

Augmented blood removal after medicinal leech feeding in congested tissue flaps

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Abstract—Reconstructive microsurgery is performed to reattach, transfer, or transplant body tissues. Venous congestion is a complication that threatens the viability of the affected tissue and is often treated with medicinal leeches. Leech therapy has two phases: active bloodletting and passive bleeding from the leech wound after detachment, which can last for several hours. Unfortunately, the small blood volumes removed by medicinal leeches are generally ineffective in decongesting tissue. Our goal was to develop a device to augment blood removal during the passive-bleeding phase of leech therapy with the use of a porcine model of venous congestion. Results indicated that the use of the device resulted in significant increases in blood retrieval relative to reports of passive bleeding alone (141%, 156%, and 155% in 1, 2, and 3 hours, respectively). These results are an encouraging first step toward development of a mechanical device that completely replaces the use of medicinal leeches in modern medicine.

Key words: *heparin, leech, mechanical device, microsurgery, reconstruction, venous congestion.*

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INTRODUCTION

Modern reconstructive surgery often involves the reattachment, transfer, or transplant of body tissues, and the anastomosis of the associated blood supply. Examples include finger replantations, fibular bone grafts to rebuild a jaw after gunshot injury or cancer, and allogenic transplants of entire hands [1–4]. The use of microsurgical techniques is growing to encompass large tissue regions and is critically important to restoration of function, rehabilitative potential, and quality of life [1]. To ensure positive outcomes, however, one must refine the postoperative care of patients undergoing microsurgical procedures to ensure viability of replanted, transferred, or transplanted tissue (i.e., tissue flaps).

Postoperatively, a complication of reconstructive surgery is blood clot formation within the venous outflow, which leads to venous congestion of the tissue [5]. Subsequently, the tissue flap becomes congested with blood, causing flow to cease. If this complication is uncorrected, cell death results and the tissue flap is lost. In cases of venous congestion, surgical exploration and revision of the venous anastomosis are always indicated [6,7]. Unfortunately, surgical revision is not always feasible and/or successful, particularly in cases of posttraumatic repair. Removing the excess blood via an alternative

method is then essential to avoid cell death and to allow time for the ingrowth of new venous outflow from the surrounding normal tissues.

When venous congestion threatens a tissue flap, live medicinal leeches (*Hirudo medicinalis*) are placed on the congested tissue to remove excess blood [7–11]. The application of medicinal leeches to a congested tissue flap reportedly increases blood flow within congested tissue directly via active feeding and indirectly by passive bleeding from the bite after the leech detaches [6,12]. The continued passive emission of blood following leech detachment assists in the decongestion process. It is facilitated by the actions and interactions among different salivary secretions of the leech, including anticoagulants, vasoactive substances, and platelet aggregation inhibitors [10,13,14]. A leech-induced skin wound on a congested fasciocutaneous tissue flap will bleed passively for a number of hours with 90 percent of blood emission within 5 hours after leech detachment [15]. However, after 3 hours, passive blood loss averages only 2.9 mL. Furthermore, increases of maximum surface perfusion only extend 8 mm from the leech head during active feeding or from the leech bite during passive bleeding [15]. As suggested by this prior report, passive blood loss from a single leech bite should not be relied on to sufficiently decongest an impaired tissue flap, even after a relatively large active blood meal.

There is the potential, however, of augmenting passive blood loss volumes with the use of a mechanical device that facilitates the antithrombogenic environment created by leech salivary secretions left behind at the bite, thus overcoming the hypercoagulable environment of a congested tissue flap. Such a mechanical device may encourage increased tissue viability. Surprisingly, little work has been done to develop a device that either assists or replaces the medicinal leech in the treatment of venous congestion. Although one literature review mentions the invention of an “artificial” leech in 1840, a concept that was again introduced in 1995, little progress has been made in modern medicine toward this end [5,6,16–18].

The goal of this study was to develop and test a working prototype of a mechanical device designed to remove blood from congested tissues after medicinal leech detachment. This device would augment blood retrieval and promote survival of the replanted or transplanted tissue. Our hypothesis was that the use of a mechanical device during the passive blood loss phase of medicinal leech therapy would increase blood loss volumes in a

compromised tissue flap. Increases in blood loss volumes may translate into improved postoperative blood perfusion in congested tissue flaps, resulting in improved tissue flap viability and survival.

