Autologous Minimally Manipulated Homologous Adipose Tissue (AMHAT) for Treatment of Nonhealing Diabetic Foot Ulcers

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Background: Diabetic foot complications are increasingly burdensome for patients, clinicians, and society. Development of innovative therapies to support good quality basic care is a priority among those with an interest in this area. One of these involves scanning and printing tissues to match and conform to a defect (so-called 3D printing).

Methods: A single-arm pilot study of ten consecutive patients with a history of a chronic diabetic foot ulcer (DFU), treated with autologous minimally manipulated homologous adipose tissue (AMHAT), dispensed by a specialized 3D bioprinter, Dr. INVIVO, was performed. Patients with nonhealing DFUs present for more than 4 weeks and refractory to standard-of-care therapies were included. Wounds were treated with a single application of AMHAT, and then followed up weekly for up to 12 weeks, or until the wounds healed. The primary outcome measure was complete epithelialization of the wound up to 12 weeks after the treatment. Secondary outcome measures included wound size and/or volume reduction, assessment of ulcer grade, and time to closure.

Results: Five wounds were healed by 5 weeks and one at 8 weeks. The mean percent area reduction at 12 weeks was 78.3% (SD: 33.23). Complete closure was achieved in 60% of wounds. The mean time to closure in these wounds was 49.1 days (95% CI, 29.9–68.3). No adverse events were reported.

Conclusions: Single treatment of bioprinted AMHAT appears to be a safe and potentially effective treatment modality for patients with chronic DFUs. Further studies are warranted to explore the full potential of 3D bioprinting for tissue repair in this high-risk population. (Plast Reconstr Surg Glob Open 2022;10:e4588; doi: 10.1097/GOX.0000000000004588; Published online 28 October 2022.)

INTRODUCTION

As of 2019, there are 463 million adults living with diabetes worldwide, and that number is expected to reach 700 million by 2045.1 The lifetime risk of developing a diabetic foot ulcer (DFU) is 34%, and an estimated 9.1–26.1 million people around the world develop a DFU every year.
In fact, greater than half of DFUs become infected, and the most common diabetes-related complication leading to hospitalization and lower limb amputation is diabetic foot infection. Therefore, the need exists to develop innovative therapies that support wound healing in patients at risk for diabetic foot complications.

Autologous fat grafting is one such modality worthy of further examination. In fat grafting, extracted adipose tissue is characterized by large numbers of extracellular substances, including growth factors such as insulin-like growth factor, hepatocyte growth factor, transforming growth factor-\(\beta\) 1, and vascular endothelial growth factor, that result in angiogenesis, epithelialization, and wound remodeling. Furthermore, anti-inflammatory cytokines, proangiogenic factors, and healing-related peptides present in autologous fat may positively affect wound healing.

In an animal study conducted by Mojallal et al, autologous fat grafting resulted in the stimulation of collagen fiber neosynthesis and improved vascularization and thickness of the dermis and subcutaneous tissue in mice. And, in a recent randomized controlled trial, improved healing rates were noted for patients with diabetes who received local injection of autologous microfragmented adipose tissue following a minor foot amputation. Dr. INVIVO (ROKIT Healthcare, Seoul, South Korea) is a customized wound healing solution based on a 3D bioprinting system platform for skin regeneration (Fig. 1).

3D printing is defined as the construction of an object from a digital file using stimuli-responsive material. The device evaluated in this study utilizes a 3D scanner to measure wound size and depth and then converts the measurements to a file that can be used in the 3D bioprinter. Dr. INVIVO manufactures customized scaffolds specific to the wound undergoing treatment. Typically, a patient’s abdominal adipose tissue is harvested and minimally manipulated to adjust to the 3D bioprinter. After micronization of the adipose tissue, the autologous dermal substitute is printed and applied directly onto the cleaned wound site (Fig. 2). (See Video [online], which displays the 3D bioprinted AMHAT treatment process.)

METHODS

A single-arm pilot study of ten consecutive patients with a history of a chronic DFU treated with autologous minimally manipulated homologous adipose tissue (AMHAT), dispensed by the Dr. INVIVO device, was performed at a single outpatient center. Dr. INVIVO obtained the international standard “Medical Device Quality Management System ISO 13485” designation. The study protocol was reviewed and approved by Advarra (Pro00046934), and informed consent was signed by all participants. Patients with nonhealing DFUs, extending to skin, subcutaneous tissue, capsule, or noninfected bone, corresponding to University of Texas Grade 1–3A or Wagner grade 1 or 2, present for more than 4 weeks, and refractory to standard-of-care therapies were included in the study. Baseline demographics, including age, height, weight, gender, race, and comorbidities, were obtained from the medical record. Wounds were treated with a single application of AMHAT, and then followed up weekly for up to 12 weeks.
or until the wounds appeared completely epithelialized and determined to be fully healed.

