

Preconditioning with Foam-mediated External Suction on Flap Microvasculature and Perfusion in a Rodent Model

Anita T. Mohan, MRCS, MBBS*†‡
 Lin Zhu, MD*
 Gregory J. Michalak, PhD§
 Alexis T. Laungani, MD*
 Andrew J. Vercnocke, BS§
 Steven L. Moran, MD, FACS*
 Michel Saint-Cyr, MD, FRCS(C)¶

Background: Foam-mediated external suction (FMES) has previously shown to improve tissue microcirculation. We hypothesized that preconditioning fasciocutaneous perforator flaps with FMES would augment perfusion and demonstrate greater capillary recruitment.

Methods: Gluteal perforator flaps were designed on sixteen 400 g rats. Continuous FMES at -125 mm Hg was applied on one side (intervention) to precondition tissue for 5 days, with the contralateral side as a paired control. In group A, we assessed changes following pretreatment, after surgery, and 7 days postprocedure, and in group B, we evaluated changes during preconditioning alone. In group A (N = 8), control and intervention flaps were assessed using laser-assisted indocyanine green fluorescence angiography. In group B, flap regions were assessed using 4-dimensional computed tomographic angiography. All flaps were analyzed for microvessel density using micro-computed tomography and histological assessment using hematoxylin and eosin and CD3 immunohistochemistry.

Results: Thirty-two flaps were included in this study (N = 16 intervention and matched controls). Four-dimensional computed tomographic angiography demonstrated 17% greater tissue perfusion in preconditioned flaps (mean, 78.7 HU; SD, 8.8) versus controls (mean, 67.3 HU; SD, 15.7; $P < 0.01$). Laser-assisted indocyanine green fluorescence angiography showed a 30% higher mean absolute intensity in preconditioned flaps versus controls ($P < 0.01$). Postsurgery mean absolute intensity in preconditioned flaps remained 21% higher than in controls ($P = 0.03$). Preconditioned flaps demonstrated a 2-fold increase in mean vessel volume of 9.1 mm^3 (SD, 7) versus 4.5 mm^3 (SD, 3) in controls ($P = 0.04$); there was a 33% higher mean area fraction of CD31 in preconditioned flaps, 3.9% (SD, 3) versus 2.9% (SD, 3) in controls ($P = 0.03$).

Conclusion: FMES preconditioning has the potential to augment vascularity of tissue for flap harvest; however, further experimental studies are required to optimize strategies and evaluate long-term effects for clinical applications. (*Plast Reconstr Surg Glob Open* 2020;8:e2739; doi: [10.1097/GOX.0000000000002739](https://doi.org/10.1097/GOX.0000000000002739); Published online 18 August 2020.)

INTRODUCTION

The blood supply to tissue and adequate wound healing potential are pivotal to successful flap harvest and reconstructive surgery. Traditionally, preoperative surgical delay of flaps and “ischemic preconditioning” are used to render tissue more resistant against ischemic insults

(including flap harvest) and extend the dynamic vascular territory of a flap. The biological mechanisms underpinning new vessel formation (vasculogenesis) and growth of preexisting vessels (angiogenesis) are subjects of current research.¹ Disadvantages of surgical delay and ischemic preconditioning include the need for additional surgical

From the *Division of Plastic Surgery, Mayo Clinic, Rochester, Minn.; †Restoration of Appearance and Function Trust (RAFT), London, United Kingdom; ‡Department of Surgery, University of Cambridge, Cambridge, United Kingdom; §Department of Radiology, Mayo Clinic, Rochester, Minn.; and ¶Division of Plastic Surgery, Baylor Scott & White, Temple, Tex.

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procedures, time, and anesthesia, and both are relatively invasive. Some factors that induce stress, such as ischemia, heat, toxins, hypoxia, and hypoglycemia,² may provide beneficial changes to the flap microcirculation. Other strategies for preconditioning include hyperbaric oxygen,³ Botox injections,⁴ heat treatment,^{2,5} isoflurane exposure,⁶ pharmacological agents,⁷ and growth factors.⁷ Successions of diverse experimental protocols have been created but not necessarily reproducible in the clinical setting.

Vascularization of tissue is essential to avoid complications in flap surgery and in the preconditioning of tissue and can have the possibility to reduce necrosis and reduce the risk of ischemic complications and the morbidity in patients.^{8–10} There is a need for targeted approaches, supported by scientific rigor, to “mimic” ischemic preconditioning or flap delay, which can enhance the safety and reliability of flap design.

Morykwas et al¹¹ introduced the concept for the potential role of topical foam-mediated negative pressure as a pretreatment to improve flap survival within a small experimental study. In more recent years, a growing body of further experimental data has proposed the use of external volume expansion, including foam-mediated external suction (FMES), as a means to increase vascularization by inducing a degree of hypoxia and mechanical stimulation that can trigger angiogenesis and cellular proliferation in wound healing models and fat grafting in small animal models.^{12–18} The role of mechanical stimulation through device approaches has promoted innovative techniques such as negative pressure therapy, with preclinical data demonstrating improved survival of axial pattern flaps.^{8,9,15,19,20} The roles of external volume expansion and foam-mediated negative pressure have been explored in both preclinical and clinical studies; its application for pre- and post-flap surgery is still largely limited to experimental data. The potential to treat compromised tissues with a modality such as negative pressure for pre- and post-flap surgery may have a greater value than more elaborate interventions and pharmacological agents.