METHODS

Mechanical Device Development

The device consisted of a multiport glass chamber, which was secured over a leech bite after leech detachment. The mechanical device is shown in a schematic diagram in **Figure 1**, while the instrumentation involved is shown in a block diagram in **Figure 2**. The mechanical device was based on three main concepts:

1. Irrigation and chemical anticoagulation: Irrigation of the leech bite with heparinized saline was accomplished via an interior capillary tube, which dripped a dilute heparinized saline directly onto the leech bite (10 U/mL sodium heparin in 0.9 percent saline; inflow rate 2 to 5 mL/min via gravity flow).
2. Pulsate suction: The blood-irrigant mixture was removed from the device chamber via an outflow port that applied software-controlled pulsate suction

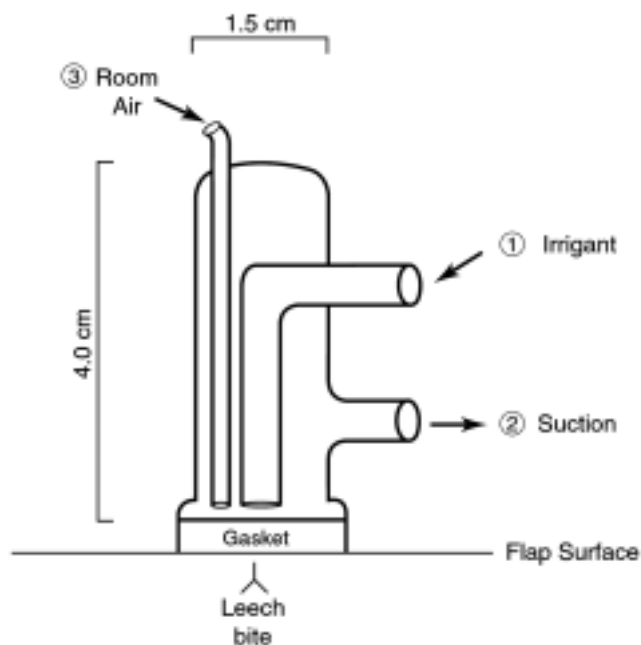


Figure 1.

Mechanical device showing ① irrigation port, ② suction port, and ③ room air entry port.

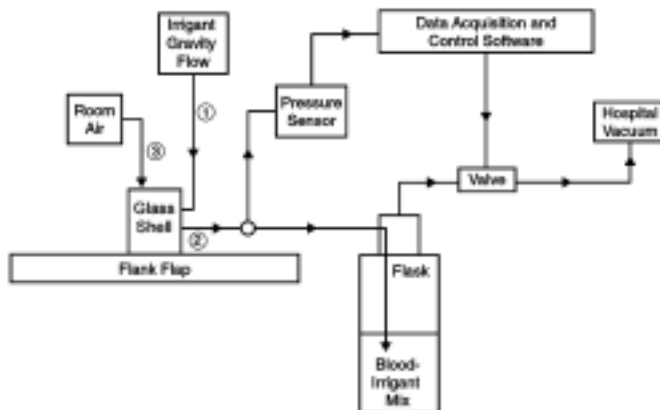


Figure 2.

Instrumentation used to create device components. ① Heparinized saline is dripped onto leech-induced skin wound. ② Suction is applied to remove blood irrigant from device chamber. ③ Room air in device chamber creates bubble turbulence to prevent thrombosis on skin surface.

for the duration of blood collection (-75 mmHg; 15 seconds on and 5 seconds off; Labview, National Instruments, Austin, Texas). Negative pressure was measured in the suction line approximately 10 cm from the glass chamber with a pressure transducer (24 PC Series, Honeywell Corp., Morristown, New Jersey). In addition to removing blood-irrigant mixture from the glass chamber, the suction may have facilitated increased bleeding from the leech bite.

3. Mechanical agitation of the irrigant: When the suction was off, the pressure in the device reverted to atmospheric pressure because of an air inlet. This air inlet also drew room air down to the skin surface and caused turbulence (bubbles) in the irrigant flowing through the device when the pulsate suction was on. These bubbles provided mechanical “agitation-anti-coagulation” of the leech bite, as shown in **Figure 3**.

