Supplement

Assisted Salvage of Ischemic Fasciocutaneous Flap Using Adipose-Derived Mesenchymal Stem Cells: In-Situ Revascularization

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Abstract

Adipose-derived mesenchymal stem cells (ASCs) have been shown to produce vascular endothelial growth factor (VEGF) and can increase perfusion in patients with critical limb ischemia. We will show that this concept can be applied to augment blood flow in zones of flap ischemia. We presented a case study of a 26-year-old man with a complex hand injury covered by a reverse radial perforator fasciocutaneous flap, which developed ischemic necrosis and was treated by debridement, transplantation of ASCs to enhance vascular support, and saline dressings. ASCs are found in the stromal vascular fraction (SVF), a heterogeneous collection of cells, including pericytes and endothelial cells, that is prepared from lipoaspirate using collagenase digestion followed by centrifugation. These were injected into the flap, the palmar tissues both subjacent and peripheral to the flap, and the skin-grafted donor site. The case was documented with photography, measurements at hand therapy, and follow-up angiography MRI. At 72 hours, new vessels appeared diffusely; at 1 week, the remaining tissues of flap were bleeding. The wound, 11 cm × 4 cm, contracted spontaneously and was healed at 21 days. The skin graft over the donor site demonstrated unusual suppleness and elasticity. 3D CT angiography disclosed a new layer of vascularity in the superficial tissues of the palm when compared with the normal side. The patient regained full composite flexion, pinch, opposition, and wrist extension. Application of ASCs into the supporting tissues surrounding the ischemic flap, and into the flap itself, constituted a form of in-situ revascularization (ISR) that was subjectively and objectively effective for this patient.

Level of Evidence: 5

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Angiogenic growth factors have long been known to induce collateral arteries; this was first appreciated in cardiac ischemia.[1] A hind limb ischemia model using femoral artery ligation has proven useful to assess the effects of various cell types: circulating endothelial cells, bone marrow mononuclear (stromal) cells (BMMCs), bone marrow mesenchymal stem cells (BMSCs), and adipose-derived mesenchymal stem cells (ASCs).[2] These studies have been summarized in the literature.[3]

The presence of mesenchymal stem cells in adipose tissue was reported in 2001.[4] Production of angiogenic factors (vascular endothelial growth factor [VEGF], hepatocyte growth factor [HGF]) by human adipose stromal cells has been well documented.[5-7] ASCs induce blood vessels in ischemic muscle through two basic mechanisms:

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(1) angiogenic factors acting through a paracrine mechanism[7,8]; and (2) cell components contributing to developing endothelial structures (inosculation).[9,10] Noting the ease of harvest of ASCs and their rapid expansion capacity (10x in I week), Nakagami et al[6] transplanted cultured adipose tissue-derived stem cells into the previously described ischemic limb model, documenting increased blood flow and capillary density. When this same model, previously explored by Murohara's group using other cells,[11-13] was tested with ASCs, the presence of multipotent mesenchymal cells were documented in stromal vascular fraction (SVF).[14] Thus, ASCs came to be recognized as a source for angiogenic factors that could be employed in humans with chronic limb ischemia, both for arteriosclerotic disease and diabetes.

The first reported trial of intramuscular ASC transplantation for critical limb ischemia in humans enrolled 15 patients with rest pain and the presence/absence of nonhealing ulcers or tissue necrosis.[15] Three patients had thromboangiitis obliterans (TAO) and 3 were diabetics. Thirteen of the fifteen patients had some degree of ischemic tissue damage. Digital subtraction angiography revealed improvement in eight out of ten TAO patients and in two thirds of the diabetics. Clinical improvement was documented in three out of three diabetic foot patients and 7/12 patients with TAO. Significant changes were noted in leg pain, ulcer size, and pain-free walking distance; these were maintained 2 years or more after surgery. Ischemic rest pain disappeared by 14 days, and the wound healing was complete by 120 days.

In 2014, Bura et al[16] reported a phase 1 clinical trial of ASCs in seven patients with nonreconstructable Rutherford stage 3 critical limb ischemia. Six patients had arteriosclerotic disease and one with TAO. At 2-year follow-up, significant improvement in leg pain, pain-free walking distance, and ulcer size was maintained, although no dimensions were given for the ulcers. The total number of ulcers decreased by an unspecified percentage, with the most significant decrease occurring in the first 90 days.