This study evaluated the role of FMES using negative pressure as a form of preconditioning, and alternative to surgical flap delay, in a perforator flap animal model. We hypothesized that the use of foam-mediated topical negative pressure to the flap region, before flap harvest, would result in greater capillary recruitment, dynamic flap perfusion, and improved flap survival when compared with control flaps.

METHODS

Flap Design

This study was carried out in accordance with our Institutional Animal Care and Use Committee (IACUC) guidelines under an approved protocol (Mayo Clinic, Minn.). Based on pilot work, including anatomical dissections, the final design for this perforator flap model was determined. Flaps were based on iliolumbar perforators arising from the deep circumflex iliac artery vessels

that appear at the cephalad edge of the gluteus maximus muscle. Two rectangular 3 × 2 cm² fasciocutaneous flaps (one control and one intervention) were marked over the gluteal region of 400 g male Sprague–Dawley rats. These flaps represented the region of interest (ROI) (Fig. 1).

Preconditioning of the Flap Area

All animals were anesthetized with 1.5% inhaled isoflurane anesthesia for induction and maintenance during the 30- to 45-minute procedure. The gluteal region and back was clipped and depilated (Nair, Church & Dwight CO., Princeton, N.J.). Flaps were marked, and negative pressure using FMES, over a nonadherent silicone dressing, was applied to the intervention side using a commercially available device (Kinetic Concepts Inc., San Antonio, Tex.). A small occlusive dressing, Tegaderm (3M Health Care, St. Paul, Minn.), was placed over the control flap (Fig. 2). Tubing was protected using a coiled spring, and dressings were protected with a soft elastic adhesive tape. The rat was able to freely walk and eat.

Topical continuous negative pressure at –125 mm Hg was continued for 5 days, and then the animals were randomized into 2 groups to assess the effects of preconditioning on the tissue following 5 days of FMES, flap harvest, and 7 days postsurgery (group A, n = 8 rats) or following 5 days of pretreatment alone (group B, n = 8 rats).

Perforator Flap Harvest

In group A, animals were induced using 1.5% inhaled isoflurane and a mixture of 0.75 ml of ketamine (100 mg/ml) and 0.075 ml of xylazine (100 mg/ml) for 1 ml/kg per rat. This allows for 45 minutes of anesthesia, with a 1- to 2-hour recovery period. A mixture

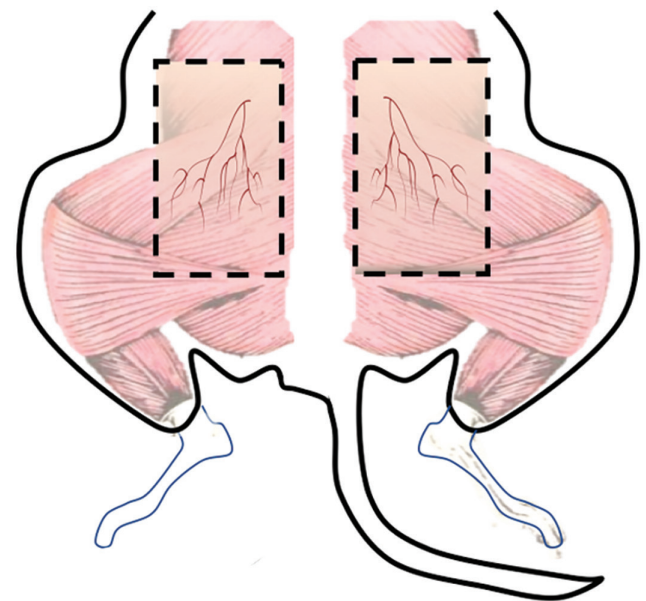


Fig. 1. Illustration of muscle anatomy and location of perforator vessel exiting proximal to the superficial gluteus and biceps femoris muscles and running in the caudal direction to be included in the 3 × 2 cm flap design (black dotted square).

of ketamine (10–20 mg/kg) + xylazine (1.25 mg/kg) provided an additional 20–30 minutes of anesthesia if required. Animals were shaved as needed and prepared with iodinated antiseptic solution. The 2 fasciocutaneous flaps were raised and islanded on the gluteal perforator. Once raised, indocyanine green laser-assisted angiography was used to assess flap perfusion before flaps were reinset.

Outcomes Measures

Outcome measures were assessed using multimodal imaging modalities to evaluate dynamic perfusion and microvessel density within the ROI, followed by histological analysis on all flaps. Figure 2 represents an overview

of the methods, animals, and outcome assessment at each stage.

Laser-assisted Indocyanine Green Fluorescence Angiography

In group A (N = 8 rats), the control and intervention flaps were compared using laser-assisted indocyanine fluorescence angiography (LA-ICGFA) to assess dynamic perfusion at 3 time points: before surgery after negative pressure wound therapy (NPWT) pretreatment period; immediately following flap harvest, and at 7 days postsurgery. Rats were anesthetized with inhaled isoflurane, and the dressing was removed. The flap outlines were remarked and a peripheral intravenous catheter was placed. The first measurement was taken 20–30 minutes following removal of the dressings.

