Medicine®

OPEN

Recombinant human granulocyte macrophage colony stimulating factor in deep second-degree burn wound healing

Dexiong Yan, MD, Sha Liu, MD, Xiaochun Zhao, MD, Huijuan Bian, MD, Xingwei Yao, MD, Jiping Xing, MD, Weijing Sun, MD, Xiangjun Chen, MD*

Abstract

Background: The aim of this study was to explore the effects of recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) on deep second-degree burn wound healing.

Methods: In this study, 95 patients with a total of 190 burn wounds were treated with either rhGM-CSF or placebo, separated into 2 groups by treatment type. Wound healing rate, wound healing time, histopathological condition, and scar scale were all compared between the 2 groups.

Results: The healing rates in the rhGM-CSF group were remarkably higher than those in the placebo group ($P < .01$). The wound healing time in the rhGM-CSF group (18.8 ± 7.6 days) was significantly shorter than that in the placebo group (25.5 ± 4.6 days, $P < .01$). On the 14th day and 28th day, the average optical density of vascular endothelial factor (VEGF) in the rhGM-CSF group was larger than that in the placebo group. Meanwhile, the average optical density of fibroblast growth factor (FGF) in the rhGM-CSF group was also larger than that in the placebo group. Furthermore, the Vancouver scar scale scores of pigmentation, pliability, height, and vascularity were notable lower in the rhGM-CSF group than those in the placebo group ($P < .01$).

Conclusion: The results suggest that rhGM-CSF can significantly accelerate deep second-degree burn wound healing.

Abbreviations: FGF = fibroblast growth factor, HE = hematoxylin and eosin, HS = hypertrophic scar, rhGM-CSF = recombinant human granulocyte macrophage colony stimulating factor, TBSA = total burn surface area, VEGF = vascular endothelial factor.

Keywords: placebo, rhGM-CSF, second- degree burn, Vancouver scale scores, wound healing

1. Introduction

Severe burns are more complicated and difficult to treat when compared to other normal traumas or burns. They can result in superabundant production of inflammatory mediators and cytokines, which can have a negative impact on several processes, increasing mortality risk.[1] Second-degree burns are sometimes a dilemma as to whether early surgery should be carried out or whether this should be delayed until remnant dermal components are re-epithelialized.[2] It has been reported that early excision and grafting of less than 20% of the total burn surface area

(TBSA) performed better than non-operative treatment due to hypertrophic scar (HS) formation and scar quality.[3] Muangman et al[4] demonstrated that dermal replacement template surgery on the 5th day after burn, followed by autografting on the 21st day after burn was safe and effective for patients with deep dermal burns to full-thickness burns and 43% TBSA.

However, many patients refuse surgery due to the financial cost and the risks involved, and in patients with large-area burns, intact epithelium is often difficult to obtain for surgery.[5] Therefore, the development of effective drugs which can be directly applied to wounds is of great importance in the treatment of deep second-degree burns.

Recently, several innovative therapies for burn treatment have been proposed, one of these was the use of recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF).[5] RhGM-CSF has been proven beneficial in healing both deep burn wounds and leprosy ulcers, indicating its potential in second-degree burns.[6] Because of rhGM-CSF can promote hematopoietic progenitor cell proliferation in bone marrow and aid the transferring of mature cells to the periphery. Meanwhile, rhGM-CSF can increase the amounts of monocytes and macrophages within tissue, eventually enhancing immune response to local wounds and accelerating wound healing.[7] Fang et al[8] and Kaplan et al[9] both found that GM-CSF could promotes wound healing, abates inflammation of local wounds, and promotes the growth of epithelial tissue in diabetic and immune deficient rats. Overall, the efficacy of rhGM-CSF in the clinical treatment of wound healing has thus gained our attention, prompting this study.