We recently reported a small open-label, nonrandomized clinical study of 10 consecutive patients with critical limb ischemia, Rutherford stages 3, 4, or 5.[17] Using a similar methodology, the posterior muscle compartments were injected with SVF, as were all wounds, if present. Not only did all patients with claudication become pain-free, but also six patients with ulcers (five of whom were diabetic) were able to achieve closure of their wounds; five of the wounds closed spontaneously, whereas the sixth patient, who had an ulcer measuring 8 cm by 6 cm, successfully supported a split thickness skin graft 5 months after the ASC procedure. These results are consistent with the studies of Lee et al[15] and Bura et al[16]; however, they differ in that the SVF, rather than being cultured, was utilized immediately.

A similar response has been documented in nonischemic tissues as well. Aged facial skin treated with subcutaneous injection of either ASCs or an autologous fat graft plus SVF demonstrated histologic and ultrastructural changes at 3 months posttransplantation. Papillary dermis with a loose arrangement of small elastic fibers preoperatively now had new elastic fibers enveloped in collagen fibers in a perpendicular orientation to the dermal-epidermal junction. The reticular dermis showed a resorption of its elastic component, better hydration, and numerous small blood vessels.[18]

The previously mentioned studies support the concept that *cell-mediated neo-angiogenesis*, driven by cytokines, such as VEGF, known to be produced by ASCs in response to ischemia, could be a useful adjunct in the treatment of flap ischemia.

CASE REPORT

A 26-year-old right-dominant man sustained a complex injury to the palmar surface of his right hand in a rollover motor vehicle accident in April 13, 2015, and he was treated at an outlying hospital. The thenar musculature and its motor branch were crushed. The flexor digitorum superficialis to the index was avulsed. Segmental losses of the digital nerves to the ulnar index and radial middle fingers were noted. He sustained zone 2 lacerations of the flexor digitorum profundus to the index finger and of both flexor tendons to the middle finger. The first reconstructive procedure addressed the tendons. The lacerations were repaired, and the thumb required a transfer of the extensor indicis proprius to the abductor pollicis brevis. At a subsequent procedure, nerve repairs were performed with 4 cm interposition sural nerve grafts. Coverage consisted of a volar forearm fasciocutaneous flap, the pedicle for which was a single perforator 2 cm proximal to the radial styloid, dissected in the suprafascial plane. The flap was rotated in-plane 180 degrees and inset. The donor site was covered with a split thickness graft from the thigh. By postoperative day (POD) 5, swelling of the flap was noted. By POD 14, the skin paddle was black and the patient was transferred to our institution (Figure 1).

Based on previous reports of autologous fat grafts for burn scars,[19] we had previously utilized subcutaneous and periarticular infiltration of SVF for the treatment of burn scar contractures of the hand and were able to document neovascularization beneath the skin grafts and around the metacarpophalangeal (MP) joints.[20] These findings were consistent with previous reports of angiogenic properties of SVF, the rationale being the known production by such cells of VEGF.[1-3] With the possibility of using SVF transfer to assist in wound healing, we obtained informed consent

