Dehydrated human amnion/chorion tissue in difficult–to-heal DFUs: a case series

Diabetic foot ulcers (DFUs) occur as a result of multifactorial complications and are commonly found in the diabetic community. Underlying disease states such as neuropathy and peripheral vascular disease can slow healing rates, potentially leading to recurrence, amputation, and increased mortality. As with many other disease processes, DFUs have several treatment options, such as debriding agents, alginate seaweed extract, hydrocolloid gels, and amniotic membrane allografts. The presented cases all used a dehydrated human amniotic/chorionic membrane allograft (dHACM; EpiFix) to aid the healing process. Human amniotic epithelial membranes have seen increased usage due to their ability to enhance the healing process and accelerate cellular regeneration. The DFUs healed in all of the five patients treated, and patients saw a full recovery in 2.5–11 weeks. In addition, the healing time decreased in spite of the non-adherence seen in three of the patients. These results suggest another possible use for dHACM; however, further studies are required to confirm these data.

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diabetic foot ulcers; dehydrated human amniotic/chorionic membrane; allograft; chronic wounds

The development of diabetic foot ulcers (DFUs) is thought to result primarily from either peripheral arterial disease or peripheral neuropathy, in addition to other factors such as deformity, callus, and trauma.[1,2] Infected DFUs are associated with significant mortality and are the leading cause of non-traumatic lower extremity amputations.[3]

While controlled blood sugar and skin maintenance are mainstays in prevention, wound healing can be aided with debriding agents, alginate seaweed extract, Hydrofiber pads, polyurethane foam, hydrogels, transparent films, hydrocolloid gels, or dehydrated human amniotic/chorionic membrane allograft (dHACM). If these therapies fail in complex wounds other possible treatments include negative pressure wound therapy (NPWT), hydrotherapy, and surgical management.[4]

Human amniotic epithelial membranes are thought to serve three major functions:

- A covering epithelium
- A secreting epithelium
- A mechanism for cellular transport.[5]

Their use has been limited as it was previously very difficult to acquire and effectively produce readily available human amniotic membranes. However, the development of stabilised and preserved dHACM led to its approval as a tissue allograft option in wound care.[2] In previous studies it has been successfully used to treat many other conditions, including osteoarthritis, keratoprosthesis implantation, and conjunctival vascular malformations.[6–8]

The use of dHACM in chronic wound care has been demostrated in several papers.[3,7–9] A recent prospective, randomised, controlled clinical trial revealed that dHACM was superior to Apligraf (a living bilayer skin substitute) in achieving complete DFU closure within 4–6 weeks.[10] A second clinical trial revealed the effectiveness of combining dHACM allografts with multilayer compression therapy in the treatment of venous leg ulcers.[11]

In this article, we attempt to discover the beneficial effects of a dHACM allograft composed of layers of epithelial cells, a basement membrane, and an avascular connective tissue matrix. Our objective was to assess dHACM as a viable treatment option for chronic DFUs, that are refractory to other forms of management.

Methods

The case series consisted of patients over the age of 45 with DFUs that were refractory to therapy. Patient selection was based on good blood perfusion with an ankle brachial pressure index (ABPI)>0.80, adequate offloading devices, and haemoglobin A1Cs≤10. We also attempted to select patients with a wide variety of wound types.

Standard care was provided in all cases. This included cleansing and debridement, application of hydrogel, alginate, and polyurethane foam dressings, along with the use of appropriate antibiotics when necessary. Each patient had presented at our clinic at least 4–5 weeks before treatment with dHACM. They received a dHACM allograft with

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Table 1. Wound measurements of cases 1–5

