

Interstitial Delivery of Vascular Endothelial Growth Factor to Skin Flaps

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Objectives: To demonstrate the feasibility of using microporous catheters to deliver a growth factor in a skin flap model, and to determine whether removal of excess fluid by ultrafiltration catheters reduces edema.

Methods: In a controlled study at a research laboratory associated with major teaching hospital, vascular endothelial growth factor was delivered to porcine skin flaps by direct infusion using hollow fiber catheters. Treated flaps received either infusion alone or infusion and ultrafiltration via hollow fibers inserted into the distal portion of the flap. Controls had neither type of catheter placed. The main outcome measure was flap survival and edema.

Results: Treated anterior flaps were found to have increased survival (mean [SD] increase, 49.9% [9.4%]) compared with control flaps (44.1% [4.5%]) for group ($P = .005$) and side ($P = .01$) but not by interaction ($P = .14$). Water content was significant by analysis of variance for group, position, and interaction (all $P < .001$, $df = 31$) for treated (55.3% [9.7%]) and control (61.9% [8.2%]) groups.

Conclusions: This study demonstrated feasibility of using hollow fiber technology to deliver a growth factor to skin flaps. Further study may yield clinical applications for human patients undergoing reconstructive procedures.

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SKIN FLAPS ARE USED FREQUENTLY for the reconstruction of various defects. Failure or suboptimal results can have serious consequences for the patient. A variety of therapies are aimed at improving these results.

Growth factors (GFs) are large polypeptide molecules whose main role is to direct the maturation of cells during the normal turnover process and stimulation of tissue repair after injury; as such, they are integral to wound healing. Basic fibroblast growth factor, platelet-derived growth factor, epithelial growth factor, and vascular endothelial growth factor (VEGF) are well studied; VEGF, the GF chosen for this study, is a potent angiogenic promoter which also increases vascular permeability.^{1,2} Prior work using VEGF to improve skin flap survival in various animal models has been promising. Padubidri and Browne³ and Tucci et al⁴ achieved results showing a significant increase in flap survival when using VEGF in a rat epigastric skin flap model. Zhang et al⁵ found that preoperative subcutaneous administration of VEGF was also beneficial.

Several routes for the administration of GFs have been investigated, including intravenous delivery, subdermal injection, subfascial injection, intra-arterial

delivery, and topical means. Kryger et al⁶ specifically examined VEGF administration in a rat model using multiple techniques and generally found significant increases in survival. Hollow fibers are a novel modality for the delivery of a GF that, to our knowledge, has yet to be studied. The fibers consist of small tubules made from a semipermeable material. The material type and pore size determine what is able to pass through the wall of the tubules. The essence of hollow fiber technology is that very large area-to-volume ratios are obtained. In addition to infusion, the same type of fiber can extract excess fluid when negative pressure is applied. This process, called ultrafiltration, has been studied in a rat model as a means of reducing edema.⁷ Odland et al⁸ found significant improvement in skin flap survival in a rat model in which multiple fibers performed ultrafiltration. The hollow fibers have been studied, in both animals and humans, in the setting of compartment syndrome as a means of edema and pressure reduction in the lower extremity.⁹ The goal of this study was to first design a model for the use of hollow fibers to both deliver GF and perform ultrafiltration and then to examine the impact on flap survival.

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Table 1. Timeline for Treated Animals and Controls

Day	Experimental Group		Control Group
	A	B	
-2 to 5	Acclimation and sling training (5 d)	Acclimation and sling training (5 d)	General acclimation
-1	Infusion of VEGF	Infusion of VEGF and ultrafiltration	
0	Skin flap surgery	Skin flap surgery	Skin flap surgery
1	Infusion of VEGF	Infusion of VEGF and ultrafiltration	Rest/recovery
2	Rest/recovery	Rest/recovery	
3	Infusion of VEGF	Infusion of VEGF and ultrafiltration	
4 to 6	Rest/recovery	Rest/recovery	
7	Flap analysis and animal is humanely killed	Flap analysis and animal is humanely killed	Flap analysis and animal is humanely killed

Abbreviation: VEGF, vascular endothelial growth factor.

METHODS

ANIMAL MODEL

Institutional guidelines for the humane care of animals were followed during the study, and the institutional animal care and use committee approved the protocol used. A porcine model was used in this study with a total of 8 Yorkshire White pigs randomly assigned to either the control group or the treated group. All animals were female and weighed approximately 10 kg.