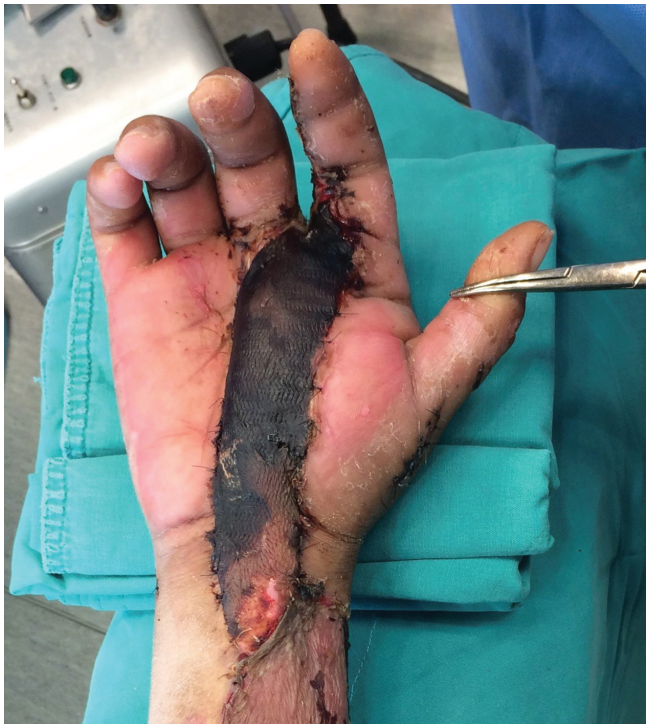

Figure 1. Radial forearm fasciocutaneous flap coverage of the palmar defect 10 cm × 4 cm. The flap was based on a single dominant perforator at the pivot point. At 14 days, the skin paddle of the flap is necrotic.

and the patient was brought to surgery. The flap was debrided down to the adipofascial layer, which was noted to be pale with little signs of vascularity (Figure 2).

SVF cells were prepared from 370 mL of adipose tissue harvested directly into a sterile tissue processing canister (The GID Group, Inc., SVF-1, Louisville, CO, USA). The lipoaspirate was washed, and then underwent collagenase digestion. After centrifugation, the SVF cells concentrated at the bottom of the device, and were removed using a 6-inch needle. The total viable SVF cell count, 6.8×10^7, was obtained using image cytometry. The cells were suspended in 30 cc of Ringer's lactate and injected into the substance of the adipofascial flap, into the subjacent palmar tissues, and beneath the previously healed split thickness skin graft covering the forearm donor site.

With the hope that the SVF-induced neoangiogenesis could help the remaining tissue of the flap to survive, we injected SVF into the subcutaneous border surrounding the fascia, the palmar tissues subjacent to the fascia, and the substance of the fascia itself. We also injected SVF into a zone of dense scar crossing the volar wrist crease and beneath the skin graft covering the volar forearm donor site.

On POD 3 (from the SVF procedure), the surface of the flap demonstrated diffuse neovascularization. The wound measured 10 cm × 3.5 cm (Figure 3). With subsequent local debridement and saline dressing changes, the width had diminished to 2.5 cm by POD 10 (Figure 4) and it was completely closed by 21 days (Figure 5), when the scar crossing from the volar forearm donor site to the palm remained dense at the wrist crease, making it impossible to extend the hand into neutral. The patient then proceeded into rehabilitation.

The initial rehabilitation evaluation on June 30, 2015 showed the following ranges of motion (in degrees, right hand vs. left hand): wrist extension 20/48, wrist flexion 70/123, index finger flexion 70/90 at the MP and 45/110 at the proximal interphalangeal joint (PIP), and middle finger flexion 90/90 at the MP and 70/110 at the PIP degrees. The patient could not perform composite flexion. The volar forearm skin graft was dry and stiff.

The initial rehabilitation evaluation on June 30, 2015 showed:

- Wrist extension: right hand 20 degrees; left hand 48 degrees
- Wrist flexion: right hand 70 degrees; left hand 123 degrees
- Finger range of motion: (1) right index finger, MP 70 degrees and PIP 45 degrees; (2) right middle finger, MCP 90 degrees and PIP 70 degrees
- Volar forearm skin graft: able to pinch the skin

Medical Ethics

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national, National Autonomous University of Nicaragua-Léon) and the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the participant in this case study. The patient provided consent to publish this case study in all formats.

