

Revascularization of Arterialized Venous Flaps through a Total Retrograde Reverse Blood Flow: Randomized Experimental Trial of Viability

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Background: Arterialized venous flaps (AVFs) have been used for reconstruction of soft tissue defects throughout the body. Several different revascularization models have been performed, but venous drainage through the arterial system has not been studied. In our total retrograde reverse blood flow (TRRBF) perfusion model, the arterial blood flow enters through the venous system and venous drainage exits through the arterial system.

Methods: We developed a novel experimental model in rabbit ears to evaluate the capacity of TRRBF perfusion pattern to allow AVF viability. The ears were assigned to 3 groups: group 1, total devascularization without revascularization (n = 3); group 2, TRRBF (n = 12); and group 3, conventional AVF (n = 12). The ears were observed during a 30-day follow-up period, and clinical serial assessment of edema, cyanosis, and necrosis was performed. Tissue oxygenation was determined at the beginning and end of the follow-up. Histological analysis was performed.

Results: Necrosis was found in 3/3 (100%) ears in group 1, 3/12 (25%) in group 2, and 0/12 (0%) in group 3 (95% CI, 0.505–0.994; P = 0.0001). In group 2, edema was higher (5/12, 41.66%) than in group 3 (0/12, 0%) (95% CI, 0.0135–0.65; P = 0.041). Cyanosis and venous congestion was of greater intensity and duration in group 2 than in group 3 (10.33±4.51 vs 4.5±2.06 d). **Conclusions:** Although evolution is torpid and prolonged in ears with TRRBF, 9/12 (75%) survived, suggesting that TRRBF can be used as a rescue method. (*Plast Reconstr Surg Glob Open 2013;1:e34; doi:10.1097/GOX.0b013e3182a4bc9d; Published online 28 August 2013.*)

n an arterialized venous flap (AVF), arterial blood flow enters through an afferent vein and venous blood flow exits through an efferent vein.^{1–3} This type of revascularization was originally introduced

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Received for publication March 26, 2013; accepted June 20, 2013.

Copyright © 2013 American Society of Plastic Surgeons. Unauthorized reproduction of this article is prohibited. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 3.0 License, where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially. DOI: 10.1097/GOX.0b013e3182a4bc9d in the late eighties. Although it is now infrequently studied, it remains in use. Clinical use of these flaps has been limited because of the high occurrence of partial or total flap necrosis and a slow healing process. Several experimental studies and clinical applications have been performed to evaluate whether different surgical procedures could enhance flap perfusion and flap survival.^{2,4–15} These experimental procedures include increasing angiogenesis by flap preexpansion,^{4,7,8} prearterialization technique,^{4,9,10} and surgical and pharmacological flap delay procedures.^{2,4,11,16–20} These procedures have been used successfully to increase flap blood flow and tissue oxygenation.

Disclosure: The authors have no financial interest to declare in relation to the content of this article. The Article Processing Charge was paid for by the authors. In 2010, Yan et al²¹ reported guidelines for improving flap survival, which included arterialization through a small afferent vein, increasing venous drainage through multiple efferent veins,²² designing the flap over a venous network, and restraining arteriovenous shunting.^{23,24} Using these guidelines can increase total flap size and survival area of AVFs.

Three types of AVFs based on perfusion patterns in relation to venous valve orientation were also reported¹⁵: anterograde perfusion pattern, retrograde perfusion pattern, and mixed perfusion pattern. The majority of reported AVFs have anterograde perfusion patterns.^{1–3,9,11–13,15,17,20,22,25–27} Although the majority of evidence suggests that perfusion pattern has no influence on flap survival, there are some reports that retrograde perfusion patterns can enhance flap perfusion. There are also other classification systems, including those based on the number, location, and configuration of flap vessels. These factors may influence flap survival.^{15,21,23}

