Effects of Vacuum-assisted Closure on Wound Microcirculation: An Experimental Study

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OBJECTIVE: To study the mechanism through which vacuum-assisted closure (VAC) induces an increase in blood flow and reduces oedema on skin wounds.

METHODS: Thirty-two Japanese large-ear white rabbits were used. A round full-thickness skin defect (retaining the perichondrium), 2 cm in diameter, was created on each dorsal ear. The wound on the left ear was assigned to the experimental group, and the wound on the right ear to the control group. In the experimental group, the sterile foam dressing was trimmed to the appropriate size and geometry for the given wound and placed into the wound defect. The surface of the wound containing the foam dressing was covered with an adhesive drape to create an airtight seal. Afterwards, negative pressures of –5, –10, –15 and –20 kPa were exerted on the same wound, each lasting for 20 minutes, at intervals of 10 minutes. In the control group, the wound was treated with petrolatum gauze only. At different time points, the microcirculation microscope and image pattern analysis were used to observe the variation in wound microcirculation through a detection window.

RESULTS: It was found that VAC promoted capillary blood flow velocity, increased capillary calibre and blood volume, stimulated endothelial proliferation and angiogenesis, narrowed endothelial spaces, and restored the integrity of the capillary basement membrane.

CONCLUSION: By increasing capillary calibre and blood volume and by stimulating angiogenesis, VAC could improve blood circulation in wounds. By narrowing endothelial spaces and by restoring the integrity of capillary basement membranes, VAC could decrease the permeability of blood vessels and wound oedema. [Asian J Surg 2005;28(3):211–7]

Key Words: blood flow velocity, microcirculation, oedema, vacuum-assisted closure, wound
operative pace, reduce the number of dressing changes and improve the success rate of treatment.\textsuperscript{7,8} So, as soon as the wound is ready for the grafting of tissue flaps, the operation should be performed immediately, before the granulation tissue begins to age.

While the clinical efficacy of VAC is widely accepted, the fundamental scientific questions regarding the mechanism by which VAC promotes wound healing remain unanswered. In this study, the rabbit injury model with acute full-thickness skin defect on each dorsal ear and microcirculation analysis system were used to observe changes in capillary calibre, endotheliocyte, blood velocity, basement membrane and endothelial spaces after VAC treatment. The morphological findings in relation to blood flow and oedema were analysed.

Materials and methods

VAC system

The VAC system used in this study consisted of a vacuum drainage apparatus (ACO-012; Ri Sheng Co Ltd, Guangzhou, Guangdong Province, China), medical grade polyurethane ether foam dressing (Wei Kong Co, Xi’an, Shanxi Province, China) and adhesive drape (3L Medical Products Co, Nanchang, Jiangxi Province, China). The foam dressing was 1.5 cm in thickness and 400–600 μm in pore size.

The instruments used for observing and analysing blood microcirculation were: microcirculation microscope (WX-753, Hongda Optic-electric Instrument Co, Xuzhou, Jiangsu Province, China), pick-up camera (TC-5202E, Sony Corp, Tokyo, Japan), videotape recorder (NV-G20HQ, National Corp, Tokyo, Japan), indicator (WV-5410, Panasonic Corp, Minoshima Hakata-ku, Japan), and color-media microcirculation detector (Dongfang Institute of Blood Disease, Wuxi, Jiangsu Province, China).

The analysis of blood velocity and flow state were performed by a microcirculation image analysis system that used a vp32 image processing software (Dongfang Institute of Blood Disease) to decode the incoming video signals. The images were processed with a computer (Benyue 2000; Lianxiang Co, Beijing, China) and indicator (BRC-2002; Dongfang Institute of Blood Disease).

Experimental animals

Thirty-two Japanese large-ear white rabbits were used. They were between 2.0 and 2.5 kg in weight and were provided by the Experimental Animal Center, Fourth Military Medical University, Xi’an, China. The animals were divided into four groups for four studies: (1) blood flow velocity and capillary calibre study \( (n = 8) \); (2) capillary density study \( (n = 8) \); (3) study of the ultrastructure of capillaries and endotheliocyte \( (n = 8) \); (4) study of the process of wound healing \( (n = 8) \). All protocols and procedures were approved by the Institutional Animal Care and Use Committee, and animals were cared for according to the Guidelines for Care and Use of Animals in Research.

Animal operations and VAC

The rabbits were anaesthetized intraperitoneally with 3% pentobarbital sodium (30 mg/kg). A round full-thickness skin defect (retaining the perichondrium), 2 cm in diameter, was created on each dorsal ear, and the whole wound was immediately covered with petrolatum gauze. The wound on the left ear was assigned to the experimental group, and the wound on the right ear to the control group. On the third postoperative day, the sterile foam dressing was trimmed to the appropriate size and geometry for the wound in the experimental group. The end of the non-collapsible evacuation tube with side ports was embedded into the foam, and the foam dressing was placed into the wound defect. The surface of the wound containing the foam dressing was covered with an adhesive drape extending 5–6 cm beyond the margins of the wound over adjacent intact skin to create an airtight seal. The other end of the evacuation tube was connected to the vacuum apparatus through a vacuum drainage bottle. Then, a given negative pressure was exerted. In the control group, the wound was treated with petrolatum gauze and absorbent gauze without exerting negative pressure. At different time points, about 0.5 cm from the wound edge on the proximal side of the wound, a round adhesive drape (1.0 cm in diameter) was cut off to form a detective window for the four studies detailed below.

