CASE STUDY

The Clinical Effectiveness in Wound Healing With Extracellular Matrix Derived From Porcine Urinary Bladder Matrix: A Case Series on Severe Chronic Wounds

Howard Kimmel, DPM^a, Michael Rahn, DPM^a, Thomas W. Gilbert, PhD^{b,*}

^aLouis Stokes Department of Veterans Affairs, Cleveland, Ohio 44106, USA; and

^bMcGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA 15219, USA

KEYWORDS:

Extracellular matrix scaffold;
Urinary bladder matrix;
Wound care

Abstract A study involving 3 patients was performed to evaluate the efficacy of Matristem, an extracellular matrix scaffold derived from the porcine urinary bladder matrix (UBM), to promote natural healing in patients with severe, chronic wounds that did not respond well to standard wound management. In all cases, the wounds closed after only a few weeks of repeated treatments with UBM, with all patients showing complete epithelialization of the wound with limited formation of scar tissue by 13 weeks after first treatment. These initial results suggest that UBM warrants further investigation for treatment of chronic nonhealing ulcers that are recalcitrant to standard wound therapies.

© 2010 Elsevier Inc. All rights reserved.

Introduction

The uses of naturally derived extracellular matrix (ECM) products as scaffolds for tissue reconstruction and wound management have been studied in both the laboratory and the clinic. ECM has been isolated from a variety of organs, including the heart, lung, trachea, and liver,^{1–7} of multiple species, but only ECM from the porcine small intestine, porcine urinary bladder, and human dermis has gained broad clinical acceptance.⁸ ECM is obtained through a process of decellularization that primarily involves soaking the tissue in acid or salt solutions, detergents, and/or enzymatic solutions.⁹ The resulting ECM largely retains its native tissue

architecture and composition. On implantation, the ECM material degrades rapidly.^{10,11} Rapid degradation is thought to be critical to the scaffold remodeling process.^{12,13}

In normal wound healing, the host ECM is vital as it acts as a support structure for cell migration.^{14,15} The basement membrane is a particular type of ECM that provides, in its native state, a suitable substrate for epithelial cell growth in vivo. In living organisms in their native state, this membrane helps with regulation of cell proliferation, migration, and differentiation during tissue development.^{16,17} In previously reported experimental work, ECM scaffolds derived from porcine liver, intestine, and urinary bladder were tested for the intactness and integrity of the basement membrane component after being prepared and processed; the urinary bladder was found to be the only tissue to retain the basement membrane.¹⁸

Matristem Devices (ACell, Inc., Columbia, Maryland) is an ECM product that consists of the basement membrane and subjacent lamina propria of the porcine urinary bladder, collectively referred to as *urinary bladder matrix* (UBM).

Conflict of interest: Thomas W. Gilbert is on the Scientific Advisory Board for ACell, Inc., and has a consulting relationship with Medline, Inc., both of whom sell Matristem for clinical use.

* Corresponding author.

E-mail address: gilberttw@upmc.edu

The material is available as a thin, single-layer, lyophilized sheet for partial thickness wounds, thick, multilaminate sheets for full-thickness wounds, and in a particulate form for tunneling wounds. This case series is a case study design to determine clinical efficacy of UBM for treatment of severe chronic wounds. The following patients were selected for treatment with UBM because of their chronic nonhealing ulcers that were recalcitrant to standard wound therapies such as offloading, moist wound environment, infection control, and sharp debridement. This case series follows 3 patients treated with the various forms of UBM. These patients all had similar comorbidities (peripheral vascular disease, type 2 diabetes with poor glycemic control, hypertension, and hyperlipidemia). The combination of all these comorbidities put these patients at significant risk for amputation.

