Lower Extremity Autologous Fat Grafting in Graft Versus Host Disease

AUTHORS:
Julia A. Cook, MD[a]; Sarah E. Sasor, MD[a]; Steven A. Mann, MD[b]; Terrence Katona, DO[c]; Sunil S. Tholpady, MD, PhD[a,d]; Michael W. Chu, MD[e]

CORRESPONDENCE AUTHOR:
Dr. Julia A. Cook
Indiana University School of Medicine
Division of Plastic & Reconstructive Surgery
545 Barnhill Drive, #232
Indianapolis, IN 46202
email: juacook@gmail.com
phone: (317) 274-5831
fax: (317) 278-8746

AUTHOR AFFILIATIONS:
a. Indiana University School of Medicine, Division of Plastic & Reconstructive Surgery
b. Indiana University School of Medicine, Department of Pathology and Laboratory Medicine
c. R. L. Roudebush Veterans Affairs Medical Center, Department of Pathology and Laboratory Medicine
d. R.L. Roudebush Veterans Affairs Medical Center, Division of Plastic & Reconstructive Surgery
e. Kaiser Permanente West Lost Angeles Medical Center, Division of Plastic & Reconstructive Surgery

Background	The late dermatologic effects of sclerotic chronic graft versus host disease (GVHD) can produce profound decreases in quality of life by causing decreased temperature regulation, skin tightening, poor wound healing, and ulceration from minor trauma. Patients with chronic wounds secondary to sclerotic chronic GVHD are a particular reconstructive challenge due to the associated dermal fibrosis and inflammatory infiltrate. Reconstructive goals of chronic lower extremity wounds are to provide wound coverage and allow for functional recovery.
Summary	We present the novel therapeutic course of a 55-year-old male with adult T-cell lymphoma in remission after donor bone marrow transplant five years prior. His recovery was complicated by chronic lower extremity ulcers due to immunosuppression, sclerotic chronic GVHD, and peripheral vascular disease. These ulcers were successfully treated in ten months with autologous fat grafting, taking advantage of the immunomodulatory and pro-angiogenic properties of mesenchymal stem cells present within the graft. It is theorized that the multipotent and immunomodulatory properties of mesenchymal stem cells aid in tissue regeneration at sclerotic chronic GVHD wounds through the promotion of collagen deposition and hindrance of proinflammatory cytokines. Our patient demonstrated complete resolution of chronic wounds and significant increases in quality of life as a result of autologous fat grafting.
Conclusion	This case illustrates a novel application of stem cell therapy for immune tolerance. Autologous fat grafting is a single-stage outpatient procedure that promotes wound healing potential with minimal donor site morbidity. Fat grafting can be considered for carefully-selected patients who are otherwise not ideal surgical candidates. Knowledge of this safe treatment may help guide difficult wound management issues that arise in sclerotic chronic graft versus host disease. Future studies may aim to replicate these results in a larger patient population, utilizing pre- and postoperative biopsies to determine histologic changes due to autologous fat grafting.

Keywords	Autologous fat graft; graft versus host disease; GVHD; lower extremity; mesenchymal stem cells; ulceration

DISCLOSURE:
The authors have no conflicts of interest to disclose.

To Cite: Cook JA, Sasor SE, Mann SA, Katona T, Tholpady SS, Chu MW. Lower Extremity Autologous Fat Grafting in Graft Versus Host Disease. *ACS Case Reviews in Surgery.* 2018;1(6):18-22.