OVERVIEW OF PROCEDURES

The overall timeline for all groups is detailed in **Table 1**, which also highlights key differences between the treated and control animals. Both groups had undergone identical skin flap surgical procedures; however, the treated animals also had 3 additional sessions during which the flaps received either VEGF infusion alone (group A) or VEGF infusion and ultrafiltration (group B).

The control group did not undergo any other procedures beyond the skin flap surgery. Those animals were simply housed and cared for in the laboratory facility before and after the surgery. It was demonstrated in a prior study⁷ that mere placement of the catheter in a control flap (without vacuum applied) increases water content by about 0.8%, so by *not* placing catheters in control flaps, control flaps endured less trauma and maintained a lower water content, resulting in a more rigorous control group. Furthermore, additional manipulation of repeated anesthetic and hours in the sling would certainly reduce flap survival in the control group. Finally, in clinical practice, there are no catheters placed into the flap. Therefore, a control group without any manipulation other than raising the skin flap is likely to have less edema, improved survival, and be more clinically relevant than a group with placement of sham catheters.

All animals had a single intramuscular dose of ketamine hydrochloride, 20 mg/kg, prior to being transferred to the operating room for any procedure. The skin flap surgery was performed with inhaled halothane. Animals in the treated groups also underwent additional procedures, which required approximately 6 to 7 hours of sedation per treatment using a combination of halothane and propofol. All animals received a single dose of postoperative antibiotics (enrofloxacin) after each procedure. On the seventh postoperative day the animals were humanely killed, after sedation, with direct intracardiac injection of potassium chloride, 40 mEq.



Figure 1. Animal restraint system. The animal is placed in the sling with hollow fiber catheters inserted and taped into position for the vascular endothelial growth factor infusion and the ultrafiltration process.

SKIN FLAP DESIGN

The skin flaps were designed to have a random blood supply with the intention of having, at baseline, the necrosis of at least one-fourth of the flap. This was needed to detect any improvement in the survival of the treated group. Typically, a random skin flap would be dorsally based in the porcine model. However, the sling system used to support the animal during the procedure made access to the distal portion of the flap difficult (**Figure 1**); thus, an alternative design was used. All animals had 4 identical, 4 × 11-cm, ventrally based skin flaps created under sterile conditions. The flaps were elevated directly under the panniculus carnosus muscle. All visible blood vessels predictably encountered at the base of the flap were cauterized in an attempt to create the desired random blood supply. The flaps were then immediately stapled back into position.

HOLLOW FIBER CATHETERS AND GF

The hollow fiber catheters were provided by the manufacturer and are shown in **Figure 2**. The 5-cm fiber, with a 0.45- μ m pore size, was attached to a stainless steel segment and plastic tubing. The VEGF was supplied by Genentech (San Francisco, California). The material was diluted in succinate buffer so that the concentration was 1 μ g/mL. Skin flaps in the treated groups each received a total of 1 μ g per therapeutic session.

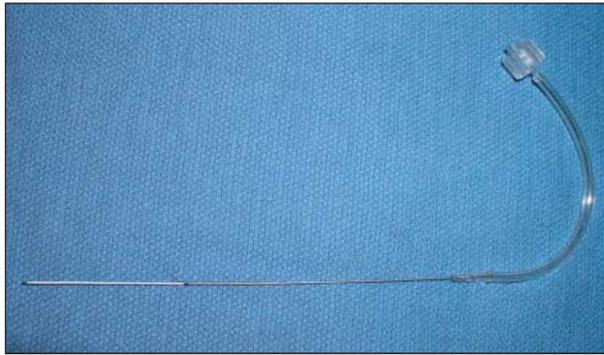


Figure 2. Photograph of hollow fiber catheter device consisting of (from left to right) the 5-cm fiber with 0.45-µm pore size, stainless steel segment, and the plastic tubing with connector.



Figure 3. Photograph of catheter placement in treated flaps. Hollow fiber catheters taped into position on the animal's left side for the vascular endothelial growth factor infusion with or without ultrafiltration.