Yoshimura et al²⁸ were the first to use AVFs clinically, and AVFs have since been used for the reconstruction of soft tissue defects of the upper extremities, face, cranium, and neck.^{2-4,13,25,27,29-33} Composite flaps have been used for reconstruction of nerves, vessels, and tendons,^{13,21,34-37} while AVFs have also been used in reconstruction of small hand defects. Small-sized AVFs (1×1 cm up to 3×10 cm) have a 95–100% survival rate. AVFs with larger surfaces often suffer partial necrosis and have a 40% failure rate. AVFs in all reported series, regardless of flap size, are at risk of sustained postoperative congestion (100%),^{4,22,29,38} partial flap necrosis (20%), and total flap necrosis (12%).²¹

There are multiple experimental and clinical models of AVF revascularization, although a total retrograde reverse blood flow (TRRBF) perfusion pattern has never been described. In TRRBF, the arterial blood flow enters the flap through the venous system and the venous drainage exits the flap exclusively through the arterial system. Thus, the aim of this novel experimental study was to evaluate the capacity of a TRRBF perfusion pattern to enhance AVF viability. The chosen experimental model was the rabbit ear because it contains a well-defined vascular pedicle and a valved venous system.^{2,10,16,17,39,40}

MATERIALS AND METHODS

This is a pilot study designed to evaluate whether this form of revascularization may maintain viable tissues. For this reason, 15 healthy female and male New Zealand rabbits, weighing 3–3.50 kg, were used. Twenty-seven ears of these rabbits were used in the experimental surgical model. Anesthesia was induced with an intraperitoneal injection of a mixture of ketamine (80 mg/kg) and epontol (20 mg/ kg) with supplemental doses administered as necessary. Preoperative antibiotic prophylaxis was administered with a single intraperitoneal injection of amoxicillin (100 mg/kg). The ears were assigned to 3 groups: group 1, total surgical devascularization (n =3); group 2, TRRBF using the right ears (n = 12); and group 3, AVF using the left ears (n = 12). The surgery was performed under sterile conditions. Surgical dissection was performed under operating microscope magnification (6×). End-to-end anastomoses using interrupted sutures with 10-0 and 11-0 nylon were performed under microscope magnification $(16\times)$. Immediate postoperative lidocaine infiltration was administered in the base of the ears for anesthesia. The same experienced microsurgical surgeon (M.I.) performed all of the surgical procedures. This protocol was approved by the Research in Animals Committee and the Investigation Department of the National Institute of Medical Science and Nutrition "Salvador Zubirán" (CEX-003-94-94-1).

Surgical Technique in Group 1

Initially, a circular incision was made at the base of the ear. Skin, cellular subcutaneous tissue, muscles, and perichondrium were cut. All vascular elements were ligated and cut. The purpose of this incision was to achieve a complete surgical devascularization of the ears. Revascularization procedures were not performed. Wounds were closed using 3-0 silk sutures (Fig. 1).

Surgical Technique in Group 2

A 2-cm incision was made over the vascular pedicle in the dorsal aspect of the right ear. The artery and the main vein were identified, dissected, and isolated within 1.5 cm. Both vessels were cut with scissors. Subsequently, a circular incision was made throughout the base of the ear, as in group 1. Revascularization of the ear was performed as follows: the proximal recipient artery was anastomosed to the central vein of the ear and the proximal recipient vein was anastomosed to the artery of the ear. The patency of both vascular anastomoses was confirmed visually for 20 minutes. Finally, the wound was closed with simple 3-0 silk sutures. This constituted an AVF with TRRBF (Fig. 2, right ear).

Surgical Technique in Group 3

Identification of vascular elements and surgical devascularization were performed in the left ears as in group 2. Revascularization of the left ear was performed as follows: the proximal recipient artery was anastomosed to the central vein of the ear and the



Fig. 1. Circular incision for total devascularization of the ear. The artery and the vein within the pedicle are ligated and cut. Revascularization was not performed. A, artery; V, vein.

proximal recipient vein was anastomosed to other vein of the ear. The patency of both vascular anastomoses was confirmed visually for 20 minutes. Finally, the wound was closed with simple 3-0 silk sutures. This constituted an AVF with retrograde perfusion pattern (Fig. 2, left ear).