Case Description

Graft versus host disease (GVHD) is a donor T-cell mediated attack on native cells in patients who have undergone allogeneic stem cell transplant.[1,2] GVHD is divided into acute and chronic forms. Acute disease is characterized by immune-mediated dermatitis, hepatitis, and enteritis.[1,2] Chronic disease occurs at a minimum of 100 days from transplantation and manifests as a pleomorphic set of symptoms mimicking autoimmune syndromes involving the skin, mucosa, eyes, lungs, gastrointestinal tract, and/or liver.[1,2] Skin is the most commonly affected organ in both acute and chronic GVHD, and dermatologic manifestations of chronic GVHD begin as an early lichenoid skin pathergy and develop into a late sclerotic form.[2-4] Sclerotic chronic GVHD demonstrates a scleroderma-like dermal and eccrine glandular fibrosis associated with poor temperature regulation, skin tightening, poor wound healing, inadequate lymphatic drainage, and ulceration from minor trauma.[2,4] We present an IRB-approved course of a patient with sclerotic chronic graft versus host disease complicated by lower extremity ulcers that were treated with autologous fat grafting.

A 55-year-old male was referred for evaluation of his chronic, non-healing, bilateral lower-extremity wounds of one-year duration. His past medical history was significant for adult T-cell lymphoma, which had been in remission for five years after being treated with allogeneic bone marrow transplant. The patient developed sclerotic chronic GVHD one year post-transplantation, manifested by keratoconjunctivitis sicca, gastroesophageal reflux, and chronic bilateral lower-extremity wounds. His chronic wounds were further complicated by chronic venous insufficiency, peripheral neuropathy, chronic lower-extremity pain, and tobacco abuse. He was maintained on sirolimus, mycophenolate mofetil, and methylprednisolone immunosuppression with acyclovir and fluconazole prophylaxis for sclerotic chronic GVHD. Medications also included over-the-counter artificial tears, pantoprazole, amlodipine, gabapentin, and twice-daily 30 mg extended-release morphine.

Patient was referred to plastic surgery after two months of conservative wound-care therapy with ointment and Allevyn adhesive foam dressings (Smith & Nephew plc, London, UK) and two months of negative-pressure wound therapy (KCI, San Antonio, TX) failed to resolve his chronic lower-extremity wounds. Physical exam demonstrated woody bilateral lower extremities with palpable dorsalis pedis and posterior tibialis pulses and stigmata of chronic venous insufficiency: atrophic skin, alopecia, and hyperpigmentation. The right distal lower extremity had four distinct wounds measuring 1.5 x 1.2 cm for the proximal lateral wound, 1.2 x 1.0 cm for the proximal medial wound, 4.0 x 2.6 cm for the anterior tibial wound, and 2.4 x 1.4 cm for the most distal wound. The left distal lower-extremity wound measured 2.5 x 2.2 cm. The wounds had scant punctate bleeding with manipulation and were not acutely infected, malodorous, or draining. The lesions were circumferentially biopsied to rule-out occult malignancy. Computed tomographic angiography of the lower extremities showed bilateral atherosclerotic disease with patent three-vessel runoff to the ankle; however, venous Duplex ultrasonography showed superficial venous insufficiency with reflux to the deep venous system bilaterally

The patient was deemed a poor surgical candidate due to tobacco abuse, chronic immunosuppression, and venous insufficiency. He was therefore treated with 5,000 units of oral vitamin A and offered autologous fat grafting for his chronic lower extremity wounds. The proposed mechanism of action, including possible worsening of his graft versus host disease, was explained at length, and the patient agreed to proceed after approval of his oncology and hematology providers.

Techniques for autologous fat grafting have been previously described.[5] Briefly, the abdomen is prepped and draped in the usual sterile fashion, and standard tumescent solution (sodium chloride with epinephrine) is injected. Suction-assisted liposuction is then performed with blunt-tip cannulas (Mentor Corp., Santa Barbara, CA) until 2-mL lipoaspirate per square centimeter of wound area is harvested. The lipoaspirate is washed with lactated Ringer solution and strained (Figure 1; Revolve System, LifeCell