Table 1

Baseline characteristics of 3 groups.

		rhGM-CSF	Placebo	P
Age		40.36±11.37	39.68±12.54	.696[*]
Sex, female: male		71: 24	71: 24	–
TBSA, %		50.3±9.8	50.1±13.0	.905[*]
Burn type	Hydrothermal burn	30	30	–
	Flame burn	62	62	–
	Chemical burn	3	3	–
Wound area, cm^2		14.9±5.6	15.5±5.2	.445[*]
Time after injury, h		5.1±1.3	4.8±2.9	.359[*]

TBSA = total body surface area, rhGM-CSF = recombinant human granulocyte macrophage colony stimulating Factor.

[*] Evaluated by the t test.

In an attempt to evaluate the treatment efforts of patients with deep second-degree burn, we planned a prospective randomized trial of rhGM-CSF versus placebo. In this experiment, we evaluated and compared the efficacy of rhGM-CSF and placebo on second-degree burn wound healing so that a more efficacious and convenient treatment can be used in clinical practice. In this study, a series of wound healing indicators have been examined to compare the effects of rhGM-CSF with placebo.

2. Materials and methods

2.1. Participants

In total, 95 large-area burn patients admitted to our hospital between December 2013 and January 2015 were enrolled in this study. The sample size we took in our study was much more than the calculation result of 48 when the power was over 90% by the power calculation. Patients were divided into 2 groups: rhGM-CSF and placebo groups. The baseline characteristics of patients in 2 groups were shown in Table 1 and all included outcomes showed no statistical difference (P > .05). A diagnosis of deep second-degree burn and large-area burn (> 50% of total body surface area [TBSA]) was made according to the 3-degree scale and the rule of nines, respectively. Participants were aged between 18 and 60, with at least 2 adjacent residual wounds (distance more than 20 cm) with similar wound area (area difference less than 4 cm^2), and with residual wound areas smaller than 25 cm^2. Patents who met any of the following conditions were excluded from this study: (1) history of medication treatment; (2) allergic to rhGM-CSF or bFGF or had allergic history with multiple medications, or recent allergy outbreak; (3) had severe combined injury, diabetes, cardiac, hepatic, or renal dysfunctions; (4) women during pregnancy and lactation. The Ethics Committee of the 253rd Hospital of PLA approved our research, and all participants were informed of the treatments and gave written consent. The number of ethical approval was 2011 (04) and the date of ethical approval was 26th July, 2015.

2.2. Treatment

A total of 190 burn wounds of 95 patients were randomly assigned into the rhGM-CSF group (using rhGM-CSF, n = 95) and control group (using placebo, n = 95) by the principle of matched pair design. Empowerstats software was used as the randomization method. When there were 2 wounds on the same participant, one was randomly chosen for the rhGM-CSF group and the other one for the placebo group. All participants underwent routine debridement. Before drug treatment, the secretions the wounds were fully cleaned and washed with physiological saline. The experimental group was treated with rhGM-CSF gelatin (GeneScience Pharmaceuticals Co., Ltd. Changchun, China) and the control group was treated with a placebo (GeneScience Pharmaceuticals Co., Ltd. Changchun, China). All wounds were covered with the rhGM-CSF gelatin or placebo of 1 to 2 mm and bandaged with 4 to 5 layers of dressings. Dry gauze was then applied and the fresh dressing was changed for each wound every day. The observation data were noted before drug treatment and on the 7th, 14th, 21st, and 28th day after injury.

2.3. Wound assessment

2.3.1. Wound healing. We measured and recorded the wound healing rate of all patients on the 7th, 14th, 21st, and 28th day to determine the treatment difference between rhGM-CSF and placebo. This was done by using a sample cloth placed on the wound after it had been cleaned and dried. The sample cloth showing the shape of the wound was then put in a grid table (the side length of each small square was 0.25 cm). The wound area and wound healing rate was calculated using the following formula: wound area = the amount of small squares × 0.0625 cm^2. Wound healing rate = (the area of wounds before the use of drugs – the area of wounds after the use of drugs)/the area of wounds before the use of drugs × 100%. Wound healing time was measured up to 28 days; we only recorded the wound healing rate when the wound healed within 28 days.

