Vacuum-Assisted Closure: A New Method for Wound Control and Treatment: Animal Studies and Basic Foundation

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A series of basic animal studies using a new subatmospheric pressure technique (The V.A.C.) to expedite wound healing are presented. The technique entails placing an open-cell foam into the wound, sealing the site with an adhesive drape, and applying subatmospheric pressure (125 mmHg below ambient) that is transmitted to the wound in a controlled manner. Utilizing a pig model, four studies were undertaken to determine the effect of subatmospheric pressure on laser Doppler-measured blood flow in the wound and adjacent tissue (N = 5), rate of granulation tissue formation (N = 10), clearance of bacteria from infected wounds (N = 5), and measurement of nutrient flow by random-pattern flap survival (N = 5). Blood flow levels increased fourfold when 125 mmHg subatmospheric pressure was applied. Significantly increased rates of granulation tissue formation ($p \leq 0.05$) occurred with both continuous (63.3% ± 26.1%) and intermittent (103% ± 35.3%) application. Tissue bacterial counts significantly decreased ($p \leq 0.05$) after 4 days of application. Random-pattern flap survival significantly increased ($p \leq 0.05$) by 21% compared to controls. We determined that the application of controlled subatmospheric pressure creates an environment that promotes wound healing.

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Galen first proposed the concepts of healing by primary and secondary intention. Healing by primary intention requires the wound edges to be opposed, minimizing blood loss and promoting restoration of an intact epithelial layer. Surgeons have used sutures for 5,000 years to oppose the wound edges. As the size of the wound increases or the mobility of adjacent tissues decreases, either larger caliber suture material or greater numbers of sutures are required to oppose the wound edges. Both increase the potential for wound infection and thus decrease healing potential [1, 2]. Wounds in which the edges cannot be opposed must heal by secondary intention, and require deposition of matrix proteins and neovascularization to form granulation tissue, followed by migration of keratinocytes across the defect, a much lengthier process than healing by primary intention.

A series of animal experiments are presented that form the foundation for a new subatmospheric pressure method for treating wounds: vacuum-assisted closure. (The vacuum-assisted wound closure device and methodology [The V.A.C.] are subject to pending United States and foreign patents, and applications assigned to Wake Forest University. A worldwide license has been assigned to Kinetic Concepts, Inc., San Antonio, TX. The V.A.C. is a trademark of Kinetic Concepts, Inc.) The technique was developed as a method to expedite the rate of healing by secondary intention, particularly in compromised and debilitated patients. The method entails placing a sterile, open-cell foam dressing into the wound defect, sealing the site with an adhesive drape, then applying controlled subatmospheric pressure to the wound. The subatmospheric pressure applies a controlled force uniformly to all tissues on the inner surface of the wound. No good animal model of chronic wounds exists, so an acute-wound swine model was used. The effects of the technique on blood flow, rate of granulation tissue formation, bacterial colonization, and changes in nutrient blood flow determined by flap survival in a porcine model are presented.

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Materials and Methods

The Device

The V.A.C. system consists of a medical-grade, open-cell, polyurethane ether foam dressing that has been approved by the Food and Drug Administration for contact with open wounds. The pore size ranges from 400 to 600 μm to maximize tissue growth [3, 4]. An evacuation tube with side ports that communicate with the reticulated foam is embedded in the foam. The open-cell nature of the foam ensures equal distribution of the applied subatmospheric pressure to all spaces within the system and thus to all surfaces in contact with the foam. The foam dressing is cut to the specific wound geometry and placed into the wound defect with the evacuation tube exiting parallel to the skin. The wound site and dressing are covered with an adhesive drape that extends 3 to 5 cm over the adjacent intact skin, thus converting an open wound into a controlled closed wound. The proximal end of the evacuation tube is connected to a canister in which effluent fluid is collected. The canister is in turn connected to an adjustable vacuum pump. The magnitude of subatmospheric pressure and whether the subatmospheric pressure application is continuous or intermittent is adjustable.

