European Review for Medical and Pharmacological Sciences 2019; 23: 5987-5993

Observation of curative effect of recombinant human basic fibroblast growth factor combined with compound polymyxin B ointment and local application of insulin on wound healing of deep second-degree burn in diabetes mellitus: a randomized study

J.-B. ZHANG, H. LI, L. ZHANG, J.-L. WANG

Department of Burn and Plastic Surgery, Cangzhou Central Hospital, Hebei, China

Abstract. – OBJECTIVE: We aimed at investigating the clinical efficacy of recombinant human basic fibroblast growth factor combined with compound polymyxin B ointment and the local application of insulin on wound healing of deep second-degree burn in diabetes mellitus.

PATIENTS AND METHODS: 80 diabetes mellitus patients complicated with deep second-degree burn treated from 2016 to December 2017 were selected and divided into observation group (n=40) and control group (n=40) using the random number method. Patients in control group were treated with compound polymyxin B ointment, while those in observation group were treated with basic fibroblast growth factor based on the treatment in control group. The time of wound pain relief, wound scarring, and wound healing was compared.

RESULTS: The number of positive bacteria in the wound was recorded and the duration of infection was also recorded. Moreover, the changes in the expressions of Advanced Glycation End products (AGEs) and Vascular Endothelial Growth Factor (VEGF) were analyzed. In observation group, the time of wound pain relief, wound scarring, and wound healing was significantly shorter than that in control group ($p<0.05$). At 3, 14, and 28 days after the treatment, the levels of AGEs in observation group were lower than those in control group, while the levels of VEGF in the observation group were higher than those in control group ($p<0.05$). The proportions of wound infection, skin necrosis, systemic blood infection, hypoglycemia, and hyperglycemia and the ineffective rate in observation group were significantly lower than that in control group ($p<0.05$).

CONCLUSIONS: The application of compound polymyxin B ointment and the local application of insulin combined with recombinant human basic fibroblast growth factor in diabetes mellitus patients complicated with a deep second-degree burn can effectively alleviate the pain, reduce the wound infection rate, promote the wound healing, and improve the overall therapeutic effect.

Key Words:

Recombinant human basic fibroblast growth factor, Compound polymyxin B ointment, Local application of insulin, Diabetes mellitus, Deep second-degree burn, Wound healing.

Introduction

The ability to repair the burn wound is closely related to the depth of the burn. In particular, there are many damaged tissues in the deep second-degree burn and above, making the corium layer lose the physiological function, so it generally takes 4 weeks or even longer to promote the wound healing only through the proliferation, differentiation, and migration of residual epithelial tissues[1]. In clinic, the skin grafting is recommended for the burn wound that is not healed after 4 weeks[2]. In diabetes mellitus, the blood glucose increases for a long time and the body's self-repair ability and immunity significantly decline. The time of wound healing after the burn is longer and during the wound healing process, the necrotic tissues adhered do not shed easily, the granulation tissue regeneration is slow with abnormal function, the epithelial differentiation rate is inhibited, and the skin basal tissues are inactive. With the prolongation of the duration of

Corresponding Author: Junling Wang, MD; e-mail: xueh92@126.com

diabetes mellitus and the unsatisfactory control of blood glucose, the burn wound healing will be more difficult and the effects of conventional conservative treatment and hypoglycemic therapy are limited[3]. The operative treatment has a low cure rate and even leads to a healing disorder at the donor site, etc.

Recombinant human basic Fibroblast Growth Factor (rh-bFGF) is a kind of physiological multifunctional cell growth factor, which possesses broad biological activity and can significantly stimulate the cell growth and reproduction in the embryonic mesoderm and neural ectoderm, thereby promoting the capillary regeneration, stimulating the epithelial, and endothelial cell proliferation, as well as improving the production of wound granulation tissues[4]. Moreover, rh-bFGF, as mitogen and chemokine, can also increase the activity and promote the aggregation of fibroblasts. The proliferation of new capillaries and fibroblasts is the most important factor for the granulation tissue regeneration and wound healing after the burn, and rh-bFGF can effectively improve both links[5]. To better improve the therapeutic effect on diabetes mellitus patients complicated with a deep second-degree burn, the clinical therapeutic value of rh-bFGF combined with compound polymyxin B ointment and the local application of insulin was mainly explored.