2.3.2. Histopathological observation. Six participants were chosen by the random number table method and tissue samples of their marginal wound areas were obtained (4 mm × 2 mm × 2 mm) on the 14th day and 28th day after drugs were applied. Samples were treated with 10% neutral buffered formalin for 48 hours. After dehydration in a graded ethanol series, the specimens were embedded in paraffin. Sections were cut and stained with hematoxylin and eosin (HE). The samples were evaluated in 5 microscopic fields (100× to 400× magnification) by 2 observers blinded. The blood capillaries and fibroblasts in granulation tissue were inspected.

The expression of VEGF and FGF was detected using the immunohistochemistry method on the paraffin-embedded tissue samples. The samples were deparaffinized in xylene and hydrated through graded ethanol to deionized water. Endogenous peroxidase activity was blocked in 3% hydrogen peroxide-methanol buffer. Serum blocking was then carried out for 20 minutes at room temperature. Mouse anti-VEGF monoclonal antibody (1:15; Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit polyclonal FGF antibody (1:10; Abcam, Cambridge) were applied for 1 hour at room temperature in 1× tris buffered saline

Table 2

Analysis of wound healing rate (%) and time in 2 groups.

Groups	Wound healing rate, %				Wound healing time, d
	7 d	14 d	21 d	28 d	
rhGM-CSF	29.5±19.4	60.3±33.9	92.2±17.6	96.8±13.9	18.8±7.6
Placebo	19.9±18.1	46.7±21.9	68.7±18.1	82.2±12.3	25.5±4.6
P	<.001*	<.01*	<.001*	<.001*	<.001*

* Evaluated by the t test.
rhGM-CSF = recombinant human granulocyte macrophage colony stimulating factor.

(TBS). After rinsing of 1×TBS, the secondary antibody was added. Samples were washed in 1×TBS 5 times and then stained for 5 to 10 minutes with DAB Kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing). Finally, hematoxylin and eosin were used to counterstain. The samples were evaluated in 5 microscopic fields (100× to 400× magnification) by 2 observers blinded.

2.3.3. Scar scaling. Scar pigmentation (0=normal, 1=hypo-pigmented, 2=mixed, 3=hyper-pigmented), pliability (0=normal, 1=supple, 2=yielding, 3=firm, 4=ropes, 5=contracture), height (0=flat, 1=< 2mm, 2=2–5mm, 3=≥ 5mm), and vascularity (0=normal, 1=pink, 2=red, 3=purple) [10] were determined using the Vancouver scar scale. Evaluation of burns was confirmed by burn experts in a blind fashion 1 year after wound healing. Finally, the scores of each scar were obtained by averaging the individual score.

2.3.4. Statistical analysis. All analyses were performed using Empower (R) (www.empowerstats.com, X&Y solutions, inc.

Boston MA), R (http://www.R-project.org) and Graphpad Prism 6.0 (GraphPad Software). The measurement data were expressed as mean±standard deviation. All variables between the 2 groups were compared using unpaired t-tests, and $P < .05$ was considered statistically significant.