Animals

Twenty-five 20 kg Chester pigs (Baux Mt Co., Baux Mt, NC) were allowed to acclimate for 1 week prior to all procedures. The animals were divided into five experimental studies: (1) blood flow studies (N = 5); (2) granulation tissue formation, continuous subatmospheric pressure (N = 5); (3) granulation tissue formation, intermittent subatmospheric pressure (N = 5); (4) bacterial clearance (N = 5); and (5) random pattern flap survival (N = 5).

On the day of surgery and when needed for measurements or biopsies, the animals were sedated with an intramuscular injection of ketamine/xylazine/acepromazine (60:12:0.6 mg per kilogram). The animals were shaved and scrubbed for surgery. Halothane (1%) was administered by inhalation for maintenance of anesthesia.

All protocols and procedures were approved by the Institutional Animal and Care Use Committee, and animals were cared for under guidelines set forth in the Guidelines for Care and Use of Animals in Research.

Blood Flow Studies

The animals were prepared for surgery as described. Two circular defects 2.5 cm in diameter were excised down to the deep fascia of the muscles over the spine. Laser Doppler needle probes (Moor Instruments, Devon, UK) were inserted into the subcutaneous tissue and deep back muscles adjacent to the defect. A foam dressing was placed into the anterior wound and covered with the adhesive drape. Subatmospheric pressure was applied to the wounds in 25-mmHg increments (range, 0–400 mmHg) for 15-minute intervals as blood flow was continuously measured. Blood flow is expressed by the Doppler as "perfusion units," and the output was continuously recorded with a strip chart recorder. The needle probes were removed and placed into the subcutaneous tissue and muscle adjacent to the posterior wound. A foam dressing was applied as described and subatmospheric pressure was applied intermittently. The "on" cycles, during which subatmospheric pressure was applied, ranged from 1 to 10 minutes and the "off" cycles ranged from 1 to 5 minutes. Blood flow measurements were again recorded on a strip chart recorder.

Granulation Tissue Formation, Continuous Subatmospheric Pressure

The animals (N = 5) were sedated, prepared for surgery, and two defects were created on the dorsal midline as described in the previous section. Previous experience with this model has shown that wounds directly over the midline do not contract during healing, and thus the defect space must fill with granulation tissue. To ensure no contraction occurred, the diameter of each wound was measured daily. Alginate impressions were made of each defect and the volume of the defect calculated by fluid displacement. A foam dressing was placed into the anterior defect, while saline-moistened gauze was placed into the posterior (control) defect. A plastic saddle (Aquaplast, WFR Aquaplast, Ramsey, NJ) was placed over the back of each animal and secured in place with Velcro straps to prevent dislodgment of the dressing and trauma to the wound site. A tubular

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bandage (Stockinette, Alba-Waldensian, Inc., Rockwood, TN) was placed on each animal to secure and prevent dislodgment of the saddle. One end of a long piece of thick wall tubing was attached to the protective garments, and the tube extending from the foam dressing was connected to the thick wall tubing. The animals were returned to their pens. The thick-walled tube was suspended from a pulley system over the top of each pen, thus the animal had full, unrestricted access to the entire pen. Subatmospheric pressure (125 mmHg) was applied to the anterior wound of each animal. This subatmospheric pressure level had been previously identified as the most efficacious from the blood flow studies conducted earlier. The animals were allowed to recover from anesthesia and given food and water ad libitum.

The animals were sedated as described 48 hours after surgery, then daily, and alginate impressions were made of each defect. This routine was continued until the defects filled with granulation tissue and were flush with the surrounding tissue. The data were expressed as cubic centimeters of granulation tissue formed per day to allow comparison between defects and between animals. The percent increase in rate of granulation tissue formation was expressed as (treated − control)/control × 100%. No attempts were made to determine degree of reepithelialization.