Patients and Methods

General Data

A total of 80 diabetes mellitus patients complicated with a deep second-degree burn treated in the Cangzhou Central Hospital from February 2016 to December 2017 were selected. All patients were diagnosed with diabetes mellitus via the past medical history and clinical manifestations and the deep second-degree burn was confirmed via physical examination. The research was approved by the Ethics Committee of Cangzhou Central Hospital and written informed consents were signed by patients and/or guardians.

Inclusion criteria: patients aged above 18 years old, patients with type 2 diabetes mellitus, and untreated patients with burn within 4 h. Exclusion criteria: patients complicated with respiratory tract burn, severe cardiopulmonary or hepatorenal dysfunction, malignant tumors, significant wound infection before enrollment, or abnormal state of consciousness and mentality. Patients enrolled were divided into observation group (n=40) and control group (n=40) using the random number method. The general data of patients in both groups are shown in Table I.

Methods

All patients enrolled underwent the conventional treatment, such as the diabetic diet, local, and systemic application of insulin to control the blood glucose, scavenging of necrotic tissues after the burn and sufficient drainage. The growth of granulation tissues was promoted combined with exposure therapy, bacteria were cultured regularly for the wound secretion or necrotic tissues, the application of antibacterial drugs was guided, the wound was observed, and the dressing was changed in time. Patients in control group were treated with the local application of compound polymyxin B ointment (Zhejiang Reachall Pharmaceutical Co., Ltd., NMPN H20061269, Zhejiang Sheng, China) for 3 times a day (7 days as one course of treatment). The ointment was applied when there were open skin defects and withdrawn after scarring. Patients in observation group, based on the treatment in control group, were treated with local external spraying of bFGF (Nanhai Longtime Pharmaceutical Co., Ltd., NMPN S20040053) once a day (20000 IU per time).

Observation Indexes

The conditions of all patients during hospitalization were observed and they were followed

Table I. Comparisons of general data between the two groups.

	Male/ female	Age (years old)	Body mass index	Burn area (%)	Duration of diabetes mellitus (years)	Glycated hemoglobin (%)
Observation group	20/20	55.6 ± 1.9	26.5 ± 2.9	21.9 ± 1.1	15.6 ± 3.5	8.1 ± 0.3
Control group	21/19	55.7 ± 2.0	26.6 ± 2.9	21.8 ± 1.1	15.7 ± 3.5	8.2 ± 0.3
t or χ^2	0.000	0.229	0.154	0.407	0.128	1.491
p	1.000	0.819	0.878	0.685	0.897	0.140

Table II. Comparisons of wound pain relief, wound scarring and wound healing between the two groups (d, $\bar{x} \pm s$).

	Time of wound pain relief	Time of wound scarring	Time of wound healing
Observation group	3.1 ±0.2	14.9 ± 2.1	24.1 ± 2.5
Control group	5.6 ± 0.5	18.9 ± 2.5	31.9 ± 2.9
t	29.361	7.748	12.884
p	0.000	0.000	0.000

up for 3 months via telephone or clinic after discharge. The time of wound pain relief, wound scarring, and wound healing was compared between the two groups. The number of positive bacteria in the wound at different time points after treatment was recorded in both groups and the duration of infection and the total times of dressing change during the treatment were also recorded in both groups. Moreover, the changes in the expressions of advanced glycation end products (AGEs) and vascular endothelial growth factor (VEGF) in both groups at different time points (at enrollment, and at 3, 14, and 28 days after treatment) were analyzed, and the complications during treatment and overall clinical therapeutic effects in both groups were detected.

Evaluation Criteria

AGEs (normal reference value: 28.8-38.7 μg/mL in adults) and VEGF (normal reference value: 43.3-58.8 pg/L in adults) were detected via enzyme-linked immunosorbent assay (ELISA). The overall clinical therapeutic effect was evaluated using the Wagner grading: ineffective (the reduction area of wound after treatment <50% or there is no improvement or even aggravation), effective (the reduction area of wound after treatment >50% but <75%, and disappearance or significant alleviation of clinical symptoms, such as pain, exudation, and infection), and cure (wound healing after treatment, and complete disappearance of clinical symptoms). Ineffective rate (%) = number of ineffective cases/total number of cases × 100%. The wound pain was evaluated using the visual analogue scale (VAS) score (0-10 points) and the higher score corresponds to the higher pain degree.

Statistical Analysis

SPSS20.0 (IBM, Armonk, NY, USA) was used for statistical processing. Measurement data were expressed as mean± standard deviation ($\bar{x} \pm s$). The *t*-test was used for the comparison of mean between the two groups and the chi-square test was adopted for the comparison of rate between the two groups. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of Wound Pain Relief, Wound Scarring, and Wound Healing Between the Two Groups

In observation group, the time of wound pain relief, wound scarring, and wound healing was significantly shorter than that in control group ($p<0.05$) (Table II).