3. Results

3.1. The wound healing rate and the wound healing time

As shown in Table 2, the wound healing rate on the 7th, 14th, 21st, and 28th day of rhGM-CSF treatment were (29.5±19.4)%, (60.3±33.9)%, (92.2±17.6)%, and (96.8±13.9)%, respectively. The wound healing rate scores of placebo were (19.9±18.1)%, (46.7±21.9)%, (68.7±18.1)%, and (82.2±12.3)%, respectively, with significant difference in comparison to the rhGM-CSF groups ($P < .01$). The average wound healing time in the rhGM-CSF group was (18.8±7.6) days, significantly shorter than that of the placebo group, which was (25.5±4.6) days ($P < .01$).
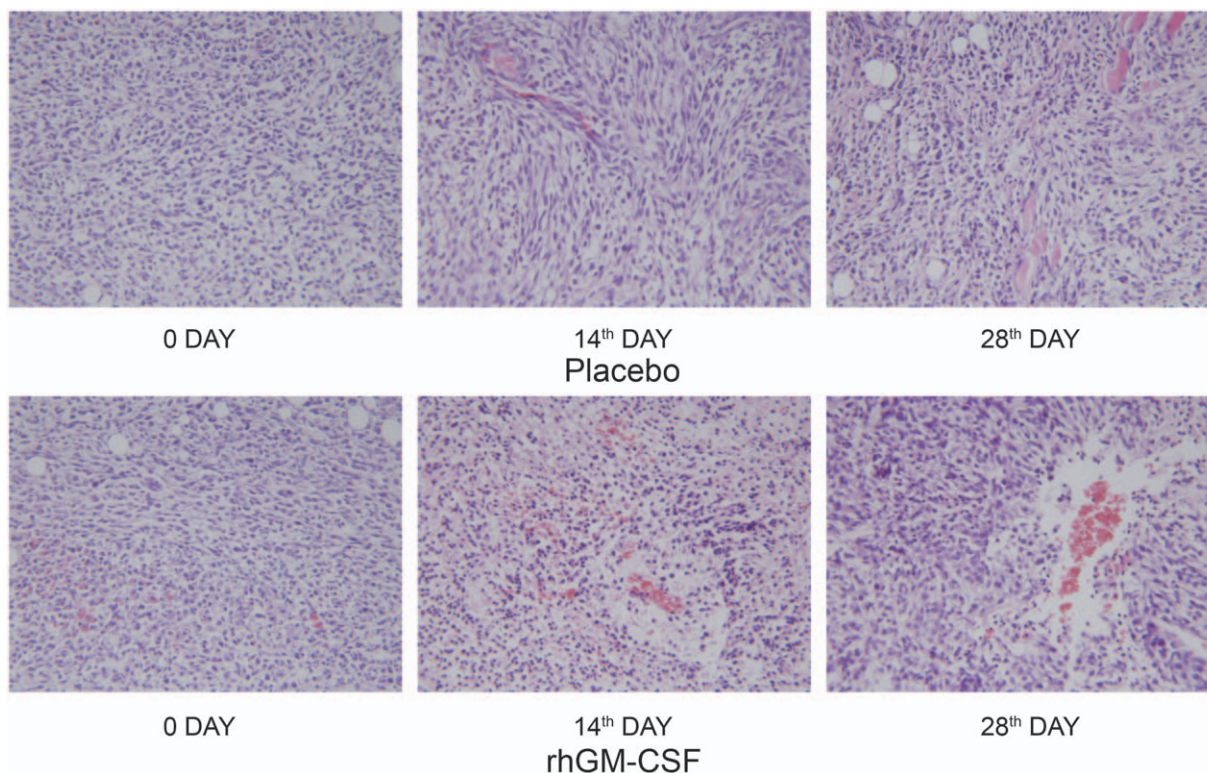

Figure 1. HE staining in wound tissues in the rhGM-CSF group and placebo group at different time points (×400). HE = hematoxylin and eosin, rhGM-CSF = recombinant human granulocyte macrophage colony stimulating factor.

Table 3

The number of blood capillaries in granulation tissue of 2 groups.

Group	Before treatment	14 d	28 d
rhGM-CSF	4.97 ± 0.58	11.29 ± 0.61	15.47 ± 1.02
Placebo	4.83 ± 0.47	7.32 ± 0.70	10.73 ± 0.88
P	.656	<.001*	<.001*

* Evaluated by the *t* test.
rhGM-CSF = recombinant human granulocyte macrophage colony stimulating factor.

