Testing a Device to Replace the Leech for Treating Venous Congestion

Gregory K. Hartig, MD; Nadine P. Connor, PhD; Thomas F. Warner, MD; Dennis M. Heisey, PhD; Majid Sarmadi, PhD; Michael L. Conforti, DVM, MS

**Objective:** To test the effectiveness of a device designed to promote decongestion and tissue survival of a fasciocutaneous flap during 15 hours of complete venous obstruction.

**Methods:** In a porcine model, a 9 × 7-cm fasciocutaneous flap was elevated and the associated veins were clamped, causing complete venous obstruction for 15 hours in 6 control and 6 treatment animals. Up to 3 devices were used to treat the flap in a predetermined pattern. Control flaps were not treated. Measures of treatment efficacy included blood volumes removed; changes in skin color, surface perfusion, and tissue oxygen tension; and end point histologic findings.

**Results:** Control flaps were characterized by progressive darkening of skin color, undetectable surface perfusion, and low levels of oxygen tension. Histologic assessment showed severe congestion and extravasation of blood and distinct signs of necrosis. In contrast, treated flaps had significant improvements in skin color, surface perfusion, and subcutaneous oxygen tension. Histologic analysis showed little, if any, congestion and no signs of necrosis. Mean blood volume removed was 29.5 mL/h.

**Conclusion:** The device was effective in decongesting a large area of tissue during 15 hours of complete outflow obstruction, based on quantitative measurements of tissue health and viability.

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USE OF the medicinal leech (*Hirudo medicinalis*) for treatment of venous congestion in transferred or replanted tissues has become the standard of care in situations in which surgical correction of a venous obstruction is not feasible.1-5 Unfortunately, there are numerous drawbacks with the use of leeches, including negative perception by patients, family, and health care workers; the possibility of infection; increased nursing care; and the possibility of unwanted leech migration or feeding on healthy tissue.6-11 Furthermore, our personal experience suggests that leeches are of limited value in situations in which significant venous congestion is present.

During the past several decades, there have been many articles5,11-13 that describe the use of medicinal leeches in clinical situations. However, the efficacy of leech use for treating congested tissue has not been documented in either clinical trials or experimental studies. Our work14 initially focused on characterizing the performance of the medicinal leech by using a clinically relevant swine model. In evaluating leech performance, we found that the average blood meal of a leech when feeding on a congested porcine fasciocutaneous flap was only 2.45 mL. Subsequent passive bleeding from the leech wound after leech detachment on the same flaps averaged only 2.21 mL during the first 2 hours after detachment. The effects of active and passive bleeding resulted in a focal improvement in flap color and perfusion, which was limited to a site only 1.6 cm in diameter, centered on the middle of the leech wound.14 Clearly, leeches have limited ability to decongest a large or severely congested flap. An alternative method for blood removal from congested tissue flaps must be developed to promote tissue survival.

In developing a mechanical device to replace the medicinal leech, we first focused our efforts on a method to improve passive bleeding from the leech wound after leech detachment.15 We developed a mechanical device that provided heparinized irrigation and mechanical agitation of the leech wound. This device increased passive bleeding by 156% during the first 3 hours after leech detachment. How-
ever, the practical effect on the congested fasciocutaneous flap was minimal. In other words, the severe global venous congestion in this extreme model was largely unchanged even with an increased volume of blood removed.

Through evaluation of medicinal leech performance and development of a first-generation device to increase passive bleeding, a second-generation device to completely replace the medicinal leech was developed. This device removed more blood from a congested flap than a leech (under identical conditions) and provided more complete tissue decongestion of larger tissue flaps than a leech.16

The goal of this study was to test the mechanical device during prolonged complete venous obstruction (15 hours) using a clinically relevant fasciocutaneous flap. Measures of flap health, including flap color, surface perfusion, subcutaneous tissue oxygenation, and end point histologic findings were used to evaluate the ability of the mechanical device to treat the venous congestion vs untreated control flaps. Our long-term goal is to develop a commercially available mechanical device with the ability to salvage congested tissue.

METHODS

Twelve mixed-breed pigs weighing 20.2 to 27.6 kg were preanesthetized using intramuscular xylazine hydrochloride (2 mg/kg), tiletamine hydrochloride and zolazepam hydrochloride (Telazol; Fort Dodge Laboratories Inc, Fort Dodge, Iowa) (6 mg/kg), and atropine sulfate (1 mL). The animals were mechanically ventilated with isoflurane (1.5%-2.5%) in 100% oxygen at a maintenance-plus-loss rate (10 mL/kg per hour). The animals were monitored continuously. Institutional guidelines for control animals and at a maintenance rate (10 mL/kg per hour) using a clinically relevant fasciocutaneous flap.