Screening of subjects occurred up to 28 days before the initiation of treatment. It included obtaining signed informed consent, taking vital signs, determination of eligibility based on inclusion/exclusion criteria, obtaining medical history and medication history, recording demographic information, digital photography of the wound, assessment of the wound, debridement of the wound if applicable, measuring of the wound site postdebridement, evaluation of arterial perfusion via Doppler or transcutaneous oxygen pressure test, Wagner grade assessment, and initiation of offloading.

On the day of AMHAT treatment, 15 ml of fat was harvested via liposuction under local anesthesia by a board-certified plastic surgeon. The AMHAT was then prepared by gently filtering the harvested fat and dispensed by Dr. INVIVO maintaining minimal manipulation. The AMHAT was applied over the cleaned DFU and covered with the primary nonadherent dressing (eg, Mepitel; Mölnlycke Health Care AB, Gothenburg Sweden), followed by a secondary dressing, a generic foam dressing followed by generic cast padding, and Coban (3M, Minneapolis, Minn.). All patients were dispensed a padded pneumatic diabetic cam walker (Darco International, Huntington, W.Va.) for offloading.

Following treatment, weekly visits included vital signs, assessment of adverse events, concomitant medications, gentle debridement of wound if applicable, digital photography and measurement of the wound postdebridement along with dressing change, wound healing trajectory, and confirmation of offloading. In addition, at each treatment visit (week 12), the wound was evaluated for wound closure/epithelialization rate.

The primary outcome measure was complete epithelialization of the wound up to 12 weeks after the treatment. Secondary outcome measures included wound size and/or volume reduction, assessment of ulcer grade/stage, and time to closure.

**Statistical Analysis**

The intent-to-treat (ITT) and safety populations comprised of patients who received at least one treatment. All analyses used the ITT approach. The last observation carried forward principle was used in regard to missing area data at study visits. Study variables were summarized as means and standard deviations (±SDs) for continuous variables as well as medians/interquartile range (IQR) for nonnormal data. Categorical variables were presented as counts and percentages. The PAR for the index wound at X weeks was calculated as 
\[
\frac{(AI - AXW)}{AI} \times 100
\]
where AI is the area of the index wound at first treatment visit and AXW the area at X weeks after first treatment date. Time to heal is the first date that the wound is considered healed (completely epithelialized, 0 cm² area, with no drainage) compared to first treatment date. Mean time to heal was calculated using the Kaplan-Meier approach.

**RESULTS**

Ten consecutive patients with a chronic DFU were included in this study. Each patient was treated with
standard-of-care therapy for at least 4 weeks before being considered for treatment with AMHAT. Patient demographics are detailed in Table 1. Patients were primarily female (60%) with a mean age of 64.2 (SD ± 7.89) and average BMI of 32.6 (SD ± 6.77). Baseline wound characteristics are shown in Table 2. Mean baseline wound area was 2.7 cm² (SD ± 2.43), and the wound size ranged from 0.1 to 6.9 cm². Wound depth ranged from 0.1 to 0.4 cm. The study ulcers varied by regional location, plane, and anatomical location (Table 2), and there were five ulcers corresponding to Wagner grade 1 (UT Grade 1A) (50%) and five Wagner grade 2 ulcers (UT Grade 2/3A) (50%). The mean number of times sharp debridement was performed was 7.6 (SD ± 4.77). Ulcers treated with AMHAT were followed up for up to 12 weeks or until wound closure was achieved. No subjects were withdrawn or lost to follow-up. Patients were monitored for adverse events at each visit, and none were observed.

Complete closure was achieved in 6 of 10 wounds (60%) in the study period. Five wounds were healed by 6 weeks and one at 8 weeks. Four wounds did not close within 12 weeks. Case examples are shown in Figures 3–5. The mean time to closure of these wounds was 49.1 days (95% CI, 29.9–68.3). [See figures, Supplemental Digital Content 1, which displays (a) the Kaplan-Meier plot of wound healing and (b) the mean PAR at 12 weeks was 78.5% (SD, 33.23), http://links.lww.com/PRSGO/C198.]

### Table 1. Key Subject-related Variables

| Variable          | Value |
|-------------------|-------|
| Patient age (y)   | 64.2  |
| BMI               | 32.6  |
| Gender            |       |
| Male              | 4     |
| Female            | 6     |
| Race/ethnicity    |       |
| White/non-Hispanic| 8     |
| Black/African American | 2 |

Continuous variables are reported as means (SD) and categorical variables as counts (percentage).