We constructed a device prototype based on these three conceptual units. The capability of the device to augment blood loss volumes during the passive phase of medicinal leech therapy was then tested with the use of a porcine model.

Animal Surgery

Seven mixed-breed pigs, weighing 18 kg to 23 kg, were preanesthetized with the use of intramuscular xylazine (2 mg/kg), telazol (6 mg/kg), and atropine (0.05 mg/kg). The animals were mechanically ventilated with isoflurane

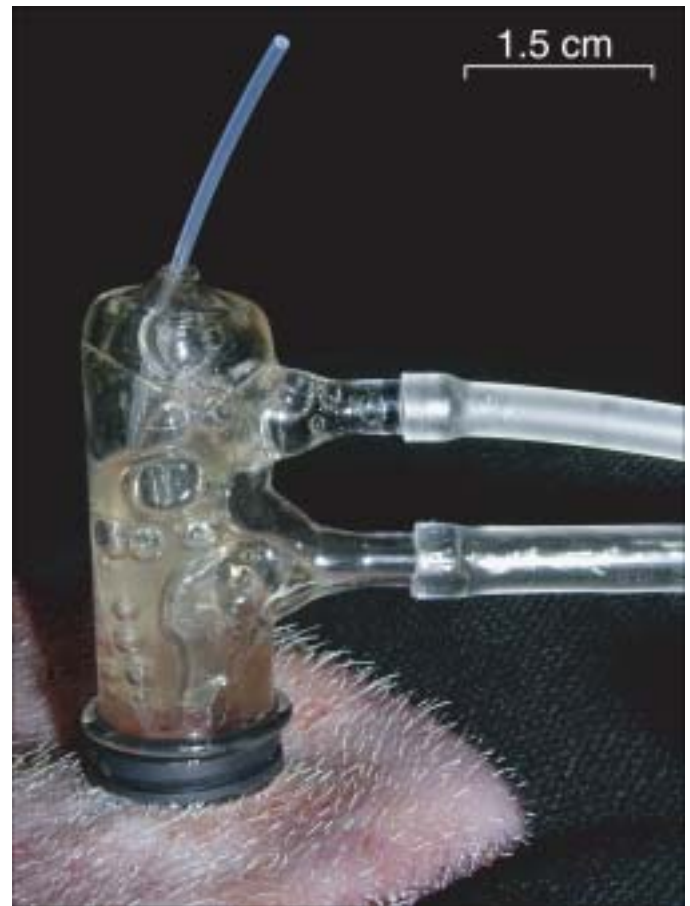


Figure 3.

Mechanical device prototype is seen in use on a congested tissue flap. Glass chambered device was fully functional in irrigating, anti-coagulating, and removing blood-irrigant mixture for all experiments. Bubble turbulence within glass shell prevents thrombosis on skin surface.

(1.5 to 2.5 percent). Isotonic fluids were administered intravenously (IV) throughout the experiment (10 mL/kg/h). Blood pressure and rectal temperature were monitored. A 10-cm \times 10-cm fasciocutaneous pedicle flap based on the superficial circumflex iliac artery and venous comitantes (VCs) was raised, one per animal, as described previously [19]. The right or left flank was used for flap elevation based on a predetermined random schedule.

Model of Venous Congestion and Medicinal Leech Application

Vascular clamps were used to occlude the VCs. The artery was then treated with 0.1 mL of topical lidocaine (4 percent) to prevent and/or treat vascular spasm. Black fabric was placed around the flap edges to isolate it from

the surrounding tissue for better imaging. The flap became visibly congested (dusky purple) 5 minutes after applying the clamps. In addition to relying on flap color, we used a Laser Doppler Perfusion Imager (Lisca, Inc., Mahwah, New Jersey) to verify congestion, which was observed as a marked drop in surface perfusion after the VCs were clamped, relative to preclamped images.

One medicinal leech (*Hirudo medicinalis*; Biopharms, Inc., Burlington, North Carolina) was allowed to attach in the center of the flap's posterior ventral quadrant. The leech fed and detached when satiated. The leech was weighed before and after feeding. Leech weight gain was converted to milliliters of blood based on the average weight of five 1-mL samples of blood (mean = 1.0 g).

