Use of Cryopreserved, Particulate Human Amniotic Membrane and Umbilical Cord (AM/UC) Tissue: A Case Series Study for Application in the Healing of Chronic Wounds

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ABSTRACT

Human amniotic membrane and umbilical cord tissues (AM/UC) are fetal tissues that contain proteins, cytokines, and growth factors that, when transplanted, can modulate inflammation and promote healing. Lyophilized, particulate AM/UC tissues can be used as wound coverings for chronic dermal ulcers or defects to promote granulation tissue formation and rapid re-epithelialization.

This study reviews a case series of 5 patients presenting with chronic nonhealing wounds that received particulate AM/UC tissues (NEOX® FLO, Amniox Medical, Atlanta, GA). For all cases, wounds were debrided in the office setting and a single application of lyophilized particulate was used with minimal additional dressings. The lyophilized AM/UC tissue was placed within the wound bed and a dressing consisting of Adaptic®, 2x2 or 4x4 (Systagenix, Quincy, MA), Kling® (Johnson & Johnson, New Brunswick, NJ), and ACE™ (3M, St. Paul, MN) wrap were applied. Dressings were kept in place until weekly follow-up appointments in which a new Adaptic, 2x2 and Kling were applied. Overall, healing of wounds was noted to have a mean of 5 weeks to complete epithelialization. Upon complete healing patients were able to return to planned postoperative care and rehabilitation.

Wound complications occur despite the best standard of care. Chronic wounds that remain weeks after surgery inhibit patients from progressing to physical rehabilitation and significantly affect patients both physically and mentally. These case presentations demonstrate how use of human AM/UC tissue may help wounds heal quickly and help patients return to normal function.

INTRODUCTION

Wound healing consists of a complex series of cellular and molecular events that act to repair damaged tissue and restore function.[1] Although the process of wound healing is continuous, in general, wound healing can be divided into four phases: (1) coagulation and hemostasis, (2) inflammation, (3) proliferation, and (4) remodeling.[2] The entire process of wound healing can take up to a year or more to complete.[3–5] Each phase of the wound-healing process involves multiple cell types and is tightly regulated by a mix of cytokines and growth factors including TGF-β, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), and keratinocyte growth factor (KGF), among others.[2] Any disruption in the wound-healing cascade may slow the course of wound healing, resulting in chronic, nonhealing wounds.

Chronic wounds are wounds that fail to progress through the normal stages of wound healing, resulting in the prevention or prolongation of the wound-healing process.[6] Chronic wounds remain in a state of pathological inflammation with disorganized and incomplete healing.[7] There are several factors that can delay wound healing including chronic disease, diabetes, neurological defects, vascular insufficiency, nutritional deficiencies, age, and obesity, among others.[8] In the United States, it is estimated that approximately 6.5 million individuals suffer from chronic wounds with an excess of $25 billion being spent annually on their treatment.[9–11] In addition to the financial burden of wound care costs in the United States, there are also significant psychological burdens, with patients with chronic wounds being unable to work and suffering from a diminished quality of life.[12] There is a significant need for improved treatment modalities in patients with chronic wounds.

The amniotic membrane is the innermost layer of the placenta and is comprised of a single layer of epithelial cells, a thick basement membrane, and an avascular stroma that is subdivided into compact, fibroblast, and spongy layers.[13] The amniotic membrane and the umbilical cord share the same cellular origin as the developing fetus. *In utero*, these tissues serve to protect the developing fetus from maternally derived immunological insults.[14] The use of the amniotic membrane therapeutically was first reported in 1910 during skin transplantation procedures.[15] Afterward, the use of amniotic membrane was reported as a biological bandage for dressing burns and nonhealing skin ulcers, and as an aid to physiological wound healing.[16–19]

Amniotic membrane/umbilical cord (AM/UC) tissues have been used more recently in a variety of ophthalmic indications as well as in lower-extremity reconstructive procedures and in the treatment of burns.[13,16,20] Cryopreserved AM/UC tissues have been demonstrated to have both anti-scarring and anti-inflammatory properties both *in vitro* and *in vivo*. AM/UC tissues decrease fibroblast proliferation and limit the differentiation of fibroblasts to myofibroblasts through the downregulation of TGF-β signaling, which is a critical signaling component in the scar formation pathway.[21,22] In addition, cryopreserved AM/UC tissues have been shown to induce the apoptosis and phagocytosis of activated neutrophils and macrophages, increase the expression of anti-inflammatory cytokines such as IL-10 while decreasing the expression of pro-inflammatory cytokines including TNF-α and IL-12, and polarize macrophages toward the M2 phenotype.[23–25] In addition to their anti-scarring and anti-inflammatory properties, both AM and UC tissues have been found to contain cytokines and growth factors that may help to modulate the wound-healing process.

