is a complex and sequential process involving multiple stages of the inflammatory response, cell proliferation, ECM remodelling, and angiogenesis [26]. This process requires precisely regulated mechanisms to ensure smooth operation. CD31, FN, and VEGF play important roles in wound healing and regulation of inflammatory responses, cell migration, cell proliferation, and angiogenesis [27–30]. CD31, also known as PECAM-1, is a member of the immunoglobulin superfamily of proteins that plays an important role in the removal of ageing neutrophils from the body [31]. CD31 is currently the single best marker of endothelial differentiation. Overexpression of CD31 during the early stages of wound healing inhibits wound healing, whereas appropriately lowered levels of CD31 expression contribute to wound healing [32]. The immunohistological results of this experiment showed that in the initial stage of wound healing, CD31 was weakly expressed in the wound tissues of all three groups, whereas in the later stage of healing, CD31 was strongly expressed in the Shixiang plaster group, with abundant neovascularisation, large vascular lumens, and vascularity extending to the surface of the wounds, suggesting that CD31 may play an important role in regulating wound healing. FN is both a regulator of cellular activity and an important scaffolding protein that maintains and directs tissue structure and ECM composition [33]. During wound healing, FN promotes cell adhesion and proliferation, thereby contributing to the regulation of inflammatory responses and angiogenesis [34]. Furthermore, FN can act as a growth factor, promoting cell growth and differentiation, and contributing to wound healing [35]. In this experiment, the serum FN of rats was in the highest expression state at all time points of wound healing compared to the model and control groups, indicating that Shixiang plaster has a good experimental effect in promoting the regeneration and repair of wound tissue. VEGF is a highly specific pro-angiogenic endothelial cell growth factor that promotes vascular permeability, ECM degeneration, vascular endothelial cell migration, proliferation, and angiogenesis [36]. VEGF expression levels change during wound healing. Appropriate levels can promote angiogenesis and provide nutritional support for wound healing. However, excessive VEGF levels may lead to abnormal angiogenesis and impede the wound healing process, the results of this experiment, Shixiang plaster at 3 days after wound modelling inflammatory phase stage, Shixiang plaster group in the three groups of the lowest level of expression, indicating that the Shixiang plaster may have a better inhibition of wound inflammation [37]. At 7 days of the remodelling stage, the rb-BFGF group had a similarly high level of expression, indicating that in the remodelling stage of the Shixiang plaster, rb-BFGF had a better promotion of vascularity and improved the effect of difficulty in healing the blood supply of wounds. In the later stage of the 14 days of wound healing, as the groups of wounds were healed, the Shixiang platter group 14 days a wound healing rate is high up to 98% or more; therefore, the expression of serum VEGF is returned to a low level. CD31 inhibits the inflammatory response and regulates the expression of FN and VEGF. Excessive inflammatory responses during wound healing may lead to tissue damage and delayed healing [38]. Thus, CD31 maintains wound homeostasis by inhibiting the inflammatory response and influencing the expression of FN and VEGF by promoting angiogenesis [39]. During wound healing, CD31 stimulates the proliferation and migration of endothelial cells, thereby promoting angiogenesis. This process helps provide the necessary nutrients and oxygen to promote wound healing [40].

In summary, CD31, FN, and VEGF constitute a complex regulatory network that is crucial for CW healing. The current experimental animal study provides preliminary insights into the effects of Shixiang plaster on the repair of these challenging wounds. One hypothesised mechanism of action suggests that FN promotes wound healing through angiogenesis and cell proliferation, whereas VEGF influences wound healing by promoting angiogenesis and inflammatory responses. Simultaneously, CD31 regulates FN and VEGF expression by inhibiting inflammatory responses and promoting angiogenesis, thereby contributing to the pivotal role of this regulatory network in regulating wound healing. The animal model used in this study proved the positive effect of Shixiang plaster on CW, opened up new ideas and methods for the treatment of CW with clinical Chinese medicine, and laid the foundation for further validation of the mechanism of action at the protein, gene, and cellular levels. However, the use of animal models to study human wounds has certain limitations. Significant differences exist between animals and humans in terms of physiology, genetic background, wound type, and severity. Although animals and humans share many biological similarities, significant physiological differences remain. Differences in human skin structure, vascular distribution, and immune system responses compared to animals may lead to significant differences in the wound-healing processes observed in animal models compared to humans. Animal backgrounds are relatively homogeneous, whereas human genetic backgrounds are complex. The wound-healing process in humans is influenced by multiple genes and environmental factors that are difficult to replicate fully in animal models. Animal models cannot fully replicate the complexity of human wounds in terms of wound type and severity. Different types of wounds, such as burns and trauma, may involve different physiological mechanisms during healing that may not be fully reflected in animal models. Given these limitations, future research could expand to the following areas: 1. Animal models should be optimised by improving the experimental conditions to make them more similar to human physiology and genetic backgrounds, thereby enhancing the accuracy of research. For example, selecting animal species that are closer to humans or using gene editing techniques to simulate the specific genetic characteristics of humans. 2. Clinical data were combined to compare and analyse the results of the animal models with clinical data to validate the reliability of the animal models. Collecting and analysing a large number of wound healing models can provide valuable references for future research. 3. Increasing in vitro cell culture by finely controlling the growth environment of cells, including the composition of the culture medium and temperature, to make the experimental results more reliable and accurate. Furthermore, some diseases and pathological processes cannot be fully replicated in animal models, and some drugs exhibit different characteristics in animal models than in humans. Therefore, in vitro cell culture can provide a model closer to the human biological system, allowing researchers to more accurately understand cell behaviour and drug responses. 4. Tissue engineering can be combined to create artificial tissues that match human wound characteristics using biomaterials and cell culture techniques. Therefore, reducing the risk of infection in patients provides a better healing environment, promotes the natural healing process, and reduces scarring. However, early experiments with laboratory animals have shown that chronic, difficult-to-heal wounds are not always dormant. Even when transitioning in a positive direction with low healing rates, it can lead to poor prognosis or healing failure. Therefore, finding better methods, such as rapid haemostasis and closure of acute wounds in the early stages of acute wound formation, maybe the focus of future research.

References