### Table 2. Key Wound-related Variables

| Variable                      | Value         |
|-------------------------------|---------------|
| Wound area (cm²)              | 2.7 (2.43)    |
|                               | Median: 2     |
|                               | IQR: 3.8      |
| Depth (mm)                    |               |
| ≤1                            | 2 (20)        |
| 1–2                          | 6 (60)        |
| 3–4                          | 2 (20)        |
| DFU regional location         |               |
| Planter                      | 5 (50)        |
| Dorsal                       | 4 (40)        |
| Anterior                     | 1 (10)        |
| DFU plane                     |               |
| Lateral                      | 5 (50)        |
| Medial                       | 5 (50)        |
| DFU anatomical location      |               |
| Toe                          | 1 (10)        |
| Forefoot                     | 4 (40)        |
| Midfoot                      | 2 (20)        |
| Hindfoot                     | 1 (10)        |
| Heel                         | 2 (20)        |
| No. sharp debridements       | 7.6 (4.77)    |
|                               | Median: 6.5   |
|                               | IQR: 10       |
| Wagner grade                 |               |
| 1                            | 5 (50)        |
| 2                            | 5 (50)        |

Continuous variables are reported as means (SD) with median/IQR additionally reported for key nonnormally distributed continuous variables, and categorical variables are reported as counts (percentage).

**DISCUSSION**

The purpose of this study was to evaluate the effectiveness of a single treatment of AMHAT, dispensed by an advanced 3D bioprinting technology, Dr. INVIVO, on wound closure in chronic DFUs. In fat grafting, large numbers of extracellular substances that promote wound healing, such as insulin-like growth factor, hepatocyte growth factor, transforming growth factor-β1, and vascular endothelial growth factor, are contained in extracted adipose tissue.7–9 The technology used in this study utilizes a 3D scanner to measure wound size and depth and then converts the measurements to a file that can be used in the 3D bioprinter. Once the patient’s adipose tissue is harvested, typically from the abdomen, it is minimally manipulated to adjust to the 3D bioprinter. Following micronization of the adipose tissue, the autologous dermal substitute is printed and applied directly onto the cleaned wound site. The technique is quite user-friendly and produces a completely customized scaffold that matches the wound exactly. Although the device is not yet commercially available in the United States, the theoretical price for the kit is estimated to be $5000 per unit. The kit, Dr. INVIVO AI Regen Kit, includes 3D bioprinting accessories and ECM bioink processing tools, all of which are disposable.

A few studies have investigated the role of autologous fat grafting for the treatment of chronic DFUs. One case report, in which autologous plantar fat grafting was used to prevent ulcer recurrence in a patient with a recently healed DFU, reported good results after 6 weeks.10 And, in another single case report, an injectable allograft adipose matrix was used to decrease pressure at the site of a healed DFU, but follow-up was limited to 2 months.20 However, in both cases, wound healing was not specifically addressed.

In a clinical study presented by Kim,21 autologous minimal manipulating autologous extracellular matrix (MA-ECM) printed with bioinks was applied to patients with chronic DFUs, and a significant reduction of wound size was noted following just 1 week of a treatment in most patients. In fact, nearly all subjects demonstrated complete closure between 2 and 5 weeks after treatment. In the pilot study presented here, wound closure was achieved in 60% of patients by 8 weeks, and the mean time to closure was 49.1 days. In addition, five wounds were healed by 6 weeks, and one at 8 weeks. Four wounds did not close within 12 weeks, which may be explained by several factors, including adherence to offloading recommendations and underlying comorbidities. No adverse events were reported during treatment.

The strength of our pilot study includes a robust trial design with appropriate screening procedures, a standardized approach to SOC, ITT analysis, and appropriate adjustment for multiple statistical testing. However, there are some weaknesses in the study, which include only one arm for treatment and no comparator as well as the need for multiple sites with geographic distributions, larger
sample size, and longer follow-up for the patients after healing to determine rate of recurrence and size of wound. Additional trials on this technology should be considered using a randomized controlled model against standard of care alone or a well-established advanced wound modality.

**CONCLUSIONS**

In this single-arm pilot study of patients undergoing treatment for chronic DFUs with AMHAT dispensed by the Dr. INVIVO device, 60% of ulcers refractory to standard-of-care treatment achieved complete wound closure by 12 weeks. Wound closure was achieved in an average of 49.1 days, and the mean PAR at 12 weeks was 78.3%. No adverse events were reported during treatment. Therefore, the use of fat grafting as a method for healing chronic DFUs should be considered as a viable therapy. Building on the success seen in this study, along with larger future studies, the 3D bioprinter may become an important tool in the patient’s pursuit of ulcer-free, activity-rich days.
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