Granulation Tissue Formation, Intermittent Subatmospheric Pressure

The animals (N = 5) were sedated and three defects per animal were created as in the previous study. Alginate impressions were taken of each defect and subatmospheric pressure devices applied to the anterior and middle wounds as described. Subatmospheric pressure (125 mmHg) was applied continuously to the anterior wound. Subatmospheric pressure (125 mmHg) was applied cyclically to the middle wound using a 5-minute-on/2-minute-off cycle. This timing regime was identified from blood flow studies as resulting in the maximum increase in local blood flow. The posterior defect was treated with saline-moistened gauze dressings. The animals were sedated at the same time points as in the previous study. Alginate impressions were taken of each wound and the volume determined. The diameter of each wound was also measured and recorded. The rate of granulation tissue formation was determined as described previously.

Bacterial Clearance

The animals were sedated, prepared for surgery, and defects created as described earlier. Each defect site was injected with 1 ml of saline containing 10^8 organisms. Two different isolates were employed: a human isolate of *Staphylococcus aureus* (RP12) (N = 3) and a pig isolate of *Staphylococcus epidermidis* (SR 5) (N = 2). Foam dressings were applied to both wounds and sealed with adhesive drape. Subatmospheric pressure (125 mmHg) was applied to the anterior defect. The animals were sedated as described at 24-hour intervals. Full-thickness punch biopsies (3 mm in diameter) were harvested in identical, controlled rotations from the base of each wound and placed in individual sterile containers. New sterile foam dressings and drapes were applied at each biopsy harvest. This routine was continued for 2 weeks.

The biopsy samples were weighed and sterile saline (99 times the biopsy weight) was added. The tissue samples were homogenized in a tissue grinder and serial dilutions were made in triplicate. One hundred microliters of each dilution were evenly distributed on the surface of a blood agar plate and the plates incubated overnight. The number of colonies on each plate were counted and the number of organisms per gram of tissue calculated. The data were recorded as the log of the number of organisms per gram of tissue.

Flap Survival

Four days prior to the surgery, the animals (N = 5) were sedated as described. The back and sides of the pigs were shaved. Two dorsally based 3 × 12-cm flaps (36 cm^2) were marked on each side of the pig in indelible ink, with 6 cm between each flap. The flaps were assigned to one of four groups: (1) the flap was exposed to reduced pressure both prior to and following surgery; (2) the flap was exposed to reduced pressure prior to surgery, but not postsurgery; (3) the flap was exposed to reduced pressure following surgery; and (4) the flap was not exposed to reduced pressure (control).

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An area on the left side of each animal that included the flaps was covered by a foam dressing and the entire area sealed with adherent sheeting. Subatmospheric pressure of 125 mmHg was continuously applied to the area for the 4 days prior to surgery. On the day of surgery, the pig was sedated as described and anesthesia maintained by 1% halothane. Random-pattern flaps were created on each side of the pig following the lines previously drawn. The flaps were raised and then sutured back in place with single, interrupted 3/0 nylon sutures. Foam dressings were placed over the anterior flaps on each side of the animal. Continuous subatmospheric pressure of 125 mmHg was applied to the anterior flaps postoperatively.

The animals were anesthetized as described 72 hours after surgery and the devices removed. Photographs of each side of the animals were taken and tracings of the flaps, including discolored areas, were made on acetate sheets to allow for calculation of percent of flap survival by planimetry. The devices were replaced and reduced pressure reapplied. This routine was continued at 48-hour intervals until no further necrosis or healing of necrotic areas occurred. The viable surface areas of each flap were expressed as a percent of the total original flap area.

Statistical Analysis

As the variability of results became known during the study, a power calculation was performed to determine the minimal number of animals required to determine statistical significance of the results. A sample of five was required for each study.

A two-sided paired *t*-test was performed to determine significance of differences between rates of granulation tissue formation for treated vs. control wounds, for the rate of bacterial clearance from the wound, and for percent flap survival. Mean values ± standard deviation (SD) are presented. Statistical significance was accepted at $p \leq 0.05$. The Bonferroni correction factor for multiple testing was used.