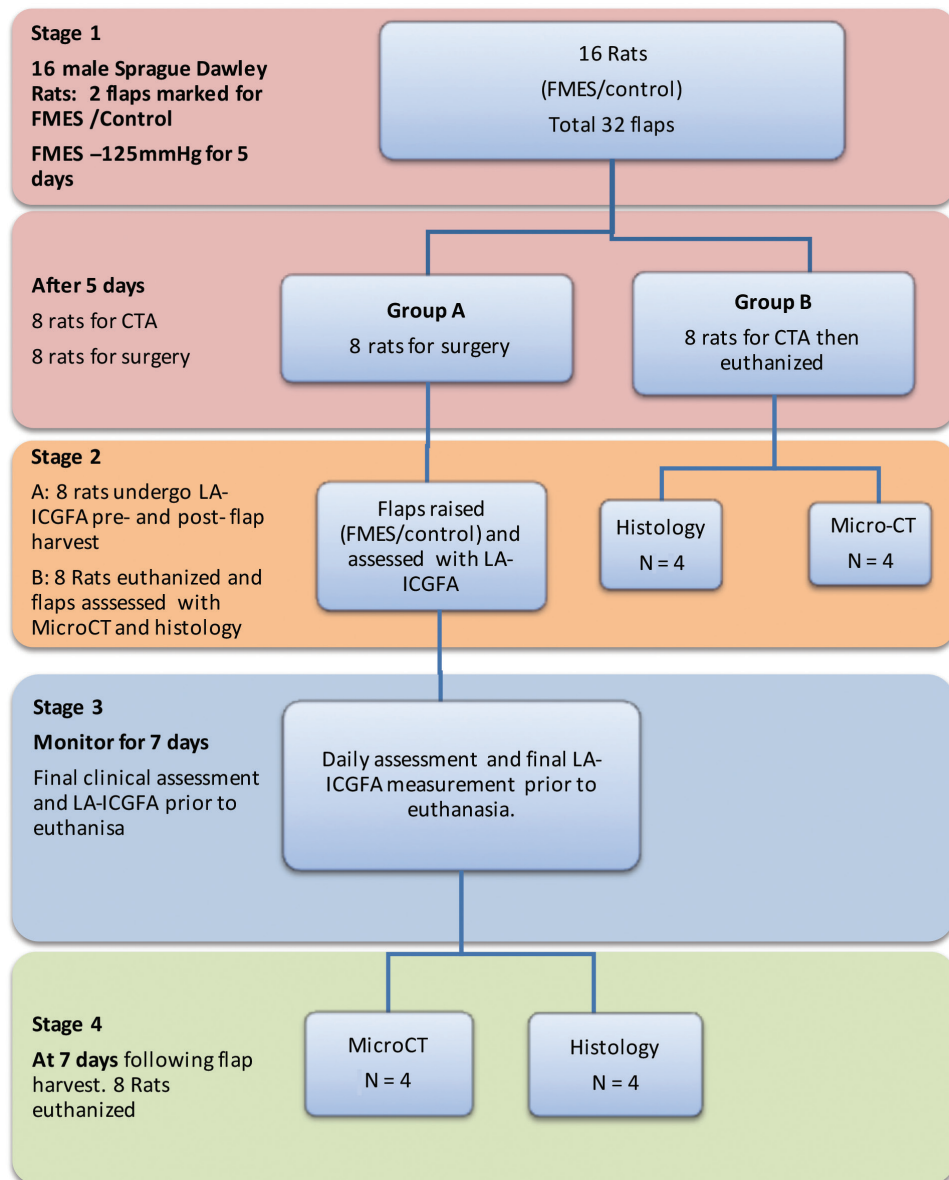


Fig. 2. Overview of methods to show animals, groups, and outcome measures used at each stage of the study.

The perfusion of the tissue was assessed using LA-ICGFA and the SPY Elite System (Novadaq Technologies Inc., Kalamazoo, Mich.). Following anesthesia, 0.1 ml of indocyanine green (2.5 mg/ml; Novadaq Technologies Inc.) was injected into the intravenous catheter. LA-ICGFA was used to assess real-time perfusion over 2 minutes and for direct comparison of control and NPWT pretreated flaps (Fig. 3). Data were acquired before (post-VAC) and after flap harvest (post-flap) and at 7 days postprocedure, before euthanasia. The ROIs for the control and NPWT-treated flap areas were marked, and the average of absolute values of indocyanine green intensity, representative of the degree of perfusion, within each ROI was recorded over all phases of angiography, allowing a comparison of perfusion across matched pairs. Data were analyzed by an independent specialist using SPY-Qc software (Novadaq Technologies Inc.).

4D Computed Tomographic Angiography

In group B (n = 8 rats), following 5 days of FMES preconditioning, the rats underwent 4D computed tomographic angiography (4D-CTA) using a body volume perfusion protocol using 1.5 ml of iodinated contrast [Omnipaque 350 (Iohexol); GE healthcare (Chicago, Ill.)] and scanned on a Siemens FLASH dual source system. The rats were continuously imaged for 97 seconds at a 7-cm range from the base of the tail to the mid-abdomen with the table moving to obtain both craniocaudal and caudocranial data. 4D data were generated by reconstructing the volume between the proximal and distal localization markers, placed at the edges of the flaps. A medium smooth kernel (B41f) was used to reconstruct the images in a field-of-view of 76 mm with a predefined abdomen window level setting of 400/40 HU. Each 5-mm slice was analyzed using the DynEval software, which generated time-attenuation curves in user-defined ROIs drawn over both skin flaps (Fig. 4). Measures across the whole region of the flaps and at intervals were performed to compare

proximal to distal portions of the flap region. Analysis was performed by an imaging specialist (G.J.M.).

