Original Article
Negative pressure wound therapy improves the quantity and quality of wound angiogenesis in diabetic rats by regulating the Ang/Tie-2 system

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Received November 10, 2015; Accepted January 23, 2016; Epub March 15, 2016; Published March 30, 2016

Abstract: Negative pressure wound therapy can accelerate wound healing by promoting angiogenesis. However, what and how the signal pathway regulated angiogenesis and vessels maturation, and how the microvessels changed at different stage after negative pressure wound therapy treatment during the wound healing remain unclear. This study aimed to investigate the underlying molecular mechanisms and signal pathways of negative pressure wound therapy on angiogenesis and vessel maturation. Deep partial-thickness wounds were created in diabetic rats, and relevant growth factors were determined. Negative pressure wound therapy increased the angiogenin-2 and α-smooth muscle protein expression levels, and decreased angiogenin-1/angiogenin-2 expression ratios and phosphorylation levels of tyrosine kinase receptor-2 at early stage (1st to 3rd day) of wound healing. At later stage (7th to 10th day) of wound healing, negative pressure wound therapy increased angiogenin-1, α-smooth muscle protein, angiogenin-1/angiogenin-2 ratios expression and phosphorylation levels of tyrosine kinase receptor-2, simultaneously increased microvessel pericyte coverage index. Negative pressure wound therapy may not only predominantly promote microvessels destabilization and thus increase the quantity of initial angiogenesis in the early stage but also preferentially promote microvessels stabilization and thus enhance the quality of formed vessels, contributing to vessels maturation. And a tyrosine kinase receptor-2 inhibitor could moderately influence angiogenesis process during the early stage but affect vascular remodeling and maturation process significantly during the later stage of wound healing. Furthermore, it demonstrated that angiogenin/tyrosine kinase receptor-2 signal pathway play a primary role in regulating the process of the vessel maturation.

Keywords: Negative pressure wound therapy, pericyte, vessel maturation, vessel stabilization

Introduction

Delayed wound healing is a severe post-operative complication in patients with diabetes mellitus. The pathology of diabetic wounds includes infections, autocrine deficiency [1, 2], vessel structure disorders, pericyte dropout [3, 4], microvascular basement membrane reduction and vascular occlusion [5, 6]. In addition, tissue necrosis can occur [7].

Wound healing is a complex and sequential process that involves the pro-angiogenesis factors and pro-maturation factors, and is pivotal for microvessel formation and maturation [8]. Angiogenin-1 (Ang-1) acts as a pro-maturation factor that promotes vessel maturation and associate with pericyte at later stage of wound healing [9]. Pericyte play a pivotal role in maintaining the functional and structural integrity of capillaries as well as in vascular remodeling and vascular maturation, and it are closely associated with the vascular endothelium and serve as a crucial marker of vessel maturation [4, 10-12]. Angiogenin-2 (Ang-2) serves as a pro-angiogenesis factor that mediates angiogenesis at early stage of wound healing [13]. Recent evidence and in vivo research have demonstrated that the tyrosine kinase receptor-2 (Tie-2) is primarily expressed in vascular endothelium and that it may affect vessel branching, vascular sprouting, remodeling, maturation, and stabilization [12].

Negative pressure wound therapy (NPWT) has been demonstrated to accelerate granulation...
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Tissue formation, thus shorten wound healing [14]. Current research has indicated that NPWT accelerates wound healing primarily through four mechanisms [15, 16]: macrodeformation, microdeformation, removal of exudate, and keep a warm and moist microenvironment. Simultaneously, NPWT induces the over expression of many growth factors that are closely connected with angiogenesis and granulation tissue formation [17]. Because granulation tissue formation is a vital process in the tissue proliferation stage of wound healing [18], NPWT increase the number of new blood vessel has been acquired demonstrated [17, 19]. Recent research suggested that NPWT could promote microvessel maturation after NPWT treatment [19], however, what and how the signal pathway regulated angiogenesis and vessels maturation, and how the microvesels changed at different stage during the wound healing remain unclear.