Comparison of the Number of Positive Bacteria in the Wound at Different Time Points After Treatment Between the Two Groups

No wound infection occurred in both groups at enrollment. In observation group, the number of positive bacteria in the wound at 3, 14, and 28 days after treatment was significantly larger than that in control group ($p<0.05$) (Table III).

Table III. Comparison of the number of positive bacteria in the wound at different time points after treatment between the two groups (n).

	At enrollment	3 d after treatment	14 d after treatment	28 d after treatment
Control group	0	9	24	31
t	–	5.600	8.584	14.679
p	–	0.018	0.003	0.000

Comparisons of the Duration of Infection and Total Times of Dressing Change During Treatment Between the Two Groups

The duration of infection in observation group [(19.3±1.5) d] was shorter than that in control group [(27.1±3.3) d] (t=13.609, p=0.000) and the total times of dressing change during treatment in observation group [(8.9±1.1) times] were fewer than those in control group [(15.3±2.5) times] (t=14.820, p=0.000) (Figure 1).

Analysis of the Expressions of AGEs and VEGF at Different Time Points in Both Groups

At enrollment and at 3, 14, and 28 days after treatment, the expression of AGEs was (56.3±3.8) μg/mL, (39.1±2.7) μg/mL, (30.4±1.3) μg/mL, and (29.1±1.0) μg/mL, respectively, in observation group. (56.4±3.8) μg/mL, (45.3±2.9) μg/mL, (42.1±1.5) μg/mL, and (40.2±1.6) μg/mL, respectively, in control group. During the same period, the expression of VEGF was (14.4±1.0) pg/L, (54.9±3.8) pg/L, (98.2±5.6) pg/L, and (78.4±4.1) pg/L, respectively, in observation group. (14.5±1.0) pg/L, (31.2±3.1) pg/L, (45.3±4.5) pg/L and (51.4±4.9) pg/L, respectively, in control group. It can be observed that at 3, 14, and 28 days after treatment, the levels of AGEs in observation group were significantly lower than those in control group (t=9.896, 37.279, and 37.207, p=0.000), while the levels of VEGF in observation group were higher than those in control group (t=30.565, 46.571, and 26.727, p=0.000) (Figures 2 and 3).

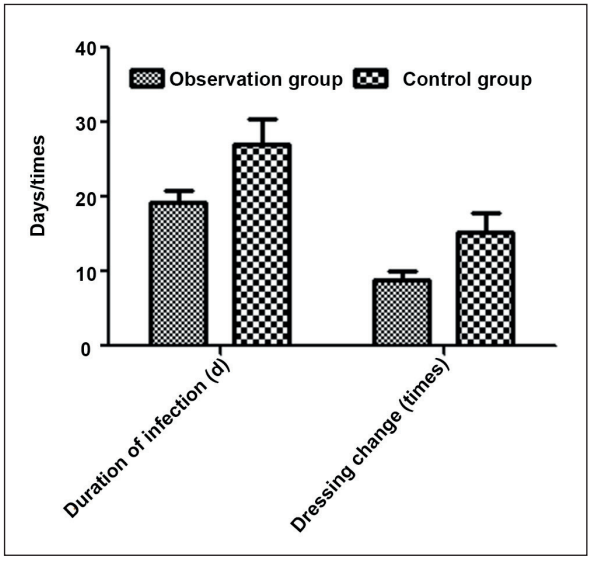

Figure 1. Comparisons of the duration of infection and total times of dressing change during treatment between the two groups. In the observation group, the duration of infection is shorter than that in control group (p<0.05), and the total times of dressing change during treatment are fewer than those in control group (p<0.05).