Results

Blood Flow Studies

Blood flow changes following application of subatmospheric pressure in both subcutaneous tissue and muscle demonstrated similar bell-shaped response curves, with peak flow of four times baseline values recorded at 125 mmHg applied vacuum. Blood flow values were depressed below baseline values at levels of subatmospheric pressure of 400 mmHg and above. The increases in local blood flow declined after 5 to 7 minutes of continuous application of subatmospheric pressure, eventually reaching baseline.

Intermittent application of subatmospheric pressure resulted in increased localized blood flow that fit a square wave function in which flow increased during application and returned to baseline during "off" times. Peak increases in flow were recorded during application of 125 mmHg, and fourfold increases in baseline values were recorded (Fig 1A). Flow again was depressed below baseline levels with the application of 400 mmHg subatmospheric pressure (Fig 1B). Peak increases in local blood flow declined with "off" intervals shorter than 2 minutes; each successive square wave was slightly shorter than the preceding recorded plateau. "Off" times longer than 2 minutes did not affect the height of the square waves. Thus a 5-minutes-on/2-minutes-off cycle was considered optimal and was used for experiments with cycled subatmospheric pressure.

Granulation Tissue Formation, Continuous Subatmospheric Pressure

The subatmospheric pressure-treated wounds filled with granulation tissue at a significantly greater rate ($p \leq 0.01$) than control wounds for all 10 animals (N = 5, continuous application study; N = 5, intermittent application study). No change in the diameter of any wound was noted during the course of the study. In one subatmospheric pressure-treated wound, the granulation tissue demonstrated hyperproliferative growth above the margins of the wound. Granulation tissue had grown into the interstices of the foam dressing for a few of the wounds, but was removed when the foam was removed and therefore could not be measured. The mean increase (±SD) in the rate of granulation tissue formation for treated wounds was 63.3% ± 26.1% (Fig 2).

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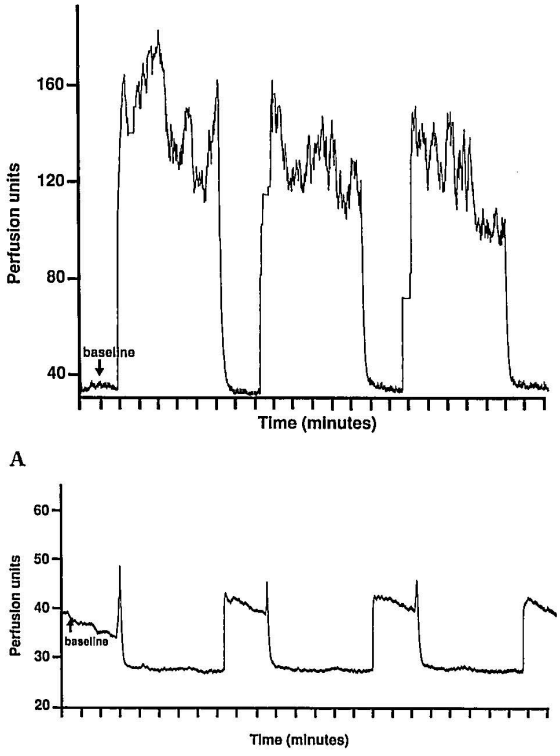

Fig 1. (A) Recording from laser Doppler needle flow probe placed into subcutaneous tissue at edge of wound. Flow (perfusion units) measured over time (minutes) increased from baseline on cyclical application of 125 mmHg and returned to baseline during off cycle (5-minutes-on/2-minutes-off cycle). (B) Recording from laser Doppler needle flow probe placed into subcutaneous tissue at edge of wound. Flow (perfusion units) measured over time (minutes) decreased from baseline levels on cyclical application of 400 mmHg and returned to baseline during off cycle (5-minutes-on/2-minutes-off cycle).