Table 4

The number of fibroblasts in granulation tissue of 2 groups.

Group	Before treatment	14 d	28 d
rhGM-CSF	77.1 ± 4.5	142.9 ± 16.8	147.9 ± 10.9
Placebo	72.3 ± 4.7	111.2 ± 17.0	124.6 ± 11.7
P	.101	<.01*	<.01*

* Evaluated by the *t* test.
rhGM-CSF = recombinant human granulocyte macrophage colony stimulating factor.

3.2. Histopathological observations

On the 14th day and 28th day after medication, the numbers of blood capillaries in granulation tissue were (11.29 ± 0.61) and (15.47 ± 1.02) in the rhGM-CSF group. This was higher than in the placebo group (7.32 ± 0.70) and (10.73 ± 0.88), respectively ($P < .01$, Fig. 1, Table 3). Table 4 shows that the fibroblasts in granulation tissue of the rhGM-CSF group (142.9 ± 16.8, 147.9 ± 10.9) were more numerous than in the placebo group (111.2 ± 17.0, 124.6 ± 11.7), ($P < .01$). No remarkable difference was observed between the rhGM-CSF group and placebo group before the drug treatment commenced ($P > .05$).

According to Fig. 2, there was no significant difference in VEGF between the rhGM-CSF group and the placebo group with both showing low expression of VEGF. On the 14th day and 28th day, levels of VEGF increased in both; however, the expressions of VEGF in the rhGM-CSF group were higher than those in the placebo group. Figure 3 demonstrates that the expressions of FGF underwent the same trend as the expressions of VEGF.

3.3. Scar scaling score

To evaluate the aesthetic outcome of the 2 groups, we calculated the scar scaling score according to pigmentation, pliability, height, and vascularity. The scores of pigmentation, pliability, height, and vascularity were (0.9 ± 0.7), (2.1 ± 0.8), (1.0 ± 0.5) and (0.8 ± 0.7) in the rhGM-CSF group, and (1.9 ± 0.9), (3.2 ± 0.7), (2.1 ± 0.6) (2.0 ± 0.9) in the placebo group. All indexes showed significant differences between the rhGM-CSF group and the placebo group ($P < .01$, Table 5).