Obtaining Adipose-Derived Stromal Vascular Fraction

Under general anesthesia, a wetting solution (1 L Ringer's lactate, 50 cc lidocaine 1%, and 1 cc epinephrine 1:1000) was infused into both the superficial and deep compartments in the zones designated for liposuction through incisions made with a #11 blade using a standard multiport infusion cannula. Liposuction was performed using a 4 mm Mercedes cannula with 370 mL of adipose tissue harvested directly into a sterile tissue processing canister (The GID Group, Inc., SVF-1, Louisville, CO, USA). The lipoaspirate was washed three times inside the canister to remove red cells and oils. Approximately 125 ml of Ringer's lactate solution was added to the adipose tissue with collagenase enzyme (Worthington

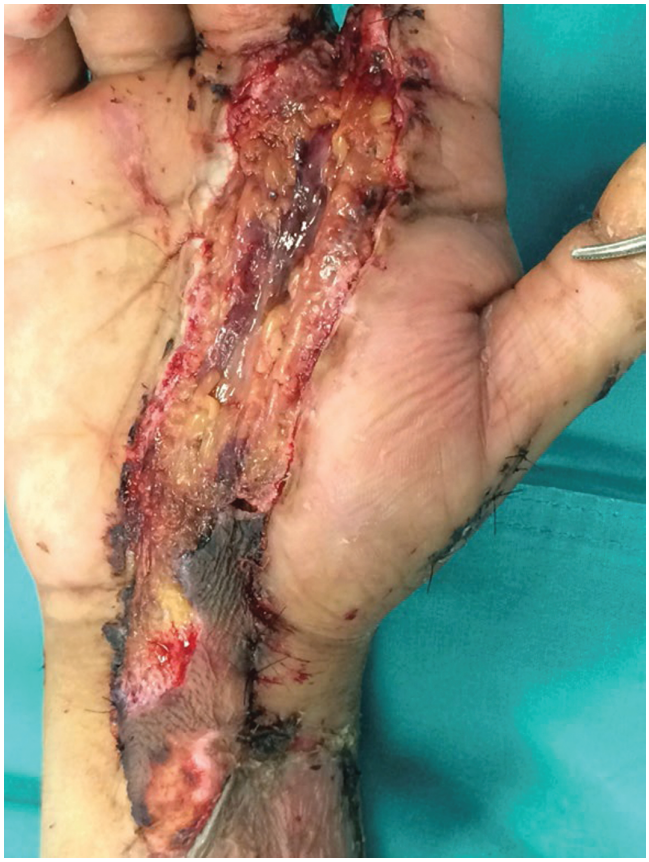

Figure 2. After debridement, the remaining adipofascial flap is pale. Despite 2 weeks of time in residence, it has been poorly vascularized from the underlying soft tissues of the hand. The flap is at this time infitrated with adipose-derived stromal vascular fraction cells.

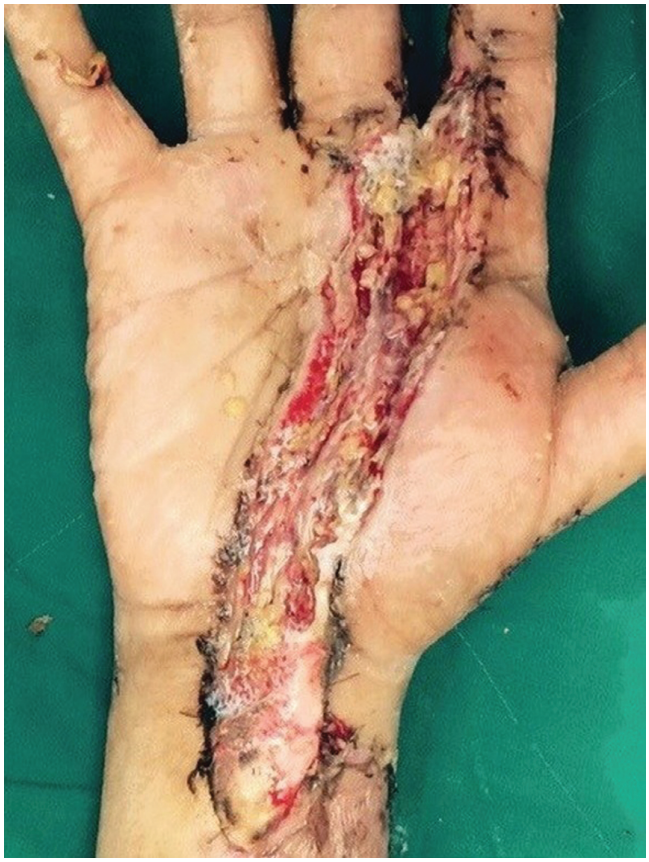

Figure 3. At 72 hours, note that bleeding vessels are more prominent throughout the flap. Also, note the change in color of the proximal dermis.

