Ready-to-Use Micronized Human Acellular Dermal Matrix to Accelerate Wound Healing in Diabetic Foot Ulcers: A Prospective Randomized Pilot Study

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ABSTRACT

OBJECTIVE: To examine and report clinical outcomes of a ready-to-use micronized dermal matrix for diabetic foot ulcers (DFUs) and compare it to treatment with conventional negative-pressure wound therapy (NPWT) only.

METHODS: The researchers randomly allocated 30 DFUs Wagner grade 2 or higher from 30 adult patients into two groups. The control group (n = 15) was treated with conventional NPWT, and the experimental group (n = 15) was treated with micronized dermal matrix and NPWT. The researchers evaluated the following outcomes: granulation tissue formation, proportion of patients with closed or granulated wounds at 42 and 120 days, achievement of complete wound healing in the 6 months of follow-up, and intervals from enrollment to final surgical procedures.

RESULTS: All 15 wounds treated with the micronized matrix showed healthy granulation tissue without noticeable complications during follow-up. At 42 days, 46.7% of wounds in the experimental group had closed compared with 28.6% in the conventional NPWT group (P = .007). At 120 days, 86.7% of the experimental group had completely closed wounds, compared with 57.1% in the conventional therapy group (P = .040). During the 6-month follow-up period, 93.3% of the experimental group achieved complete wound healing compared with 85.7% of the conventional therapy group (P = .468).

CONCLUSIONS: The healing outcomes for DFUs in the experimental group were superior when micronized matrix treatment was combined with NPWT.

KEYWORDS: acellular dermal matrix, advanced therapy, cellular and/or tissue-based product, diabetic foot ulcer, micronized human acellular dermal matrix, negative-pressure wound therapy, wound healing

INTRODUCTION

Diabetic foot ulcer (DFU) is a chronic, refractory, and easy-to-recur disease in need of multidisciplinary approaches for treatment. It is the principal cause of hospitalization and nontraumatic lower extremity amputation. In recent years, several advanced wound healing modalities such as negative-pressure wound therapy (NPWT), topical growth factor therapies, hyperbaric oxygen therapy, and bioengineered cellular and/or tissue-based products have brought about new opportunities for patients with DFUs. Among these, NPWT is well established for managing complex diabetic foot wounds. Supporting evidence for NPWT in treating DFUs includes numerous randomized controlled trials.

Acellular dermal matrices (ADMs) have long been used as soft tissue replacements and are commonly used in wound healing and tissue repair and reconstruction. Human ADM is decellularized human dermis devoid of immunogenic components. The epidermis and the cellular components of the dermis are removed by appropriate processing, leaving collagen, elastin, proteoglycan, laminin, and the basement membrane. Collagen and elastin control tensile strength and elasticity, proteoglycans induce angiogenesis, laminin maintains binding with the connective tissues, and the basement membrane consists of collagen type IV. The extracellular matrix is crucial for wound healing because it provides structural support and modulates cellular responses. When placed in vivo, it first biointegrates by cellular repopulation and revascularization and then becomes vascularized and remodeled with host tissue after implantation.

Micronized ADM is a commercially available graft material developed to improve wound healing. Its composition is similar to that of sheet-type ADMs such as AlloDerm. The ADM is micronized by using a cutting mill to produce microfractures with particles of less than 750 μm (rather than shredding the ultrastructure). Micronized
ADM can be filled in with a syringe, but it needs to be rehydrated for 10 to 15 minutes before application. A gel-type micronized dermal matrix that preserves the structural component of the dermal matrix under aseptic tissue processing has been recently developed. It can be applied easily and directly onto the wound site because the materials are provided in syringes that are ready to use without the need for rehydration.

The authors hypothesized that micronized ADM would be an effective treatment for DFUs and aimed to evaluate the advantages of ADM for chronic wound healing and the micronized form’s capacity for three-dimensional molding onto full-thickness wound beds of irregular surfaces. In this study, the authors present their preliminary results, including a comparison of clinical efficacy of micronized human ADM (MHADM) and NPWT for DFUs.