4. Discussion

Burn healing is a complex physiological process, whereby residual wounds often undergo additional adverse changes such as infection and inflammation.[11,12] Due to its outstanding performance in wound healing, rhGM-CSF has been introduced in treating large-area burn wounds at clinically,[5] and its promotion of the effective healing of infected burn wounds along with infection preventive effect via immune activity modulation have been verified.[13] RhGM-CSF was thought to promote burn wound healing and prevent bacterial infections
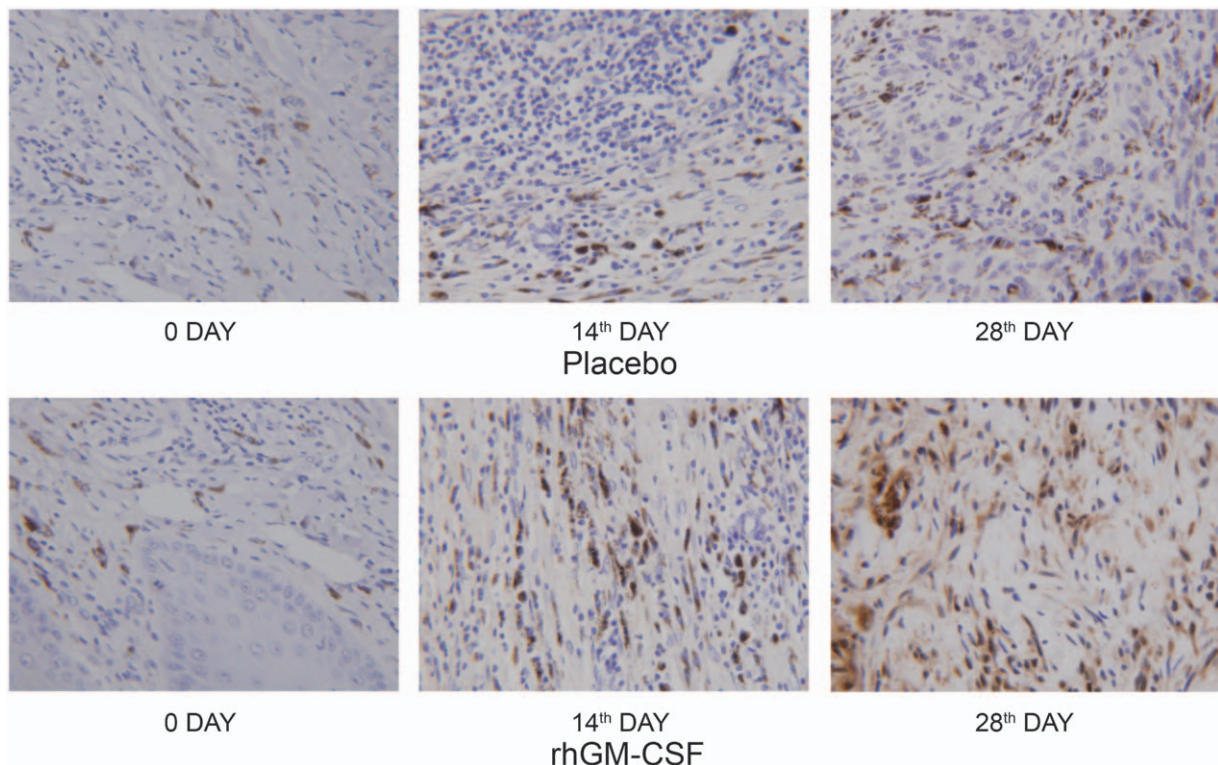

Figure 2. Expression of VEGF in wound tissues of rhGM-CSF group and placebo group at different time points (×400). VEGF = vascular endothelial factor, rhGM-CSF = recombinant human granulocyte macrophage colony stimulating factor.
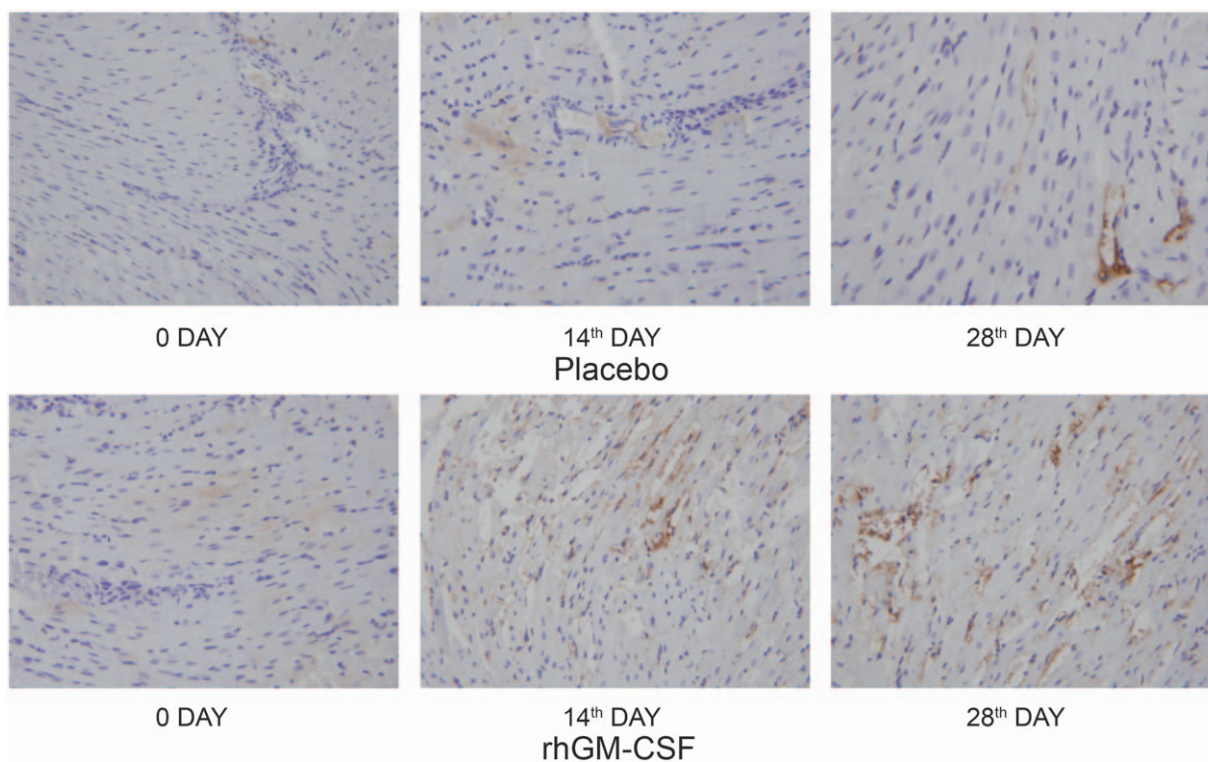
Figure 3. Expression of FGF in wound tissues of rhGM-CSF group and placebo group at different time points (×400). FGF = fibroblast growth factor, rhGM-CSF = recombinant human granulocyte macrophage colony stimulating factor.