Blood flow velocity and capillary calibre

Liquid paraffin was dropped onto the skin through the detective window, and the area was examined under a microcirculation microscope. In the experimental group, negative pressures of \(-5, -10, -15\) and \(-20\) kPa were exerted on the wound, each lasting for 20 minutes and kept up at an interval of 10 minutes. The microcirculation microscope and image analysis system were used to detect blood flow velocity and capillary calibre before negative pressure was applied, and every 2 minutes from 2 to 20 minutes after treatment.

Capillary density

In the experimental group, \(-15\) kPa negative pressure was exerted immediately after wound creation and continuously...
to the wound for 8 hours every day until the wound healed. At 6 and 24 hours and on days 3, 6 and 9 after the wounds were created in both the experimental and control groups, the capillary density (capillary intercrossed number/mm²) in the wound edge was detected, and the mean value of the data in four random fields for each wound was calculated.

Ultrastructure of capillaries and endotheliocytes
After the wounds were created, and at 2, 10 and 30 minutes and 3 and 6 days after treatment (in the experimental group, –15 kPa negative pressure was exerted after wound creation until the wound healed), skin and subcutaneous tissue from the wound edge were harvested and ultrathin sections were prepared to observe the morphological changes in the capillary lumina, basement membrane, endotheliocyte and endothelial spaces.

Process of wound healing
Photographs of the wound were taken immediately after they were created and on postoperative days 3, 6 and 9 (Minolta-700 camera, close-up lens, aperture 8, speed 1/60 second, distance 20 cm; Konica Minolta, Tokyo, Japan). Wound and wound-edge specimens were harvested and 4-µm sections were prepared (haematoxylin and eosin stain) for observing granulation tissue, oedema, cellular infiltration, epithelial proliferation and transmigration under light microscopy. The area of wound surface was measured with image analysis software. The healing rate of the wound was calculated according to the formula: Healing Rate = (1 – Area of wound surface/Original area) × 100%. Wound healing was confirmed if the healing rate was over 90%.

Statistical analysis
For all data, the mean ± standard deviation was calculated using SPSS 10.1 (SPSS Inc, Chicago, IL, USA). Two-tailed independent sample t tests were used to compare blood flow velocity in the microcirculation, capillary diameter and density, and rate of wound healing between VAC-treated and control groups and between before and after VAC treatment. Statistical significance was accepted at p less than 0.05.

Results
Change of microcirculation in wound
In the VAC-treated group, when negative pressure greater than –10 kPa was exerted, blood flow velocity in the capillaries immediately increased and reached its peak value in the fourth minute. When negative pressure greater than –15 kPa was exerted, velocity remained at a high level even 20 minutes after treatment (Figure 1). When the negative pressure increased to –15 and –20 kPa, capillary calibre increased significantly compared to that pre-treatment (p < 0.01) (Table). At 6 and 24 hours and days 3 and 6, capillary density in the VAC group was significantly higher than that in the control group (p < 0.01). However, on day 9, no difference was seen between the two groups (p > 0.05) (Figure 2).

VAC also changed the ultrastructure of capillaries and endotheliocytes. Before VAC treatment, the capillaries looked flat (Figure 3), the mitochondria were swollen, and the cell bodies projected into the blood vessels. Meanwhile, basement membranes were not integral, the endothelial spaces were wide, cell junctions were few, and many pinocytotic vesicles could be seen in the cytoplasm. Two minutes after VAC treatment, capillaries became almost round (Figure 4), the endotheliocyte became cubic, and the continuity of the base-

Table. Comparison of capillary calibre before and after vacuum-assisted closure (VAC)*

<table>
<thead>
<tr>
<th>Negative pressure, kPa</th>
<th>Capillary calibre, µm</th>
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<tbody>
<tr>
<td></td>
<td>Pre-VAC</td>
</tr>
<tr>
<td>–20</td>
<td>12.43 ± 1.88</td>
</tr>
<tr>
<td>–15</td>
<td>11.98 ± 1.38</td>
</tr>
<tr>
<td>–10</td>
<td>12.28 ± 1.97</td>
</tr>
<tr>
<td>–5</td>
<td>12.14 ± 1.46</td>
</tr>
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*Data are expressed as mean ± standard deviation; †p < 0.01.
Figure 2. Effect of vacuum-assisted closure (VAC) on capillary density: in the VAC treatment group, at 6 and 24 hours, and 3 and 6 days post-operation, capillary density was significantly greater than before VAC treatment ($p < 0.01$), and also significantly greater than in the control group ($p < 0.01$).