Methods

The preparation of UBM has been described previously.¹⁸ Porcine urinary bladders were harvested from market-weight pigs (approximately 110–130 kg) immediately after sacrifice at an abattoir and transported to the lab on ice. The urothelial layer of the bladders was removed by soaking the material in saline. The tunica serosa, tunica muscularis externa, tunica submucosa, and most of the muscularis mucosa were mechanically delaminated from the bladder tissue. The remaining basement membrane of the tunica epithelialis mucosa and the subjacent tunica propria were collectively termed UBM. UBM was decellularized and disinfected by immersion in mild peracetic acid with ethanol and deionized water, followed by rinses in phosphate-buffered saline and deionized water.¹⁸ For single-layer sheets, the UBM was immediately lyophilized.¹⁹ To manufacture particulate UBM, the lyophilized material was chopped into small sheets and fed through a rotary knife mill with a screen to control the collected micronized particle size.²⁰ For multilaminate sheets, the hydrated UBM was layered and vacuum pressed to dehydrate the material and form a mechanical bond between the layers.²¹ All materials were terminally sterilized with electron beam irradiation.²²

Patient Details

A 67-year-old man presented with a past medical history of type 1 diabetes, hypertension, and recovering alcohol abuse. The patient had poor glycemic control, with a hemoglobin A1C of 8.1. As a result of his diabetes and vessel calcification, he had undergone a right below-knee amputation 7 years ago. The patient presented to our clinic with Charcot deformity and calcified vessels with an ankle-brachial index (ABI) of 1.2. A Charcot reconstruction surgery was performed on his left foot. Staples were removed from the wound 4 weeks after the surgery. There

remained a central aspect of dehiscence to the lateral surgical wound that was present for 6 weeks and was nonresponsive to standard postoperative wound care of moist gauze dressings (Figure 1A). The wound was probed deeply at central portion. UBM micronized particles were chosen for wound treatment because of the patient's delayed wound healing and the deep nature of his wound. The matrix particles were poured into the tunneling aspect of the wound and covered with a nonadherent dressing and sterile gauze (Figure 1B). The patient presented to

Figure 1 A, Dehiscence to the lateral surgical wound that was present for 6 weeks and was nonresponsive to standard postoperative wound care of moist gauze dressings. B, UBM micronized particles were poured into the tunneling aspect of the wound and covered with a nonadherent dressing and sterile gauze. C, The wound was healed after 4 weeks of treatment.

Figure 2 A, A necrotic diabetic ulcer to the medial aspect of the patient's right foot. B, Debridement in the operating room down to joint capsule. C, A thick UBM sheet was hydrated in normal saline, fenestrated, and then sutured to the wound base. D, The full thickness wound was healed in 13 weeks from the date of surgery.

clinic on weekly intervals for dressing changes and reapplication of UBM micronized particles. Debridement of the UBM particles was avoided. Over the course of treatment, the micronized particles were noted to completely assimilate into the wound. There were no signs of infection or local inflammation during the course of treatment. The wound was healed after 4 weeks of treatment (Figure 1C).

An 85-year-old man with a past medical history of type 2 diabetes, hypertension, thrombocytopenia, and tibial calcified vessels (ABI of 1.32) presented with a necrotic diabetic ulcer to the medial aspect of his right foot that had been present for 4 weeks (Figure 2A). The wound was debrided in the operating room down to joint capsule (Figure 2B). A thick UBM sheet was hydrated in normal saline, fenestrated, and then sutured to the wound base. The graft was then covered with petrolatum-impregnated gauze, sterile gauze, and elastic wrap (Figure 2C). The dressing was changed at week 1, with a new application of petrolatum-impregnated gauze to the UBM, covered by a dry sterile dressing. The wound was assessed at 2 weeks. The UBM was noted to be incorporating into the wound, but there was still a deep tissue deficit due to the depth of the original wound. A lyophilized thin sheet of UBM was placed over the incorporating graft, cut to size, hydrated with saline, and covered with a nonadherent dressing. The patient was seen weekly in clinic, proper local wound care was provided, and a thin UBM sheet was added. At each week, UBM matrix was noted to be absorbed into the wound, with no signs of infection or local inflammation. The full thickness wound was healed in 13 weeks from the date of surgery (Figure 2D).

A 61-year-old man with a past medical history of type 2 diabetes, coronary artery disease, peripheral vascular disease (ABI of 0.47), and alcohol dependence presented with an ulceration to the lateral aspect of his right foot. The wound was probed to bone and fifth metatarsal head. The patient underwent a right partial fifth ray resection that was left open to allow further drainage of infection (Figure 3A). Negative pressure therapy was used for 1 month, with some improvement in wound size. Negative pressure therapy was then discontinued, and the patient was started on once-a-week UBM treatments (Figure 3B). The thin lyophilized UBM sheets were applied in the same manner as above without debriding the previous sheets. The wound was completely healed in 9 weeks (Figure 3C).

