A Retrospective Analysis of a Human Cellular Repair Matrix for the Treatment of Chronic Wounds

Matthew Regulski, DPM; Douglas A. Jacobstein, MD; Russell D. Petranto; DPM, Vincent J. Migliori, DPM; Girish Nair, DPM; and Darelle Pfeiffer, DPM

Abstract
Despite the introduction of advanced wound care modalities over the last 15 years, chronic wounds are an increasing problem. Few single options are available for clinicians to treat recalcitrant wounds such as diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs). A retrospective, single-center study was conducted at an outpatient wound care center to evaluate the clinical effect of a human cellular repair matrix (h-CRM) on chronic wounds that had failed to heal. Data from all patients who had received this treatment modality during a period of 2 years were abstracted. Standard care included weekly visits, regular debridement, offloading DFUs, compression for VLUs, and h-CRM for wounds >4 weeks duration. A total of 66 patients (30 male, 36 female, mean age 71.1 [± 8.8] years) received h-CRM treatment for 67 wounds (34 VLUs, 27 DFUs, and six other chronic wounds). The average wound size at baseline was 6.65 (± 9.68) cm², and the average wound duration before h-CRM treatment was 38 (±70.8) weeks. Fifty (50) patients (74.6%) had failed to heal using other advanced therapies. After 12 weeks of care, 51 of the 67 wounds (76.1%) were healed: 23 of 34 (67.6%) VLUs and 23 of 27 (85.2%) DFUs. Average time to closure in these wounds was 5.8 (±2.5) weeks. No significant differences were found between proportions of VLUs and DFUs healed. No adverse events or recurrences occurred during an average follow-up time of 20.4 months (range 11 to 32 months). Overall, patients received an average of 3.8 applications of h-CRM, and 3.2 applications were used among patients that healed. The study results suggest h-CRM may benefit patients with chronic wounds. Prospective, randomized clinical studies are warranted.

Keywords: retrospective study, wound care, venous leg ulcer, diabetic foot ulcer, cellular matrix

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Chronic, nonhealing wounds, including venous leg ulcers (VLUs), diabetic foot ulcers (DFUs), pressure ulcers, and traumatic wounds, are a growing problem in the United States, affecting more than 6.5 million people annually. These wounds, which represent a disruption in the normal healing process, are rarely seen in individuals who are otherwise healthy and often occur due to underlying conditions such as diabetes, vascular insufficiency, and obesity. Hallmarks of impaired wound healing, including chronic inflammation and increased local injury, lead to failure of the wound site to transition through the normal coordinated sequence of events from hemostasis through tissue regeneration. A 2009 review suggests that chronic wounds represent a tremendous financial burden on the US healthcare system, with estimated treatment costs in excess of $25 billion annually. In addition, chronic wounds impact the quality of patients’ lives and interfere with normal physical and social functioning.

Although standard treatment modalities, including debridement and offloading for DFUs and compression dressings for VLUs, are typically employed first, many wounds still require advanced therapies in addition to these standard treatment modalities to achieve complete wound closure. Options including topical dressings, ointments, and acellular matrices may offer some benefits depending on the wound environment. Bioengineered skin substitutes have demon-
conducted in laboratories, the repair matrix has been shown to be beneficial for the treatment of chronic wounds by inhibiting inflammation and supporting transition of chronic wounds from the inflammatory to the regenerative phase; stimulating cell migration, proliferation, and differentiation, leading to wound vascularization, granulation and reepithelialization; and preventing scar formation.

The purpose of this retrospective study was to evaluate the initial clinical experience with this h-CRM for the treatment of a large, single-center cohort of recalcitrant, chronic wounds.

Methods

Data were collected retrospectively through chart review by the treating physicians at Ocean County Foot and Ankle and The Wound Institute of New Jersey, which provides podiatric care, including chronic wound care, to patients with a variety of foot, ankle, and leg problems. Data from all wound care patients seen in the clinics between April 2010 and March 2012 who received at least one application of h-CRM were abstracted.

During that time, patient eligibility for h-CRM treatment was based on wound type, wound duration of 4 weeks or greater, and adequate resolution of underlying morbidity. Standard office consent for treatment with available, marketed products, which includes use of de-identified data for medical research or education, was provided by all patients. Patients with clinical signs of acute infection, including cellulitis, wound infection, or osteomyelitis, did not receive h-CRM until the infections were adequately treated; h-CRM also was not used in the presence of maceration. Additionally, patients with underlying ischemia or poor nutrition were not treated until they underwent work-up when indicated including Doppler ultrasound, skin perfusion pressure, angiography, and serological evaluation for malnutrition (comprehensive metabolic profile, prealbumin, and complete blood count) for underlying etiology and a treatment plan.