To further study the change of angiogenesis and vessel maturation after NPWT treatment, we investigated the effect of NPWT on the overall process in the deep partial-thickness wounds in diabetic rats, including angiogenesis and endothelial tube formation during the early stage of wound healing, and the vessels maturation and stabilization during the later stage of wound healing. We also explored whether the Ang/Tie-2 signaling pathway could affect angiogenesis and the vessel maturation process, and investigated vascular change at different stage of wound healing after NPWT treatment.

Materials and methods

Animals

Ninety-six adult sex-matched Sprague-Dawley rats were shaved dorsally in preparation for the experiment. The rats were administered general anesthesia through intraperitoneal injection of 350 mg/kg chloral hydrate (7%). The rat dorsa were sterilized with povidone-iodine and 75% alcohol, and a 3.5 cm×3.5 cm area of skin including the panniculus carnosus was removed, producing a full-thickness diabetic wound to the deep fascia of the dorsum. All animals were randomly divided into the following four groups: NPWT (group A), NPWT+Tie-2 inhibitor (group B), control (group C), and control+Tie-2 inhibitor (group D). Groups A and B included 48 diabetes mellitus rats whose wounds were covered with a 3.5 cm×3.5 cm area of Duoderm foam (VSD Medical Technology Co, Ltd, Wuhan, Hubei, China), followed by therapy using a vacuum-assisted closure (VAC) device with the constant negative pressure value set continuously at 125 mmHg. The VAC device did not affect ambulation or the diets or lifestyles of the treated animals. Groups C and D included 48 diabetes mellitus rats that were treated...
with petrolatum gauze. In addition, 48 diabetes mellitus rats in groups B and D were injected intraperitoneally with 50 mg/kg Tie-2 kinase inhibitor (Selleck Chemicals, Shanghai, China) twice a week. The inhibitor was dissolved in 1 ml of vehicle (5% ethanol, 5% Cremophor, and 90% distilled water) according to the supplier’s instructions.

The rats were euthanized by cervical dislocation on the 1st, 3rd, 7th, and 10th days after operation (24 rats [6 from each group] were killed on each selected day). Samples were aseptically harvested from the peripheral skin, central part of the wound and underlying muscle tissue.

**Immunohistochemistry**

Samples were fixed in 4% neutral formalin and embedded in paraffin wax. Then, 5 µm thick serial sections were cut. Slice were dewaxed, rehydrated and stained with standard hematoxylin and eosin (H&E) stain for observation of histological changes.

Primary antibodies directed against rat Ang-1 (Santa Cruz Biotechnology, 1:200), Ang-2 (Santa Cruz Biotechnology, 1:150), Tie-2 (Santa Cruz Biotechnology, 1:200), and α-SMA (Abcam, 1:200) were used. The sections were dewaxed and hydrated for immunohistochemical staining. First, 3% hydrogen peroxide was used to quench the activity of endogenous peroxidases. Citrate buffer was used for antigen retrieval, and the sections underwent microwave treatment at 500 W for 5 minutes. The sections were then incubated with primary antibodies at 4°C overnight followed by three washes in PBS. Next, the sections were incubated with goat anti-rabbit antibody (Invitrogen, 1:500) for approximately 30 minutes, followed by incubation with an avidin-biotin complex (Elite ABC kit; Vector Laboratories) for approximately 30 minutes. Finally, the reaction was visualized using 3’3′-diaminobenzidine (Dako), with hematoxylin stain for the nuclei (Sigma). The negative controls for the staining experiments were prepared by incubating each section with 1:1000 normal goat serum instead of primary antibody. All images were captured using a light microscope (Olympus BX51 Wi).