In the current study, we discuss the use of a cryopreserved, lyophilized, particulate form of AM/UC tissues (NEOX® FLO, Amniox Medical, Atlanta, GA) for the treatment of chronic, nonhealing wounds. A total of 5 patients presenting with various chronic wounds and medical histories were treated with a single application of particulate AM/UC tissue.

CASE REPORTS

Case Report #1: Post-digital Amputation with Wound Dehiscence

A 72-year-old male patient with a long history of rheumatoid arthritis and alcoholism underwent amputation of the left second and third digits secondary to infection. The patient had previous amputations and similar difficulty in healing wounds. Postsurgery, the patient went on to wound dehiscence. The patient received several treatment modalities including repeated debridement with local wound care consisting of cadexomer iodine gel and collagen-based dressing with no improvement in healing outcomes. At this time, it was decided to use particulate AM/UC tissue. The initial wound measured 1.0 cm x 0.3 cm x 0.3 cm (Fig. 1A). Prior to application of the AM/UC tissue particulate, the wound was sharply debrided to good, healthy granulation tissue (Fig. 1B). A total of 50 mg of lyophilized AM/UC tissue was applied to the wound (Fig. 1C). After application of the particulate AM/UC tissue, a dressing consisting of Adaptic® nonadherent dressing and Kling® compression dressing was applied. Dressings were kept in place until a one-week follow-up visit at which time the wound was evaluated and new dressings were placed. At the one-week follow-up, the wound measured 0.6 cm x 0.3 cm x 0.1 cm (Fig. 2A), and no drainage, purulence, or peri-wound erythema were noted. No further application of the particulate AM/UC tissue was necessary, and the wound went on to complete epithelialization by day 13. The wound

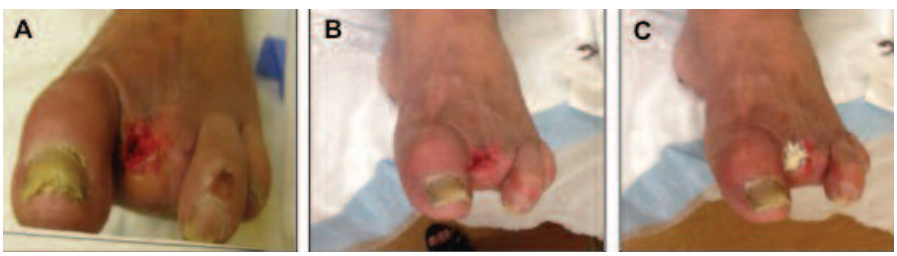

Figure 1. Patient presented with wound dehiscence at the second toe area following second and third toe amputation. (A) Wound presentation at first visit. (B) Wound following debridement. (C) Application of AM/UC particulate.

was healed completely by 5 weeks post-AM/UC application (Fig. 2B).

Case Report #2: Posterior Heel Wound after Achilles Tendon Repair

A 46-year-old male patient underwent insertional Achilles tendon repair. Eight weeks following surgery, the patient presented with a new wound, drainage, and pain (Fig. 3A). The patient's medical history was fairly benign, with only hypertension noted. Prior to application of particulate AM/UC tissue, the wound was debrided down to good, healthy tissue weekly and received antimicrobial alginate dressing, along with negative-pressure wound therapy. On the day of particulate AM/UC application, the wound measured 1.0 cm x 0.5 cm x 0.4 cm (Fig. 3B). The wound was sharply debrided, and a total of 50 mg of particulate AM/UC tissue was applied directly to the wound bed. Following application, a wound dressing consisting of Adaptic and Kling was applied and kept in place for one week. At a one-week follow-up visit, there were minimal edema and erythema noted surrounding the wound and it was decided not to debride the wound again. A fresh dressing consisting of Adaptic was applied and kept in place for an additional week. At the two-week follow-up visit, there was a dry eschar (Fig. 4A) and by 4 weeks post-AM/UC application, the wound had near complete re-epithelialization (Fig. 4B). The wound went on to heal completely in 7 weeks following only a single application of particulate AM/UC tissue.