CATHETER PLACEMENT, VEGF INFUSION, AND ULTRAFILTRATION

All treated flaps had 3 identical catheters placed by insertion into the distal portion of the flap (**Figure 3**). **Figure 4** illustrates the arrangement of the catheters in both group A and group B. Angiocatheters were used to insert the hollow fibers into the treated skin flaps and were then withdrawn, leaving just the fiber. Infusion catheters were connected to plastic tubing and syringes containing VEGF. A pump was used to infuse the VEGF at a specific rate: 1 hour for group A and 2 hours for group B. Ultrafiltration catheters were connected to wall suction throughout the procedure. The goal for each treated animal was to have a total of 4 hours of ultrafiltration. The animals tolerated the sling system (described in the subsection "Skin Flap Design" in this section) for an average of 3.5 hours.

STATISTICAL ANALYSIS

The skin flaps were analyzed on the seventh postoperative day. Photographs were taken of the skin flap and used to determine survival percentage. A blinded observer (V.Y.) later used the BIOQUANT software program (Nashville, Tennessee) to analyze the photographs and classify areas of the flap as "alive," "dead," or "intermediate." This program performs the calculation of the respective percentages in each category as outlined by the ob-

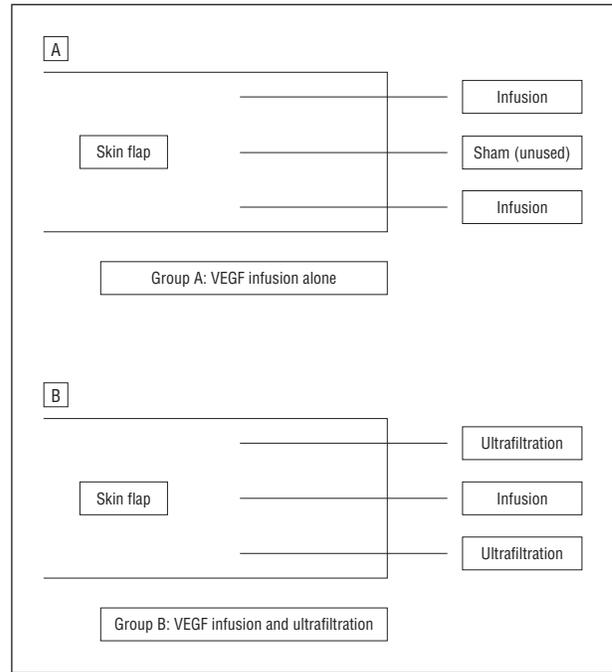


Figure 4. Schematic illustrating catheter arrangement in skin flaps of the treated groups. A, Group A. Infusion without ultrafiltration; the sham catheter was a capped hollow fiber that was not connected to any tubing. B, Group B. Infusion with concurrent ultrafiltration. VEGF indicates vascular endothelial growth factor.



Figure 5. A typical skin flap at time of analysis. Skin flap displays areas with all 3 categorizations: alive (normal color), dead (blackened areas with overlying eschar), and intermediate (discolored but not blackened areas). (Ruler units are in centimeters.)

server. Obviously blackened areas with thickened eschar were considered dead, portions of the flap with entirely normal coloration were classified as alive, and the remaining areas with various degrees of discoloration were deemed intermediate. **Figure 5** is representative of a flap with all 3 categorizations. After photographs had been completed, the skin flaps were harvested, and 5-mm punch biopsy specimens were immediately taken from the midline of the flap at 1 cm, 3 cm, 5 cm, and 9 cm from the distal margin of the flap. The punch biopsy specimens were used to determine water content by the wet-dry method.¹⁰⁻¹³

Punch biopsy specimens were weighed immediately after harvest and placed on an impermeable surface in a climate-controlled laboratory for 1 week, and the dry specimens were weighed again. The difference in wet and dry weight divided by the wet weight was used to calculate the percentage of water content. Finally, the area of the flaps was quantified as cal-

Table 2. Analysis of Variance From Tissue Water Content Analysis

Source of Variation	SS	df	MS	F Value	P Value	F _{crit}
Group	0.13	3	0.04	16.76	<.001	3.00
Position	0.04	1	0.04	13.68	.001	4.26
Interaction	0.05	3	0.02	7.05	.001	3.01
Within	0.06	24	0.002	NA	NA	NA
Total	0.30	31				

Abbreviations: F_{crit}, F critical; MS, mean of squares; NA, not applicable; SS, sum of squares.

