Initial report of the use of an injectable porcine collagen-derived matrix to stimulate healing of diabetic foot wounds in humans

WILLIAM A. MARSTON, MD[a]; ANTON USALA, MD[b]; RONALD S. HILL, PhD[b]; ROBERT MENDES, MD[a]; MARY-ANN MINSLEY, RN[a]

A novel injectable scaffolding matrix (E-Matrix™) has been developed to accelerate wound healing in diabetic foot ulcers. This porcine collagen-derived matrix is designed to mimic tertiary embryonic connective tissue and to stimulate fetal wound repair mechanisms including angiogenesis. In vitro and animal studies have indicated a beneficial effect on tissue growth and an acceptable safety profile. In this report, we describe the initial use of this product in a pilot study of six humans with chronic nonhealing diabetic foot ulcers. A dramatic initial response to injection was seen, with an average wound size reduction of 72% 2 weeks after injection. Randomized trials are underway to define the potential benefit of this new treatment modality for diabetic foot ulcers. **(WOUND REP REG 2005;13:243–247)**

Progressive diabetic foot ulceration is the leading cause of lower limb amputation in the United States.[1] These lesions are associated with peripheral neuropathy and microcirculatory compromise, resulting in the breakdown of dermal integrity.[2,3] Commonly used treatments for diabetic foot ulcers (DFUs) include debridement, pressure off-loading, and moist wound healing. These treatments are inadequate, as evidenced by the high incidence of healing failure and recurrent ulceration after ulcer closure.[4]

Recent active techniques reported to increase the rate of wound closure have included growth factor application[5] and cultured human tissue equivalents.[6,7]

DFU	Diabetic foot ulcer
NO	Nitric oxide

In prospective randomized trials, these treatments have each shown an increase of 10–20% in wound closure rates after 12 weeks of treatment.

A novel injectable matrix scaffolding (E-Matrix™ Encelle, Inc., Greenville, NC) has been developed to accelerate wound healing in DFUs. E-Matrix is designed to stabilize denatured single stranded collagen, gelatin, by copolymerization with a high molecular weight polysaccharide, dextran (Figure 1). The ionic and hydrophobic interactions that maintain the copolymer structure are further stabilized by addition of the polar amino acids cysteine, glutamic acid, arginine, and lysine. This open, polar copolymer structure makes available amino acid sequences of gelatin that are entwined within triple stranded native collagen. This open polar structure was designed to mimic the open nature of early embryonic dermis.[8] It is hoped that this matrix will stimulate faster wound healing and a better tissue quality at the healed site to reduce the risk of recurrent ulceration.

E-Matrix has been tested in vitro and in vivo in various animal models of wound healing. These studies have shown that wounds treated with E-Matrix are associated with significantly increased angiogenesis,

From the Department of Surgery[a], University of North Carolina School of Medicine, Chapel Hill, and Encelle, Inc.[b], Greenville, North Carolina.

Presented at the 15th Annual Symposium on advanced wound care; April 27th, 2002, Baltimore, MD.

Manuscript received: January 31, 2003

Accepted in final form: December 8, 2004

Reprint requests: William A. Marston, MD, Department of Surgery, University of North Carolina; Campus Box 7212, 210 Burnett-Womack, Chapel Hill, NC 27599–7212. Fax: (919) 966-2898; Email: sky@med.unc.edu.

ISSN: 1067-1927.

reduced wound contraction, and increased levels of growth factor production, including transforming growth factor-β3 and vascular endothelial-derived growth factor receptors.[9] It is believed that the interaction of host cells at the wound site with E-Matrix leads to altered cellular responses, ultimately improving wound healing and stimulating the tissue regeneration observed in these preclinical studies. In addition, extensive preclinical studies of E-Matrix in animals were performed to show the safety of E-Matrix for use in this clinical trial.[10] Based on these data, a feasibility study was designed to study the use of E-Matrix in human DFUs. This article summarizes the design and results of this initial experience.

MATERIALS AND METHODS

E-Matrix is a biocompatible scaffolding for cellular attachment composed of gelatin alpha chains derived from porcine skin collagen stabilized by copolymerization with a high molecular weight polysaccharide (500 kDa dextran). The matrix scaffolding is designed to maximize the polar amino acid hydrogen bonding sites found in the open polar alpha chains (Figure 1). This open polar structure is stabilized by copolymerization with dextran while the monomer is heated.[11] Polar amino acids (lysine, arginine, glutamic acid and cysteine) are added which stabilize the copolymer by supplying additional hydrogen bonding sites and disulfide linkages. EDTA is added to enhance these structural linkages and also may transiently inhibit superoxide formation at the time of E-Matrix injection. Aminoguanidine at a low concentration (800 μM) is added as a nitric oxide (NO) inhibitor to reduce potentially disruptive oxidative activity.[12] Aminoguanidine inhibits NO production by acting as a competitive inhibitor of inducible nitric oxide synthase. E-Matrix also contains cysteine (0.8 mM), which binds NO and may reduce NO released during injection.