Case Report #3: Medial Ankle Wound after Total Ankle Replacement

A 58-year-old male patient with a history of post-traumatic arthritis to the right ankle presented with a wound on the medial aspect of the ankle following total ankle replacement (Fig. 5A). The patient's medical history included gout along with arthritis. Initial treatment of the wound included compression dressings, antimicrobial alginate dressings, and collagen dressings for a period of 4 weeks. At the time of AM/UC application, the wound was sharply debrided and a total of 50 mg of particulate AM/UC tissue was applied directly to the wound bed (Figs. 5B & 5C). At the time of AM/UC application, the wound measured 2.5 cm x 1.8 cm x 0.1 cm.

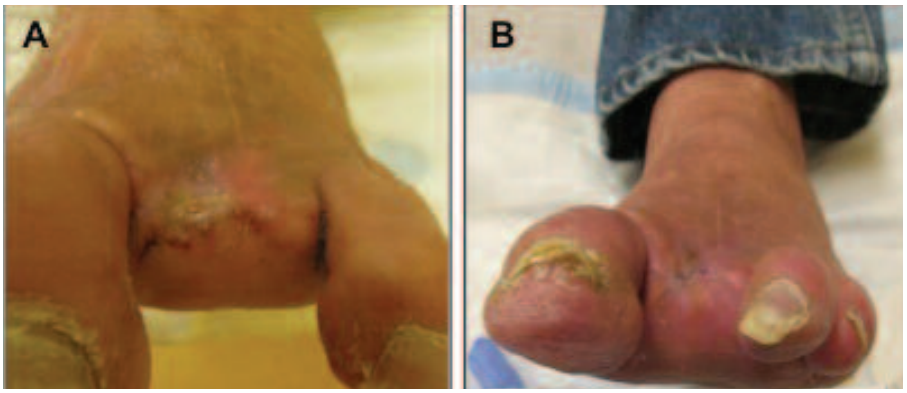

Figure 2. Follow-up images after application of AM/UC particulate. (A) 7 days, and (B) 5 weeks showing complete epithelialization of the wound.

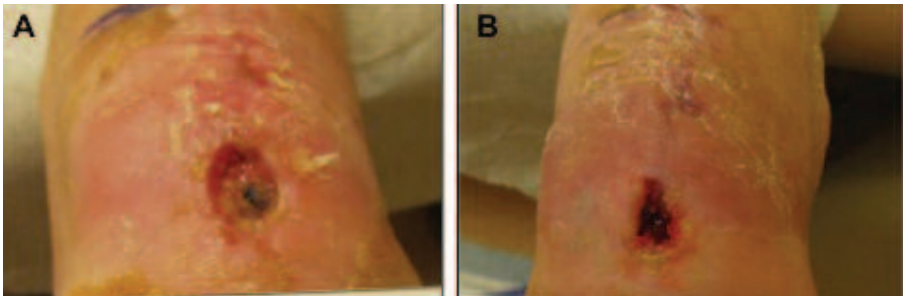

Figure 3. Patient presented with posterior heel wound following Achilles tendon repair. (A) Initial wound presentation. (B) Wound presentation following 2 weeks treatment with Silvercel® (Systagenix, Quincy, MA), wound V.A.C.® (KCI, San Antonio, TX), and Prisma® (Systagenix, Quincy, MA). Wound received application of particulate AM/UC at this time.

Postapplication, a dressing consisting of Adaptic, Kling, and Coban™ self-adherent wrap (3M, St. Paul, MN) was applied. One-week after AM/UC application, the wound was significantly reduced in size and showed markedly less inflammation (Fig. 6A). No further debridement or application of particulate AM/UC were performed. The wound went on to complete re-epithelialization in 16 days after application of AM/UC tissue (Fig. 6B) and remained fully healed out to a one-month follow-up visit (Fig. 6C).