Table 3. Repeated Measure Analysis of Variance From Anterior Flap Survival Analysis

Source of Variation	SS	df	MS	F Value	P Value	F _{crit}
Group	0.08	3	0.03	19.07	<.001	4.07
Side	0.01	1	0.01	10.19	.01	5.32
Interaction	0.01	3	0.003	2.41	.14	4.07
Within	0.01	8	0.001	NA	NA	NA
Total	0.12	15				

Abbreviations: F_{crit}, F critical; MS, mean of squares; NA, not applicable; SS, sum of squares.

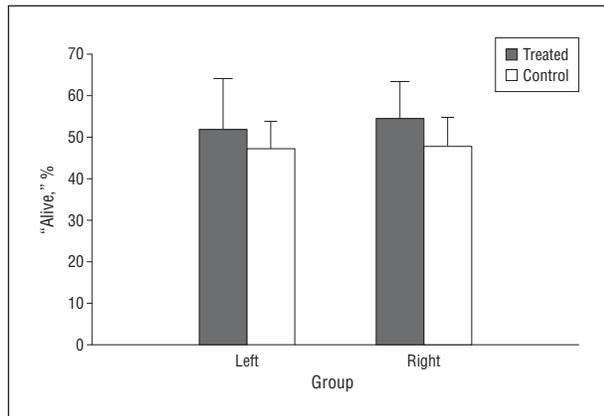


Figure 6. Results for skin flap survival in anterior flaps. Skin flap survival by a blinded observer using the “alive” tissue category. The error bars indicate means (SDs).

culated based on the photographs. Results are reported as mean (SD). $P < .05$ was considered significant.

RESULTS

WATER CONTENT

Treated flaps had reduced water content, which was significant by analysis of variance (ANOVA) for group, position, and interaction ($P < .001$ for all comparisons, $df = 31$) (**Table 2**). Treated flaps had a water content of 55.3% (9.7%), while control flaps had a water content of 61.9% (8.2%). There was no significant difference in mean dry weights between treated and control animals (79 [15] mg vs 75 [17] mg, respectively; $P = .72$ by t test.)

SURVIVAL

Previous work had shown that in the porcine model with multiple skin flaps there was typically better survival of

posterior skin flaps. This proved to be true in this study as well. Posterior control flaps had 89% survival, which was also related to an inability to identify and cauterize all perforating vessels. This high degree of flap survival among posterior control flaps did not allow for a reasonable opportunity to improve survival in the treated groups. Thus, the posterior skin flaps were excluded from the survival analysis. In contrast, anterior flaps had considerably less survival in both groups and thus served as a better model. Repeated-measure ANOVA showed that anterior treated flaps had a significant increase in survival for group ($P = .005$) and side ($P = .01$) but not by interaction ($P = .14$) (**Table 3**). **Figure 6** illustrates the flap survival results.

EFFECT OF ULTRAFILTRATION ON FLAP WATER CONTENT AND SURVIVAL

Ultrafiltration was added to half of the flaps for comparison of findings in the prior rat skin flap studies⁷ that showed reduction of water content owing to ultrafiltration. In the treated flaps, those with ultrafiltration had a mean (SD) water content of 54.9% (9.9%) compared with those treated only with infusion (55.7% [9.5%]). However, this aspect of the study was underpowered and was therefore not statistically different. Survival of flaps that had ultrafiltration in addition to infusion had a trend toward higher survival, but, again, this aspect of the study was underpowered and not statistically significant ($P = .74$).

COMMENT

To our knowledge, this is the first use of hollow fibers to deliver a therapeutic agent such as VEGF. There are theoretic advantages for use of the hollow fiber compared with other techniques. Systemic and intra-arterial routes will often not achieve an acceptable therapeutic

index; thus, direct infusion of the agent into the targeted tissue has been studied. However, drawbacks to conventional means for direct injection include inhomogeneous distribution, backflow along the needle tract, and shearing injury. Alternatively, hollow fibers offer several potential advantages over other systems. Hollow fiber catheters used for drug delivery have been shown to achieve better distribution and higher concentration than other methods.⁹ Improved drug delivery is a result of several characteristics of hollow fiber in tissue, including (1) redundancy of pores, (2) minimal interstitial flow velocity for equivalent volumetric flow rates, and (3) maximal permeable surface area to catheter volume ratios. This reduces backflow and prevents shear injury while allowing for a more homogeneous distribution. Targeting appropriate tissue levels will be a function of concentration, infusion rates, and tissue distribution.