**Immunofluorescence analysis**

A double-labeling immunofluorescence technique was applied to analyze endothelial cell proliferation using anti-CD31 (Abcam, 1:200) and anti-Ki67 (Abcam, 1:600) antibodies. Simultaneously, pericytes covering microvessels tube were detected using anti-CD31 and anti-α-smooth muscle actin (α-SMA) (Abcam, 1:400) antibodies. The sections were blocked with 5% BSA for 2 hours, followed by incubation with primary antibodies at 4°C overnight. The sections were washed three times in PBS and then incubated with secondary goat anti-rabbit antibodies (Invitrogen, 1:400) for 1 hour in the dark. Afterward, the nuclei were stained using 4’6-diamidino-2 phenylindole (DAPI). All images were captured using a fluorescence microscope (Nikon, Tokyo, Japan) and were merged using Image-Pro Plus v.6.0 software.

**Quantification of the proliferating capillary index (PCI)**

The PCI was used to calculate endothelial cell proliferation. The ratio of the proliferating cells to microvessels was determined by calculating the number of proliferating endothelial cells (Ki67) divided by the total number of microvessels (CD31) (Ki67/CD31) [20]. The counting and analysis of the sections were performed independently by three investigators. The PCI was quantified in vascular hot spots, which were identified by screening for the areas with the highest vessel density under power field of 10×20.

**Quantification of microvessel density (MVD)**

MVD was quantified as the average number of microvessels per viewing field, and the counting was performed as described previously [20-23]. CD31 was used as a marker of endothelial cells. Simultaneous CD31-positive endothelial cells and vessels with or without lumen in 5 randomly selected fields from 3 separate sections of each experimental sample were screened and counted under a power field of 10×20.

**Quantification of the microvessel pericyte coverage index (MPI)**

The MPI, which was used to evaluate the maturity of new blood vessels, was expressed as the number of microvessels stained with α-SMA divided by the number of microvessels stained with CD31 (α-SMA/CD31), as described previously [21, 23-26]. The MPI was established by quantifying the percentage of CD31-positive microvessels that showed colocalization of
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Figure 1. Histological changes of wounds in the different groups at day 10. In the group A the close-knit and regular collagen fibrils distribute on the wound. And inflammatory cells infiltration and lesser vessel in groups B, C, D. Scale bar = 50 um. Group A, NPWT; group B, NPWT+Tie-2 inhibitor; group C, control; group D, control+Tie-2 inhibitor.

endothelial cell staining (CD31) and pericyte staining (α-SMA) under 200× magnification. A single endothelial cell was regarded as a unit of quantification, regardless of whether it formed a tube. A pericyte was defined as a single layer of α-SMA-positive cells colocalized with CD31-positive cells. For MPI quantification, at least 5 standalone microscopic fields were chosen per section, and the sections were independently analyzed by three different investigators.

Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted using an RNeasy Mini Kit (Qiagen) and was reverse-transcribed into cDNA using a thermocycler (S1000, Bio-Rad) and a First Strand cDNA Synthesis Kit (Fermentas) according to the supplier’s instructions. The primer sequences were as follows: Ang-1 forward primer, 5′-TAA CCT CGC CCT GCA AAG AG-3′, reverse primer, 5′-CTG TAT GCT TGC AGG TGG TAT-3′; Ang-2 forward primer, 5′-AAT AAG CCA GTC TCC CTT CCAG-3′, reverse primer, 5′-CAG GCA AGC CAT TCT CAC AG-3′; α-SMA forward primer, 5′-CAA CCC CTA TAC AAC CAT CAC AC-3′, reverse primer, 5′-CCC AAA CTC CTG CCG TAA CC-3′; and GAPDH forward primer, 5′-CGC TAA CAT CAA ATG GGG TG-3′, reverse primer, 5′-TTG CTG ACA ATC TTG AGG GAG-3′.

Real-time RT-PCR was performed using a SYBR qPCR mix (Toyobo) and Real-Time PCR Detection System (Bio-Rad iQ5). The thermocycling profile for SYBR Green RT-PCR was as follows: initial denaturation at 95°C for 1 minute, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 15 seconds, and elongation at 72°C for 60 seconds. Each sample was run in triplicate. The relative mRNA expression was analyzed using the 2^ΔΔCt method.