Granulation Tissue Formation, Intermittent Subatmospheric Pressure

The mean increase in rate of granulation tissue formation for intermittently treated subatmospheric pressure-treated wounds (103.4% ± 35.3% [mean ± SD]) was at a significantly greater ($p \leq 0.01$) rate than control wounds for all 5 animals. No changes in the diameter of any wounds was noted. Again, for a few of the intermittent subatmospheric pressure-treated wounds hyperproliferative granulation tissue was present, and ingrowth into the interstices of the foam dressing was present at some dressing changes.

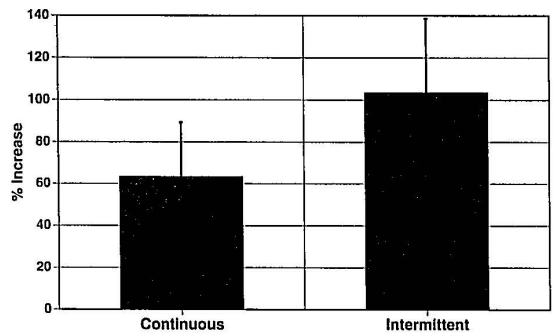

Fig 2. Percent increase (mean ± standard deviation) in the rate of granulation tissue formation of acute wounds in pigs compared to conventional wet-to-moist saline gauze dressing changes (control). Both continuous (N = 10) and intermittent (N = 5) application of subatmospheric pressure to the wounds resulted in a significant increase ($p \leq 0.01$) in the rate of granulation tissue formation.

Bacterial Clearance

Both organisms were readily able to infect the wounds, with levels of approximately 10^8 organisms per gram of tissue, well above the 10^5 organisms per gram of tissue accepted as the clinically infected level. The total number of organisms per gram of tissue present in both wounds decreased slightly for all 5 animals over the first 4 days postinoculation (Fig 3). There was no discernable difference between rate of clearance of RP12-infected wounds vs. SR 5-infected wounds in all studies performed. The number of organisms per gram of tissue decreased significantly between days 4 and 5 for subatmospheric pressure-treated wounds in all 5 animals, with levels falling below the traditionally accepted level of infection of 10^5 organisms per gram of tissue. Bacterial levels remained below 10^5 organisms per gram of tissue in all treated wounds. Levels in control wounds peaked at day 5 and dropped below 10^5 organisms per gram of tissue after a mean of 11 days. The difference in mean log organisms per gram of tissue for subatmospheric pressure-treated wounds is significantly less ($p \leq 0.01$) than in control wounds at day 5 (see Fig 3).

Flap Survival

The distal portions of all flaps were discolored 72 hours postsurgery, with treated flaps less discolored than untreated flaps. The distal ends of all

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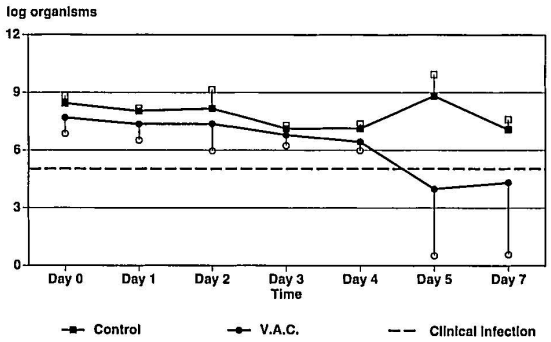

Fig 3. Daily quantitative bacterial loads (mean ± standard deviation) (log number of organisms per gram of tissue) of deliberately infected wound tissues. Vacuum-assisted closure-treated wounds exhibited a significant decrease in the number of microorganisms following 4 days of treatment (N = 5).

flaps appeared to necrose and an eschar formed over the distal portion of each flap. Over time the eschar spontaneously exfoliated, exposing the outline of the original flap. The eschar over the flaps not treated postoperatively was drier and was dislodged consistently earlier than the flaps treated postoperatively with subatmospheric pressure. Control flaps contracted to a Y shape. The pre- and posttreated flaps contracted slightly and appeared as long, thin rectangles following dislodgment of the eschar. The pretreated flaps and posttreated flaps survived to a length intermediate between the control and pre- and post-subatmospheric pressure-treated flaps.