Discussion

This case series showed rapid healing via the use of UBM devices in 3 severe, recalcitrant, chronic wounds that were nonresponsive to standard wound care therapy. The first patient's wound was difficult to heal because of its tunneling nature. UBM worked well for this wound as it was able to physically reach the area of delayed healing to promote constructive remodeling of the site. Standard packing dressings for tunneling wounds, such as calcium alginate rope or iodophor packing, allow drainage and keep the wound base clean but lack biologic factors that may promote healing. The second patient's wound involved the joint capsule. Wounds of this depth have difficulty forming granulation tissue and healing because of the absence of an

Figure 3 A, An ulceration to the lateral aspect of this patient's right foot probed to bone. Radiographs were suggestive of osteomyelitis to the fifth metatarsal head. Right partial fifth ray resection was performed and left open to allow further drainage of infection. Negative pressure wound therapy was used for 1 month, improving wound size. B, Negative pressure wound therapy was discontinued and once-a-week UBM treatments were initiated. C, The wound was completely healed in 9 weeks.

organized vascular tissue bed. It is possible that the thicker variety of UBM, which degrades more slowly, served as a framework for revascularization in this particular patient.

It appears that all 3 patients healed in a similar manner with the use of UBM even though 3 different forms of the material (micronized particles, thin matrix, and thick matrix) were used. Each patient had a significant increase in granulation tissue within the first 2 weeks of application of the UBM. Compared with the effects of UBM sheets, use

of the micronized particles led to an additional significant decrease in the depth of the wound. Based on the clinical observations of the 3 patients that were studied, the UBM-based ECM allowed natural epithelialization to occur in these 3 wounds that were stalled in the healing process. All the patients recruited into this study had diabetic complications, with comorbidities including peripheral vascular disease and atherosclerotic occlusive disease. Such clinical conditions, in our opinion, put these patients at an increased risk for wound infection and delayed wound healing.

This product has multiple regenerative properties that have been shown to be important in wound healing. UBM uses a largely and optimally intact ECM from porcine urinary bladder. As such, UBM differs from other available products that are synthesized from purified type I collagen in that it contains a complex mixture of proteins, glycosaminoglycans, and other biological molecules that are basically present in their normal concentrations and distributions. Furthermore, because of the specific layers of the tissue selected from this material, the basement membrane component of the ECM is naturally present on the original tissue, and studies have shown that the basement membrane is preserved through the manufacturing process.¹⁸ The distinctive ECM composition and ultrastructure of UBM appears to affect the response of host cells, thereby guiding the wound-healing response by providing a complex molecular architecture that appears to aid in biological tissue repair.^{18,23}

ECM scaffolds contain the same structural and functional proteins as the host tissue, so they are subject to processing with the usual body proteases. Nonchemically cross-linked scaffolds have been shown to be resorbed into the body within 60 to 90 days.^{10,11} The degradation of the scaffold unlocks properties not associated with intact ECM; for example, studies have shown collagen degraded by proteases to have regenerative capacity.²⁴ Degradation of the UBM was one of the earliest events observed after device placement.

The case series presented herein shows promising healing results in 3 patients who suffered from recalcitrant wounds. Based on these results and the extensive preclinical and clinical results available for this material and other ECM-derived scaffolds, UBM-derived ECM has the ability to alter the default wound-healing process from one that leads to a stalled, chronic wound to one that shows relatively rapid wound closure. Future studies in a randomized prospective regime can clarify to a higher degree the abilities of interesting materials such as the ECM derived from UBM.

References

1. Wainwright JM, Czajka CA, Patel UB, et al: Preparation of cardiac extracellular matrix from an intact porcine heart. *Tissue Eng Part C Methods*. 2010;16(3):525–532.
2. Uygun BE, Soto-Gutierrez A, Yagi H, et al: Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. *Nat Med*. 2010;16:814–820.