![Graph showing capillary density over postoperative time](image)

Effects on process of wound healing

Immediately after the wounds were created, no obvious differences in shape or size were seen between the experimental and control wounds. The skin within the limits of the wound was completely removed and the yellowish perichondrium became visible (Figure 12). In the VAC-treated group, much granulation tissue appeared on the third day, the epithelium on the wound edge began to transmigrate, and abundant neogenetic vessels were found (Figure 13). On the sixth day, fibroblasts had proliferated markedly, some of which had turned into spindle fibrocytes, and there were abundant extracellular matrixes. The surface area of the VAC-treated wound became significantly smaller than the control wound (Figure 14). On the ninth day, the wound had basically healed (Figure 15). In four hours later, endotheliocyte proliferation started in the capillary buds (Figure 8), and there were fewer pinocytotic vesicles. Three days later, capillary morphology returned to normal (Figure 9).

In the control group, 24 hours after wound creation, capillary lumina were still stenosed and irregular (Figure 10), and no capillary buds were seen. It was not until the third day that a few buds began to emerge, but the capillary basement membrane was still not integrated, the endothelial spaces were wide, and there were many pinocytotic vesicles in the cytoplasm (Figure 11).

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contrast, there was no evidence of granulation tissue, no marked epithelium hyperplasia in the hair follicles and sebaceous glands, and oedema and inflammatory infiltration remained severe in the control group on the third day. On the ninth day, only a few neogenetic epithelium had formed, and the number of fibroblasts and extracellular matrixes remained few.

Discussion

Using laser Doppler flowmeters, Morykwas et al and Xu et al observed changes in blood volume in swine models with acute skin defects or granulation wounds.3,8 They demonstrated that blood volume in the wounds significantly increased after VAC treatment, but did not discuss the possible reasons in
Figure 12. The wounds were created by removal of the full-thickness skin on the dorsal ears of the rabbits. There were no differences in the shapes and sizes of the wounds between the experimental and control groups. a = experimental wound; b = control wound.

Figure 13. Granulation tissue began to form 3 days after wound creation in the experimental wound (a), while in the control wound (b), the perichondrium was still laid bare.

Figure 14. The surface area of the experimental wound (a) was much smaller than that of the control wound (b) 6 days after wound creation.

Figure 15. By 9 days after wound creation, the experimental wound (a) had almost healed, but the control wound (b) mainly consisted of granulation tissue and the surface area of the wound was larger.

depth; they also observed the healing process for only 30 minutes rather than the whole process of wound healing.\textsuperscript{3,8} We found that after VAC treatment, wrinkles in the skin appeared around the wound, most likely the result of a reduction in oedema, but this needs to be further studied.

In this study, it was demonstrated that the increase in blood flow was related to the increase in capillary calibre and density, and also with angiogenesis. Owing to negative pressure intrinsically bringing mechanical stress into play, the possible cause for the increase in blood flow may be that a pressure gradient of blood flow was formed between the wound and surrounding tissues by VAC, with blood surging to the wound to increase perfusion pressure of the tissue, which in turn promoted blood flow velocity and passively dilated the capillaries and opened up the capillary beds.\textsuperscript{9–12} The existence of mechanical forces, change in endothelial morphology, and increase in blood flow stimulated endothelial proliferation, capillary budding and angiogenesis.\textsuperscript{13} These events were also important factors in increasing capillary density.\textsuperscript{14} Several days after VAC treatment, capillary density decreases, and probable reasons for this are that the small arterial and venous pressures are maintained at a new equilibrium, and that as the neogenetic capillaries mature, some degenerate.

Angiogenesis is a complicated biological process involving angiogenetic and inhibitory factors, cells that secrete or synthesize these factors, the extracellular matrix and the local microenvironment.\textsuperscript{12,13,15} Although at present, the relationship between endothelial morphological change and angiogenesis remains unclear, it has been reported that the cytoskeleton is the vital receptor of mechanical stimulation and plays a very important role.\textsuperscript{16,17}
The capillary wall is mainly composed of endotheliocytes and basement membrane. The basement membrane is an important barrier of intra- or extracellular substance exchange, and the endothelial space is the main passage for macrocellular substances. This study demonstrated that after the wound was created, the integrity of the capillary basement membrane was destroyed and the endothelial space became enlarged. After VAC treatment, the integrity of the basement membrane was restored and the endothelial space was reduced. These results conform with the results from our previous experimental study in which the permeability of blood vessels and wound oedema were markedly decreased after VAC treatment. These findings suggest that by increasing capillary calibre and blood volume and stimulating angiogenesis, VAC can improve blood circulation in wounds, narrow endothelial spaces, and restore the integrity of the capillary basement membrane. VAC can also reduce the permeability of blood vessels and, hence, the degree of wound oedema. These constitute a possible scientific interpretation for our clinical findings, and it is hoped that our results have clarified the mechanism by which VAC accelerates wound healing.

The rabbit ear model and the method of research used in this study has many advantages: (1) we can conduct noninvasive, continuous and dynamic observations of the complete course of healing in the same wound; (2) the cartilage support of the ear and tight adhesion between skin and cartilage could dispel the influences of skin contracture on the assessment of wound healing; and (3) the very thin skin of a rabbit’s ear and the fact that the ear may be easily fixed to a plate facilitated microcirculation observation.

References