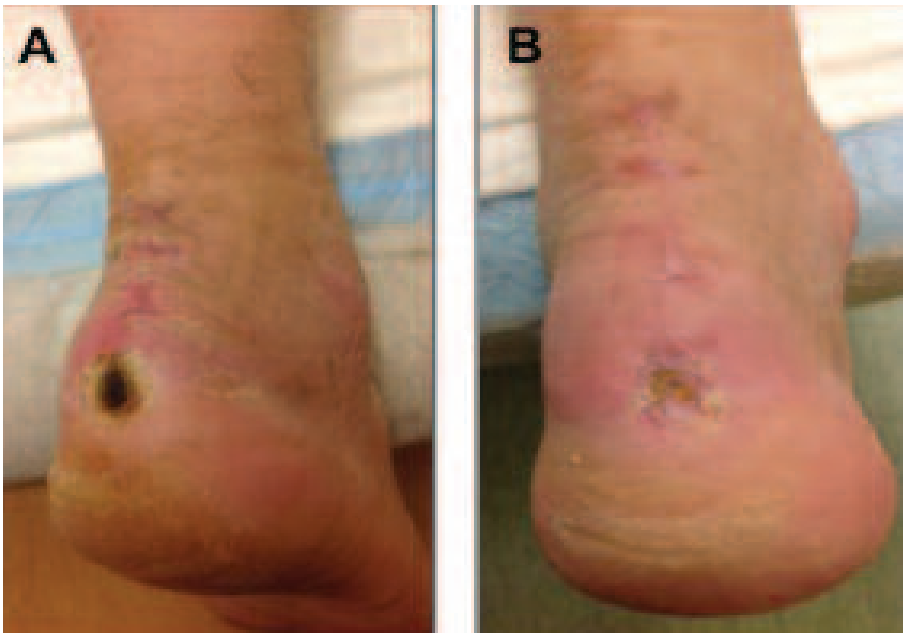

Figure 4. Follow-up images after application of AM/UC particulate. (A) 2 weeks, and (B) 4 weeks showing complete epithelialization of the wound.

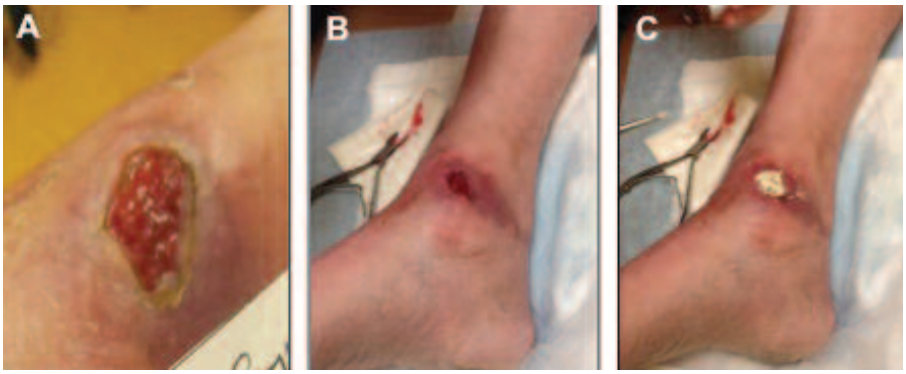

Figure 5. Patient presented with wound on the medial aspect of the right ankle following TAR. (A) Initial wound presentation following 4 weeks treatment with Silvercel® and Prisma®. (B) Wound following debridement. (C) Application of particulate AM/UC.

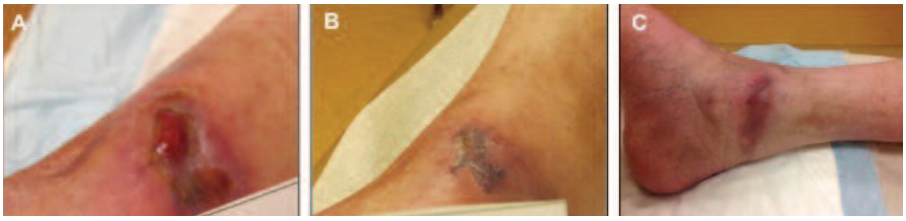

Figure 6. Follow-up images after application of AM/UC particulate: (A) 5 days, (B) 16 days, and (C) 1 month showing complete epithelialization of the wound.