The E-Matrix injection process around and under the wound exposes polar sites on the tissue for E–Matrix interaction. Once host tissues interact with the E-Matrix film, they undergo changes in gene activity[13] that putatively activate a fetal-like wound healing cascade theoretically involved in the recreation of complete skin architecture.

Study protocol

The study protocol was reviewed and approved by the Center for Devices and Radiological Health at the Food and Drug Administration. Informed consent was obtained from each screened patient according to the 1975 Declaration of Helsinki ethical guidelines.

Patients aged 21–75 with documented type I or II diabetes and foot ulcers of greater than 2 months' duration were eligible for enrollment. Wagner stage 1 ulcers measuring at least 1 cm in diameter and no greater than 6 cm in diameter were eligible for enrollment. Hemoglobin A1c values of 7–12 were specified as well. Exclusion criteria are outlined in Table 1, including those typical for a DFU trial.

Patients satisfying all inclusion and exclusion criteria were skin tested for sensitivity to E-Matrix. E-Matrix (0.1 cc) was injected intradermally into the forearm 1 week prior (day −7) to ulcer treatment. Extensive wound debridement and pressure offloading were performed at this visit as well. Blood was drawn for metabolic assessment at several intervals during follow-up.

Patients returned 3 days after screening for examination of the test injection site. There were no patients who experienced an allergic response to the test injection site.

At day 0, wound debridement and E-Matrix injection were performed. Sharp debridement was performed to remove all callus and necrotic material from the wound base, after which wound measurements and photographs were obtained. E-Matrix is a

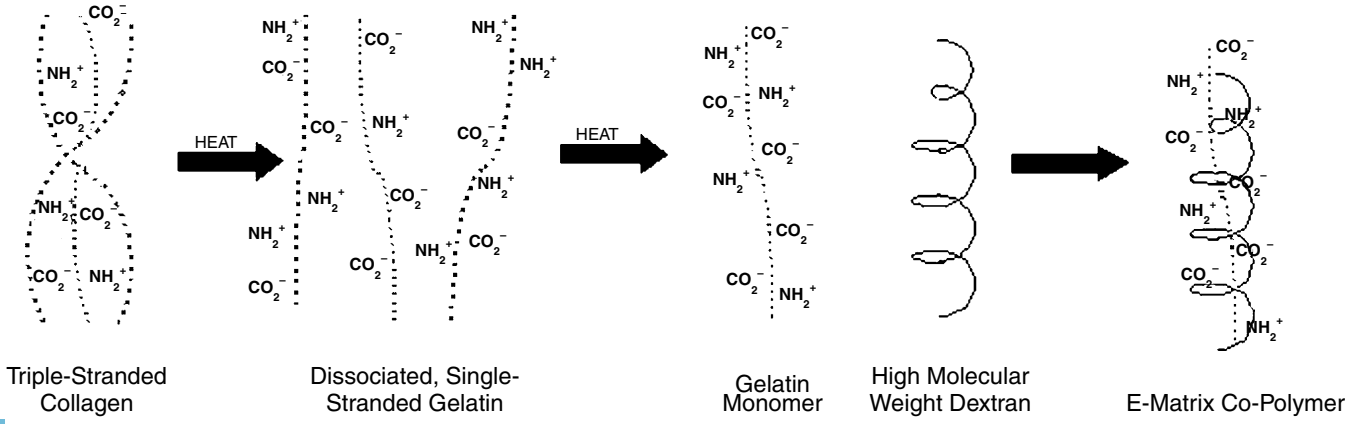

FIGURE 1. Schematic diagram of the E-Matrix.