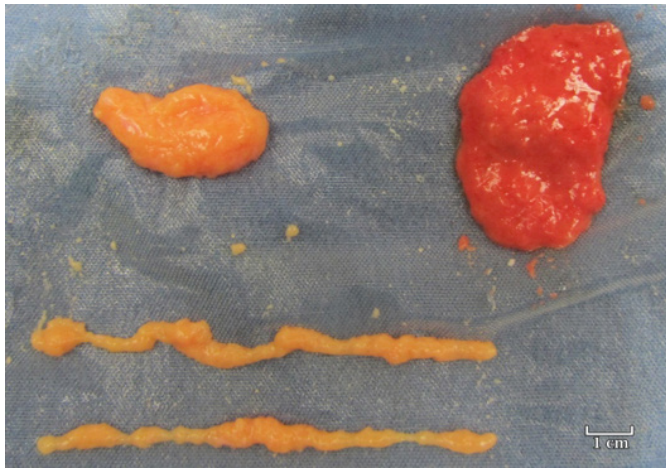

Figure 1. Autologous Fat Grafting. Autologous fat grafting pre- (upper right) and postwashing and processing (upper left). Fat post-processing is placed in 10 mL syringes for transfer (bottom).

Corp., Bridgewater, NJ). A blunt tip, 17 gauge injection cannula (Coleman Infiltration Cannula Style I; Mentor, Corp., Santa Barbara, CA) is attached to 10 mL syringes and is used to inject the lipoaspirate in a layered, cross-hatch fashion in multiple passes through an access incision one centimeter from the wound edges retrograde towards the wound. The amount of fat injected is controlled by observing the skin to avoid blanching and fat starting to fill the ulcer surface.

Our patient underwent outpatient debridement, fat harvesting, and injection of a total 17 mL of autologous fat into the periphery of the wound. Petroleum-impregnated gauze and negative-pressure wound therapy (KCI, San Antonio, TX) was applied to the wounds for six days. Following negative-pressure wound therapy, petroleum-impregnated gauze coverage was continued for one week, after which non-adherent gauze dressing changes were continued until the wounds were epithelialized. Surgical biopsy specimens demonstrated ulceration with underlying mixed acute and chronic inflammation, dermal fibrosis, and capillary proliferation consistent with venous stasis (Figure 2).

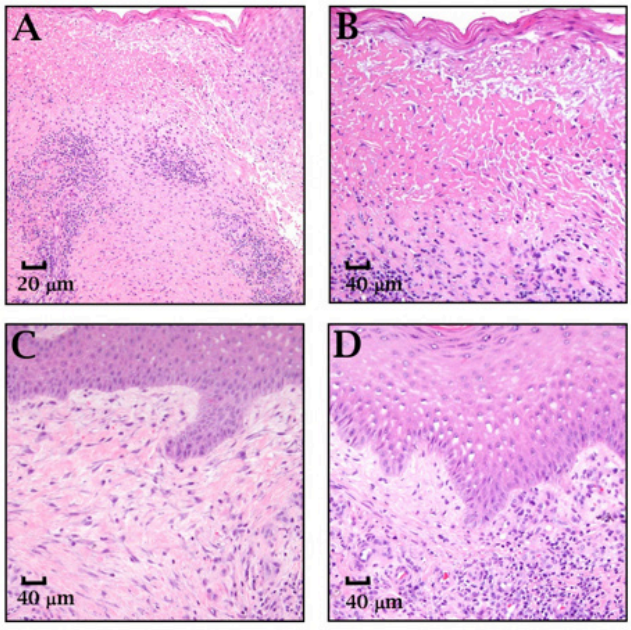

Figure 2. Sclerotic chronic Graft versus Host Disease Pathology. (A) 100x ulceration (left side of image) with adjacent zone of nearly-confluent epidermal necrosis surfaced by parakeratotic stratum corneum. Intact viable epidermis present toward right portion of image. Underlying dermal fibrosis with predominantly lymphoplasmacytic inflammatory cell infiltrate and extravasated erythrocytes. (B) 200x magnification of Image A. (C) 200x variably acanthotic epidermis with subjacent sclerotic dermis and superficial subcutis containing reactive fibroblasts and perivascular and interstitial lymphocytes and plasma cells. Perivascular hemosiderosis also noted. (D) 200x variably acanthotic epidermis, lobular proliferation of capillaries and small blood vessels within superficial dermis consistent with chronic stasis. Admixed lymphocytes and plasma cells with hemosiderin deposition and extravasated red blood cells.