Follow-up and Evaluation

The rabbits were maintained under daily observation for 30 days. The animals were housed in individual boxes with temperature control (20–24°C). Ad libitum access to water and food was provided. Postoperative treatment included intraperitoneal administration of amoxicillin (100 mg/kg) every 24 hours for 3 days and 5 drops of metamizole in the rabbit's drinking water every 12 hours for analgesia.

Oxygen saturation (PaO_2) was determined in all ears with a transcutaneous oximeter placed at the most distal region of the ear. Measurements were taken immediately after anesthesia (day 0) and just before euthanasia (day 30). Both measurements

were recorded without supplementary oxygen administration. The purpose of these evaluations was to assess for differences in PaO_2 between the different forms of revascularization.

Viability of the ears was evaluated clinically every 24 hours until the end of the study. Tissue viability was determined by the color of the ear. A color scale was established and printed (pink = normal, blue and violet = mild cyanosis, purple = intense cyanosis, and black = necrosis). Observers at each assessment used this printed scale. There were only 2 observers. These changes were registered as: normal = 0, mild cyanosis = 1, severe cyanosis = 2, and necrosis = 3. The duration (in days) of the coloration changes was recorded. The necrotic area was expressed as a percentage of the total ear surface. A graduated pattern was used to assess the necrotic area in the ear. The necrotic tissue was defined when the tissue perfusion was not detected, with loss of firmness and warmth, and when the color skin changed to black. Failure was defined as necrosis of more than 50% of the ear



Fig. 2. Right ear revascularization with total retrograde reverse blood flow. Left ear revascularization as an arterialized venous flap with retrograde perfusion pattern. Red arrows show the direction of blood flow. Blue arrows show the direction of venous drainage. A, artery; V, vein.

surface. Success with partial necrosis was defined as necrosis of less than 50% of the total ear surface.

Edema of the ears was also recorded. The edema was evaluated using a clinical scale: grade 0 = without edema, grade 1 = bulking edema without the loss of the shape and creases, and grade 2 = bulking and deforming edema with loss of creases. Duration of edema was recorded. The clinical changes of the ears were documented by clinical photography.

At the end of the follow-up, all rabbits were euthanized with an intraperitoneal lethal overdose of sodium pentobarbital. Biopsies of the arterialized vein of the main pedicle were taken. To evaluate tissue biopsy away from the main vessels, full thickness biopsies, including skin, subcutaneous cellular tissue, cartilage, and vascular network, were taken from the middle of the ear in the area located between the central pedicle and the lateral edge of the ear. Ears that showed necrosis were not sent to pathology.

Sections were stained with hematoxylin and eosin and examined. Endothelial hyperplasia was evaluated and classified: grade 1 = vascular occlusion less than or equal to 25%, grade 2 = vascular occlusion between 25% and 50%, grade 3 = vascular occlusion between 50% and 75%, and grade 4 = vascular occlusion greater than 75%.

Statistics

The results were expressed as percentages, means, and standard deviations. Mann–Whitney and Friedman–Kaplan tests were used. In the comparison of proportions we used χ^2 tests (with adjustments to several if necessary). Differences were considered significant at P < 0.005. Confidence intervals (CIs) were constructed at 95%. Statistical analysis was performed using Minitab statistical software version 13.1.

RESULTS

The necrosis rates were 3/3 (100%) for group 1, 3/12 (25%) for group 2, and 0/12 (0%) for group 3 (95% CI, 0.505–0.994; P = 0.0001). Necrosis was significantly different between group 2 and group 3 (95% CI, 0.005–0.494; P = 0.046). This statistical difference was small because of the sample size. The χ^2 test was used.

O₂ Saturation

The difference between initial and final PaO₂ measurements was minimal for group 3 and greatest for group 1. The drop of PaO₂ measured in group 2 was between groups 1 and 3. There was a significant overall difference in the final PaO₂ measurements among groups 1–3 (0, 55.6±41, and 91.3±35, respectively) (95% CI, 2.06–81.3; P = 0.0001). The initial mean PaO₂ in group 2 was 94% (90–97%) and

the final measure was 84% (73–90%). The initial mean PaO₂ in group 3 was 95% (90–99%) and the final measure was 91% (86–99%). For each one of the surviving ears of group 2 and group 3, the difference (delta) between initial PaO₂ and final PaO₂ was calculated. The mean value of the delta was significantly higher in group 2 than in group 3 (10.44% ± 5.2% vs 3.41% ± 1.78%; 95% CI, 2.99–9.99; P = 0.002).