The 12 animals were randomly assigned to control and treatment groups based on a predetermined randomization schedule. The period of complete venous obstruction for control and treatment flaps was 15 hours, which included 30 to 40 minutes just after clamping the VCs during which the flap was sutured back into place and digital photographs and initial physiologic measurements were acquired. The control flaps were not treated for the duration of the experiment. For treatment flaps, up to 3 mechanical devices were used sequentially in a predetermined pattern to treat the flap. At the end of the 15-hour experimental period, all 12 animals were humanely euthanized.

In treatment animals, the first mechanical device was attached 30 to 40 minutes after clamping the VCs. During the first 5 hours after clamping the VCs, this device was used to decongest the flap. At the onset of hour 5, a second device (if needed) was attached and a third device (if needed) was attached at a new site alongside the second device. Through evaluation of medicinal leech performance.

MECHANICAL DEVICE

The device consisted of a handblown glass shell (1.5 cm in diameter and 2.5 cm high) adhered to the skin over a bleeding wound using veterinary-grade adhesive (Nexaband; Closure Medical Corp, Raleigh, NC). The shell had inflow and outflow ports that allowed suction, anticoagulation, and air intake into the glass shell. A full schematic of the device is shown in Figure 2. In total, 6 functional concepts were integrated into the design of this device:

Concept 1: A surgically created bleeding wound. Two crossed stab incisions, each 6 to 8 mm long, were made through the epidermis and dermis using a No. 15 blade. An area, 8 to 10 mm in diameter (centered on the crossed stab incisions), was undermined using a 3.2-mm angled ophthalmic slit knife (Alcon Inc, Fort Worth, Tex). This undermining occurred between the dermal and hypodermal layers.

Concept 2: Suction. Computer-controlled suction was kept constant at –50 mm Hg (LabVIEW; National Instruments, Austin, Tex).

Concept 3: Surface chemical anticoagulation via irrigation. Heparinized isotonic sodium chloride solution (10 U/mL heparin, 200 mL/h) was dripped directly onto the bleeding wound via a 20-gauge hypodermic needle inserted through a port in the glass shell.

Concept 4: Surface mechanical anticoagulation via irrigant turbulence. The suction that was applied to the shell drew in room air via a 22-gauge hypodermic needle inserted through a port.
in the glass shell. This caused turbulence (bubbles) in the irrigant-blood mixture pooled at the skin surface within the shell and created mechanical anticoagulation at the incision surfaces.

Concept 5a: Subcutaneous chemical anticoagulation via injection. Six bolus intradermal injections (0.2 mL each) of concentrated heparin (1000 U/mL) were given just before wound creation (3 equidistant injections within 5 to 8 mm of the proposed wound and 3 equidistant injections at sites 15 mm from the glass shell).

Concept 5b: Subcutaneous chemical anticoagulation via infusion. Concentrated heparin (1000 U/mL per hour) was continuously infused into a hydrogel-impregnated microporous polyethylene disk (6 mm wide \times 2.5 mm high) (Porex Corporation, Fairburn, Ga) that had been inserted subcutaneously into the stab incisions during device attachment. The heparin was delivered to the disk via a stainless steel 15-gauge hypodermic tube. The disk and tubing were bonded using urethane adhesive.

Concept 6: Subcutaneous mechanical anticoagulation via disk agitation. The stainless steel hypodermic tubing attached to the subcutaneous disk was manually turned 360° every 5 to 15 minutes as needed, thus rotating the disk. The hydrogel that impregnated the microporous disk consisted of 10% polyvinyl alcohol (hot soluble) and 2% sodium heparin mixed in sterile water. The microporous disk was impregnated with 0.5 mL of hydrogel under negative pressure. Sigma-Aldrich Corp (St Louis, Mo) manufactured all of the chemicals.

**BLOOD VOLUME REMOVED**

The amount of blood removed from the congested flap was measured at the end of hours 5, 10, and 15. Blood volume was measured by subtracting the total device effluent from the known quantities of irrigant and concentrated heparin infused during 5 hours of collection. Hematocrit and hemoglobin concentrations were monitored in all animals at 4 times: just after VC clamping and at the end of hours 5, 10, and 15. Systemic coagulation function (prothrombin time and partial thromboplastin time) was monitored at 2 times: just after VC clamping and at the end of hour 15.