Biochemical Corporation, CLS-1, Lakewood, NJ, USA) at a concentration of 200 CDU/ml of total volume. The collagenase was injected into the canister through a sterile 0.22-micron filter (Millex-MP, Millipore, Cork, Ireland). The mixture was dissociated for 40 minutes in an incubated shaker table at 38°C and 150 RPM. After dissociation, human albumin was added to achieve a concentration of 2.5 and thereby stopping the reaction through the binding by albumin of calcium and magnesium ions essential for enzyme function. The mixture was centrifuged for 10 minutes at 800x gravity. The SVF cells concentrated at the bottom of the device, and were removed using a 6-inch needle (Abbocath-T, Hospira Ireland, Sligo, Ireland). The cells were resuspended in Ringer's lactate solution to a total of 20 cc. Ten microliters of SVF were taken from the final suspension and submitted for differential staining. Two samples were then passed through an image cytometer (ADAM-MC, Portsmouth, NH, USA) for the counting of mononucleated cells and to assess cell viability. The viable index was 184,000 cells per gram of dry fat. The total viable cell count was 6.8×10^7.

Hand Therapy Regimen

The hand therapy regimen consisted of passive motion, active motion, and desensitization twice a week.

CT Angiography

Contrast agent was injected into the left arm and allowed to circulate. Images were taken in the sagittal plane of the right index finger to correlate with the plane of the flap.

Rehabilitation

The rehabilitation evaluation was conducted on September 8, 2015, which was 4 months after the application of SVF cell therapy, showed that the cutaneous tissues of the palm were pliable. This was remarkable, because, given the length of time involved in the reconstruction, we were expecting a great deal more fibrosis and possible involvement of the flexor tendons. The scar crossing the volar

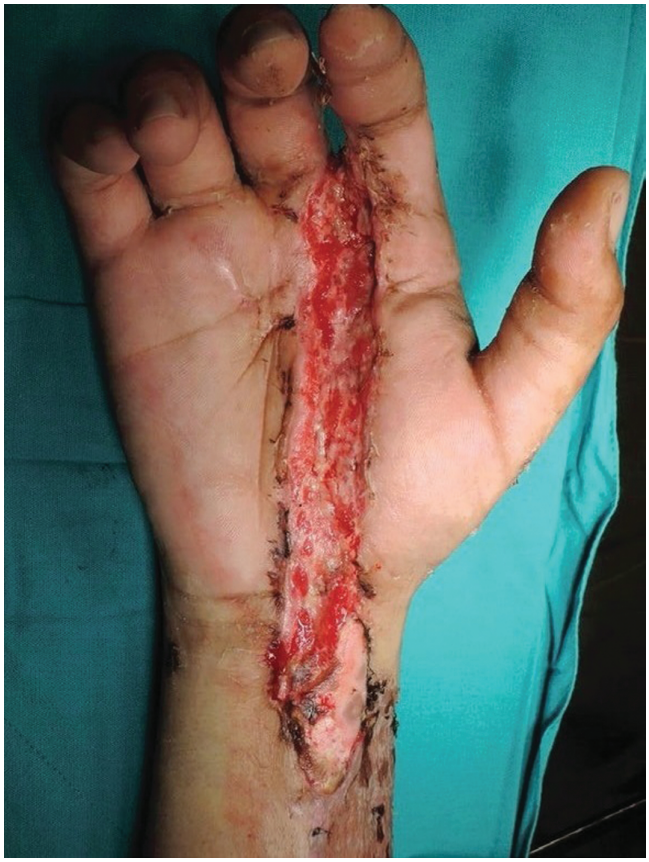

Figure 4. At 10 days, the width of the wound has diminished by 50%. Interim treatment consisted of local debridement and saline dressings.