Western blot analysis

The samples were homogenized, and then total proteins were extracted using radioimmunoprecipitation assay (RIPA) buffer (Beyotime Biotechnology). A BCA kit (Beyotime Biotechnology) was used to determine the protein concentration. The proteins were subjected to 10% SDS-PAGE and then transferred to nitrocellulose membranes. The membranes were blocked in nonfat dry milk (5%) at room temperature for 2 hours and then incubated overnight at 4°C with primary antibodies against Ang-1 (1:1000), Ang-2 (1:1000), pTie-2 (Santa Cruz Biotechnology, 1:500), α-SMA (1:2000), and GAPDH (1:5000; Bioworld Technology). Finally, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1 hour and then detected using an enhanced chemiluminescence substrate (Beyotime Biotechnology).

Statistical analysis

Data were presented as mean ± standard deviation. Statistical significance was assessed by one-way analysis of variance (ANOVA). All statistical analyses were performed using SPSS 18.0 software (SPSS, Chicago, IL, USA). Differences between groups were considered statistically significant at P<0.05 or P<0.01.

Results

Histopathological assessments

The granulation tissue and new microvessels were more abundant in the group A on the 10th day, simultaneous accompany with close-knit and regular collagen fibrils distribute on the wound. However, in other groups the wound granulation tissue oedema, and the new blood
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Figure 2. Immunofluorescence assay of proliferating endothelial cells in the wounds of diabetes mellitus rats. The graph shows double staining of Ki67 and CD31 (red, CD31; green, Ki67; nucleus, blue) in wound tissue sections in different groups at day 3 post-operation. Original magnification, ×200. Group A, NPWT; group B, NPWT+Tie-2 inhibitor; group C, control; group D, control+Tie-2 inhibitor.

Figure 3. Proliferating capillary index was quantified and compared in different groups during wound healing. In group A, the PCI value was significantly increased from the 1st to the 3rd day (\( {P<0.05} \)), and sharply decreased from the 7th to the 10th day and was significantly lower than other groups (\( {P<0.05} \)). In group C, the PCI value gradually decreased on the 10th day, but it was higher than groups B and D during this time point. All statistical analysis results are expressed as the mean ± SD (\( {P<0.05} \), **\( {P<0.01} \)).

Group A, NPWT; group B, NPWT+Tie-2 inhibitor; group C, control; group D, control+Tie-2 inhibitor.

The PCI was quantified by calculating the percentage of microvessels proliferating. CD31 was used as a marker of endothelial cells, and Ki67 was used as a marker of proliferating cells (Figure 2). In group A, the PCI value significantly higher compared with the other groups at day 1 and day 3 (\( {P<0.05} \), **\( {P<0.01} \)), however, it was sharply decreased and was significantly lower than other groups from the 7th to the 10th (\( {P<0.05} \), **\( {P<0.01} \)) (Figure 3). In group C, the PCI value gradually decreased until the 10th day, and same as groups B and D.

Endothelial cell proliferation in diabetic wounds

vessels were reduced, simultaneous collagen fibrils clutter and broken (Figure 1).
Figure 4. Immunofluorescence assay of microvessel density (MVD) and microvessel pericyte coverage index (MPI) in the wound at day 7 and day 10. The double stained to identify endothelial cells by CD31 expression (red) and pericytes by α-SMA expression (green); a typical graph of expression on days 7 and 10 post-operation is shown. The microvascular endothelial cells tube were covered markedly with pericytes on the 10th day in the group A. Original magnification, ×200. Group A, NPWT; group B, NPWT+Tie-2 inhibitor; group C, control; group D, control+Tie-2 inhibitor.
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Microvessel density and microvascular pericyte coverage