Hollow fibers have the additional capability to perform ultrafiltration, which, alone, has been shown to improve skin flap survival. Fluid removal would, hypothetically, (1) prevent increases in interstitial pressure; (2) enhance convective flow, thereby improving distribution of the agent; and (3) remove excess fluid, metabolites, and solvent. Removal catheters may allow for higher infusion rates and thereby improved mass delivery.

Despite theoretic advantages of the catheter treatment, there were several factors in this model that reduced survival in the treated group. Treated animals underwent 3 additional procedures, which were a stress to the animals. Each of these sessions lasted 7 to 8 hours, much of it while the animals were under deep sedation. Postoperatively, there were periods during which the animals were often restless and agitated as they awoke. This created the potential for trauma to the flap despite efforts to limit such injury. Treated animals also had their diet withheld before procedures, which could be detrimental to nutrition and healing. In addition, repeated placement of the hollow fiber catheters introduced local trauma to the tissues.⁷ These differences, and certainly others, were all possible means by which the treated flaps were "disadvantaged" compared with the controls.

We deliberately aimed to have a rigorous control group, choosing not to subject those animals to the identical stressors of the treated animals. The primary rationale for this was that the control group would then more closely resemble the clinical practice in actual human patients. Patients undergoing a skin flap procedure do not generally undergo other treatments. Thus, control animals did not have sham catheters or any extended sessions of sedation to mimic the treated group. In addition, the goal of this study was primarily to test the overall model (hollow fibers, VEGF, ultrafiltration, etc) rather than to specifically compare the presence or absence of any isolated component (eg, the presence or absence of VEGF or ultrafiltration).

Evaluation of flap survival in this model was fairly straightforward because it was a simple matter of looking for areas of normal color. These areas were categorized as obviously alive, providing a consistent basis for flap survival analysis. The impact of edema on skin flaps has been controversial over the past century with a wide range of opinions as to whether it is detrimental.¹⁴⁻¹⁶ Ul-

trafiltration decreases skin flap edema. Water content, as measured from the punch biopsy sites, is a reflection of edema in the skin flaps. Treated flaps did have a statistically significant decrease in water content ($P = .001$), which may potentially benefit the skin flap.

The inflammation and hyperosmolarity that occur in an acute skin flap result in progressive swelling and a decrease in compliance. In a noncompliant state, the tissue pressures increase rapidly as further edema accumulates.¹⁷ Previous work⁸ has shown that a 10-mm Hg increase in external pressure will lead to a 50% reduction in capillary blood flow. Conversely, a decrease in edema carries the potential for clinically significant improvements in microcirculation. In our study, simply the placement of hollow fibers alone results in some minor trauma and associated edema.⁷ Yet, there was a net decrease in overall water content, which is most likely attributable to the ultrafiltration. In addition, there was improved survival in the random pattern anterior flaps.

This study had some limitations. First, the skin flap design was problematic owing to the fact that the posterior control flaps had very low necrosis (11%); thus, there was little opportunity for improvement. Our efforts to cauterize the visible vessels supplying the flap did not predictably result in the baseline necrosis we expected. A true random flap (dorsally based) in this model had previously shown a rate of necrosis of around 35%. Future study would likely require a true random flap and alterations of the harness system or catheter insertion scheme. Another limitation is that it is difficult to draw conclusions about the benefit or detriment derived from any isolated component of the treatment model.

In conclusion, this pilot study demonstrated the feasibility of using hollow fiber technology to deliver a GF, such as VEGF, to a skin flap. Treated flaps underwent considerably more physical manipulation than control flaps yet showed advantages in terms of decreased water content, less wound contraction, and improved survival. Future studies would require alterations to this model to result in a greater and more predictable necrosis of the control flaps. With further study, this device may have clinical applications in human patients undergoing reconstructive procedures.

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Author Contributions: *Study concept and design:* Anderson, Hom, and Odland. *Acquisition of data:* Anderson and Yu. *Analysis and interpretation of data:* Anderson. *Drafting of the manuscript:* Anderson. *Critical revision of the manuscript for important intellectual content:* Anderson, Yu, Hom, and Odland. *Statistical analysis:* Anderson. *Obtained funding:* Anderson and Hom. *Administrative, technical, and material support:* Anderson and Yu. *Study supervision:* Odland.

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Additional Contributions: Mary Helen Schmidt, BA, assisted with the editorial preparation of the manuscript.

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