Viability

In group 1, 3/3 (100%) ears reached the worst possible color grade (necrosis), whereas only 3/12(25%) of group 2 ears and 0/12 (0%) of group 3 ears reached this grade. There was a significant overall difference between these groups (95% CI, 0.505– 0.994; P = 0.00001). Group 1 ears and group 2 ears reached their worst color grade on different days, although this difference was not statistically significant $(2.66 \pm 0.57 \text{ dvs } 10.33 \pm 4.51 \text{ d}; 95\% \text{ CI}, -18.93 \text{ to } 3.63;$ P = 0.10). This statistical difference was low because of the sample size. In group 2, 3 ears developed partial necrosis (10-20%) of up to 20% of the ear's total surface. This necrosis was exclusively localized to the proximal region where the vein was arterialized. A 15% necrosis was also seen in the same place in 1 ear from group 3. In group 2, grade 1 and grade 2 ears recovered normal coloration (grade 0) at 10.6 ± 6.09 days. In group 3, grade 1 and grade 2 ears recovered normal coloration (grade 0) at 4.5 ± 2.06 days. Grade 3 coloration (necrosis) never occurred in group 3.

Edema

Edema was not recorded in group 1. In group 2, grade 2 edema occurred in 5/12 (41.66%) ears compared with 0/12 (0%) in group 3 (95% CI, 0.0135– 0.65; P = 0.041). In group 2, 2/5 (40%) ears that had grade 2 edema experienced necrosis, while the other three survived (95% CI, -0.40 to 0.80; P = 0.05). Grade 2 edema developed in the early recovery days and evolved with necrosis of the ear. The necrotic ears reached maximum edema at 1.33 ± 0.23 days and the surviving ears at 6 ± 1 days (95% CI, -0.40 to 0.80; P = 0.148).

Histology

The histological results of the surviving ears are shown in Tables 1 and 2. There was no significant difference between the groups. In group 2, the reduction in PaO_2 allowed ear survival, but was accompanied by 5 cases of skin atrophy, 2 cases of chronic inflammation, and 2 ears with normal skin characteristics (Table 1). Subendothelial hyperplasia (SH) of the arterialized vein was found in both groups. SH was mostly grade 1. Grade 3 SH was identified in only 1 ear in group 2 compared with 2/12 ears in group 3 (Table 2).

Skin Quality	Group 2	Group 3	Р	95% CI
Normal	2/9 (22.2%)	02/12 (16.6%)	0.751	-0.288, 0.331
Atrophy	5/9(55.5%)	10/12 (83.4%)	0.160	-0.664, 0.109
Chronic inflammation	2/9 (22.2%)	0/12 (0%)	0.109	-0.049, 0.493

Table 1. Cutaneous Histology of Viable Ears (Group 1 vs Group 2)

DISCUSSION

In all clinical and experimental AVFs, the entry of arterial blood flow occurs through an afferent vein and the venous drainage occurs through one or more efferent veins. AVFs have demonstrated their clinical applicability, therefore expanding the possibilities of transfer and replantation of artery-free tissues.⁴¹⁻⁴⁴ The physiologic perfusion and survival mechanisms of AVFs remain controversial. Currently, 3 main theories have been postulated as to the physiology of the venous flap. These include "A-V shunting," "reverse flow," and "capillary bypass." Neovascularization and perivenous areolar tissues were also hypothesized to play a role in the survival of venous flaps.4,45

To evaluate the capacity of the TRRBF to allow AVF survival, we developed a novel experimental model in rabbit ears. The experimental model in rabbit ears is ideal, as shown in previous studies.⁴⁶ Revascularization was performed in previously devascularized ears, mimicking an AVF with a retrograde perfusion pattern and thereby forcing blood flow through the entire venous system of the ear. In this manner, the efferent blood flow exits the flap through the only available efferent vessel, which is the artery of the flap that had been anastomosed to the recipient vein.