Blood Collection and Quantification

Upon leech detachment, we attached the glass housing and underlying rubber gasket over the leech bite using veterinary-grade adhesive (Nexaband). The associated inflow and outflow tubes were connected, and the device was turned on. Intravenous blood samples were taken pre- and postmechanical blood collection. An automated cell counter (Cell-dyne) measured red blood cell counts (RBCC) (per milliliter) on the IV blood samples. We also took RBCCs on the diluted blood-irrigant aliquots that were obtained at 1-hour intervals using a filtration flask. These hourly RBCCs were used to determine the amount of whole blood (milliliters) mechanically collected from the leech bite, based on the following equation:

$$\text{Blood}_A = \frac{\text{RBCC}_A \times \text{Volume}_A}{\text{RBCC}_{IV}}$$

In this equation, Blood_A represents the volume of whole blood collected (milliliter) in an aliquot; RBCC_A represents the RBCC in the blood-irrigant aliquot; Volume_A represents the volume of blood-irrigant collected (milliliter) in an aliquot; and the denominator, RBCC_{IV} , represents the RBCC in the IV blood samples obtained either pre- or postblood collection. For the first hourly aliquot, the precollection IV sample RBCC was used for RBCC_{IV} . For the 2-hour aliquot, the average of the pre- and postcollection IV sample RBCC was used for RBCC_{IV} , and for the 3-hour aliquot, the postcollection IV sample RBCC was used. Three 1-hour blood-irrigant aliquots were collected from all animals, with the exception of two animals, from which blood was collected hourly at hours 1 and 2 only.

Using identical experimental conditions, we calculated the percent increase in blood retrieval using the mechanical device for each hourly aliquot relative to values for passive blood loss alone obtained previously at this site following leech detachment [15]. Specifically, the cumulative volumes of blood collected via passive bleeding alone were 1.66 mL at hour 1, 2.51 mL at the end of hour 2, and 2.93 mL at the end of hour 3.

RESULTS

As shown in **Figure 4**, the average volumes of blood retrieval observed for our device at 1, 2, and 3 hours were significantly greater than volumes retrieved with passive bleeding alone under identical experimental conditions [15]. For each of the hourly time points, the blood retrieval of the device surpassed medicinal leech volumes by at least 141 percent ($t = 3.66$, degrees of freedom (df) = 6, $p = 0.01$).

For passive bleeding following leech detachment, we had previously observed a moderate correlation between the postfeeding leech weight (prefeeding weight plus blood-meal weight) and passive blood loss ($rs = 0.62$, $p = 0.001$) [15]. Because the average leech weights for the passive bleeding experiments were somewhat less than those used

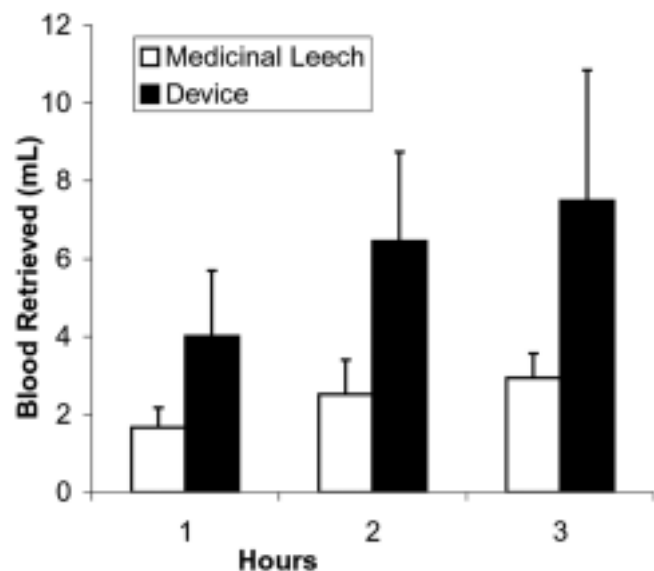


Figure 4.