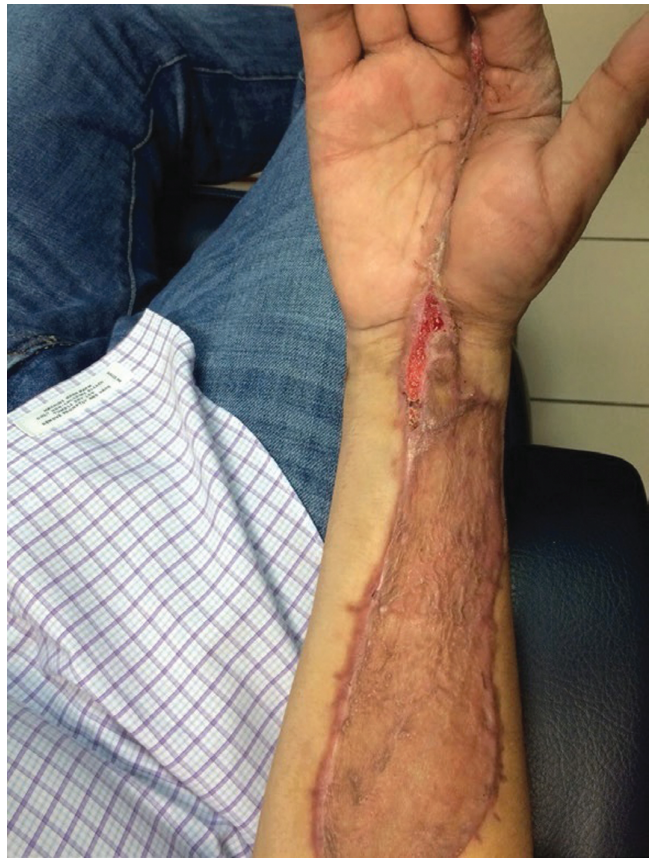

Figure 5. At 24 days, the wound has achieved closure.

wrist crease had softened by a considerable degree, allowing for increased wrist extension. Sensation was restored to the radial middle finger and proximal ulnar index finger but not yet to the pulp of the index. Full composite flexion has been achieved. There is a 10-degree loss of extension at the PIP level of the middle finger. The patient gained control and strength of the thumb through the tendon transfer with restoration of abduction in all planes, and opposition pulp-pulp to all fingers. The strength of the key pinch and chuck pinch continued to improve. The donor site skin graft demonstrated suppleness to pinch, with wrinkles forming with normal motion (Figure 6).

The final rehabilitation evaluation at 6 months showed the following changes in range of motion (in degrees, right hand only):

- Wrist extension: right hand 48 degrees (previously 20 degrees)
- Wrist flexion: right hand 110 degrees (previously 70 degrees)
- Index finger flexion: unchanged, will need z-plasties
- Middle finger flexion: MCP 90 degrees (unchanged) and PIP 90 degrees (previously 70 degrees)
- Full composite flexion achieved
- Thumb: restoration of full chuck and key pinch, with a full range of motion
- Volar forearm skin graft showed significant improvement in overall softness and elasticity (Figure 7).

Angiography

In the 3D angiography CT scan, the contrast agent disclosed a separate layer of enhancement in the immediate subcutaneous tissues of the right palm and wrist. Vessels were observed to be in continuity with the palmar arch and with the digital arteries of the index finger (video, available as Supplementary Material online at www.aestheticsurgeryjournal.com).

DISCUSSION

The traumatic defect in this patient with a distally based fasciocutaneous flap was based on a large perforator vessel of the radial artery leaving the radial artery itself intact.