Micro-computed Tomography

All rats were subsequently euthanized and flaps were excised and analyzed using micro-computed tomography (micro-CT) and histology. Micro-CT was used to assess the microvessel density and vessel volume per flap to compare the capillary density in the entirety of the flaps (n = 8 rats). The rat was injected with 1000 units of heparin intravenously and allowed to circulate for 5 minutes before euthanasia. Rats were dissected in preparation for sequential flushes of heparinized saline (10,000 units/1000 ml) and 10 ml of 10% formalin, followed by injection of the MicroFil (Flow Tech, Inc Carver, Mass.) that was mixed with diluent (50/50 ratio), making up a 2-ml preparation with an added 5% curing agent. The MicroFil was slowly injected and then allowed to set for 120 minutes. The marked or previously raised skin flaps were then excised and placed in 10% formalin solution for micro-CT. The specimens were placed in paraffin to stabilize the skin flap for the CT scan. The specimens were mounted on a 360-degree rotating stage and scanned with a micro-CT scanner (reference) using an x-ray source with a molybdenum anode and zirconium filter to produce 17 keV x-ray photons. The resulting 3-dimensional images consisted of 20 μ m cubic voxels and were analyzed using the Analyze 12.0 software package (Analyze 12.0; Mayo Clinic College of Medicine, Rochester, Minn.) by an independent reviewer (A.J.V.).

Histological Assessment

Histologic examination was carried out in all flaps. Longitudinal 5- μ m paraffin cross-sections of the tissue were prepared for histologic assessment with hematoxylin and eosin and immunohistochemistry (IHC) staining for the platelet endothelial cell adhesion molecule (PECAM-1; also known as cluster of differentiation 31 [CD31]), an endothelial cell marker for vasculature staining. Tissue sectioning and IHC staining were performed and optimized at the Pathology Research Core (Mayo Clinic, Rochester, Minn.) using the Leica Bond RX stainer (Leica, Buffalo, Ill.). Sections were evaluated using an Olympus BH-2 microscope (Olympus, Lake Success, N.Y.), and images were captured through an Olympus DP72 camera (Olympus, Lake Success, N.Y.). Measurements of epidermal and dermal thickness were assessed in all specimens. Quantitative analyses were performed using the CellSens Dimension software version 1.11 (Olympus, Lake Success, N.Y.). Six different random high-power fields-of-view were examined at $\times 20$ magnification, and thresholds were optimized for identification of the stain. The total summed area and percentage area fraction occupied by vessels were recorded for each ROI. Statistical analyses were performed using a 2-tailed paired *t* test.

Flap Survival

Digital photographs were taken following removal of FMES, immediately following surgery (flap harvest and inset), and at 7 days postsurgery. Flaps were monitored, and Flap survival, degree of observed necrosis, and

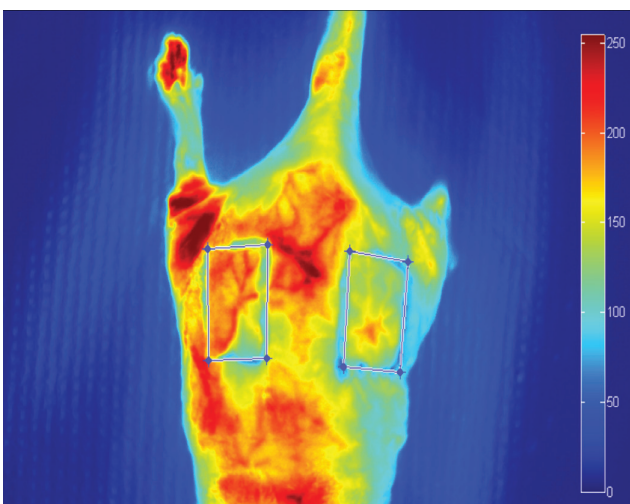


Fig. 3. Flap outlines and SPY imaging demonstrating perfusion during image acquisition for control on the rat's left side and the preconditioned side on the rat's right-hand side.

complications were recorded using digital photographs. Flap necrosis was documented as a percentage of the total superficial flap area.

Statistical Analysis

Rats were alternatively assigned to specific treatment groups. Quantitative variables were summarized with means and SDs or proportions and frequencies as appropriate. For normally distributed, data were analyzed with a paired sample *t* test. A *P* value <0.05 denoted a significant difference. Skin necrosis and complications were assessed using a χ^2 test or Fisher’s exact test as appropriate. Statistical analysis was performed using JMP Statistical Software Version 11. 0.

RESULTS

Sixteen rats were included in the experimental study (32 flaps). Following 5 days of FMES pretreatment on the intervention side, rats were subsequently divided into 2 groups as previously described.