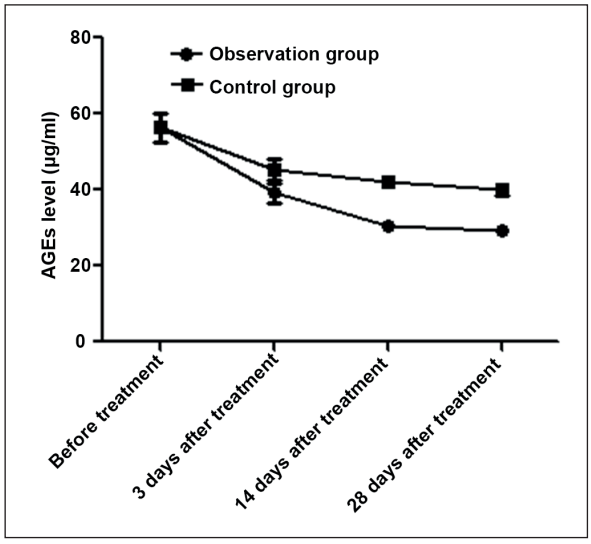

Figure 2. Changes in the levels of AGEs at different time points in both groups. At 3, 14, and 28 days after treatment, the levels of AGEs in observation group are significantly lower than those in control group (p<0.05).

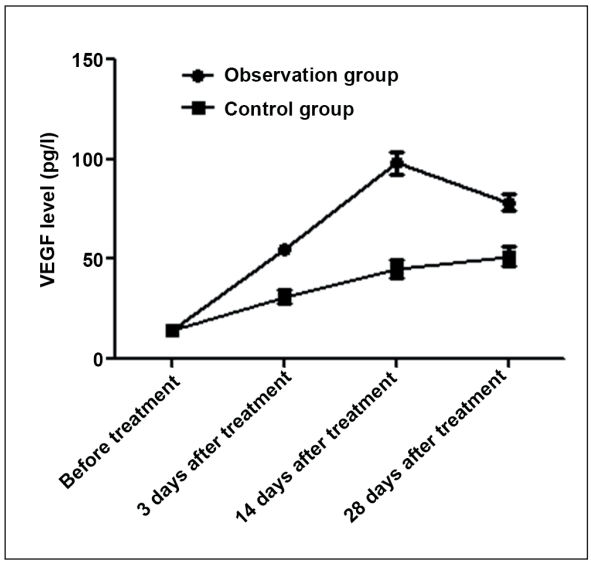

Figure 3. Changes in the levels of VEGF at different time points in both groups. At 3, 14, and 28 days after treatment, the levels of VEGF in observation group are significantly higher than those in control group (p<0.05).

Table IV. Comparison of complications during treatment between the two groups.

	Wound infection	Skin necrosis	Systemic blood infection	Hypoglycemia	Hyperglycemia
Observation group	14	3	2	1	1
Control group	31	14	10	9	9
χ^2	14.679	7.470	4.804	5.600	5.600
p	0.000	0.006	0.028	0.018	0.018

Comparison of Complications During Treatment Between the Two Groups

During the treatment, the proportions of wound infection, skin necrosis, systemic blood infection, hypoglycemia, and hyperglycemia in observation group were lower than those in control group (p<0.05) (Table IV).

Comparison of the Overall Clinical Effect Between the Two Groups

The ineffective rate in observation group (2.5%) was markedly lower than that in control group (22.5%) (χ^2=5.600, p=0.018) (Table V).

Discussion

Diabetes mellitus leads to lesions in the peripheral vessels and nerves in the body, significantly delaying the body's sense of high-low temperature and tenderness. When the deep burn is combined, the body's immunity will decline due to the peripheral circulation hypofunction[6]. Therefore, the self-repair ability of the wound becomes significantly worse. Moreover, the diabetic diet often results in malnutrition. So, the granulation tissue growth and epithelial expansion of the wound are slow, causing wound disunion[7]. The patients with an extensive burn, especially those complicated with deep second-degree burn and above, suffer from the significant stress hyperglycemia[8]. Besides, several degenerated necrotic tissues and protein exudate in the burn wound, causing the damage to the complete physiological barrier impairment of skin, leading to the local circulatory dysfunction and providing the pathway and medium for the invasion of pathogenic microbes[9]. In this report, diabetes mellitus patients complicated with deep second-degree burn in observation group were treated with compound polymyxin B ointment and the local application of insulin and rh-bFGF was used for intervention. The wound pain relief, wound scarring, and wound healing were compared between the two groups and it was found that the time of wound pain relief, wound scarring, and wound healing in observation group was significantly shorter than that in control group, indicating that the combined application of rh-bFGF can significantly alleviate the wound pain and promote the wound healing of diabetes mellitus patients complicated with deep second-degree burn. The number of positive bacteria in the wound at different time points after treatment was also compared between the two groups and it was found that the number of positive bacteria in the wound in observation group at 3, 14, and 28 days after treatment was significantly larger than that in control group, suggesting that the combined application of rh-bFGF can effectively reduce the risk of wound infection and promote the tissue healing of diabetes mellitus patients complicated with a deep second-degree burn. At the same time, the duration of infection and total times of dressing change during treatment were compared between the two groups. The results revealed that the duration of infection in observation group was shorter than that in control group and the total times of dressing change during treatment in observation group were fewer than those in control

Table V. Comparison of the overall clinical effect between the two groups.