**FLAP COLOR QUANTIFICATION VIA REFLECTANCE SPECTROPHOTOMETRY**

The flap was visually divided into 4 quadrants, and a color measurement was made in the center of each quadrant (Minolta 508d spectrophotometer; Minolta Co, Ramsey, N J) at 5 separate times during the experiment: before any surgical manipulation (presurgery, baseline color), 20 to 25 minutes after clamping the VCs, and at the end of hours 5, 10, and 15. The spectrophotometer used xenon illumination and measured wavelengths of light (400-700 nm) reflected by 8-mm-diameter areas of skin. The spectrophotometer also measured the L*, a*, b* coordinates of the 3-dimensional color space put forth by the Commission Internationale de l’Eclairage in 1976 (L* indicates lightness; a*, red-green component of the color space; and b*, yellow-blue component).

To evaluate the change in color from the presurgery, baseline skin color, the spectrophotometer was used to quantify ΔE*, which is the Commission Internationale de l’Eclairage standard measure for evaluating total change in color. In general, the ΔE* is the Euclidean distance between 2 color points in the 3-dimensional color space. Values for ΔE*, which incorporated change from presurgery, baseline skin color, for each of the 4 quadrants and for the average of the 4 quadrants, were acquired at 4 different times (20-25 minutes after clamping the VCs and at the end of hours 3, 10, and 15).

**FLAP SURFACE PERFUSION MONITORING VIA LASER DOPPLER IMAGING**

Laser Doppler imaging (LDI) provided a 2-dimensional color-coded image and quantification of the flap’s surface perfusion. The LDI instrument (Perimed Inc, North Royalton, Ohio) used a monochromatic (laser) light source directed at the skin surface. Frequency shifts (Doppler shifts) in the laser light occurred when the light hit moving red blood cells. Detectors measured these shifts within the backscattered light (raw data=voltage) and used this information to produce the 2-dimensional color-coded image of relative perfusion, which was proportional to the number and velocity magnitude of the moving blood cells. The surface perfusion of the entire flap (up to 4096 individual readings) was measured, and the raw voltage data for the entire flap were averaged. All images were taken with the mechanical device turned off so that there were no vibration artifacts in the measurements.

**SUBCUTANEOUS OXYGEN TENSION AND TEMPERATURE MONITORING**

The anterodorsal quadrant was monitored for subcutaneous tissue oxygen tension and temperature using the Licox MCB universal oxygen and temperature monitor. The Licox MCB unit used a temperature-compensated, electrochemically reversible, polarographic microprobe to monitor oxygen tension over a 14-mm² area. A type K thermocouple probe is used to monitor temperature in the same proximity as the oxygen tension probe. Oxygen tension and temperature data were recorded every 5 minutes for the first 60 minutes after ligating the VCs and every 15 minutes thereafter until the end of the experiment.

**HISTOLOGIC EVALUATION**

Two punch biopsy specimens (8 mm in diameter), one from the anterodorsal quadrant and one from the posteroverentral quad-
rant, were acquired from each flap after the 15-hour control and treatment periods. The biopsy samples were fixed in 10% buffered formalin, paraffin embedded, sectioned (at 5 µm), and stained with hematoxylin-eosin. The integrity of the tissue was determined by examining stained sections for signs of (1) epidermal vacuolation, degeneration, or necrosis; (2) vascular congestion and thrombosis; (3) sweat gland necrosis; and (4) hemorrhage. An experienced pathologist (T.F.W.) masked to the experimental condition performed all of the histologic evaluations.

All statistical analyses were performed using SAS statistical software (SAS Institute Inc, Cary, NC).

RESULTS

DIGITAL PHOTOGRAPHY

Figure 3 contains representative images of control and treatment flaps after 15 hours of complete venous obstruction. The mechanical device decongested the flap (except for the posterodorsal corner), as evidenced by the minimal color difference between the flap and the surrounding normal skin. Without treatment, the control flap color progressively darkened, as observed by the very dusky, deep purple color compared with the surrounding normal skin.

BLOOD VOLUME REMOVED AND HEMATOLOGIC EVALUATION

The mean ± SD blood volume removed by each mechanical device was 29.5 ± 24.7 mL/h (range, 13.3-104.0 mL/h) during 15 hours of treatment. Figure 4 shows the volume of blood removed per collection period for all 6 treatment experiments. Hematocrit and hemoglobin concentrations were significantly decreased during the 15-hour experimental period in control animals (15.1%; \( P = .04 \) and 13.4%; \( P = .03 \), respectively) and treatment animals (33.1%; \( P = .003 \) and 32.3%; \( P = .004 \), respectively), with treatment animals more severely affected. Coagulation function was normal for all animals at the end of the 15 hours.