METHODS

The present study was a prospective, randomized trial performed by a single investigator at a single center designed to determine the efficacy of MHADM. The study protocol was approved by the authors’ institutional review board (AJIRB-MED-OBS-16-017), and written consent was obtained from all participants prior to any study-related procedure. The patient described in the case report also provided written informed consent to study-related procedure. The patient enrolled in the intention-to-treat analysis.

Patients with DFUs in an inpatient setting at the authors’ institute between April 2016 and December 2016 were included in the study. The inclusion criteria were 19 years or older, and diagnosis of type 1 or type 2 diabetes with at least one nonhealing neuropathic or vasculopathic foot ulcer that failed to heal following a minimum of 4 weeks of documented conservative care prior to study enrollment. Patients had DFUs of stage 2 or higher on the Wagner scale, and surgery was indicated. If a single patient had multiple wounds, the researchers selected the deepest wound for inclusion.

Exclusion criteria were low level of serum albumin (assessed by a serum albumin level <2.0 g/dL), malignancy, autoimmune disease, use of long-term corticosteroids or immunosuppressants, and uncontrolled hyperglycemia (preoperative hemoglobin A1c [HbA1c] level >15.0%). Patients whose ulcer healed or who reached the 6-month follow-up visit were included in the intention-to-treat analysis.

The researchers randomized participants into either the experimental group (MHADM and conventional NPWT treatment) or the control group (conventional NPWT treatment) using sequentially numbered, opaque, sealed envelopes that a statistician randomly generated before enrollment to avoid selection bias. The researchers collected demographic data including age, sex, comorbidities, HbA1c level (at study enrollment and at 120 days), albumin, angioplasty, and glucose control methods. An- giographic and wound details were also collected. An attending professor evaluated the wounds every other day until complete wound closure was observed or until completion of the 6-month follow-up visit for each participant.

Digital photographs for documentation were taken at a distance of 35 cm by single-lens reflex camera with constant lighting and shooting conditions. The researchers measured wound surface area with a portable three-dimensional camera (InSight; eKare Inc, Fairfax, Virginia) that enables digital measurement of ulcerative wounds including area, depth, and volume. The researchers photographed wounds of hospitalized patients every other day and at weekly follow-up visits postdischarge.

As a treatment protocol after study enrollment, all patients underwent conventional angiography for screening of lower extremity vascular stenosis or occlusion, and if either was verified, patients underwent percutaneous transluminal angioplasty on their lower extremity arteries prior to surgical debridement. Arterial stenosis of more than 50% vessel caliber was used as an indication for balloon angioplasty. The researchers confirmed patency of the recanalized artery by final angiography after the procedure.

To assess regional tissue perfusion, ankle-brachial index and transcutaneous oxygen tension on the ipsilateral side were measured in all patients. For patients with peripheral arterial disease requiring balloon angioplasty, both measurements were performed before and after the procedure.

All patients underwent operative debridement until healthy, viable tissue was visible in their wounds before applying the NPWT or MHADM graft. Necrotic bone was also debrided. The primary investigator performed all debridement. Patients were treated with culture-directed IV antibiotic therapy for infected wounds according to the recommendations of the International Working Group on the Diabetic Foot. Wounds in both groups were dressed with NPWT until surgical coverage or complete healing occurred. In select wounds, the surgeon achieved surgical coverage with primary repair, skin graft, or flap reconstruction. The indications for surgical coverage were less than 25% reduction of wound size during 2 weeks of treatment.

The NPWT dressings were changed every other day during hospitalization until the study wound was covered surgically or wounds showed sufficient healing.
progress. Patients were discharged from hospital when sutures were removed from surgical wounds or when NPWT dressings were not required. After discharge, the wound was dressed with polyurethane dressing. Patients were evaluated weekly in the clinic after discharge for wound assessment for up to 6 months of follow-up.

For patients in the control group, study wounds were covered with NPWT (CURAVAC; CGBio Co Ltd, Hwaseong, Korea) after surgical debridement and changed every other day. For patients assigned to the experimental group, MHADM (CG-PASTE; CGBio Co Ltd) was placed on the wound bed to cover the entire wound surface, and the volume placed on each wound was recorded. The MHADM was applied repeatedly, once a week during NPWT dressing change until wound reconstruction or complete healing occurred. As initial therapy, MHADM was molded to fit the three-dimensional shape of the wound, placed under a silicone-based nonadherent dressing, and covered with NPWT as in the conventional therapy group.