Table 1. Exclusion criteria for patient enrollment in this clinical trial

Clinical wound infection or osteomyelitis
Active charcot arthropathy
Surgical procedure on study foot within 56 days of screening
Significant arterial insufficiency (ankle brachial index < 0.7 or toe pressure < 50 mmHg)
Chronic venous insufficiency
Severe hypertension (> 200 systolic or 120 diastolic)
Renal insufficiency (serum creatinine > 2 mg/dL)
Hepatic insufficiency (LFTs greater than 2.5 times normal)
Serum albumin < 3.0 g/dL
History of active collagen vascular disease
Use of chemotherapy, radiation, or immune suppressants within 1 year of screening
Immune deficiency syndromes
Pregnancy
Treatment of ulcer with growth factor or human tissue substitute within 28 days of screening

semisolid gel at room temperature and requires warming to physiologic temperature (30 minutes at 39 °C) where it is a moderately viscous liquid. E-Matrix was then injected using a standard 22 gauge needle circumferentially around the perimeter and intralesionally under the base of the ulcer (Figure 2). The target location of the injection was the area of the dermal/subdermal tissue junction. The volume of E-Matrix delivered depended on the ulcer size and ranged from 1.3–9.4 mL.

Post-injection protocol

Daily wound dressings were performed after day 3 using hydrogel and gauze on the ulcer site. The patients returned at day 3, 7, 14, 21, 28, 42, 56, and 84 for follow-up. This involved physical examination, sharp wound debridement, ulcer tracings, photographs, and assessment of pressure offloading at each visit. At days 14, 28, 56, and 84, blood samples were obtained for metabolic follow-up and 24-hour urine for creatinine clearance was collected.

At the conclusion of the 12-week study protocol, two patients remained with nonhealed ulcers measuring greater than 1 cm^2. Permission was obtained through the Institutional Review Board and Food and Drug Administration to retreat these two patients with E-Matrix 4–5 months after the initial procedure. The same protocol was followed with E-Matrix reinjection, with a 12-week follow-up time.

RESULTS

Six patients with diabetes and nonhealing foot ulcers were recruited satisfying the inclusion/exclusion criteria. Patient demographics are listed in Table 2. Of note, the group included an average wound duration of 3.5 years prior to enrollment with a range of 6 months to 12 years. The patients had all been treated in wound management clinics prior to enrollment, most with growth factors, human tissue substitutes, or hyperbaric oxygen. Injection of E-Matrix was well tolerated, with one of six patients reporting pain with injection, described as moderate. There were no other noted complications related to the procedure.

During follow-up, there was one patient death, occurring due to medical complications related to his diabetes. At the last clinic evaluation within 1 week of death, the ulcer was 89% healed with no evidence of infection or other complication. The medical team treating the patient reported no evident association between the patient's death and his foot ulcer or the study treatment. One patient experienced mild and transient erythema. No other adverse events were reported that were deemed related to the study procedure or treatment.

Medically, one patient experienced an increase in serum creatinine from 1.1 to 1.7 mg/dL, and two patients had small decreases in creatinine clearance during follow-up. In one of six patients, rheumatoid factor was detectable at day 56. In all other cases, no

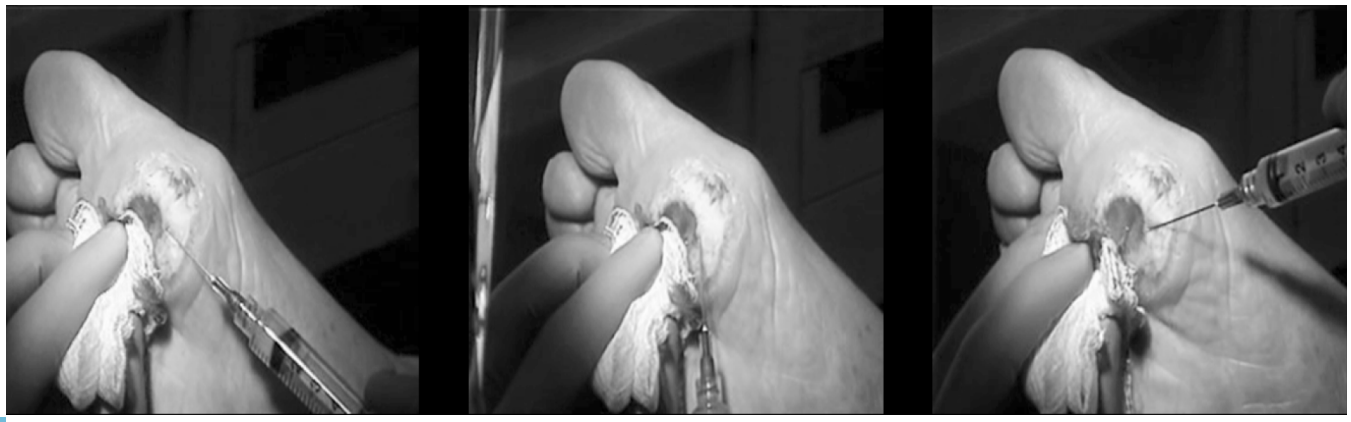

FIGURE 2. Technique of E-Matrix injection.