The patient was seen ten months postoperatively for routine follow-up (Figure 3). His wounds healed and his pain resolved. He was able to wean off of scheduled morphine, likely due to the lack of exposed underlying tissue and a healed wound. He was also able to return to physical activities and play soccer with his grandchildren.

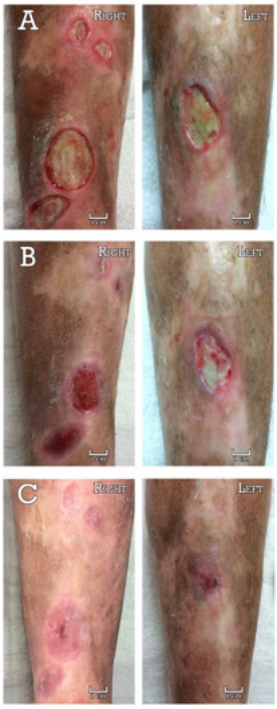

Figure 3. Chronic Wound Healing Progression. (A) Progression of right and left anterior tibial chronic wound immediately postoperatively, (B) five weeks postoperatively, and (C) final follow-up visit 10 months postoperatively.

Discussion

Graft versus host processes are all too common and create unintended secondary problems in the pursuit of cancer treatment. Skin manifestations can be particularly troublesome, as they may greatly reduce quality of life. Chronic lower extremity wounds are a particularly complex clinical problem, as they accumulate proinflammatory cytokines including tissue necrosis factor-α, Interleukin-1, and neutrophil elastase. Additionally, these cytokines tend to create oxygen radicals that cause senescence of structural dermal elements and cell membranes.[6] In this patient, the lower-extremity wound was a result of GVHD and was exacerbated by superficial venous reflux and atherosclerotic disease.

Autologous fat grafting (AFG) has numerous aesthetic and reconstructive applications, yet it has not been described in the treatment of GVHD. Subcutaneous fat has several properties that make it amenable to chronic wound healing; however, its mechanism is still a topic of research.

Subcutaneous fat is the largest known depot of multipotent adipose tissue-derived mesenchymal stem cells in the human body.[7] As multipotent cells, adipose tissue-derived mesenchymal stem cells have the potential to mature into adipocytes, fibroblasts, mesocytes, and vascular endothelial cells.[8] As progenitor cells, adipose tissue-derived mesenchymal stem cells secrete multiple cytokines including vascular endothelial growth factor, placental growth factor, transforming growth factor-β, angiopoietin-1, and fibroblast growth factor-2.[8-10] These cytokines aid in tissue regeneration through the promotion of angiogenesis and collagen deposition, even in hypoxic conditions.[6,9.10] Furthermore, mesenchymal stem cells express proliferative surface markers for hematopoietic and endothelial cell lineages including CD14, CD34, CD45, and CD144.[9] Adipose tissue-derived mesenchymal stem cells also lack human leukocyte antigen-DR expression, which decreases the risk of tissue rejection post-transplantation.[8] Interestingly, these stem cells have demonstrated immunosuppressive characteristics that subdue the proliferation of activated lymphocytes in vivo.[6,8,11] Therefore, the multipotent, angiogenic, and immunomodulatory effects of ADSCs transplanted in AFG contribute to the treatment of sclerotic chronic GVHD and poor perfusion. Injection of adipocytes in the sclerotic wound periphery provides structural support for mesenchymal stem cells. In addition, multiple passes of the cannula through the sclerotic wound edges mechanically breaks fibrotic tissue and increases the surface area of exposed tissue to mesenchymal stem cells for neovascularization and new collagen deposition.