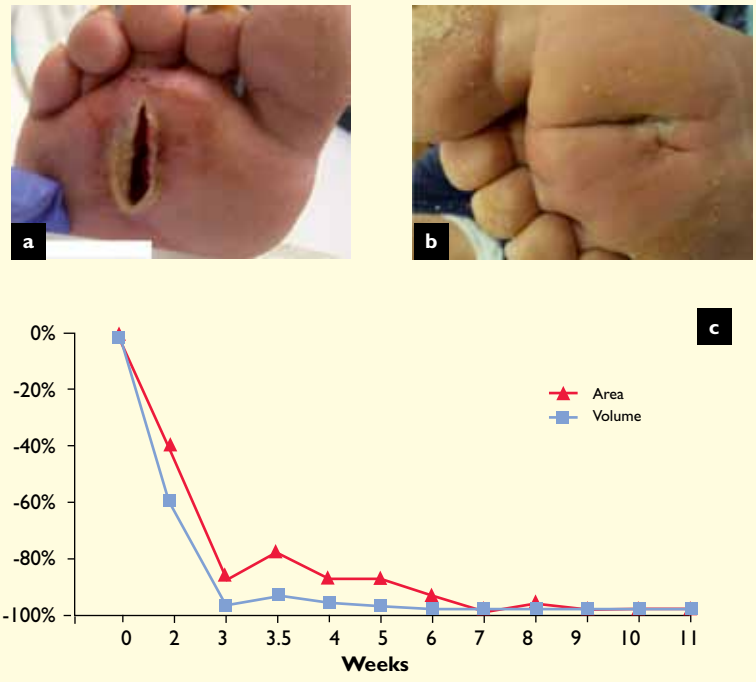
Date	Week	Length	Width	Depth	Area	Volume	Area change	Volume change
Case 1								
25-Jun	0.0	4.0	1.0	3.3	4.00	13.20	0%	0%
9-Jul	2.0	3.5	0.7	2.4	2.45	5.88	−39%	−55%
16-Jul	3.0	1.3	0.4	1.0	0.52	0.52	−87%	−96%
19-Jul	3.5	2.0	0.5	1.2	1.00	1.20	−75%	−91%
23-Jul	4.0	1.5	0.4	1.2	0.60	0.72	−85%	−95%
30-Jul	5.0	1.2	0.5	0.8	0.60	0.48	−85%	−96%
6-Aug	6.0	1.2	0.2	1.0	0.24	0.24	−94%	−98%
20-Aug	8.0	0.5	0.1	1.8	0.05	0.09	−99%	−99%
27-Aug	9.0	0.5	0.3	1.7	0.15	0.26	−96%	−98%
3-Sep	10.0	0.2	0.3	0.7	0.06	0.04	−99%	−100%
10-Sep	11.0	0.0	0.0	0.0	0.00	0.00	−100%	−100%
Case 2								
6-May	0.0	9.0	0.5	0.5	4.50	2.25	0%	0%
13-May	1.0	4.5	0.4	0.2	1.80	0.36	−60%	−84%
27-May	3.0	1.0	0.4	0.1	0.40	0.04	−91%	−98%
2-Jun	4.0	0.5	0.2	0.1	0.10	0.01	−98%	−100%
10-Jun	5.0	0.0	0.0	0.0	0.00	0.00	−100%	−100%
Case 3								
7-Mar	0.0	1.7	0.8	0.3	1.36	0.41	0%	0%
14-Mar	1.0	1.6	0.6	0.2	0.96	0.19	−29%	-53%
21-Mar	2.0	1.4	0.4	0.1	0.56	0.06	−59%	−86%
1-Apr	3.5	1.4	0.4	0.0	0.56	0.00	−59%	−100%
13-May	9.5	0.0	0.0	0.0	0.00	0.00	−100%	−100%
Case 4								
25-Feb	0.0	1.2	1.1	0.1	1.32	0.13	0%	0%
4-Mar	1.5	0.7	0.9	0.0	0.63	0.00	−52%	−100%
11-Mar	2.5	0.0	0.0	0.0	0.00	0.00	−100%	−100%
Case 5								
7-Mar	0.0	1.5	0.7	0.7	1.05	0.74	0%	0%
18-Mar	1.5	1.0	0.6	0.2	0.60	0.12	−43%	−84%
25-Mar	2.5	1.0	0.6	0.2	0.60	0.12	−43%	−84%
8-Apr	4.5	0.8	0.3	0.1	0.24	0.02	−77%	−97%
15-Apr	5.5	0.2	0.2	0.0	0.04	0.00	−96%	−100%
29-Apr	7.5	0.3	0.2	0.0	0.06	0.00	−94%	−100%
6-May	8.5	0.0	0.0	0.0	0.00	0.00	−100%	−100%

subsequent applications as required. The method is in accordance with Sheehan et al.[12] and our own experience of initially using basic standard wound care products rather than advanced therapies.

dHACM was applied as per the manufacturer's instructions (EpiFix, MiMedx Group Inc, Marietta, GA) with Steri Strips (3M) to anchor the graft, with the addition of Mepitel One (non-adherent dressing: Mölnlycke Health Care) and Aquacel Ag dressings (silver dressing; Convatec). Based on the authors' experience, when more moisture control was necessary the non-adherent and silver dressing combination was changed to Sorbion Sana (absorbent non-adherent dressing; H&R Healthcare). A secondary dry dressing was then applied.