Preconditioned Flaps with Foam-mediated Negative Pressure Therapy Demonstrated Greater Perfusion on LA-ICGFA

In group A, average intensity curves and images of both control and FMES flap ROIs were captured during the different angiographic phases (Fig. 5). Following pretreatment and before flap harvest, there was an average increased ratio of perfusion in pretreated ROIs of 1.3 compared with controls that was statistically significant (*P* = 0.03). The average ingress rate before surgical intervention was 26.5 and 40.0 in FMES pretreated versus control flaps, demonstrating faster rate of blood inflow into the

preconditioned flaps (*P* = 0.03). Following flap harvest and inset, this ratio reduced to 1.1 with no significant statistical difference between intervention and controls (*P* = 0.31). At 7 days postsurgery, only data from 5 animals were suitable for analysis. There was an increase in mean absolute intensity values in both groups, with an average increase of 53.3 in the FMES group compared with 50.2 in controls when compared with the preoperative values. At 7 days postprocedure, the absolute perfusion in the intervention (mean, 189; SD, 27) flaps versus controls (mean, 157; SD, 31) was not statistically significant (*P* = 0.13). Figure 6 shows LA-ICGFA assessment at 7 days postsurgery. Pretreatment with FMES before flap harvest consistently demonstrated a better perfusion ratio at the time of surgery compared with controls; however, both groups demonstrated increased perfusion postsurgery, and perfusion between the groups were comparable 7 days postprocedure.

Preconditioned Flaps with Foam-mediated Negative Pressure Therapy Demonstrated Greater Dynamic Perfusion on 4D-CTA Compared with Controls

Seven rats (14 flaps) were successfully injected for assessment using 4D-CTA. Matched paired analysis demonstrated a 17% increase in tissue perfusion in intervention versus matched control ROIs (*P* = 0.001). Comparison of peak Hounsfield Units (HU) values reached during the time of data acquisition showed a mean peak value of 63 HU (SD, 17) in FMES-treated ROI versus 56 HU (SD, 13) in matched controls (*P* < 0.001).

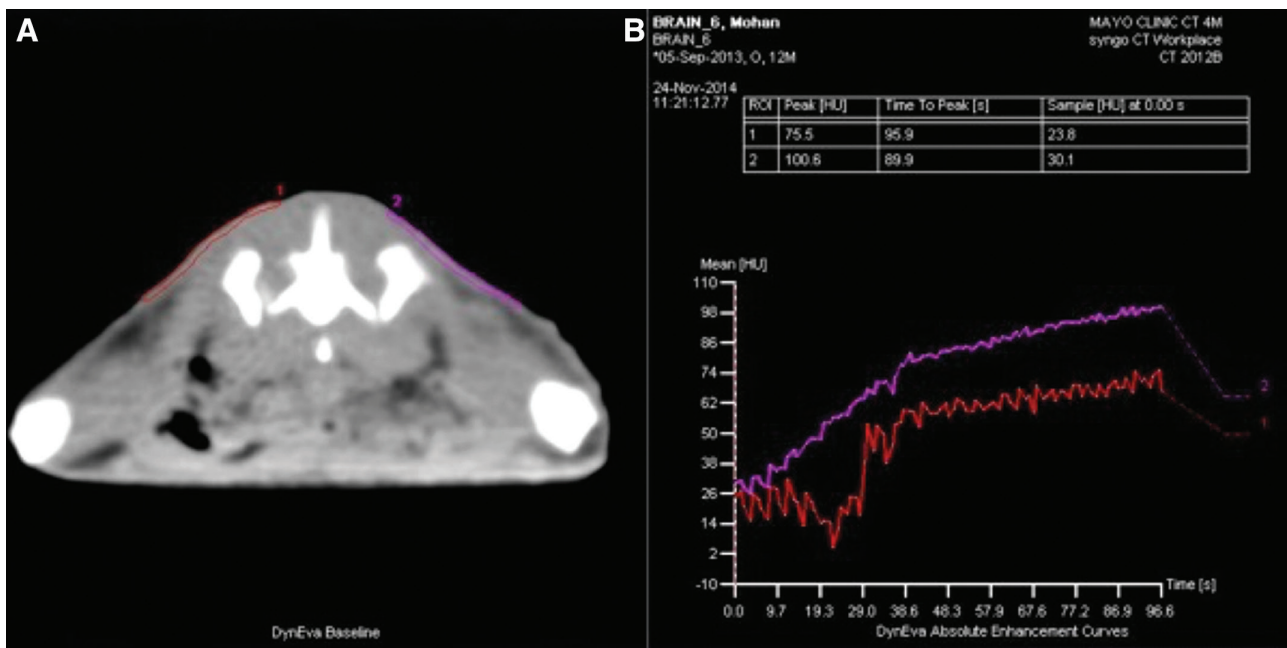


Fig. 4. Example of dynamic computed tomographic imaging assessment in rodent model. A, An axial image of the 4D-CT with a region of interest (ROI) drawn over the preconditioned area (pink) and control area (red). B, Graphs represent the absolute quantity of contrast detected in Hounsfield units, in the respective ROI, against time in seconds.