The pre- and posttreated flaps had the greatest survival in terms of percent survival (72.2% ± 10.0% [mean ± SD]; 26.0 cm^2), followed by the posttreated flaps (67.4% ± 4.0%; 24.3 cm^2), and then the pretreated flaps (64.8% ± 10%; 23.3 cm^2). The control flaps had the least flap survival (51.2% ± 6.9%; 18.4 cm^2). The pre- and posttreated flap survival was significantly greater than the control flap survival ($p \leq 0.01$). The survival of pre- and posttreated flaps was not significantly greater than either the pretreated flaps ($p \leq 0.07$) or the posttreated flaps ($p \leq 0.08$). The survival of both pretreated and posttreated flaps was significantly greater than survival of control flaps ($p \leq 0.01$). There was no significant difference in percent survival when pretreated flaps were compared to posttreated flaps. Progression of flap discoloration, necrosis, and contraction is shown in Figure 4.

Discussion

Healing is an intricate, interdependent process that involves complex interactions between cells, the cellular microenvironment, biochemical mediators, and extracellular matrix molecules that usually results in a functional restoration of the injured tissue [5, 6]. The goals of mammalian wound healing are to minimize blood loss, replace any defect with new tissue (granulation tissue followed by scar tissue), and restore an intact epithelial barrier as rapidly as possible. The rate of wound healing is limited by the available vascular supply and the rate of formation of new capillaries and matrix molecules [7]. These events are heavily influenced by locally acting growth factors that affect various processes including proliferation, angiogenesis, chemotaxis and migration, gene expression, proteinases, and protein production [5, 8–11]. Disruption of any of these factors may adversely affect the healing process, resulting in a chronic or nonhealing wound.

Blood Flow

Chronic wounds, and to a lesser extent subacute and acute wounds, are characterized by localized peripheral edema that compresses the microvasculature and lymphatic system [12, 13]. This peripheral edema is similar to the "zone of stasis" surrounding burns. The application of subatmospheric pressure to wounds results in fluid egress from the wound and diminution of local tissue turgor. The increase in localized blood flow following application of subatmospheric pressure is postulated to be due to active removal of the excess interstitial fluid from the tissues immediately surrounding the wound, decompressing small blood vessels and restoring flow.

Granulation Tissue Formation

The 63.3% increase in the rate of granulation tissue formation of wounds treated with continuous subatmospheric pressure application is significantly higher than the rates reported for human wounds treated with growth factors. Compared to saline dressing-treated wounds, wounds treated with platelet derived growth factor decreased in size 19.6% more rapidly and wounds treated with basic fibroblast growth fac-