Each wound was assessed for percentage of non-viable tissue, exudate volume, and signs of infection. Wounds were measured at each visit. Images were

Fig 1. Case 1 wound located on plantar side of right foot in the 2nd sub-metatarsal region. At initial presentation (a) at wound closure (b) and percentage reduction in wound area and volume (c)



taken before and after treatment. Follow-up assessments were performed 1 year post wound closure. Non-adherence was also assessed and recorded. When necessary, patient treatment plans were altered.

Results

There were five patients recruited with the following wound types: post-surgical debridement, non-healing surgical wound, traumic wound, diabetic ulcer secondary to Charcot arthropathy, and a pressure ulcer. The use of dHACM led to full wound closure after other treatment options failed. The average wound took 7.3 weeks to heal (range: 2.5–11 weeks). Prolonged healing times were due to patient non-adherence with treatment regimens. These issues were seen in case 1, who refused to continue with contact-casting, and in cases 3 and 5, where some dressings were macerated after patients refused to sponge bathe, leading to extremely wet casts. Table 1 shows wound measurements and results for all the presented cases. Previous wound treatments included alginate seaweed extract, hydrogels, and standard debridement.

Case 1

Patient 1 was a 60-year-old male with type 2 insulin-dependent diabetes, peripheral diabetic neuropathy, hypertension, hyperlipidaemia and with no history of smoking. His primary care physician (PCP) said he was non-adherent to treatment. He had been hospitalised for a plantar ulcer with cellulitis that was followed by a complete surgical debridement down to the plantar fascia, with no significant improvement.

After 4 weeks of treatment, he was referred to our clinic, having refused to wear a negative pressure wound therapy (NPWT) device. He was initially treated with Silvasorb Hydrogel Silver Gel (silver gel dressing; Parthenon) with saline packing and fitted with an IPOs forefoot relief shoe (Bird & Croni).

At the first application of dHACM the wound measured 4.0 x 1.0 x 3.3cm (Fig 1a). The patient was contact casted along with the dHACM application due to his long-standing history of non-adherence. The graft was covered with non-adherent and an absorbent non-adherent dressing for additional moisture control with a dry dressing. After 1 week of contact-casting, the patient refused to wear another contact cast and was issued a manual scooter (since he could not use crutches). Follow-up appointments revealed that the patient admitted going back to work against the doctors' orders, using his diabetic shoes while at work and only using his IPOs forefoot offloading shoe when he was at home. He also admitted not using the manual off-weight bearing scooter.

Full wound closure (Fig 1) was achieved after three applications of dHACM (Table 1) and took 11 weeks. A graph of the patient's healing time frame is provided in Fig 1c. Non-adherence led to multiple office visits and a longer healing time.

Case 2

Patient 2 was a 45-year-old male with history of obesity, lymphoedema and peripheral neuropathy of unknown aetiology. The patient smoked ten cigarettes a day. He was referred to our service due to a 5 month-old infected non-healing surgical wound, post fracture of the tibia and fibula, and dislocation of the left ankle.

It was recommended the hardware be removed from the left fibula. He was referred back to the surgeon for hardware removal and cellulitis treatment. Based on wound culture results the patient was given Levaquin (500mg, 10 days). Once the infection cleared and the hardware was removed, it was decided to proceed with dHACM in an attempt to heal the full-thickness wound with a small amount of exposed tendon.

The patient was immobilised and protected with a removable CAM walker fracture boot (United Surgical). His initial measurement at the first application was 9.0 x 0.5 x 0.5cm (Fig 2a). Wound healing took 5 weeks, with a total of 4 applications (Table 1; Fig 2b). A graph of the patient's healing time frame is provided in Fig 2c. Due to drainage issues, an absorbent non-adherent dressing and a non-adher-

ent dressing were used after the application of dHACM. It was then covered with a dry dressing.