Autologous fat grafting is a short, single-stage, outpatient procedure with low demands on wound healing, minimal donor site morbidity, and avoidance of flap reconstruction, which would be difficult in a poorly-perfused sclerotic extremity. Potential complications of fat grafting include damage to the viscera, lungs, or surrounding tissues during harvest, infection, subcutaneous contour deformities, donor site pain, necrosis, and need for repeat procedures.[12] These complications were not encountered in our patient. The patient declined biopsy to determine the histologic architecture and composition of the regenerated tissue. However, we believe that the use of autologous fat grafting is safe and effective, and that it helped promote resolution of the previously chronic wounds of the sclerotic chronic GVHD patient. Fat grafting can be considered for carefully-selected patients who are otherwise not ideal surgical candidates. Future studies may aim to replicate these results in a larger population, utilizing pre- and postoperative biopsies to determine histologic changes as a result of AFG.

Conclusion

We report the first case of the successful treatment of chronic lower extremity wounds in a sclerotic chronic GVHD patient with AFG. Knowledge of this safe treatment may help guide difficult management issues that arise in sclerotic chronic GVHD. Further research with control treatments, long-term follow-up, and post-treatment histologic examination is warranted to further develop treatment protocols with AFG for this subset of patients.

Lessons Learned

AFG may be used to treat lower extremity chronic ulceration due to sclerotic chronic GVHD. We present the novel application of autologous fat grafting for treatment of chronic lower extremity wounds as a result of sclerotic chronic graft versus host disease.

References

1. Shlomchik WD. Graft-versus-host-disease. *Nat Rev Immunol.* 2007;7:340-352.
2. Horwitz ME, Sullivan KE. Chronic graft-versus-host-disease. *Blood Rev.* 2006;20:15-27.
3. Brüggen MC, Klein I, Greinix H, et al. Diverse T-cell responses characterize the different manifestations of cutaneous graft versus host disease. *Blood* 2014;123:290-299.
4. Jagasia MH, Greinix HT, Arora M, et al. National institutes of health consensus development project on criteria for clinical trials in chronic graft versus host disease: I, the 2014 diagnosis and staging working group report. *Biol Blood Marrow Transplant.* 2015;21:389-401.
5. Coleman SR. Long-term survival of fat transplants: controlled demonstrations. *Aesthetic Plast Surg.* 1995;19:421-425.
6. Fromm-Dornieden C, Koenen P. Adipose-derived stem cells in wound healing: recent results in vitro and in vivo. *Mol Cell Biol.* 2013;1:8-13.
7. Hsu VM, Stransky CA, Bucky LP, Percec I. Fat grafting's past, present, and future: why adipose tissue is emerging as a critical link to the advancement of regenerative medicine. *Aesthet Surg.* 2012;32:82-89.
8. Schaffler A, Buchler C. Concise review: adipose tissue-derived stromal cells – basic and clinical implications for novel cell-based therapies. *Stem Cells.* 2007;25:818-827.
9. Nakagami H, Morishita R, Maeda K, Kikuchi Y, Ogihara T, Kaneda Y. Adipose tissue-derived stromal cells as a novel option for regenerative cell therapy. *J Atheroscler Thromb.* 2006;13:77-81.
10. Stasch T, Hoehne J, Huynh T, De Baerdemaeker R, Grandel S, Herold C. Debridement and autologous lipotransfer for chronic ulceration of the diabetic foot and lower limb improves healing. *Plast Reconstr Surg.* 2015;136:1357-1366.

11. Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant severe, acute graft-versus-host-disease: a phase II study. *Lancet.* 2008;371:1579-1586.
12. Luu CA, Larson E, Rankin TM, Pappalardo JL, Slepian MJ, Armstrong DG. Plantar fat grafting and tendon balancing for the diabetic foot ulcer in remission. *Plast Reconstr Surg Glob Open.* 2016;4:e810.