The main outcomes were (1) granulation tissue formation during 120 days of the follow-up period (defined as healthy red tissue covering 75% or more of the raw surface without infection) and (2) achievement of complete wound healing in 6 months. Complete healing was defined as an epithelialized wound with no raw surface and no requirement for additional wound dressings. Secondary outcomes were the proportions of wounds that were surgically closed or covered without complications or that were completely healed at 42 and at 120 days. Adverse events including wound infection, surgical complications, and any complications associated with the interventions were also evaluated during the follow-up period.

Statistical Analysis

The researchers tabulated baseline patient and wound characteristics for categorical and continuous variables and used the χ² test or Fisher exact test to examine for differences in categorical characteristics between the two groups. They used a two-sample t test or Wilcoxon rank sum test to examine differences in continuous characteristics. Values for the numbers of cases, persons, years, days, and months were expressed as original value and percentage (including one decimal place). The data were fit using the R program v. 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria), and P < .05 was considered statistically significant. The researchers used Kaplan-Meier analysis to investigate differences in complete healing and granulation tissue formation between the two groups.

RESULTS

The researchers enrolled a total of 30 participants randomized to either the experimental (n = 15) or the control (n = 15) group. One patient in the control group withdrew 2 days after enrollment because of major amputation. A total of 29 patients were included in the intention-to-treat analysis. One patient died because of exacerbation of existing medical morbidities after the study period. The cases of major amputation and death were not related to the study treatment.

Patient demographics were similar at enrollment (Table 1). The study population consisted of 21 men and 8 women, with a mean age of 49.9 years (range, 22-80 years). The two groups were comparable in terms of age, sex, co-morbidities, diabetes duration, wound duration, wound location, glucose control method, HbA1c level, and albumin level, without significant differences. The baseline ulcer size was also similar for each group, with no observed statistically significant difference (Table 2). Thirteen patients had peripheral artery occlusion as a complication of diabetes and had undergone angioplasty involving the ipsilateral extremity prior to surgical debridement. All patients tolerated the surgical procedures and dressing changes well, with no perioperative mortality or serious systemic morbidity. The number of infected wounds, baseline and postangioplasty ankle-brachial index, baseline and postangioplasty transcutaneous oxygen tension, and method of offloading were not significantly different. The location and cause of DFU were not different for both the groups. After surgical debridement, an average of 22.4 days of NPWT was applied to all participants. In the experimental group, the average amount of MHADM applied for each person was 3.2 mL per week.
Ten patients (66.7%) in the experimental group and seven (50.0%) in the control group were treated surgically (Table 3). Among them, all patients (100%) in the experimental group achieved complete healing. Complete healing was achieved in five patients (71.4%) in the control group, and two patients needed further wound management because of incomplete healing. There was no significant difference between groups in the proportion of patients who achieved complete healing ($P = .154$). The mean interval between study enrollment and surgical coverage was 21.8 days in the experimental group and 16.0 days in the control group, which was not significantly different ($P = .468$).

All wounds in the experimental group and nine (64.3%) in the control group showed granulation tissue formation during the 120-day follow-up, and this difference was significant ($P = .017$; Table 1). At 42 days, 46.7% ($n = 7$) of the MHADM-treated wounds had closed compared with 28.6% ($n = 4$) of the wounds treated with NPWT alone ($P = .007$; Table 2). At 120 days, 86.7% ($n = 13$) of the MHADM-treated wounds had closed completely, compared with 57.1% ($n = 8$) of the wounds that received NPWT alone ($P = .468$). On Kaplan-Meier analysis, there was significant difference in granulation tissue formation between the two groups ($P = .038$; Figure 1). There was no significant difference in complete healing after 120 days ($P = .329$; Figure 2).