Use of mechanical device significantly increased blood retrieval relative to passive bleeding alone ($p < 0.05$). Blood volumes are expressed in cumulative milliliters retrieved at end of hours 1, 2, and 3. Error bars represent standard deviations.

in this experiment (3.09 versus 3.83, respectively), we were concerned that leech weight differences might bias a direct comparison of the blood losses. Accordingly, we performed an analysis to determine the importance of this potential bias. We chose the cumulative blood loss at 2 hours for this analysis as a reflection of an intermediate value of cumulative blood retrieval. The best fit regression equation predicting passive 2-hour blood loss was determined to be

$$\text{Passive blood loss} = 0.338 + 0.609 \bullet \text{postfeeding weight.}$$

We used this equation to predict the expected passive blood loss at 2 hours as a function of postfeeding leech weight. We then expressed the observed blood loss at 2 hours with the mechanical device as a percent of the expected passive blood loss for a leech of this weight. When we adjusted for weights in this manner, the weight-adjusted increase in 2-hour blood loss was observed to be 144 percent, only slightly less than the unadjusted increase of 156 percent. Therefore, it is unlikely that leech weights introduced any important biases into our results.

DISCUSSION

Venous congestion is identified in clinical situations by the dusky purple of the tissue flap following microsurgical procedures. For one to treat this condition and to temporarily reestablish blood perfusion within the flap, medicinal leeches are applied to congested tissue flaps where surgical microvascular revision has failed. The protocol typically used in a hospital setting is to place one or multiple leeches on the congested flap, allow them to feed until they detach, and then allow blood to passively flow from the leech bites until cessation. For maximum passive blood loss, the leech bites are frequently wiped with a damp sponge to discourage clot formation, which would halt passive bleeding. Nursing care is vital in this process and can occupy a great deal of time and focus [20]. Unfortunately, despite dedicated staff attention, medicinal leech feeding followed by passive blood emission is often inadequate to decongest large areas of affected tissue [12,21–23]. For tissue death to be avoided, improved postoperative treatment of venous congestion must be developed. As the use of microsurgical replantations and transplantations increases, development of new technology is imperative.

The goal of this study was to develop an automatic device for use in the passive bleeding phase of medicinal leech therapy. Such a mechanical device was designed in

our laboratory to overcome some of the inherent obstacles in medicinal leech therapy, such as clotting of the leech bite (cessation of passive bleeding) and the intense staff time that must be devoted to wiping the leech bite. In our experiments, use of the mechanical device allowed continuous irrigation (chemical anticoagulation), as well as mechanical agitation-anticoagulation of the leech bite. Furthermore, the pulsate suction applied by the device, along with the anticoagulation techniques just described, allowed for a significant increase in blood removed over a 3-hour period as compared to passive bleeding alone. The continuous, automatic nature of the anticoagulation effects tended to surmount the extreme hypercoagulable conditions typical of congested tissue flaps. In a clinical situation, the automated features of the device would translate into a reduced nursing time devoted to wiping and monitoring the leech bites during the passive bleeding phase of therapy. In this sense, further development and use of a mechanical device for removing excess blood appear worthwhile and may contribute to increased flap survival.

We demonstrated that the use of a mechanical device increased blood retrieval by an average of 157 percent, in comparison with blood loss obtained solely via passive emission following medicinal leech detachment [15]. This increase in blood loss suggests increased tissue perfusion relative to purely passive blood loss. However, because we did not directly address perfusion in this study, future work must determine if temporary increases in perfusion are a reality during blood collection via a mechanical device. More importantly, future work must determine if improvements in tissue viability are observed via the use of a mechanical device versus medicinal leech therapy alone.

The success of this study is an encouraging first step toward development of a mechanical device that completely replaces the use of medicinal leeches in modern medicine. Medicinal leeches have limited effectiveness in decongesting a tissue flap and have several additional characteristics that detract from their clinical use, including—

- The negative psychological or emotional impact on patients and families [20,24,25].
- The possibility of infection via bacteria found within the leech [25–27].
- The tendency of leeches to migrate from the desired tissue target [24,25].
- The expense incurred because of the necessity of using multiple leeches over several days [25].
- Costs for continuous staff monitoring of the leeches.

Development of a device that more effectively treats venous congestion will have far-reaching implications for many areas of surgical reconstruction and rehabilitation. Accordingly, after hundreds of years of use in medicine, a replacement for the use of live leeches is warranted and would contribute significantly to modern postoperative patient care.

CONCLUSION

A mechanical device was designed and tested for the removal of excess blood within congested tissues. This device functioned to augment blood removal volumes seen during the passive blood loss phase of medicinal leech therapy. Significantly, larger blood volumes were retrieved via this mechanical device, relative to prior reports of passive blood loss alone. These findings are encouraging in the development of more effective treatments for venous congestion.

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