3. Petersen TH, Calle EA, Zhao L, et al: Tissue-engineered lungs for in vivo implantation. *Science*. 2010;329(5991):538–541.
4. Ott HC, Clippinger B, Conrad C, et al: Regeneration and orthotopic transplantation of a bioartificial lung. *Nat Med*. 2010;16(8):927–933.
5. Ott HC, Matthiesen TS, Goh SK, et al: Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med*. 2008;14:213–221.
6. Macchiarini P, Jungebluth P, Go T, et al: Clinical transplantation of a tissue-engineered airway. *Lancet*. 2008;372(9655):2023–2030.
7. Remlinger NT, Czajka CA, Juhas ME, et al: Hydrated xenogeneic decellularized tracheal matrix as a scaffold for tracheal reconstruction. *Biomaterials*. 2010;31(13):3520–3526.
8. Badylak SF, Freytes DO, Gilbert TW: Extracellular matrix as a biological scaffold material: structure and function. *Acta Biomater*. 2009;5(1):1–13.
9. Gilbert TW, Sellaro TL, Badylak SF: Decellularization of tissues and organs. *Biomaterials*. 2006;27(19):3675–3683.
10. Record RD, Hillegonds D, Simmons C, et al: In vivo degradation of ¹⁴C-labeled small intestinal submucosa (SIS) when used for urinary bladder repair. *Biomaterials*. 2001;22(19):2653–2659.
11. Gilbert TW, Stewart-Akers AM, Simmons-Byrd A, Badylak SF: Degradation and remodeling of small intestinal submucosa in canine Achilles tendon repair. *J Bone Joint Surg Am*. 2007;89(3):621–630.
12. Crisan M, Yap S, Casteilla L, et al: A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*. 2008;3(3):301–313.
13. Agrawal V, Johnson SA, Reing J, et al: Epimorphic regeneration approach to tissue replacement in adult mammals. *Proc Natl Acad Sci U S A*. 2010;107(8):3351–3355.
14. Hackam DJ, Ford HR: Cellular, biochemical, and clinical aspects of wound healing. *Surg Infect (Larchmt)*. 2002;3(suppl 1):S23–S35.
15. Mendonca RJ, Coutinho-Netto J: Cellular aspects of wound healing. *An Bras Dermatol*. 2009;84(3):257–262.
16. Ortega N, Werb Z: New functional roles for noncollagenous domains of basement membrane collagens. *J Cell Sci*. 2002;115(Pt 22):4201–4214.
17. Vracko R: Basal lamina scaffold-anatomy and significance for maintenance of orderly tissue structure. *Am J Pathol*. 1974;77(2):314–346.
18. Brown B, Lindberg K, Reing J, Stolz DB, Badylak SF: The basement membrane component of biologic scaffolds derived from extracellular matrix. *Tissue Eng*. 2006;12(3):519–526.
19. Freytes DO, Tullius RS, Valentin JE, Stewart-Akers AM, Badylak SF: Hydrated versus lyophilized forms of porcine extracellular matrix derived from the urinary bladder. *J Biomed Mater Res A*. 2008;87(4):862–872.
20. Gilbert TW, Stolz DB, Biancaniello F, Simmons-Byrd A, Badylak SF: Production and characterization of ECM powder: implications for tissue engineering applications. *Biomaterials*. 2005;26(12):1431–1435.
21. Freytes DO, Badylak SF, Webster TJ, Geddes LA, Rundell AE: Biaxial strength of multilaminated extracellular matrix scaffolds. *Biomaterials*. 2004;25(12):2353–2361.
22. Freytes DO, Stoner RM, Badylak SF: Uniaxial and biaxial properties of terminally sterilized porcine urinary bladder matrix scaffolds. *J Biomed Mater Res B Appl Biomater*. 2008;84(2):408–414.
23. Brown BN, Barnes CA, Kasick RT, et al: Surface characterization of extracellular matrix scaffolds. *Biomaterials*. 2010;31(3):428–437.
24. Bellon G, Martiny L, Robinet A: Matrix metalloproteinases and matrikines in angiogenesis. *Crit Rev Oncol Hematol*. 2004;49(3):203–220.