	Cure	Effective	Ineffective
Observation group	35	4	1
Control group	23	8	9

group. This further indicated that the combined application of rh-bFGF can also reduce the wound exudate and shorten the time of wound infection control. Moreover, the expressions of AGEs and VEGF at different time points in both groups were analyzed and the results showed that at 3, 14, and 28 days after treatment, the levels of AGEs in observation group were significantly lower than those in control group, while the levels of VEGF in observation group were higher than those in control group. This suggested that the combined application of rh-bFGF can effectively reduce the level of damage-related factor AGEs, improve the levels of tissue healing, and angiogenesis-related VEGF, as well as benefit the wound repair more in diabetes mellitus patients complicated with deep second-degree burn. Finally, the complications and overall clinical effect were detected in both groups. It was found that the proportions of wound infection, skin necrosis, systemic blood infection, hypoglycemia, and hyperglycemia in observation group during treatment were lower than those in control group, and the ineffective rate in observation group was lower than that in control group, while the effective rate in observation group was higher than that in control group. These findings suggested that compound polymyxin B ointment and the local application of insulin combined with rh-bFGF can significantly reduce the complications during the treatment and improve the overall clinical therapeutic effect on diabetes mellitus patients complicated with a deep second-degree burn. The mechanism of rh-bFGF applied in diabetes mellitus patients complicated with a deep second-degree burn is mainly divided into the following three aspects: first, it is an exogenous chemical chemotactic agent, which can promote the chemotaxis of inflammatory cells in the burn wound and improve the local tissue differentiation ability[10]. Second, it directly acts on the burn wound to enhance the activity of tissue repair-related factor receptors and improve the mitosis of tissue cells, thereby promoting tissue regeneration and accelerating wound healing[11]. Third, it can also accelerate the cell repair-related signal transmission and better improve the clinical therapeutic effect combined with effective regulation of blood glucose[12]. The application of rh-bFGF in diabetes mellitus patients complicated with a deep second-degree burn can better promote the healing below the wound scab[13], scab shedding, and granulation tissue regeneration[14], thus repairing the wound rapidly, filling the dermal defects and restoring the skin integrity[15]. The early healing of burn wound can effectively reduce the risk of infection[16], shorten the duration of hospitalization, and improve the overall clinical therapeutic effect[17]. Moreover, rh-bFGF is the colorless clear liquid[18] with irritating odor, the wound is clean and tidy after application[19], and it is simple in operation and highly accepted among patients[20].

Conclusions

The application of compound polymyxin B ointment and the local application of insulin combined with rh-bFGF in diabetes mellitus patients complicated with a deep second-degree burn can effectively alleviate the pain, reduce the wound infection rate, promote the wound healing, and improve the overall therapeutic effect.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

The study was funded by Cangzhou City key R & D Program guidance Project (NO. 172302011).

Authors' Contribution

JZ wrote the manuscript. JZ and HL were responsible for observation indexes analysis. LZ and JW helped with statistical analysis. All authors read and approved the final manuscript.

References

1) Oryan A, Alemzadeh E, Eskandari MH. Kefir Accelerates burn wound healing through inducing fibroblast cell migration in vitro and modulating the expression of IL-1ß, TGF-ß1, and bFGF genes in vivo. Probiotics Antimicrob Proteins. 2018 Jun 8. doi: 10.1007/s12602-018-9435-6. Epub ahead of print]

2) Yuan B, Broadbent JA, Huan J, Yang H. The effects of adipose stem cell-conditioned media on fibrogenesis of dermal fibroblasts stimulated by transforming growth factor-β1. J Burn Care Res 2018; 39: 129-140.

3) Yang L, Zhang D, Wu H, Xie S, Zhang M, Zhang B, Tang S. Basic fibroblast growth factor influences epidermal homeostasis of living skin equivalents through affecting fibroblast phenotypes and functions. Skin Pharmacol Physiol 2018; 31: 229-237.

4) Alemzadeh E, Oryan A. Effectiveness of a crocus sativus extract on burn wounds in rats. Planta Med 2018; 84: 1191-1200.

5) Pallua N, Grasys J, Kim BS. Enhancement of progenitor cells by two-step centrifugation of emulsified lipoaspirates. Plast Reconstr Surg 2018; 142: 99-109.