Case Report #4: Wound Dehiscence after Metatarsophalangeal Joint Fusion in a Rheumatoid Arthritis Patient

A 53-year-old female patient with a history of rheumatoid arthritis, smoking, and steroid use including Infliximab and Methotrexate presented with wound dehiscence following first MTP joint fusion surgery. The patients' medical history included a Clayton-Hoffman procedure with wound dehiscene requiring a revision surgery. Prior to treatment with AM/UC particulate tissue, the wound was treated using cadexomer iodine gel, oral antibiotics, and negative-pressure wound therapy. The wound initially presented with some fibrotic material within the wound bed with no peri-wound erythema, no ascending cellulitis, and no exposure of hardware or bone from the MTP joint fusion surgery (Fig. 7A). Following sharp debridement, the wound initially measured 4.7 cm x 2.0 cm x 0.3 cm (Fig. 7B). A total of 50 mg of particulate AM/UC tissue was applied to the wound (Fig. 7C). After application of the AM/UC tissue, the wound was dressed using compressive dressing to maintain adequate decompression and decrease the amount of edema. At one week, the patient returned for a follow-up visit at which time the wound measured 4.3 cm x 1.5 cm x 0.3 cm (Fig. 8A). A fresh dressing was placed over the wound with no further debridement being performed. At two weeks post-AM/UC application the wound was evaluated and measured 4.2 cm x 1.5 cm x 0 cm (Fig. 8B). The wound was noted to consist of healthy hyper-granulation tissue. At this time, the wound was debrided, and a fresh dressing was placed. At three-weeks, the wound continued to show healing with hypergranulation tissue (Fig. 8C). The proximal aspect of the wound measured 1.9 cm x 0.8 cm x -0.3 cm while the distal aspect of the wound measured 2.1 cm x 1.6 cm x 0 cm. The wound was again debrided, and a fresh dressing was placed. The patient continued to be seen at weekly intervals, with the wound showing continued improvement in healing. By 11 weeks post-AM/UC application, the wound showed complete healing (Figs. 8D, 8E & 8F).

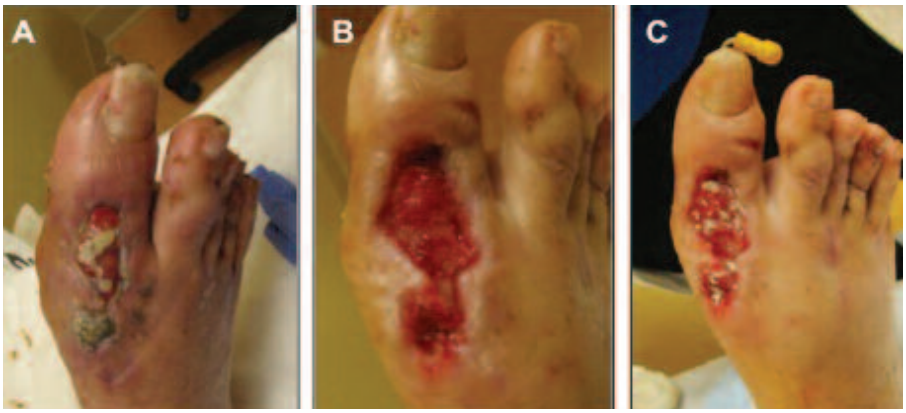

Figure 7. Patient presented with wound dehiscence following revision MTP joint fusion surgery. (A) Initial wound prior to debridement, (B) wound following sharp debridement down to granulation tissue, and (C) application of AM/UC particulate.

Case Report #5: Post-traumatic Degenerative Joint Disease

A 58-year-old female patient initially presented with right ankle pain. The patient had previously had an ankle fracture with ORIF in 2004. The patient developed post-traumatic degenerative joint disease and underwent total ankle replacement. The patient's medical history included depression and sleep apnea, but was otherwise benign. The distal aspect of the incision had difficulty healing throughout the entire postoperative course (Fig. 9A). Initial wound treatment consisted of cadexomer iodine gel; however, the wound persisted with no acute signs of infection. Prior to application of particulate AM/UC tissue, the wound was debrided and measured to be 0.8 cm x 0.8 cm x 0 cm with no signs of infection and good granulation tissue (Fig. 9B). A total of 50 mg of AM/UC tissue was applied directly to the wound bed. After application, a compression dressing was placed over the wound. No further debridement or application of AM/UC tissue was necessary, and the wound showed complete epithelialization two weeks after treatment with particulate AM/UC tissue (Figs. 9C & 9D).