Table 2. Demographics of patients enrolled in this clinical trial

Patient #	Gender	Age (years)	Ulcer duration	Previous treatment
103	Male	62	4 years	Vein revascularization, Dermagraft® × 3, Regranex®
104	Male	69	2 years	Betadine soaks, Hydrogel® dressings
105	Male	68	6 months	Apligraf® × 3, Dermagraft®
106	Male	63	1 year	Regranex®
108	Male	60	12 years	Regranex®
109	Male	58	1.5 years	Apligraf® × 4, Regranex®, hyperbaric oxygen

rheumatoid factor was detectable after injection. No other evidence of immunologic response was noted.

In all cases, an immediate response was noted to E-Matrix injection, with a 72% average decrease in wound size over the first 2 weeks after treatment (Figure 3). The average wound closure was 61% at 1 week, 72% at 2 weeks, and 77% 4 weeks after injection. After 4 weeks, healing progress slowed, with the average closure 78% at 8 weeks. Two wounds experienced a small increase in size between 56 and 84 days. After reinjection, these two wounds improved markedly, from 9 to 2.8 cm^2 and from 2.5 to 0.6 cm^2 at 12 weeks, but neither had completely closed by day 84 (Figure 4). At 6 months after reinjection, both of these wounds were completely closed. No moderate or severe study-related adverse events were experienced after E-Matrix reinjection.

DISCUSSION

As noted in the introduction, DFUs remain a critical problem resulting in limb loss in a significant number of cases. A recent study examined the expected healing rates for DFUs through a person-level meta-analysis from published, randomized clinical trials.[14] Evaluating a database that included 586 individuals, the author concluded that 24% of ulcers healed after 12 weeks and 33% healed after 20 weeks of standard wound care. Unfortunately, re-ulceration after closure is common due in part to the poor quality of scar tissue in the area of healing.

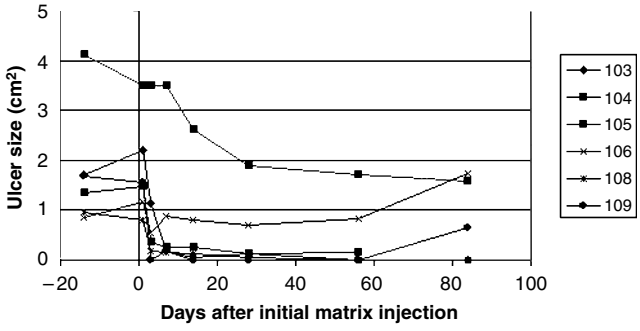

FIGURE 3. Wound sizes for each patient before and after E-Matrix injection.

Early in fetal development, a more open form of collagen (compared to tightly bound mature collagen) is associated with large carbohydrate molecules, and serves as the predominant tissue scaffolding.[9,13,15] It is postulated that the attachment of undifferentiated, or incompletely differentiated cells to the polar collagen scaffolding results in a specific host tissue response. This response during fetal development is to guide the differentiation of mesenchymal tissue into various skin structures, including endothelial cells. Subsequently, these organize into blood vessels and other critical structures in the development of new skin. It is possible that the induction of fetal-like wound healing may lead to more rapid wound healing, and these hypotheses have been supported by the results of preclinical evaluation of E-Matrix.[8,10,12] It is also expected that wounds closing with tissue that is characterized by increased vascularity and decreased scarring may be more resistant to ulcer recurrence.

In this initial feasibility study, E-Matrix was injected into six human DFUs. No treatment-related severe adverse events were noted. Patients were followed closely for renal, hepatic, immunologic, or other toxicities. There appeared to be no significant trends related to treatment. Clearly, this feasibility study is a small sample size, and must be followed with larger controlled clinical studies.