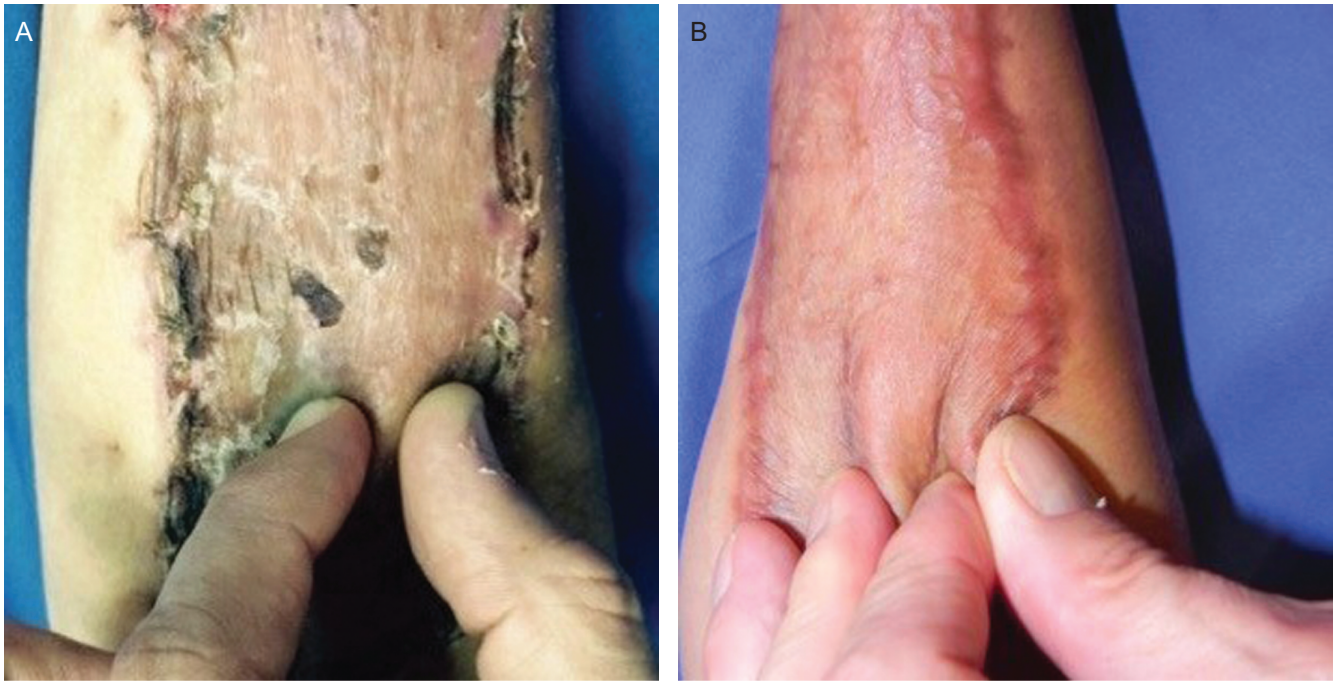

Figure 6. (A) The split thickness skin graft over volar forearm flap donor site at 2 weeks (photograph was taken immediately post-stromal vascular fraction treatment) shows typical stiffness and coloration. The proximal forearm is inferior. (B) The split thickness skin graft over the volar forearm donor site at 5 months post-stromal vascular fraction treatment shows color change, smoothness of contour, and improved flexibility. The proximal forearm is inferior.

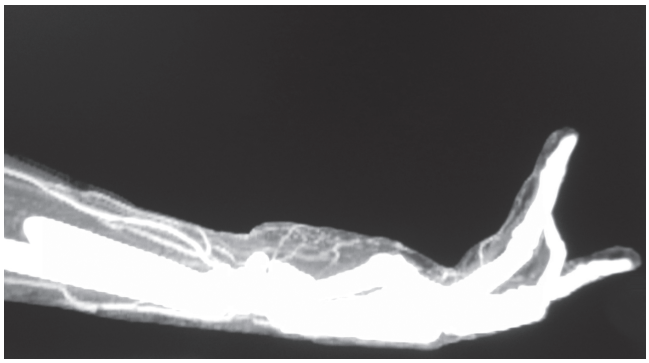

Figure 7. Angiography CT scan at 5 months shows in-situ revascularization of the palmar flap with vessels in continuity with underlying arterial arcade and distally with the digital arteries.