DISCUSSION

Chronic wounds represent a significant healthcare burden around the world. In developed countries, it is estimated that 1–2% of the total population will experience a chronic wound during

their lifetime.[26] In the United States alone, chronic wounds affect 6.5 million patients with an annual treatment cost of $25 billion.[12] In addition to the direct healthcare costs of chronic wounds, the prolonged healing process can affect patients' quality of life with impaired mobility and loss of productivity.

Wound healing involves a coordinated process of cellular and molecular events that involve multiple cell types, cytokines, and growth factors. Immediately following injury, coagulation and hemostasis take place to stabilize the bleeding wound.[27–29] These events trigger a release of cytokines and growth factors including PDGF, TGF-β, and EGF that act to promote the migration of neutrophils and macrophages.[30] During the inflammatory phase, the recruited neutrophils and macrophages serve to clear the wound of foreign particles, bacteria, and damaged tissue via phagocytosis.[27] In addition to their phagocytic role, these cells release growth factors including TGF-β. VEGF, FGF, EGF, and KGF that serve to activate keratinocytes, fibroblasts, and endothelial cells.[27,31,32] These activated cells then shift the wound toward tissue repair during the proliferative phase of wound healing with the formation of granulation tissue, which is subsequently remodeled leading to final scar formation. The entire process of wound healing may take up to a year or more to complete.[33] Any disruption in the normal wound healing process resulting in a pathological state of nonhealing inflammation leads to the development of chronic wounds.[6]

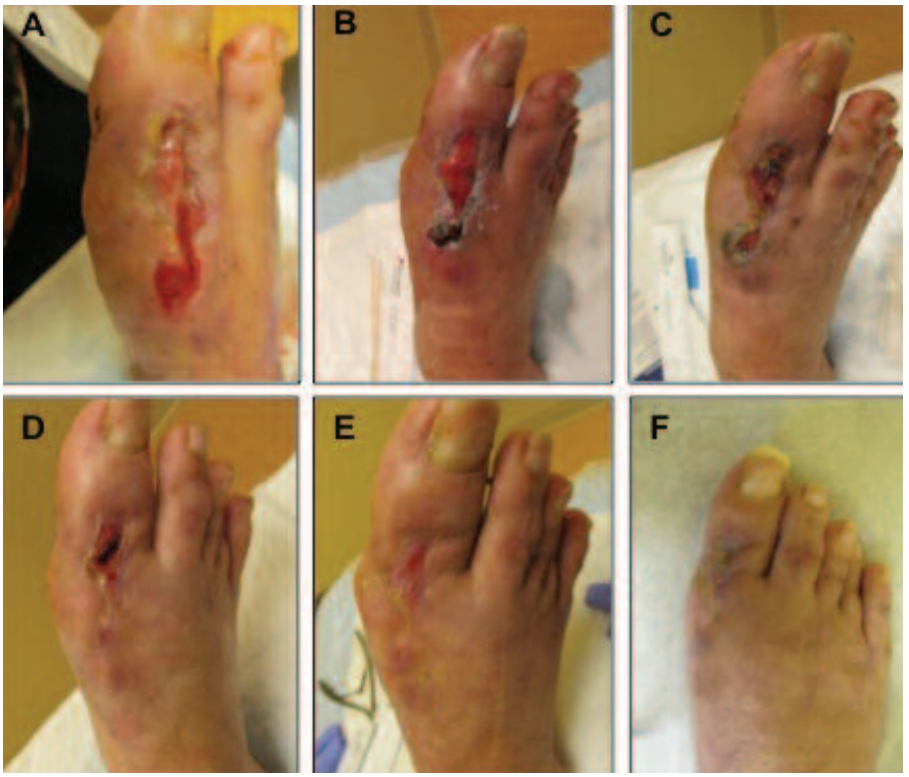

Figure 8. Follow-up images after application of AM/UC particulate: (A) 1 week, (B) 2 weeks, (C) 3 weeks, (D) 8 weeks, (E) 10 weeks, and (F) 11 weeks.