by improving immune potency.[6] For instance, rhGM-CSF was reported to suppress sepsis (a whole-body inflammatory response to infection) in burnt mice by restoring T cell and IL-2.[14] Meanwhile, Yuan et al[5] reported that the healing time in the rhGM-CSF group (17.28±6.70 days) was shorter than in the mupirocin ointment group (23.8±4.6 days). Consistently, we found that compared with the placebo group, rhGM-CSF exerted far better efficacy both at the outcomes of wound healing rate and wound healing time. We noted that patients in the group of treated with rhGM-CSF, the wound healing time was shorter (18.8±7.6 days) when compared to the placebo group (25.5±4.6 days). Besides, RhGM-CSF repairs wounds not only by accelerating the proliferation and differentiation of hematopoietic progenitor cells into neutrophils, eosinophils, and macrophages, but also by promoting the migration and differentiation of epithelial cells and keratinocytes, which spread across the wound area. And the main function of epithelial cells and keratinocytes is to re-epithelialize and form a neuroepithelial layer.[13] Fang et al[8] discovered that exogenous GM-CSF promoted wound healing in diabetic mice and raised the level of some cytokines which promote

neovascularization and infiltration of macrophages as well as neutrophils.[15] Mann et al[7] also suggested that rhGM-CSF can promote vascular endothelial cell differentiation and accelerate the generation of new blood vessels, which was also supported by our experiment. In our research, we discovered that blood capillary and vascularity distinctively improved in the rhGM-CSF group in comparison with the placebo group. The results of our study indicated that rhGM-CSF can promote both re-epithelization and angiogenesis in the burn wound area, which then could facilitate a more rapid wound recovery.

In our experiment, we compared the scar scaling scores of patients treated with rhGM-CSF and placebo. The results demonstrated that patients treated with rhGM-CSF showed lower scores, indicating better recovery condition, whereas those treated with placebo showed inferior recovery conditions to those treated with rhGM-CSF. The scaling results suggested an outstanding performance of rhGM-CSF in reducing scar formation, which also contributes to a better treatment outcome of large-area burns.

Our research consisted of a comparison between rhGM-CSF and placebo confirmed the effectiveness of rhGM-CSF in the treatment of burn injuries. However, the participators in our study only included hydrothermal burn, flame burn, and chemical burn. Moreover, the effect of rhGM-CSF on purulent secretion of burn wounds needs further investigation.