The original design of the reverse radial forearm flap, first described by Lu et al in 1982,[21] involved an obligatory sacrifice of the radial artery but proved simple and reliable, and it quickly became a first-line choice for the reconstruction of hand defects, both volar and dorsal. However, reports soon emerged regarding consequent risks for the viability of the hand or chronic ischemia.[1,22]

These concerns prompted a search for a clinical alternative. In 1988, Chang first reported 10 successful cases of coverage using fasciocutaneous flaps harvested without radial artery compromise[23] based on 10 branches (from 0.3 cm to 0.8 cm) centered between 1.5 cm proximal to the snuff box to the bifurcation of the radial artery.[21,24] These arteries traverse the septum, reach the fascia, and then link together to form a longitudinal plexus in parallel with the radial artery. Subsequent reports using either an island adipofascial flap or a fasciocutaneous flap focused on further defining the ideal pivot point of the flap.[23,25-33] Contrast studies by Saint-Cyr et al[34] in 26 cadaver forearms describe the presence of a major, clinically relevant (>0.5 cm) perforator from the radial artery within 2 cm of the radial styloid in 100% of the specimens.

In this particular case, the fasciocutanous flap was of standard design, based on the perforator described by Saint-Cyr. Vascular compromise of the flap was apparently evident by POD 4 and was of venous origin, proceeding on to full necrosis of the skin paddle at 2 weeks. Despite this length of time, after we had taken down the dead skin, we did not find much evidence for vascular ingrowth into the remaining fascia from the underlying palmar tissues. Because of the need to protect the tendon repairs and nerve grafts, stabilizing the coverage was a clinical priority. Could this have been achieved with dressing changes alone? For this reason, an alternative means to provide vascular support for this tissue was a clinical priority. We decided to institute this therapeutic measure based on our experience with the treatment of ischemic extremities with adipose-derived

SVF cells, our experience with burn scars, the medical literature, having obtained approval from the Medical Ethics Committee at the National Autonomous University of Nicaragua-Léon, and having obtained informed consent.

We think that the response of the fascia to infiltration with ASCs is consistent with the literature, follows a cytokine mechanism, and is very rapid. In our experience with lower extremity injections of SVF in small volumes, significant swelling of the leg soft tissues was noted within the first hour and persisted for approximately 48 to 72 hours, thus indicating an immediate tissue response to the cells.

Could the fascia have survived based solely on secondary vascular invasion originating from the subjacent tissues of the palm? Given that the fascia had already been in direct contact with well-vascularized palmar tissues for 14 days, and that it still remained hypoperfused at the time of the debridement, we considered dressing changes alone to be a risky option. We think that the improvement in blood supply at 72 hours was attributable to SFV cytokines causing a "jump-start" of the vessels in the tissue bed beneath and surrounding the flap, and extending outward to connect with precursors within the flap itself with a resulting restitution of flap integrity.

The rapidity of closure of the wound after ISR merits comment. Did additional growth factors along the wound edges accelerate side-to-side approximation in 21 days? Experimental design to test ISR in test wounds with serial biopsies and growth factor assays could generate useful answers to questions of "accelerated" wound healing vs the traditional time course.

Finally, the physical changes in the donor-site skin graft should be noted. The texture was smooth. Hyperpigmentation in the graft seen at 2 weeks after surgery was dramatically lightened. Although the skin was previously hard and stiff, it could be readily pinched. These are completely consistent with the findings of Charles-de-Sa[18] and Rigotti.

The significance of this study is as follows. Although a number of studies have been published regarding injecting chronic wounds with mesenchymal stem cells, photographic evidence of serial changes in wound size has not been reported. This case study gives preliminary evidence regarding the rapidity of response of tissues to SVF. The video (available as Supplementary Material online at www.aestheticsurgeryjournal.com) illustrates the following two points: (1) The changes in scar thickness at the proximal rotation point of the flap at the wrist crease are consistent with the antifibrotic properties of SVF; and (2) the volar forearm donor site skin graft demonstrates qualitative changes in color and flexibility, which are also consistent with the antifibrotic and angiogenic effects of paracrine factors secreted by SVF cells.

CONCLUSION

We report a case of salvage of an ischemic adipofascial flap supported by infiltration with adipose-derived SVF. Our clinical observations support the concept that the treatment of ischemic tissue, regardless of type, with adipose-derived SVF can enhance vascularity, provided that the surrounding supportive tissues are also treated. The technique is simple and can be employed as a first line of defense. These findings also support the concept that the prophylactic administration of SVF into a prospective flap can enhance its vascularity.

Supplementary Material

This article contains supplementary material located online at www.aestheticsurgeryjournal.com.

Disclosures

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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