There was an apparent initial benefit to E-Matrix injection resulting in a decrease in wound size in all patients in the first 2 weeks after injection. The

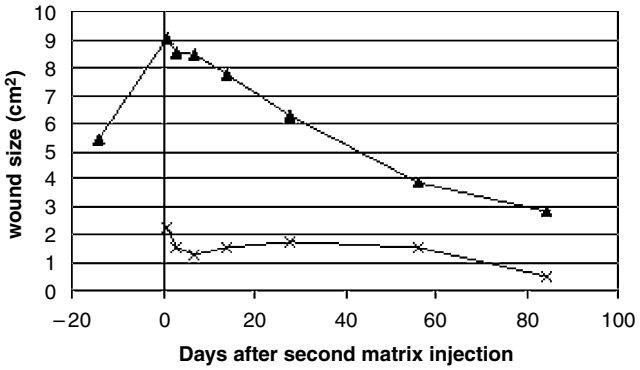

FIGURE 4. Wound area of Patients 105 and 106 after the second application of E-Matrix (second application is day 0 on this graph).

stimulatory response apparently slowed after several weeks, possibly due to degradation of the matrix, collagen turnover, or other factors. In preclinical studies, transforming growth factor-β3 gene expression was enhanced by E-matrix, and this effect may be transient, limited to 2–4 weeks.[9] Based on these results, and the lack of procedure-related adverse events, we believe that further study is warranted, including larger trials randomizing patients to control treatment compared to E-Matrix treatment. In these studies, repeated injections will be performed to assess the sustainability of the healing response.

Several issues concerning the optimal use of E-Matrix must be determined during further studies. The exact method and site of injection of E-Matrix must be determined. Also, it appears that patients might benefit from repeated applications of E-Matrix during application, but the optimal re-treatment time and the safety of multiple applications must be determined.

In conclusion, we believe that the injection of E-Matrix into chronic nonhealing diabetic foot wounds may induce fetal-like wound healing mechanisms, improving the ability of these wounds to close. This feasibility study provides promising results that argue for larger trails to further examine the benefit of this new method of treatment.

ACKNOWLEDGMENTS

This clinical study was supported by an educational grant from Encelle, Inc., Greenville, NC.

REFERENCES

1. Armstrong DG, Lavery LA. Diabetic foot ulcers: prevention, diagnosis, and classification. Am Fam Physician 1998;57:1325–32.
2. Veves A, Akbari CM, Primavera J, Donaghue VM, Zacharoulis D, Chrzan JS, DeGirolami U, LoGerfo FW, Freeman R. Endothelial dysfunction and the expression of endothelial nitric oxide synthetase in diabetic neuropathy, vascular disease, and foot ulceration. Diabetes 1998;47:457–63.
3. Jorneskog G, Brismar K, Fagrell B. Skin capillary circulation is more impaired in the toes of diabetic than non-diabetic patients with peripheral vascular disease. Diabet Med 1995;12:36–41.
4. Ramsey SD, Newton K, Blough D, McCulloch DK, Sandhu N, Reiber GE, Wagner E. Incidence, outcomes, and cost of foot ulcers in patients with diabetes. Diabetes Care 1999;22:382–7.
5. Weiman TJ, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (Becaplermin) in patients with chronic neuropathic diabetic ulcers. Diabetes Care 1998;21:822–7.
6. Veves A, Falanga V, Armstrong DG, Sabolinski ML. Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. Diabetes Care 2001;24:290–5.
7. Gentzkow GD, Jensen JL, Polak RA. Improved healing of diabetic foot ulcers after grafting with a living human dermal replacement. Wounds 1999;11:77–84.
8. Smith LT, Holbrook KA. Development of dermal connective tissue in human embryonic and fetal skin. Scan Electron Microsc 1982;4:1745–51.
9. Klann R, Lloyd W, Lacy S. A novel hydrogel copolymer reduces scar formation and increases TGF β3 gene expression. Presented at the 15th Annual Symposium on Advanced Wound Care. Baltimore, MD, April 29, 2002.
10. Safety data on file in FDA, submission. Encelle, Inc.
11. Usala AL. Methods for increasing vascularization and promoting wound healing. US Patent #6,261,387; issued July 17, 2001.
12. Ribbons KA, Currie MG, Conner JR, Manning PT, Allen PC, Didier P, Ratterree MS, Clark DA, Miller MJ. The effect of inhibitors of inducible nitric oxide synthase on chronic colitis in the rhesus monkey. J Pharmacol Exp Ther 1997;280:1008–15.
13. Usala AL, Dudek R, Lacy S, Olson J, Penland S, Sutton J, Ziats NP, Hill RS. Induction of fetal-like wound repair mechanisms in vivo with a novel matrix scaffolding. Diabetes 2001;50 (Suppl. 2):A488.
14. Kantor J, Margolis DJ. Expected healing rates for chronic wounds. Wounds 2000;12:155–8.
15. Lovvorn HN, Cheung DT, Nimni ME, Perelman N, Estes JM. Relative distribution and cross-linking of collagen distinguish fetal from adult sheep wound repair. J Ped Surg 1999;34:218–23.