Key Points

- The authors of this retrospective study abstracted data from 66 patients with chronic wounds who had received treatment with a human cellular repair matrix (h-CRM).
- Wounds had been refractory to previous treatments for an average of 38 weeks.
- The majority of wounds were healed after 12 weeks with regular debridement, supportive care, and application of h-CRM.
- Controlled clinical studies are warranted to evaluate the efficacy of h-CRM compared to other topical treatment modalities.
was established.

**Procedure.** The h-CRM was applied at each visit based on physician assessment of the wound status. Before treatment, sharp debridement of the wound margins and wound bed was performed as indicated. Matrix preparation followed standard instructions, including thawing of the product followed by direct rinse in a saline bath. After applying the matrix directly to the wound surface, a nonadherent dressing (Adaptic Touch® or Adaptic® [Systagenix, Gatwick, UK]), saline-moistened gauze, and dry gauze dressing were applied. Proper offloading utilizing total contact cast, surgical shoe, or DH Offloading Walker® (Össur Americas, Foothill Ranch, CA), and/or multilayer compression bandaging for venous ulcers were provided as clinically indicated. Treatment algorithms indicated weekly follow-up and routine wound care at the clinic, including assessment. Wounds were measured with a standard wound ruler at each visit using the wound’s greatest length and width. Wounds were considered closed when 100% reepithelialization and no evidence of drainage were determined by the treating physician. Closure was documented and confirmed with PictZar® Digital Planimetry Software (Biovisual Technologies, Elmwood Park, NJ). No additional applications of the matrix occurred after wound closure or, in the case of nonhealed wounds, after 12 weeks of care per clinic protocol for advanced therapies.

**Data collection and analyses.** Data were retrieved from the charts, dictated, and entered into a Microsoft Excel spreadsheet by a dedicated data entry person. A single investigator reviewed all entered data for comparison with the medical record and confirmation of accuracy. All data were de-identified for analysis. The identifying patient information was maintained in a secured location at the wound care center.

Data collected and analyzed included patient demographics (gender and age); wound type, location, size, and duration; previous therapies received; h-CRM application by visit; number of h-CRM applications; wound disposition including whether wound was healed or not; and recurrence (if known). Safety outcomes abstracted included the occurrence of any adverse event following application of h-CRM. Wound measurements were calculated using greatest length and width multiplied to obtain area and confirmed with PictZar software. Descriptive statistics were used to summarize patient and wound variables. Nominal wound closure endpoints, including the proportion of patients with wound closure by 12 weeks and 26 weeks and time to wound closure, were assessed using

<table>
<thead>
<tr>
<th>Table 1. Study demographics and baseline wound characteristics</th>
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<tr>
<td><strong>All wounds (N=67)</strong></td>
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<tr>
<td>Gender, n (%)</td>
</tr>
<tr>
<td>Male</td>
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<tr>
<td>Femaleb</td>
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<td>Patient age, years</td>
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<tr>
<td>Range</td>
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<tr>
<td>Wound age, weeks</td>
</tr>
<tr>
<td>Mean±SD (range)</td>
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<tr>
<td>Wound surface area, cm²</td>
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<tr>
<td>Median, 25%, 75%</td>
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<td>Previous advanced therapiesa</td>
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aOther wounds include pressure ulcers, postsurgical wounds, and non-venous vascular wounds
bOne female VLU patient had two separate wounds
cIncludes collagen dressings, acellular matrices, skin grafts, cellular skin substitutes, hyperbaric oxygen, wound vacuum, and topical growth factors

Figure 1. Proportion of DFUs and VLUs healed at 12 weeks by wound size. 
Quartile 1: 0.42 cm²–1.80 cm²; Quartile 2: 1.81 cm²–3.37 cm²; Quartile 3: 3.38 cm²–6.56 cm²; Quartile 4: 6.57 cm²–99.18 cm².
Fisher Exact test, and continuous variables were analyzed using one-way analysis of variance (ANOVA). Kaplan-Meier time-to-event analysis was performed to assess the probability of closure.

**Results**

Sixty-six (66) patients with 67 full-thickness chronic wounds received at least one application of h-CRM between April 2010 and March 2012. Participants included 36 women (54.5%); average age of all patients was 71.1 years (range 41–88) (see Table 1). Two patients required surgical intervention unrelated to the application of h-CRM that resulted in discontinuation of treatment, one patient had failure of a femoral popliteal bypass graft during week 4, and one patient developed critical limb ischemia during week 1.