In this study, the double immunofluorescence markers CD31 and α-SMA were combined to detect vascular endothelial cells and microvessel pericytes (Figure 4). The results showed that a few CD31-positive cells were detected in groups A and C on the 1st day. A small number of CD31-positive endothelial cells were identified in the group A on the 3rd day. On the 7th day, a large number of endothelial cells were detected in group A, and the number of endothelial cells continued to increase as well as peaking on the 10th day. Moreover, a number of endothelial cells were detected in the group C on the 3rd and 7th days; however, fewer endothelial cells were detected in this group compared with group A. In groups B and D, the numbers of endothelial cells were lesser compared with group A. The MVD was used to assess the number of microvessels; in the group A was found to have a significantly higher MVD than other groups from the 1st to the 10th day (*P<0.05, **P<0.01) (Figure 5A). Pericytes were detected using a green marker for α-SMA-positive pericytes, and a small number of pericytes appeared on the 3rd day in groups A and C. However, a few pericytes were detected in groups B and D. Many pericytes were detected in group A, and these pericytes discontinuously covered endothelial cells on the 7th day. Subsequently, the microvessel endothelial cells were encircled and covered with pericytes on the 10th day. However, a small number of microvessels were covered with pericytes in other groups at that time point. Our results showed that group A had the highest number of pericytes of all of the groups from the 3rd to the 10th day post-operation (*P<0.05, **P<0.01) (Figure 5B), and the MPI was significantly higher in group A than in the other groups at the corresponding time points (*P<0.05, **P<0.01). All statistical analysis results are expressed as the mean ± SD (*P<0.05, **P<0.01).

Change of Ang-1, Ang-2, and Ang-1/Ang-2 expression

The qRT-PCR was used to quantify alterations in Ang-1 and Ang-2 mRNA expression levels between the four groups at different time points (Figure 6A). In group A, the level of Ang-1 mRNA expression was gradually decreased from the 1st to the 3rd day post-operation, and difference was statistically significant compared with group C (*P<0.05), and groups B and D (**P<0.01). However, the level of Ang-1 mRNA expression was significantly increased at day 7 and 10 (*P<0.05). In groups B and D, the expression trend of Ang-1 was contrary to group A, and the levels of Ang-1 mRNA expression were significantly lower compared with group A (**P<0.01).

Western blots were used to quantify the levels of Ang-1 protein expression in different groups (Figure 6B). The levels of Ang-1 mRNA and protein expression, as detected by qRT-PCR and Western blot, respectively, exhibited the same trends of alteration during wound healing. Statistical analysis of Ang-1 protein expression is shown in Figure 6C. Immunohistochemical analysis demonstrated that Ang-1 was primarily present in pericyte, and the Ang-1-positive staining was greater in group A than in the other groups on the 7th day (Figure 6D).
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The levels of Ang-2 mRNA expression are shown in Figure 6E. The results suggested that level of Ang-2 mRNA expression was significantly higher in group A than in the other groups on the 1st and 3rd days (*P<0.05, **P<0.01), however it was sharply decreased from the 7th to the 10th day, and difference was statistically significant compared with group C (*P<0.05). The trend of Ang-2 mRNA expression in groups B and D were similar to that in group A; however, significant differences were still detected on the 7th day (*P<0.05) and on the 10th day (**P<0.01).

The levels of Ang-2 protein expression are shown in Figure 6F, and are consistent with the levels of Ang-2 mRNA expression. Statistical
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Figure 6G. The expression ratios of Ang-1/Ang-2 were shown in Figure 6H. In group A, the expression ratio of Ang-1/Ang-2 was much lower compared with other groups on the 1st and 3rd days of wound healing. The expression ratios of Ang-1/Ang-2 exhibited gradually and significantly increases from the 7th to the 10th day in groups A (*P<0.05, **P<0.01).

The immunohistochemical staining results suggested that Ang-2 is primarily expressed in endothelial cells and capillary walls and that Ang-2-positive staining was markedly lower in group A than in the other groups on the 7th day (Figure 6I).

Change of pTie-2 and α-SMA expression

The tyrosine phosphorylation of Tie-2 (pTie-2) was detected by Western blot as shown in Figure 7A. Statistical analysis of the levels of pTie-2 protein expression is shown in Figure 7B. The results showed that expression of pTie-2 was gradually decreased from the 1st to the 3rd day and sharply increased from the 7th to the 10th day in group A. The expression of pTie-
2 was significantly lower in group A compared with in the other groups (*P<0.05, **P<0.01) on the 1st and 3rd days, and was significantly higher than in the other groups on the 7th and 10th days (*P<0.05, **P<0.01). In group C, the expression trend of pTie-2 was identical to that of group A. However, in groups B and D, the level of pTie-2 expression was gradually decreased from the 1st to the 10th day. Immunohistochemical staining showed that Tie-2 was primarily expressed in endothelial cells and that Tie-2-positive staining was markedly higher in group A compared with in the other groups on the 7th day (Figure 7C).