6) Liu G, Lu P, Chen L, Zhang W, Wang M, Li D, Zhang X. B-cell leukemia/lymphoma 10 promotes angiogenesis in an experimental corneal neovascularization model. Eye (Lond) 2018; 32: 1220-1231.

7) Chakrabarti S, Islam J, Hazarika H, Mazumder B, Raju PS, Chattopadhyay P. Safety profile of silver sulfadiazine-bFGF-loaded hydrogel for partial thickness burn wounds. Cutan Ocul Toxicol 2018; 37: 258-266.

8) Zhao L, Chen D, Yao Q, Li M. Studies on the use of recombinant spider silk protein/polyvinyl alcohol electrospinning membrane as wound dressing. Int J Nanomedicine 2017; 12: 8103-8114.

9) Gholipourmalekabadi M, Samadikuchaksaraei A, Seifalian AM, Urbanska AM, Ghanbarian H, Hardy JG, Omrani MD, Mozafari M, Reis RL, Kundu SC. Silk fibroin/amniotic membrane 3D bi-layered artificial skin. Biomed Mater 2018; 13: 035003.

10) Du HC, Jiang L, Geng WX, Li J, Zhang R, Dang JG, Shu MG, Li LW. Evaluation of xenogeneic extracellular matrix fabricated from CuCl2-conditioned mesenchymal stem cell sheets as a bioactive wound dressing material. J Biomater Appl 2017; 32: 472-483.

11) Monsuur HN, van den Broek LJ, Jhingoerie RL, Vloemans A, Gibbs S. Burn eschar stimulates fibroblast and adipose mesenchymal stromal cell proliferation and migration but inhibits endothelial cell sprouting. Int J Mol Sci 2017; 18(8). pii: E1790.

12) Zhang L, Qian Z, Tahtinen M, Qi S, Zhao F. Prevascularization of natural nanofibrous extracellular matrix for engineering completely biological three-dimensional prevascularized tissues for diverse applications. J Tissue Eng Regen Med 2018; 12: e1325-e1336.

13) Simonetti O, Lucarini G, Orlando F, Pierpaoli E, Ghiselli R, Provinciali M, Castelli P, Guerrieri M, Di Primio R, Offidani A, Giacometti A, Cirioni O. Role of daptomycin on burn wound healing in an animal methicillin-resistant staphylococcus aureus infection model. Antimicrob Agents Chemother 2017; 61(9). pii: e00606-17.

14) Butt H, Mehmood A, Ali M, Tasneem S, Anjum MS, Tarar MN, Khan SN, Riazuddin S. Protective role of vitamin E preconditioning of human dermal fibroblasts against thermal stress in vitro. Life Sci 2017; 184: 1-9.

15) Xu HL, Chen PP, ZhuGe DL, Zhu QY, Jin BH, Shen BX, Xiao J, Zhao YZ. Liposomes with silk fibroin hydrogel core to stabilize bFGF and promote the wound healing of mice with deep second-degree scald. Adv Healthc Mater 2017; 6 (19). doi: 10.1002/adhm.201700344.

16) Herold C, Engeli S, Beckmann B, Vogt PM, Rennekampff HO. Adipokine concentrations in lipoaspirates may have a role in wound healing. Indian J Plast Surg 2017; 50: 56-63.

17) Peng Y, Li P, Zhao ZA, Chen L, Zhao XG, Chen X, Zhao Y, Xiong RP, Ning YL, Yang N, Ye J, Zhou YG. Comparative evaluation of the wound-healing potency of recombinant bFGF and ski gene therapy in rats. Growth Factors 2016; 34: 119-127.

18) Hayashida K, Fujioka M, Morooka S, Saijo H, Akita S. Effectiveness of basic fibroblast growth factor for pediatric hand burns. J Tissue Viability 2016; 25: 220-224.

19) Mitsukawa N, Higaki K, Ito N, Muramatsu H, Karube D, Akita S, Kubota Y, Satoh K. Combination treatment of artificial dermis and basic fibroblast growth factor for skin defects: a histopathological examination. Wounds 2016; 28: 158-166.

20) Shi H, Xie H, Zhao Y, Lin C, Cui F, Pan Y, Wang X, Zhu J, Cai P, Zhang H, Fu X, Xiao J, Jiang L. Myoprotective effects of bFGF on skeletal muscle injury in pressure-related deep tissue injury in rats. Burns Trauma 2016; 4: 26.