As seen in the baseline wound characteristics, the distribution of chronic wound types was approximately evenly divided between DFUs (27 wounds, 40.3%) and VLUs (34 wounds, 50.7%). Six (9%) other wounds (pressure ulcers, traumatic and postsurgical wounds, and non-venous vascular wounds) also received h-CRM. The average wound size at baseline was 6.65 ± 9.68 cm² for all wound types (median 3.29 cm²). The average VLU was 8.66 ± 12.02 cm² (median 4.07 cm²), and the average size of DFUs was 3.97 ± 3.08 cm² (median 3.11 cm²). Wound duration before treatment with h-CRM across all wound types was 38 weeks (range 4–416 weeks; VLUs had a longer average wound duration (51.5 weeks) than DFUs (24.5 weeks). The majority of patients (50, 74.6%), had failed prior advanced wound therapies, including porcine small intestine submucosa (25, 50%), skin autograft (13, 26%), bilayered cell-based product (eight, 16%), collagen matrix (eight, 16%), becaplermin (six, 12%), and human-fibroblast-derived dermal substitute (five, 10%).

Overall, 51 of the 67 wounds (76.1%) achieved complete closure within 12 weeks (range 1–12 weeks). The average time to wound closure was 5.8 weeks ± 2.5 weeks. Fifty-four (54) of the 67 wounds (80.6%) healed within 26 weeks without additional therapy beyond 12 weeks. At 12 weeks, 23 of 27 (85.2%) DFUs and 23 out of 34 (67.6%) of VLUs were closed, which was not a statistically significant difference (Fisher’s exact test, P >0.05) (see Table 2). Nor was there a statistically significant difference in the time to closure of DFU and VLU wounds that healed (one-way ANOVA, P >0.05). In this study, an average of 3.8 matrices per wound were applied and among wounds that healed by 12 weeks, an average of 3.2 matrices were used.

Analysis of the closure rates in terms of baseline characteristics demonstrated longer healing times for wounds with longer duration but little effect of initial wound size on the proportion of healed wounds. Among wounds of >4 weeks and <6 months duration, 35 of 45 (77.8%) healed, 13 of 14 (92.9%) of wounds with a history of at least 6 months and <2 years healed, and three of eight (37.5%) wounds present 2 years or longer (range 2–8 years) healed. The proportion of wounds healed by baseline wound size varied little, ranging from 73.3% to 80.0% for DFUs and VLUs combined and from 62.5% to 77.8% for VLUs alone (see Figures 1 and 2).

The probability of closure for all wounds at 12 weeks was 82.6%, with a median time to closure of 49 days (see Figure 3). The probability of closure by 12 weeks was 71.5%, (median 49 days) for all VLUs and 89.7% (median 42 days) for all DFUs (see Figure 4).

No adverse events related to the application of the h-CRM were reported. Follow-up data available on 47 of the 54 wounds that were 100% reepithelialized with a mean follow-up time of 20.4 months (range 11–32 months) demonstrated no wound recurrences. No wound-associated amputations were reported at any time during treatment or in the follow-up period post-treatment. One amputation occurred in a patient who developed critical limb ischemia; the patient did not receive further treatment with the matrix after week 1.

**Table 2. Wound closure outcomes**

<table>
<thead>
<tr>
<th></th>
<th>All wounds (N=67)</th>
<th>Diabetic foot ulcers (n=27)</th>
<th>Venous leg ulcers (n=34)</th>
<th>Other wounds (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete wound closure at 12 weeks Number of wounds, n (%)</td>
<td>51 (76.1%)</td>
<td>23 (85.2%)</td>
<td>23 (67.6%)</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>Time to complete wound closure, weeks (Mean±SD)</td>
<td>5.8±2.5</td>
<td>6.2±2.6</td>
<td>5.3±2.5</td>
<td>5.8±2.4</td>
</tr>
<tr>
<td>Complete wound closure at 26 weeks, n (%)</td>
<td>54 (80.6%)</td>
<td>24 (88.9%)</td>
<td>24 (70.6%)</td>
<td>6 (100.0%)</td>
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Figure 2. Proportion of venous leg ulcers healed at 12 weeks by wound size. Quartile 1: 0.42 cm²–1.89 cm²; Quartile 2: 1.90 cm²–3.74 cm²; Quartile 3: 3.75 cm²–9.23 cm²; Quartile 4: 9.24 cm²–59.18 cm²
Discussion

A retrospective study was conducted to assess the clinical use and outcomes of a h-CRM used to manage chronic wounds. No adverse events occurred, and with an average of approximately four applications, 85.2% of DFUs and 67.6% of VLUs were healed after 12 weeks after being refractory to a variety of previous treatments for an average of 24.5 and 51.5 weeks, respectively.