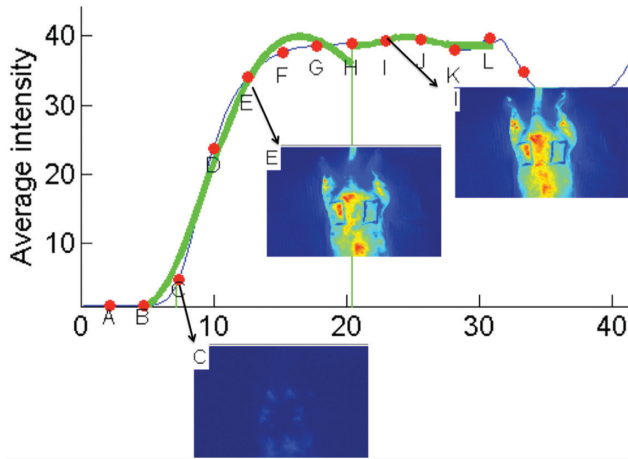


Fig. 5. Example of an average intensity curve and images of control and NPWT flap captured during the different phases of LA-ICGFA assessment following application of FMES before flap harvest.

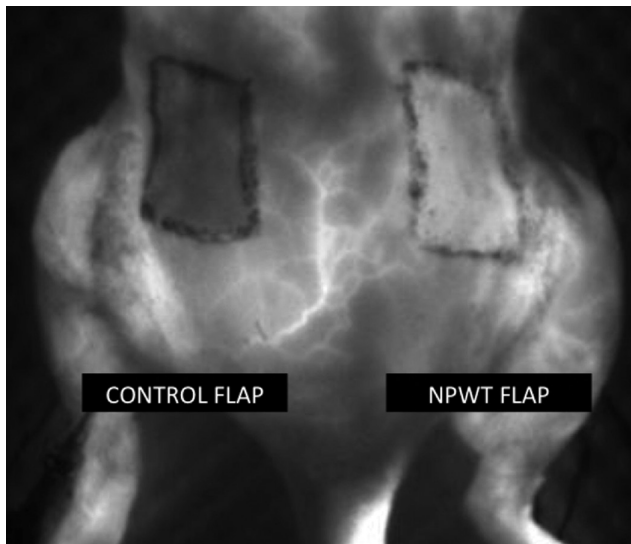


Fig. 6. Video capture using SPY Elite during LA-ICGFA assessment at 7 days following flap procedure. Area of lower or slower perfusion is represented by the darker region (over the control flap) compared with FMES preconditioned flap.

Preconditioned Flaps with Foam-mediated Negative Pressure Therapy Demonstrated Greater Degree of Microvasculature Compared with Controls on Micro-CT

Figure 7 illustrated an example of micro-CT 3-dimensional volume rendering of the control and intervention skin flaps. Overall, the mean vessel volume (mm^3) in FMES-treated flaps were 9.1 mm^3 (SD, 7) versus 4.4 mm^3 (SD, 3) in control flaps ($P = 0.04$). Subgroup analysis demonstrated that vessel volume in the intervention group after treatment (group B rats) was 73% greater (mean, 4.4 mm^3 ; SD, 3) than that in control flaps (mean, 2.6 mm^3 ; SD, 2), but this difference was not statistically significant ($P = 0.23$). At 7 days after flap surgery (group A rats), there was an average vessel volume of 12.3 mm^3 (SD, 8) compared with 5.7 mm^3 (SD, 3) in FMES and in controls, respectively, $P = 0.06$. Overall, there was

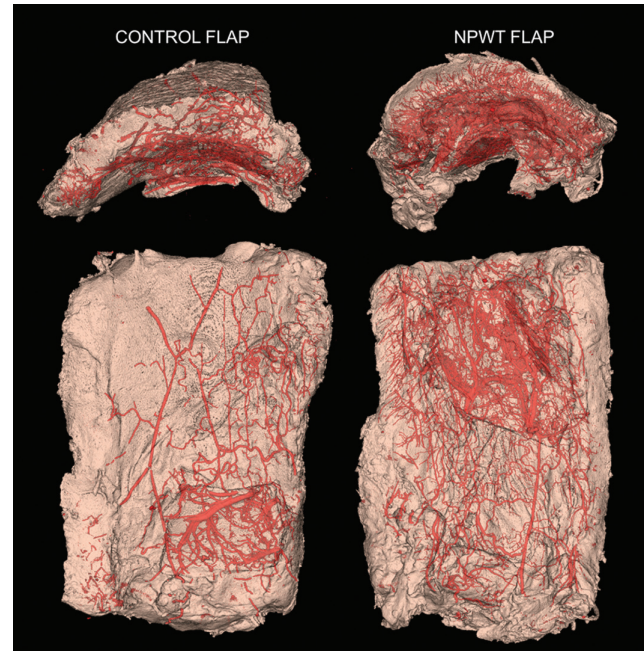


Fig. 7. Example of assessment of Micro-CT using 3-dimensional volume-rendered skin flaps with microvasculature demonstrating transverse cross-section and longitudinal views of control and FMES skin flaps.

significant increased capillary density in the FMES compared with control groups. However, attempted subgroup analysis did not yield any statistical differences between the 2 groups.