COLOR QUANTIFICATION

The average total color change for the 6 control and treatment experiments at each time are shown in Figure 5. A repeated-measures analysis of variance with a multivariate error structure, followed by linear contrasts, was used to analyze the total color change. A significant time by group interaction was observed (\( P < .001 \)). There was no detectable difference in total color change (mean \( \Delta E^* \) for the 4 quadrants of 1 flap) between the control and treatment at minute 25 (\( P = .32 \)), but there were significant differences at hours 5, 10, and 15 (\( P < .001 \)). Although total color change of the treatment flaps had decreased substantially by 15 hours (ie, the color of the flap was more similar to the presurgery, baseline color), it was still significantly different from baseline color (ie, \( \Delta E^* = 0 \)) (\( P < .001 \)). Furthermore, regression analysis, with a repeated-measures error structure to accommodate the 3 within-pig samples (hours 5, 10, and 15), indicated a highly significant association between the total color change and the cumulative amount of blood removed during the previous 5-hour collection period (Figure 6) (\( r = -0.84; P < .002 \)). The relationship was such that the
more blood removed, the more closely the intratreatment flap color resembled the presurgery, baseline color.

**SURFACE PERFUSION: LDI**

*Figure 7* shows representative LDI scans of control and treatment flaps. The dark blue regions of the flap image are reflective of severely limited surface perfusion, and light blue, green, yellow, red, and brown indicate progressive increases in tissue perfusion levels. Gray regions on the scan are indicative of very dark purple flap coloration where perfusion could not be measured by the LDI instrument because of low levels of backscattered light. Compared with normal levels of perfusion (*Figure 7A*, presurgery scan), severe reduction in blood perfusion was found after the VCs were clamped for 25 minutes (*Figure 7B*). Similarly, almost the entire control flap (*Figure 7C*) had undetectable perfusion after 15 hours of complete venous obstruction, as indicated by the gray area. In contrast, the treatment flap (*Figure 7D*) showed increased surface perfusion after 15 hours of complete venous obstruction (14.5 hours of treatment using 3 mechanical devices) relative to the initial clamped scan (*Figure 7B*).

The averaged raw voltage data for the 6 control and treatment experiments at each time are shown in *Figure 8*. There were no significant differences between mean voltage data for control and treatment flaps when comparing presurgery and 25-minute data (*P* > .14). There were significant differences between mean voltage data for control and treatment flaps when comparing data from hours 5, 10, and 15 (*P* < .005). There were also significant increases in perfusion for the treatment flaps at hours 5, 10, and 15 relative to 25-minute data (*P* < .001). Furthermore, the perfusion in treatment flaps at hour 15 was not significantly different than the presurgery levels (*P* = .20). Regression analysis, with a repeated-measures error structure to accommodate the 3 within-pig samples, indicated a significant association between the magnitude of surface perfusion and the amount of blood removed in the previous 5-hour collection period (*r* = .59; *P* = .009). For control flaps, at hours 5, 10, and 15, the LDI scans progressively showed more areas of undetectable perfusion (ie, there was not enough backscattered light to detect perfusion because the flap color was very dark).

**SUBCUTANEOUS OXYGEN TENSION**

After the first mechanical device was attached, the subcutaneous tissue oxygen tension between the control and treatment flaps diverged (*Figure 9*). A repeated-measures analysis of variance with first-order autoregressive error structure was used to analyze oxygen tension during the 15-hour clamped period. There was a significant time by group interaction (*P* < .001). The 95% confidence intervals were computed at each time for both groups. At 390 minutes, the curves separated with nonoverlapping 95% confidence intervals, and the 95% confidence intervals remained nonoverlapping until 555 minutes, suggesting significant differences in subcutaneous oxygen tension during these times.

**HISTOLOGIC EVALUATION**

In 11 of 12 control flap biopsy samples, there was evidence of vacuolization and degeneration of the epidermis (*Figure 10A*). All 12 control flap biopsy samples showed mild to severe congestion with extravasation of blood in the dermis and hypodermis as well as mild to severe necrosis of sweat glands in the dermis (*Figure 10A and C*). In contrast, after 14.5 hours of treatment, all 12 treatment flap biopsy samples showed no signs of necrosis or congestion in the epidermis and dermis (*Figure 10B and D*). Three treatment flap biopsy samples showed mild congestion in the hypodermis (2 were from the same flap) and only 1 showed moderate congestion in the hypodermis.