Case 3

Patient 3 was an 86-year-old female with type 2 diabetes, hypertension, obesity, hypercholesterolaemia, arthritis, peripheral vascular disease, lymphoedema, and osteoporosis. She had no history of smoking. The wound occurred after she bumped into a car door. The wound extended completely down to the periosteum. She self-treated for 2 weeks with Neosporin cream, after which her PCP treated her with saline gauze and dry dressings twice a week for 6 weeks before being referred to our clinic.

On presentation at the wound clinic, she was treated for 2 weeks with a silver gel dressing, moistened gauze, and dry dressings with a Tubigrip to control her lymphoedema. This treatment was chosen rather than a split-thickness skin graft to avoid a donor-site wound and due to the patient's desire for a less invasive option.

When this was unsuccessful, dHACM was used covered with a non-adherent and absorbent non-adherent dressing to cover the graft. Sana Sorbion with dry dressings were used to address the drainage issue. Tubigrip was used to control her lymphoedema. At first application the wound dimensions were 1.7 x 0.8 x 0.3cm (Fig 3a). Wound healing took three applications of dHACM over 9.5 weeks (Table 1). In the final 3 weeks the wound epithelialised (Fig 3b). A graph of the patient's healing time frame is provided in Fig 3c.

Case 4

Patient 4 was a 66-year-old male with type 2 insulin-dependent diabetes, hypercholesterolaemia, hypertension, ferrous sulfate deficiency, folic acid deficiency, and Charcot joint disease (neuroarthropathy) of the foot. He originally presented with a chronic wound, caused by ill-fitting shoes, that was refractory to previous treatment for over 4 weeks. The patient was treated with silver, alginate, and foam dressings.

We decided the patient would be contact casted with dHACM due to the prolonged healing time and previous non-adherence as determined by his PCP and previous medical history. The category II pressure ulcer initially measured 1.2 x 1.1 x 0.1cm upon first application (Fig 4a) and took 2.5 weeks to heal (Table 1). The graft was covered with non-adherent dressing and an absorbent non-adherent dressing, and a dry dressing. After the wound healed (Fig 4b), the patient was contact casted one more time to ensure that the wound would stand up to weight bearing without breaking down. A graph of the patient's healing time frame is provided in Fig 4c. The patient was placed into a DH Off-Loading Walker until fitted with a new pair of diabetic shoes.

Fig 2. Case 2 wound located on lateral side of left leg. At initial presentation (a), at wound closure (b), and percentage reduction in wound area and volume (c)

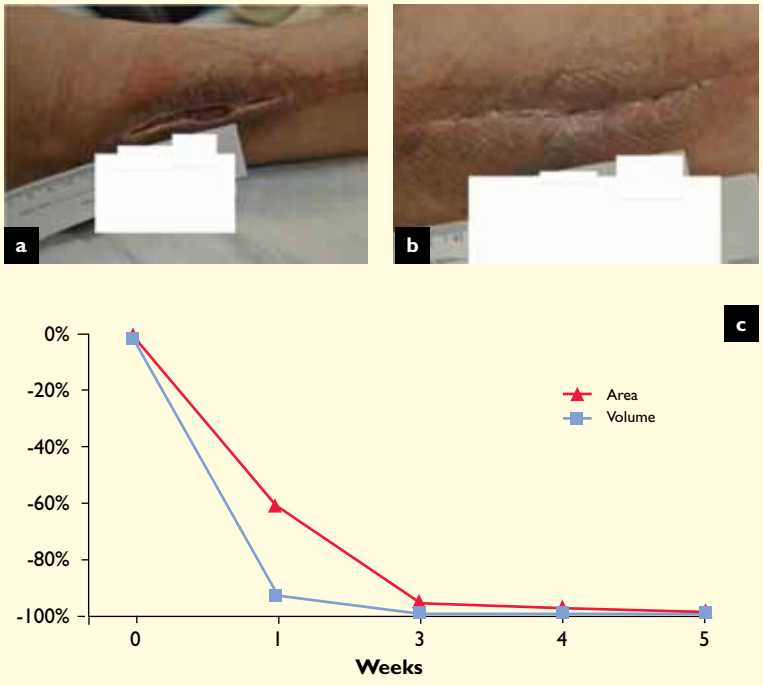


Fig 3. Case 3 wound located on anterior side of left leg. At initial presentation (a), at wound closure (b), and percentage reduction in wound area and volume (c)