The levels of α-SMA mRNA expression are shown in Figure 7D. The results shown in group A the levels of α-SMA mRNA expression were sequentially and significantly increased from the 3rd day to the 10th day (*P<0.05, **P<0.01). In groups B and D, the levels of α-SMA mRNA expression were maintained at signifi-

Discussion

Diabetes mellitus is known to affect the process of wound healing [27-29], resulting in ulcers and lower limb necrosis, often requiring amputation [28]. The causes of diabetic complications are unclear; however, these complications may lead to cellular apoptosis and reduced angiogenesis and collagen formation [30]. NPWT has been demonstrated to accelerate vascular endothelial cells proliferation and new blood vessels formation and thus accelerated diabetic wound healing [31-33]. Previous research found that microvessels became gradually mature after NPWT treatment [19], however, what and how the signal pathway regulate the angiogenesis and microvessels maturation during the wound healing and the underlying mechanism remain unclear.

In the early stage of wound healing, the vasculo-
genesis was depended on the degradation of extracellular matrix, pre-existing blood vessels and their extensively mutual neovascular anastomosis, subsequently the cascaded growth factors and various cytokines orchestration contribute to accomplish the process of angiogenesis and vessel maturation [34, 35]. Recent study demonstrated that angiogenesis and vessel maturation were associated with Ang family [11, 21]. The Ang family of secreted growth factors consists of the following four ligands, all of which bind to endothelial tyrosine kinase Tie-2 receptor: Ang-1, Ang-2, and their orthologs Ang-3 (in mouse) and Ang-4 (in humans) [11, 12, 21]. Ang-1 is primarily expressed in pericyte-like perivascular mural cells (pericyte), suggesting a paracrine effect on new vascular function [11, 21], and it serves as a vascular pro-maturation factor that facilitates vascular remodeling and maturation [9]. In contrast, Ang-2 is primarily expressed in the endothelium itself, suggesting an autocrine function, and it serves as an antagonist that inhibits Ang-1-induced [11, 12], and it serves as a pro-angiogenesis factor that promotes vessel endothelial sprouting and pericyte detachment from the endothelium lumen [11, 21, 36]. Ang-3 has been shown to act as an agonist for tyrosine kinase Tie-2 receptor and to be particularly expressed in mouse endothelium. Ang-4 acts as an agonist for Tie-2 and it was specifically expressed in human lung tissue [21].

For this reason, vascular sprouting, vascular remodeling and vascular maturation are primarily regulated by Ang-1, Ang-2 and pTie-2 [11, 21]. In this study, the expression of Ang-1 and pTie-2 gradually decreased during the first 3 days in group A, however the Ang-2 was sharply elevated at the same time. The results suggested that Ang-1, which can be bound to and thus phosphorylated by the activation of Tie-2 receptor, might serve as a pericyte-derived microves-

el pro-maturation and stabilizing factor. In groups B and D, the expression of Ang-2 was changed weakly, but the expression of Ang-1 and pTie-2 were changed markedly at early stage. Results suggested that angiogenesis might be affected weakly, and the Ang-1 would fail to activate the phosphorylation of Tie-2 receptor when the latter was blocked by Tie-2 inhibitor at early stage of wound healing, as well as combing with MPI and the expression of α-SMA could demonstrate this phenomenon. As well as destabilize microenvironment, thus leading to angiogenesis [9, 13]. Results showed that the quantitative comparison ratios of Ang-1/Ang-2 were sequentially over expression at early stages in the groups B, C, D, while it were no statistical significant in the above
groups. However, the ratios of Ang-1/Ang-2, Ang-1 and pTie-2 remained at a lower level, and the Ang-2 and PCI were highly expressed in the early stage in the group A. Because of the role of Ang-2, results suggested that the microvascular destabilization, endothelial cell proliferation and microvessel tube formation were the primary events occurred during the early stage of wound healing. We also found that the NPWT can preferentially promote vessel destabilization and thus increase angiogenesis in the early stage.