Preconditioned Flaps with Foam-mediated Negative Pressure Therapy Demonstrated Greater Degree of Microvessel Density Identified on Histology and CD31 Staining

A total of 32 flaps were fixed in 10% formalin and subsequently paraffin embedded. Full longitudinal cross-sections were cut, and final sections were stained with hematoxylin and eosin and IHC with CD31. On histologic analysis, there was a significant statistical increase in the epidermal thickness ($P < 0.001$) but comparable dermal thickness ($P = 0.34$) between intervention versus control flaps. FMES pretreatment demonstrated greater more dense organization of the collagen than controls. Quantitative analysis showed a total mean area of CD31 stain per high-power field and percentage mean area fraction were higher in FMES compared with control flaps within the entire cohort ($P = 0.03$ and $P = 0.04$, respectively). Analysis of matched pairs at 7 days post-FMES pretreatment period showed a percentage mean area fraction of CD31 stain per high-powered field at 4.1% (SD, 2.8) in the intervention flaps compared with 2.6% (SD, 1.4) in controls ($P = 0.03$). However, assessment in flaps at 7 days postsurgery showed that this difference was reduced, with a mean area fraction of 3.7% (SD, 3.6) in FMES pretreated and 3.3% (SD, 3.2) in matched controls and not statistically significant, $P > 0.05$ (Fig. 8).

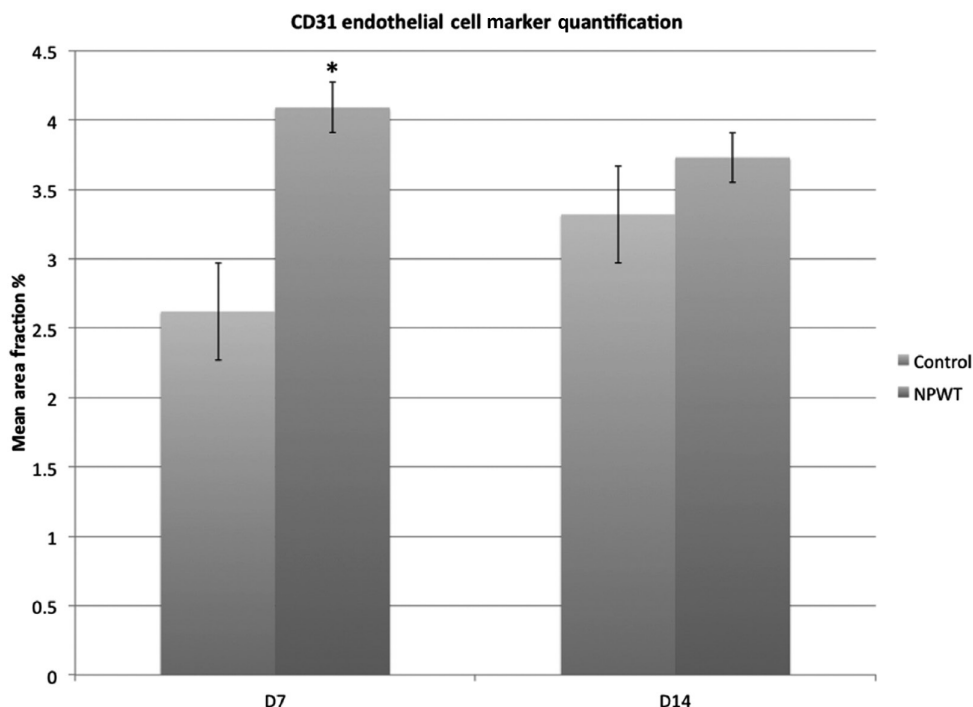


Fig. 8. CD31 quantification based on mean area fraction percent per high-power field showing an increase in the marker following FMES removal at day 7, but this difference was smaller 7 days following surgery, at day 14. *Significance level set at $P = 0.05$, Wilcoxon sign-ranked test. Data presented with mean and standard error (SE) bars.

Flap Viability

There were no major complications directly associated with the application of FMES. Minor skin changes noted as a result of FMES included erythema over the flap region and occasional superficial skin blistering seen at the periphery of the dressings used to secure the FMES, but away from the site of flap. In group A, 3 flaps within the control group experienced partial necrosis, which were recorded as 40%, 60%, and 70% of the total flap area, whereas 1 flap in the FMES group experienced partial necrosis, representing <5% area. Table 1 provides a summary of results and outcome measures to compare control and FMES pretreatment.

DISCUSSION

NPWT or FMES has been well established in clinical practice over the last 20 years.^{21,22} Mechanisms of action in wound healing include increased blood flow,^{18,23} promotion of circulating stem cells into the wound bed,²⁴ fluid removal,²⁵ and stimulation of cellular proliferation promoting wound size reduction and maintenance of wound homeostasis.²⁶ The application of FMES has demonstrated increased capillary vessel caliber, capillary density, and increased blood flow.²⁷

Angiogenesis is an intricate process involving the interaction of multiple genes expressed by a variety of cell types that interact in a complex process. Hypoxia is thought to play a pivotal role as a regulator for the expression of hypoxia-induced factor-1 α and the physical interactions among cells,

extracellular matrix, and angiogenic factors, including vascular endothelial growth factor.^{19,28,29} Microdeformational transduction forces on cells are thought to be influential on the microenvironment and stimulate transcriptional cellular mechanisms that control angiogenesis and vessel remodeling.^{29,30} Static forces applied to skin in murine or rodent models have been shown to induce vascular modeling and promote angiogenesis through increased vessel density, epidermal proliferation and hyperplasia, and increased expression of hypoxia-induced factor-1 α and vascular endothelial growth factor.^{13,17,18,30}