**COMMENT**

The goal of this study was to test the effectiveness of a mechanical device to treat venous congestion in a large fasciocutaneous flap model. Development of such a device...
is necessary owing to the known limitations of medicinal leech therapy for the treatment of venous congestion. Previous modern work in this area is limited to 2 externally applied mechanical devices. One device significantly increased flap survival after 5 hours of venous congestion compared with medicinal leech therapy. The other device increased flap perfusion compared with medicinal leeches during 1 hour of venous congestion.
However, although encouraging, these studies involved techniques applied for a limited time in a small animal model. Until this study, use of an externally applied mechanical device to decongest large volumes of congested tissue over a long period has not been attempted in a clinically relevant animal model, to our knowledge.

In testing the mechanical device, 4 areas of device performance or tissue health were evaluated: blood volume removed, clinical appearance (flap color), physiologic tissue characteristics (surface perfusion and subcutaneous oxygen tension), and histologic assessment. The average volume of blood removed by the mechanical device (29.5 mL/h) demonstrated a tremendous increase over the medicinal leech (0.99 mL/h). The larger blood volumes removed by the device were presumably due to the mechanical device tapping into a greater number of larger blood vessels, which are located between the dermis and the hypodermis.

Systemic hematologic evaluation of the animal reflected the large blood volumes removed. The decreases in hematocrit and hemoglobin values in treated animals during the 15-hour experimental period were statistically significant. Therefore, in the future, the amount of blood removed by the device must be controlled at a minimal therapeutic level to reduce the risk of hypovolemia and the consequent need for blood transfusions. However, systemic anticoagulation due to heparin application should not be considered a risk factor for device operation given that coagulation function did not change for control and treatment animals after the 15-hour experimental period.

Currently, flap color is the most relevant clinical measure of venous congestion. In clinical settings, a flap with a dusky, purple coloration is considered congested, whereas normal skin tone is indicative of proper venous drainage. At each measurement time, the mechanical device consistently affected treatment flap color so that the total color change was more characteristic of the presurgery, baseline color than control flaps. This global effect was not observed with medicinal leech therapy. Furthermore, the significant negative correlation between total color change and blood volume removed, although intuitive, can be used for future device development. For example, the amount of blood removed by the mechanical device could be modulated automatically during treatment, based on measures of total color change, to re-
move minimal therapeutic levels of blood and reduce the risk of and need for transfusions.

Although flap color assessment is simple and is the most clinically relevant monitoring tool, the use of physiologic tissue variables such as surface perfusion and subcutaneous oxygen tension is also beneficial in quantifying tissue flap health.20,21 The surface perfusion and subcutaneous oxygen tension data suggested that decongestion therapy using the mechanical device had a significant beneficial effect on flap health by increasing perfusion and improving tissue oxygenation.

The 15-hour testing period ensured that histologic signs of venous congestion and tissue necrosis, such as vacuolated and pyknotic epidermal cells and necrosis of sweat glands in the dermis, were readily apparent in control flaps. The histologic results (along with blood volume removed, clinical appearance, and physiologic tissue features) were encouraging with regard to the ability of the mechanical device to promote tissue survival in conditions that would otherwise lead to severe venous congestion and tissue necrosis, that is, 15 hours of venous obstruction.

Future development and optimization of this mechanical device will include (1) optimization of the subcutaneous disk for simplicity, ease of use, noninvasiveness, bleeding longevity, and bleeding efficiency; (2) optimization of heparin and other pharmacologic agents administered before device attachment and during therapy; and (3) development of automated device control to achieve minimal therapeutic blood removal. By systematic evaluation of these device variables, we believe that a clinically applicable commercial product will be available in the near future. Given the limitations and drawbacks of medicinal leeches, the development of a mechanical device will include (1) optimization of the subcutaneous disk for simplicity, ease of use, noninvasiveness, bleeding longevity, and bleeding efficiency; (2) optimization of heparin and other pharmacologic agents administered before device attachment and during therapy; and (3) development of automated device control to achieve minimal therapeutic blood removal. By systematic evaluation of these device variables, we believe that a clinically applicable commercial product will be available in the near future.

In conclusion, the device was effective in decongesting and surviving a large area of tissue during 15 hours of complete outflow obstruction. This level of effectiveness is unparalleled compared with the current use of medicinal leeches. Considering the many drawbacks of medicinal leech therapy, standard postsurgical treatment of venous congestion may be greatly improved with the development of a mechanical device to replace the use of live leeches.

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