Amniotic membrane and umbilical cord tissues have been used clinically in several indications in ophthalmology where it has been shown to facilitate regeneration with minimal inflammation.[13] AM/UC tissues have been found to contain cytokines and growth factors including EGF, KGF, TGF-β, VEGF, FGF, PDGF, and IGF[34,35] indicating the potential for AM/UC tissues to mediate the healing of chronic wounds. Several studies have explored the use of amnion tissues for use as a treatment in burn patients,[16,20] in the treatment of venous leg ulcers,[36,37] and in the treatment of ankle wounds[38] with good success. In this study, a lyophilized, particulate form of AM/UC tissue as a treatment for chronic nonhealing wounds was exam-

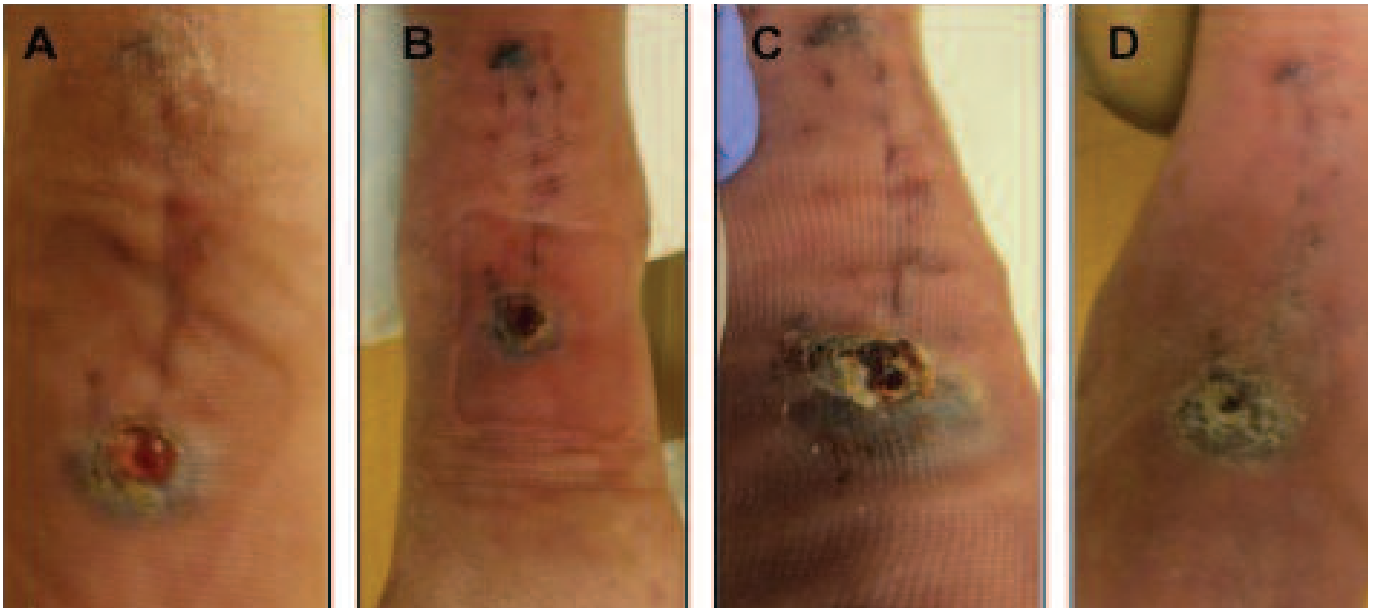

Figure 9. Patient presented with a nonhealing surgical incision wound after TAR. (A) Debrided wound prior to application of particulate AM/UC, (B) wound presentation 5 days after AM/UC particulate application, (C) 10 days, and (D) 21 days post-AM/UC application.

ined. Chronic wounds of the lower extremities in patients with medical conditions including smoking, arthritis, gout, and hypertension received a single dose of particulate AM/UC tissue. In all cases, wounds were found to heal within an average of 5 weeks, indicating the potential of AM/UC tissue to help difficult-to-heal chronic wounds.

CONCLUSIONS

Wound complications occur despite the best standard of care. The present study presents a case series of the use of cryopreserved, lyophilized, particulate AM/UC tissues for the healing of various lower-extremity chronic wounds with different etiologies. In all cases, wounds healed following a single application of the particulate AM/UC tissue. Chronic wounds that remain weeks after surgery inhibit patients from progressing to physical rehabilitation and significantly affect patients both physically and mentally. These case presentations demonstrate how use of human AM/UC tissue may help wounds heal quickly and help patients return to normal function. STI

AUTHOR'S DISCLOSURES

Jennifer Swan is a consultant for Amniox Medical, Inc.

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