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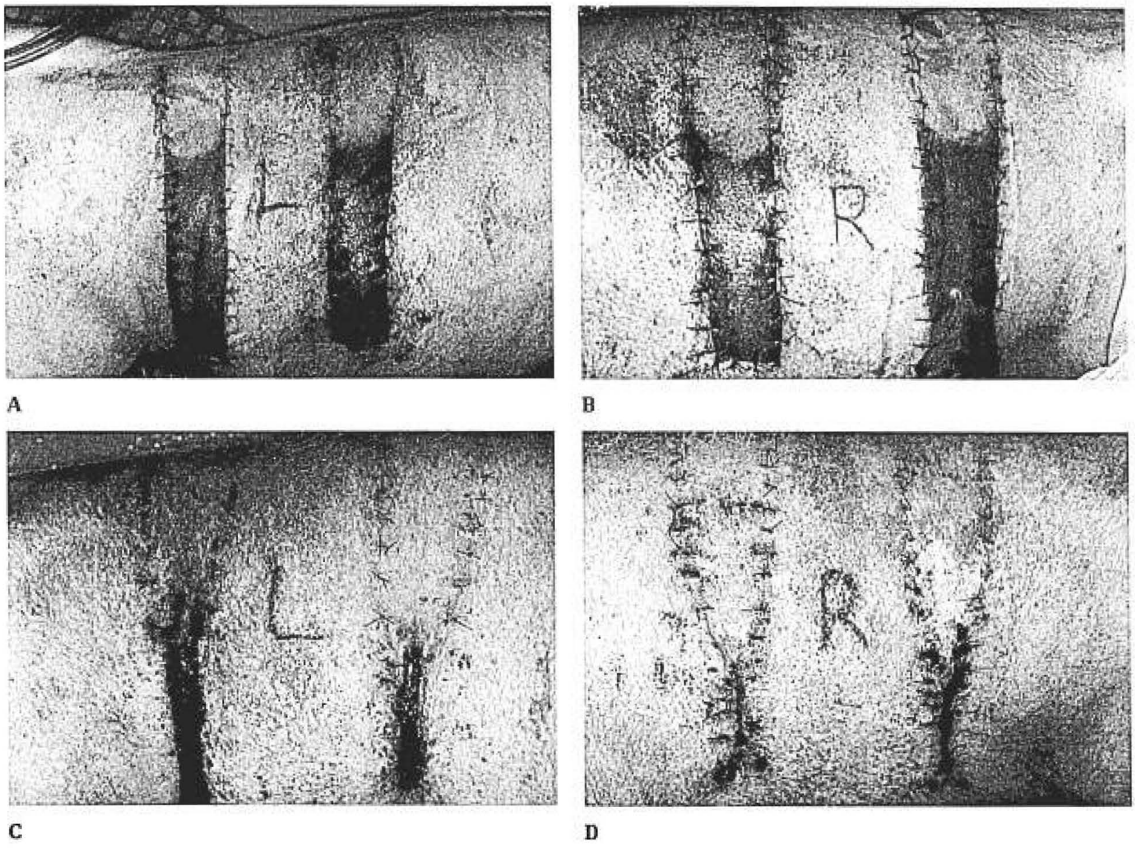

Fig 4. Appearance of 3 × 12-cm dorsally based random-pattern flaps in pigs (N = 5). (A) Appearance of flaps 72 hours postcreation (flap sites pretreated for 4 days with 125 mmHg). (B) Appearance of flaps 72 hours postcreation of control side. (C) Appearance of flaps at 1 month postcreation. Left flap was exposed to subatmospheric pressure pre- and postcreation; right flap was exposed to subatmospheric pressure precreation. (D) Appearance of flaps at 1 month postcreation. Left flap was never exposed to subatmospheric pressure (control); right flap was exposed to subatmospheric pressure postcreation. Significantly more ($p \leq 0.01$) of the pre- and posttreated flaps survived compared to other treatments.

tor decreased in size 10% more rapidly [14, 15]. The highly significant increase in the rate of granulation tissue formation of subatmospheric pressure-treated wounds is postulated to be due to transmission of the uniformly applied force to the tissues on the periphery of the wound. These forces both recruit tissues through viscoelastic flow and promote granulation tissue formation.

Thoma [16] first postulated that application of mechanical stress would result in angiogenesis and tissue growth. This original hypothesis has been supported by recent in vivo studies that examined mechanochemical switching as a mechanism to increase cellular proliferation and angiogenesis [17–20]. Currently, the Ilizarov technique and soft-tissue expanders both apply mechanical stress to tissues to increase mitotic rates [21–26]. In vitro, the basic mechanisms of action by which mechanical forces promote cell proliferation and protein production have been conducted using deformable bottom tissue culture plates [27, 28].