Total devascularization of the ear without revascularization procedures caused a 100% necrosis rate (group 1). The 3 ears in the study all showed very similar clinical evolution and complete necrosis at 2.66 ± 0.57 days, suggesting that this would be typical even with a larger sample size. These data suggest that arterial afferent blood flow is required for flap survival. The surgical revascularization performed in AVF ears (group 3) exhibited a significantly lower rate of necrosis than total devascularization without revascularization (group 1) (P = 0.0001).

To our knowledge, AVF with TRRBF has not been previously reported. The position of the venous valves impedes the advancement of retrograde blood flow in the venous system, and thus hinders the perfusion and viability in the distal tissue. We developed our model in this way to evaluate its feasibility. Although a higher necrosis rate was observed in group 2 ears than in group 1 and 3 ears, this rate was within the ranges previously reported with traditional AVFs (0-42.3%).²¹ Our 100% success rate with AVFs with a retrograde perfusion pattern is identical to the success rates reported by Koch et al⁶ and Woo and Seul.8

The clinical course of TRRBF ears (group 2) was characterized by edema and cyanosis occurring over a 10-day period, compared with the 4.5-day period observed in the AVF ears (group 3). The edematous and cyanotic period observed in group 3 ears is within the 3- to 6-day period reported by other authors.8 Higher intensity edema and cyanosis was observed in group 2 ears than in group 1 and 3 ears, which is attributable to the high resistance to flow within the artery. These signs were more evident in the vicinity of the arterialized efferent veins, causing partial necrosis proximal to the wound site in 3 ears of the group 2 and 1 ear in group 3. After 2 weeks, necrotic areas healed by secondary intention. The percentage of partial necrosis was similar to the results previously reported.^{4,44} There was no significant difference in the clinical courses of group 2 and group 3.

The TRRBF ears (group 2) showed a significant reduction in PaO₉ at the end of the study when compared with AVFs with retrograde perfusion patterns (group 3). The results observed in group 2 are consistent with previous studies of AVFs examining fibrosis and atrophy as primary endpoints.^{5,6,15} It was also suggested that the presence of a good recipient bed where revascularization will take place after 3 days might prevent the appearance of alterations like fibrosis and atrophy. In our experimental model, the survival of the ears depended exclusively on the re-

Table 2.	Subendothelial	Hyperi	plasia Grade
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Grade	Group 2	Group 3	Р	95% CI
1	4/9 (44.4%)	7/12 (58.3%)	0.52	-0.566, 0.289
2	4/9(44.4%)	3/12(25.0%)	0.34	-0.212, 0.601
3	1/9 (11.1%)	2/12 (16.6%)	0.71	-0.349, 0.238

Grade 1: <25% occlusion of the lumen. Grade 2: 25-50% occlusion of the lumen. vascularization. However, there was no recipient bed in our model.

Our experimental model did not fulfill the previously reported requirements for improving AVFs survival.⁴ The arterialization of the flap was performed in the main vein of the ear instead of using a small diameter vein. Additionally, there was only 1 efferent vessel with high resistance to flow. However, the survival rate at the end of the follow-up was 75% for TRRBF. Flap survival increased to 100% when we had endowed ears with an efferent vein with low resistance to flow (P = 0.046). For both groups, the survival rates were similar to those previously reported.^{4,21,22,29,38}

If a total vascular occlusion develops, collateral circulation would already be formed and may allow flap survival. Evaluation of this concept requires long-term follow-up. AVFs with TRRBF have high probabilities of surviving, even though their clinical evolution is torpid and prolonged. In this study, 9/12 ears with TRRBF survived. We demonstrated that if we had not vascularized these ears, they would have been lost. Despite the fact that our sample number is small, we conclude that TRRBF could be used as a rescue method.

CONCLUSIONS

This is only a pilot study to evaluate whether this form of revascularization may maintain viable tissues. Our model requires more experimental work. Nevertheless, we consider that reconstructive microsurgeons should be familiarized with TRRBF to have them succeed and should also be familiar with their prolonged clinical evolution.

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