However, vascular remodeling, stabilization, and maturation occurred as the primary events after endothelial lumen formation during the later stage of wound healing [21]. One study has shown that the pericytes serve as a crucial substance of vascular maturation structures that maintain vascular endothelial stabilization [37]. Therefore, the structural characteristics of mature vasculature should include the endothelial tube surface, which is covered with pericytes [21]. In this study, results showed that ratios of Ang-1/Ang-2, the expression of Ang-1 and pTie-2 were gradually increased, and sustained at a high level in the later stage in the group A, simultaneous the expression of Ang-2 was sharply decreased. The results suggested that Ang-1 can preferentially bind to Tie-2 and activate it by inducing its phosphorylation in the later stage of wound healing and thus effect vessel maturation after NPWT treatment, in the other hand, the Ang-2 lost its predominant position in the later stage. Furthermore, the results showed that the ratios of Ang-1/Ang-2 were sequentially over expressed and the α-SMA, the number of pericyte, MVD and MPI were gradually increased in the groups A and C at later stage. Because of the role of Ang-1, the results suggested that the microvessels were gradually stabilized and mature, and the microvascular stabilization and maturation became the primary feature during the later stage of wound healing, as well as suggested that stabilized wound microenvironment might contribute to vessel maturation.

Our study showed that the PCI, the expression of Ang-1 and pTie-2 was gradually reduced during wound healing in groups B and D, while the expression of Ang-2 was the same as the groups C, simultaneous the expression of α-SMA, MVD and MPI were gradually increased but at a lower level at later stage compared with group A. It is possible that Ang-1 induced phosphorylation of Tie-2 was dramatically inhibited by the inhibitor and thus vascular remodeling and maturation processes were obviously deferred at later stage. And the ratios of Ang-1/Ang-2, α-SMA, number of pericytes, MVD and MPI were significantly increased in the groups A than group C. Results suggested that a Tie-2 inhibitor could significantly affect vascular remodeling and maturation during the later stage of wound healing, and the NPWT can preferentially promote microvessel stabilization, maturation and integrity in the later stage, and the vessel maturation process was primarily regulated by the Ang/Tie-2 signaling pathway.

Recent research has demonstrated that immature vessels could induce vessel leakage, tissue edema, and vessel occlusion, leading to reduction of oxygen and nutrient supplies [38]. Some research found that the number of new blood vessels increased in tumors, however, the blood vessel structures are inclined toward destabilization and immaturity because of lack pericytes in new formative vessels, leading to vessel hemorrhage [39], and previous study demonstrated that inhibition of Ang-2 induces a reduction in tumor angiogenesis and an increase in pericytes, leading to vascular stabilization and tumor vascular normalization [40], however, this study suggested that the relative increase of pericytes is caused by the reduction of angiogenesis in tumor.

Conclusion

In summary, NPWT may not only predominantly promote microvessels destabilization and thus increase the quantity of initial angiogenesis in the early stage but also preferentially promote microvessels stabilization and thus enhance the quality of formed vessels, contributing to vessels maturation. And a Tie-2 inhibitor could moderately influence angiogenesis process during the early stage but affect vascular remodeling and maturation process significantly during the later stage of wound healing. Furthermore, it demonstrated that Ang/Tie-2 signal pathway play a primary role in regulating the process of the vessel maturation.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81572163) and by Hubei National Natural Science
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Fund projects (No. 2014CFB751). The funders had no role in the study design, date collection and analysis, decision to publish or preparation of the manuscript. We would like to acknowledge the Wuhan VSD Medical Science & Technology, Co., Ltd. (Wuhan, China) for supplying the vacuum material. We also thank the Medical Science Experimentation Center of Wuhan University providing the experiment equipment.

Disclosure of conflict of interest

None.

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