Comparison of “external volume expansion” and “cupping” techniques to apply a negative pressure to wound have demonstrated similar effects of cellular proliferation and vessel remodeling based on mechanical stretch that leads to modulation at a cellular level.³¹ There is growing body of evidence in small animal preclinical models on the impact of wound healing and skin expansion models that has demonstrated the stimulation of external suction on increased angiogenic and adipogenic potential.^{10,12,13,16,19,31,33} External volume expansion has previously demonstrated an influence on histological structure of tissues, and has shown to increase adipogenic potential^{10,17} in fat grafting, and potential utility in diabetic and radiation in small experimental animal models.^{8,13,34}

In contrast, the use of FMES has been described to paradoxically exhibit a positive tissue pressure to the applied area, although this effect becomes less pronounced over time.^{31,35} However, in animal models, the application of foam and sustained or cyclical micromechanical forces has

Table 1. Summary of Outcomes Measures Comparing Control and NPWT Pretreatment of Perforator Flaps before and following Flap Harvest

Outcome Measure	Control Mean (SD)	NPWT Mean (SD)	P
LA-ICGFA			
Absolute perfusion intensity value after NPWT	131 (24)	172 (33)	0.03*
Average ingress after NPWT	26.5	40	0.03*
Absolute perfusion intensity value after flap harvest	67 (27)	99 (63)	0.31
Absolute perfusion intensity value 7 days postoperative	157 (31)	189 (27)	0.13
4D-CTA			
Mean peak value, HU	56 (13)	63 (17)	<0.001*
Adjusted perfusion metric, HU	67 (16)	79 (9)	0.001*
Micro-CT			
Total mean vessel volume, mm ³	4 (3)	9 (7)	0.04*
Total vessel volume, %	0.005(0.004)	0.009(0.006)	0.09
Post NPWT mean vessel volume, mm ³	3 (2)	4 (3)	0.23
7 days postsurgery mean vessel volume, mm ³	6 (3)	12 (8)	0.06
Histology			
Epidermal thickness, µm	38 (9)	60 (9)	<0.0001*
Dermal thickness, µm	1316 (408)	1381 (495)	0.27
Overall CD31 % mean area fraction	3 (2)	4 (3)	0.03*
CD31 % mean area fraction after NPWT	3 (1)	4 (3)	0.03*
CD31 % mean area fraction 7 days postsurgery	3 (3)	4 (4)	0.62

been shown to stimulate proliferative growth and vascular remodeling, including improved survival of axial pattern flaps.^{8,9,15,19,20} There have been a few small experimental animal models that have tried to compare cyclical versus continuous therapy and degree of negative pressure, which both consistently show upregulation of angiogenesis and vessel volume compared to no therapy.^{8,15} The use of FMES approach has been developed in closed incision negative pressure therapy to stimulate healing of surgical defects following primary closure, and there has been recent recognition of FMES benefits in preconditioning tissue in diabetic murine models.⁸

In many studies, a period of 5 days was deemed adequate to see incremental changes of increased angiogenesis following therapy.⁸ Investigation in small experimental animal models has been attempted to evaluate the interface and compare settings of applying external suction to the tissue, but translation to clinical practice will still require further optimization.⁸

This study focused on perfusion and micro-vessel density within the flaps pretreated with FMES compared with controls. In both groups, there was an increased average perfusion after flap harvest following surgery, which may be attributed to a delay effect on the flap. However, we observed a consistent trend of average improved perfusion and increased microvessel density, suggestive of augmented vascularity in the integument following preconditioning with FMES. This difference was statistically significant immediately following the removal of NPWT, although before flap harvest, this difference diminished over time when evaluated at 7 days after flap surgery.

Limitations of this pilot study include the sample size and potential size of the animal model, which may have influenced some of the results. Although there is possibility that FMES may influence surrounding tissue, previous murine models have used similar sample sizes and smaller models that demonstrated a local effect with minimal effect to surrounding tissues.¹³ New protocols were developed

for dynamic imaging using 4D-CTA and LA-ICGFA, which required adequate peripheral intravenous access, but if access was lost, we avoided increasing morbidity with central venous cut-down procedures previously described. However, at every time point, each intervention was perfectly matched to the control flap, which eliminated additional confounding factors. Flap size to assess flap survival was another limitation in this initial experimental model: a perforator flap that extended to induce flap necrosis at the tip would potentially allow a better comparison of flap complications to assess the impact of FMES preconditioning on flap survival. Small animal models are traditionally used to investigate biological phenomena and validate proof of concept prior to large animal or clinical patient studies. Future work includes investigation of defining optimizing degree of negative pressure, timing and duration of treatment.

CONCLUSIONS

The clinical application for the use of noninvasive techniques that are already safe and well-established in practice can provide a direct translational tool in augmenting flap vascularity, which may be of particular significance in high risk patients or when larger loco-regional flaps are required for reconstruction. This pilot experimental study, using multimodal assessment, provides assessment of FMES in a small model of perforator flap and supports the literature on the impact of FMES on tissue vascularity previously described for wound healing and random flap models. It provides the basis to develop further experimental models to validate the developed methodology, with the potential translation to clinical trials and practice.

Michel Saint-Cyr, MD, FRCS(C)

Division of Plastic Surgery
Wigley Professorship in Plastic Surgery
Baylor Scott & White
2401 S. 31st Street
Temple, TX 76508

E-mail: msaint-cyr@BSWhealth.org

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