These results are encouraging. A meta-analysis\(^\text{17}\) of standard DFU care (offloading, debridement, and moist gauze) showed that only 24.2% of DFUs were healed at 12 and 30.9% were healed at 20 weeks. This study established an early benchmark for the likelihood of closure utilizing commonly used care practices such as wet-to-dry dressings,\(^\text{17}\) confirming that gauze-type dressings are associated with poor healing outcomes. Letendre et al,\(^\text{18}\) in a pilot evaluation of devitalized placental membranes, showed closure rates among 14 patients with a DFU of 55.5% after 12 weeks. Similarly, results of a large, retrospective study\(^\text{19}\) evaluating the use of a biologically active, cryopreserved human skin allograft showed a closure rate of 60.38% by 12 weeks for 54 DFUs with an average wound age of 20.6 weeks and 60.77% by 12 weeks for 134 VLUs with an average wound age of 17.9 weeks. These results were considered encouraging, but the history of these wounds was only 18.7 weeks, compared to 38 weeks in the current study.

The current results also indicate a clinical benefit when compared with published results from studies involving bioengineered skin equivalents. The Veves et al\(^\text{11}\) study, which evaluated the use of a bilayered, cell-based product in 208 patients with DFUs present for at least 2 weeks, reported closure of 56% of wounds at 12 weeks. The average wound size in that study was 2.97 cm\(^\text{2}\). Similar results were reported in DFU trials utilizing a bilayered, cell-based product by Steinberg et al\(^\text{20}\) and Edmonds et al,\(^\text{21}\) with healing rates of 55.2% and 51.5%, respectively, within 12 weeks. In 2003, Marston et al\(^\text{10}\) reported results from a DFU trial utilizing a human-fibroblast-derived dermal substitute; among the 130 patients who received the skin substitute, 30% achieved complete wound closure at 12 weeks.

Among VLU trials involving a bilayered, cell-based product, Falanga et al\(^\text{9}\) reported the healing of 63% of VLUs (N = 293) at 6 months, which is slightly lower than the 70.6% of closed wounds at 26 weeks observed in this analysis. Additionally, the healing rates obtained in this retrospective study were largely achieved within a much shorter period of time, as 67.6% of VLUs had healed by only 12 weeks. A second study of 120 patients conducted by Falanga and Sabolinski\(^\text{22}\) showed healing rates of VLUs of 47% within 6 months. A large VLU trial conducted by Harding et al\(^\text{23}\) using a human fibroblast-derived dermal substitute in 366 patients showed a much lower proportion of wounds healed (34% at 12 weeks in the treatment group of 186 patients) and no statistically significant difference between the treatment and standard compression therapy alone.

Three quarters of patients in the current study (including 85% of DFU patients and 65% of VLU patients) received at least one advanced therapy before receiving the cellular repair matrix. This includes 50% of patients who received porcine small intestine submucosa and 52% of patients who received a cell-based product (skin graft, bilayered skin substitute, or human fibroblast-derived dermal substitute). Additionally, of patients who failed to heal using prior advanced therapy, 37.3% failed more than one type of treatment, indicating the serious, refractory nature of the wounds studied. Importantly, follow-up of 47 (87%) of those patients that healed showed no wound recurrence with an average follow-
up of 20.4 months. This compares favorably to published recurrence rates of 12% for VLUs at 12 months and 6% for DFUs at 6 months in patients who received the bilayered skin substitute in clinical trials.24 Furthermore, no patients in this study required a wound-related amputation.

When comparing initial wound sizes among patients who received the treatment, no differences in the proportion of wounds healed based on size were observed. This differs from published studies, including the Reyzelman et al study of 86 DFU patients randomized to receive an acellular dermal tissue matrix or control dressings who were followed for 12 weeks. The authors reported that wound size at presentation may affect the proportion of wounds healed within 12 weeks. However, this study was not designed to test the effect of wound size on outcome, and the grouping by wound size may have masked any potential wound size effect.

Finally, the treatment was well tolerated by all patients.

Limitations
Missing data is an important limitation of retrospective study designs. In this study, adequate follow-up data was not always available. The absence of a control group further limits the ability to draw conclusions about the effectiveness of the treatment.

Conclusion
Despite advances in the treatment of chronic wounds, patients continue to develop significant long-term morbidities. The results of this retrospective study of the use of a h-CRM in chronic refractory wounds, including DFUs and VLUs, suggests that the combination of supportive care and the application of the matrix may help these wounds heal. More than 75% of wounds were healed after 12 weeks of care following previously being treated unsuccessfully for an average of 38 weeks. Given the positive results of this retrospective analysis, the h-CRM shows promise in the treatment of various chronic wounds and prospective, randomized studies in chronic DFUs and VLUs are warranted to evaluate the efficacy of this treatment modality compared to other advanced wound care therapies.

References