In a static state, external forces applied to cells through the extracellular matrix are balanced by intracellular cytoskeletal forces, with integrins acting as transmembrane bridges. Application of subatmospheric pressure changes the external forces and perturbs the integrin bridges and cytoskeleton, causing the release of intracellular second messengers (prostaglandins, inositol phosphates, protein kinase C, and intracellular calcium) [29, 30]. These messengers cause

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changes in expression of immediate-early genes, with subsequent matrix molecule synthesis and cell proliferation [31–36]. It is logical that in vivo stretching of cells following collapse of the foam dressing would also result in up-regulation of the growth- and healing-associated oncogenes.

While a single mechanical stretch causes an up-regulation of cells [37], intermittent application of subatmospheric pressure results in repetitive release of second messengers. This continual stimulation is reflected in the more rapid deposition of granulation tissue in wounds exposed to intermittent subatmospheric pressure (103%) compared to wounds exposed to continuous subatmospheric pressure (63.3%). Permutations in cellular shape result in an increase in proliferation and protein and matrix molecule synthesis, thus promoting granulation tissue formation.

Bacterial Clearance

As has been demonstrated, blood flow (flux) increases and bacterial colonization of wound tissues decreases following the application of subatmospheric pressure to wounds. Any increase in circulation and oxygenation to compromised or damaged tissue enhances the resistance to infection [38]. Successful, spontaneous healing and healing following surgical intervention are correlated with tissue bacterial counts of less than 10^5 organisms per gram of tissue [39]. Higher levels uniformly interfere in wound healing. Increases in local tissue oxygen levels reduce or eliminate the growth of anaerobic organisms, which have been correlated to decreased healing rates [40, 41]. Additionally, the increased flux should make greater amounts of oxygen available to neutrophils for the oxidative bursts that kill bacteria [42].

Flap Survival

The measurement of flap survival in controlled models has been used in various mechanical and pharmacological manipulations to increase nutrient blood flow. The current model uses flaps that are known to survive to a known length of 50% [43]. Various manipulations can be attempted to increase nutrient blood flow that translates to increased flap survival.

Numerous pharmacological interventions have been promoted to increase flap survival, including sympatholytics, vasodilators, and free radical scavengers [43–47]. Pharmacological agents have limitations that include potential systemic effects and limited delivery to the flap tissues themselves if the flap is edematous or vascular inflow is compromised. Physical modalities have also been used in attempts to increase survival, including low-energy lasers, hyperbaric oxygen, irradiation, and soft-tissue expansion [19, 48–51]. Of the physical modalities, only tissue expansion has been shown to have a statistically significant increase in the amount of flap survival (117% over nonexpanded flaps). Of the pharmacological modalities tried, application of nitroglycerin ointment resulting in a 24% increase in flap survival over untreated controls has been the most efficacious. However, this modality has not gained wide clinical acceptance.

The efficacy of subatmospheric pressure to increase local blood flow has distinct advantages over previous modalities. While it is worthwhile to pretreat flaps with modalities such as tissue expansion before transfer, such modalities are of little use after the fact. Modalities that can be administered to compromised tissues such as distally based traumatic flaps, partial tissue avulsions, complex lacerations, and tissue that has been compromised by chronic infection and edema are of greater value. Unfortunately, pharmacological agents administered intravenously usually do not reach such tissues because of the compromised nature of the underlying microvasculature. Treatment of compromised tissues with a modality that can be applied to a limited area has great appeal and potential. The 21% increase in survival of treated flaps rivals the success of nitroglycerin without the concomitant potential for side effects possible with pharmacological agents.

In summary, application of a controlled subatmospheric pressure (The V.A.C. technique) to wounds results in an increase in local functional blood perfusion, an accelerated rate of granulation tissue formation, a decrease in tissue bacterial levels, and an increase in nutrient blood flow as determined by the survival of random-pattern flaps. Multiple mechanisms are postulated to be responsible for the effects observed: removal of excess interstitial fluid decreases localized

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edema and increases local blood flow; increased local blood flow decreases tissue bacterial levels; and mechanical deformation of cells results in an increase in protein and matrix molecule synthesis, and also an increase in the rate of cell proliferation. These factors combine to accelerate the rate of wound healing.

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