**CASE REPORT**

A 67-year-old woman with a history of type 2 diabetes mellitus and peripheral arterial occlusive disease was admitted to the hospital with a necrotic DFU on her right foot (Figure 3). After revascularization by an interventional radiologist on her ipsilateral popliteal and anterior tibial arteries, the wound was surgically debrided, and 5 mL of MHADM and NPWT was applied for 2 weeks. The patient was discharged and followed up as an outpatient. The wound healed completely at 10 weeks of treatment.

**DISCUSSION**

The authors report the usefulness of MHADM in treating diabetic ulcers. The MHADM, a micronized form of an allogeneic ADM with an intact basement membrane,
provides a collagen scaffold that promotes cellular migration, growth factor retention, and tissue integration without rejection or foreign body reaction. To the authors’ knowledge, this is the first randomized controlled study comparing the efficacy and safety of gel type-MHADM in the management of DFU. This experience suggests that micronized ADM has positive effects on DFU treatment outcomes.

Dermal matrices are known to become integrated with and often become histologically indistinguishable from native tissue, producing pliable and functional tissue.11 Acellular dermal matrices, in both sheet and micronized forms, are increasingly used in reconstructive surgery and wound management because they serve as scaffolds for the ingrowth of native cellular elements over time. The sheet form of ADM has demonstrated excellent results in complex wounds of the lower extremities.12,13 The sheet form is used as a substitute for dermal loss or to reinforce soft tissue, whereas the micronized form is used in a paste form to fill complex defects with uneven surface. Early retrospective case studies using these micronized ADMs suggest that they may effectively promote healing in refractory ulcers and recalcitrant sinus tract wounds without surgery and without complications such as rejection or foreign body reaction.14,15

In this study, the authors compared MHADM plus NPWT treatment with NPWT treatment alone, with a focus on wound preparation and achieving complete wound healing during the follow-up. Baseline parameters were similar in both groups, including average wound area. As a parameter of wound preparation, the researchers assessed granulation tissue formation, which is meaningful for infection control and readiness for surgical coverage. The combined treatment appeared to be more effective at forming granulation tissue than NPWT alone: by 42 days, 46.7% of the DFUs had closed in the experimental group compared with 28.6% in the control group (P = .007). After 120 days, 86.7% of the MHADM-treated wounds had closed completely compared with 57.1% in the control group (P = .040). Although this statistically significant difference was not maintained at study completion, the MHADM appeared to help control infection effectively in the early phase of wound healing and thus helped to positively influence the healing process. As with conventional cellular and/or tissue-based products, the micronized ADM appears to recruit healing materials at early time points, which could be useful in the clinical setting for treating DFUs and for wound bed preparation for other surgical procedures.

**Limitations**

The present study has some limitations. First, the sample size of each arm was relatively small. Second, although the investigator in the study judged complete wound healing as objectively as possible, a common limitation in wound healing studies is the absence of a blinded, quantitative analysis of healing. In addition, the investigator decided to perform surgical coverage in select wounds. A surgically closed wound might accelerate

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**Figure 1. **KAPLAN-MEIER PLOT FOR GRANULATION TISSUE FORMATION
Dotted line, control; solid line, matrix.

**Figure 2. **KAPLAN-MEIER PLOT FOR COMPLETE WOUND HEALING
Dotted line, control; solid line, matrix.
healing time in larger wounds. However, because surgical closure was possible only when enough vascularization of surrounding tissue was achieved, the secondary outcomes included the proportion of wounds that were surgically covered.

Third, the control arm was treated with NPWT because this is the best current treatment protocol for deep ulcers. Ethically, a control group without NPWT was not considered possible. Further, although statistically insignificant, the average wound size was different between the two groups. Given that wound size also has an impact on wound healing, a larger sample size would resolve this bias.

Future studies with larger samples should focus on clarifying the superior outcomes of combination treatments to single treatment methods. Finally, the cost-effectiveness of such treatments should also be considered. Accordingly, additional studies to estimate total expenses are also needed to illustrate the clinical value of the study method adopted.

CONCLUSIONS

Based on the results of this randomized study, the authors believe that MHADM is effective for treating DFUs when combined with NPWT. The healing progress in the experimental group was significantly more rapid than in the control group. These data indicate the need for adequately designed studies to elucidate the full potential of MHADM as an adjunctive treatment for DFUs.

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