In conclusion, our study demonstrated that rhGM-CSF generally outperformed placebo in burn wound healing. The good performance of rhGM-CSF confirms its role in burn healing and could provide a practical method in the treatment of burn wounds.

Table 5				
Scar scaling score.				
Group	Pigmentation	Pliability	Height	Vascularity
rhGM-CSF	0.9±0.7	2.1±0.8	1.0±0.5	0.8±0.7
Placebo	1.9±0.9	3.2±0.7	2.1±0.6	2.0±0.9
P	<.001*	<.001*	<.001*	<.001*

* Evaluated by the t test.
rhGM-CSF = recombinant human granulocyte macrophage colony stimulating factor.

References

[1] Liu LY, Hou YS, Chai JK, et al. Basic fibroblast growth factor/vascular endothelial growth factor in the serum from severe burn patients stimulates the proliferation of cultured human umbilical cord mesenchymal stem cells via activation of Notch signaling pathways. J Trauma Acute Care Surg 2013;75:789–97.

[2] Akita S, Akino K, Imaizumi T, et al. Basic fibroblast growth factor accelerates and improves second-degree burn wound healing. Wound Repair Regen 2008;16:635–41.

[3] Engrav LH, Heimbach DM, Reus JL, et al. Early excision and grafting vs. nonoperative treatment of burns of indeterminant depth: a randomized prospective study. J Trauma 1983;23:1001–4.

[4] Muangman P, Deubner H, Honari S, et al. Correlation of clinical outcome of integra application with microbiologic and pathological biopsies. J Trauma 2006;61:1212–7.

[5] Yuan L, Minghua C, Feifei D, et al. Study of the use of recombinant human granulocyte-macrophage colony-stimulating factor hydrogel externally to treat residual wounds of extensive deep partial-thickness burn. Burns 2015;41:1086–91.

[6] Zhang L, Chen J, Han C. A multicenter clinical trial of recombinant human GM-CSF hydrogel for the treatment of deep second-degree burns. Wound Repair Regen 2009;17:685–9.

[7] Mann A, Breuhahn K, Schirmacher P, et al. Keratinocyte-derived granulocyte-macrophage colony stimulating factor accelerates wound healing: Stimulation of keratinocyte proliferation, granulation tissue formation, and vascularization. J Invest Dermatol 2001;117:1382–90.

[8] Fang Y, Shen J, Yao M, et al. Granulocyte-macrophage colony-stimulating factor enhances wound healing in diabetes via upregulation of proinflammatory cytokines. Br J Dermatol 2010; 162:478–86.

[9] Kaplan G, Walsh G, Guido LS, et al. Novel responses of human skin to intradermal recombinant granulocyte/macrophage-colony-stimulating factor: Langerhans cell recruitment, keratinocyte growth, and enhanced wound healing. J Exp Med 1992;175:1717–28.

[10] Baryza MJ, Baryza GA. The Vancouver Scar Scale: an administration tool and its interrater reliability. J Burn Care Rehabil 1995; 16:535–8.

[11] Fujii T. Local treatment for extensive deep dermal thickness burn and follow-up study. Acta Chir Plast 1990;32:46–56.

[12] Barret JP, Herndon DN. Effects of burn wound excision on bacterial colonization and invasion. Plast Reconstr Surg 2003;111:744–50. discussion 751-742.

[13] Hu X, Sun H, Han C, et al. Topically applied rhGM-CSF for the wound healing: a systematic review. Burns 2011;37:729–41.

[14] Yu C, Wang J, Fu Y, et al. Treatment of skin injury due to vinorelbine extravasation using bFGF and rhGM-CSF: an experimental study in a murine model. Biol Res Nurs 2011;13:32–7.

[15] Baker EA, Leaper DJ. Proteinases, their inhibitors, and cytokine profiles in acute wound fluid. Wound Repair Regen 2000;8:392–8.