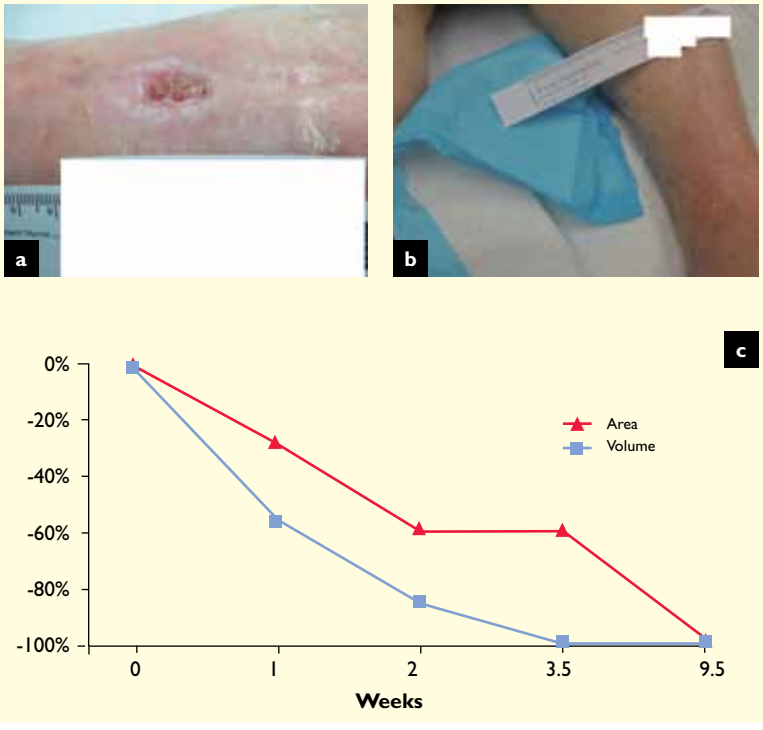


Fig 4. Case 4 wound located on plantar medial arch of left foot. At initial presentation (a), at wound closure (b) and percentage reduction in wound area and volume (c)

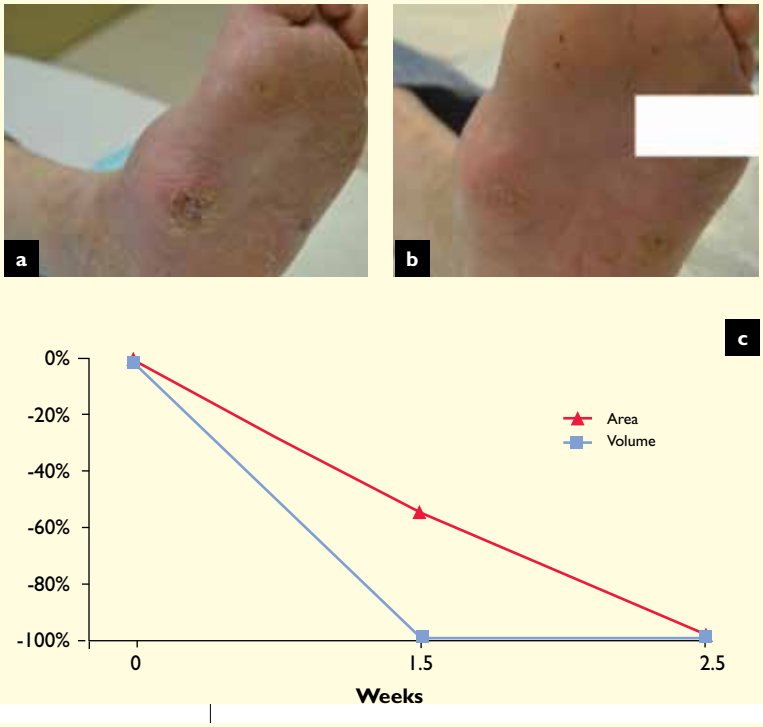
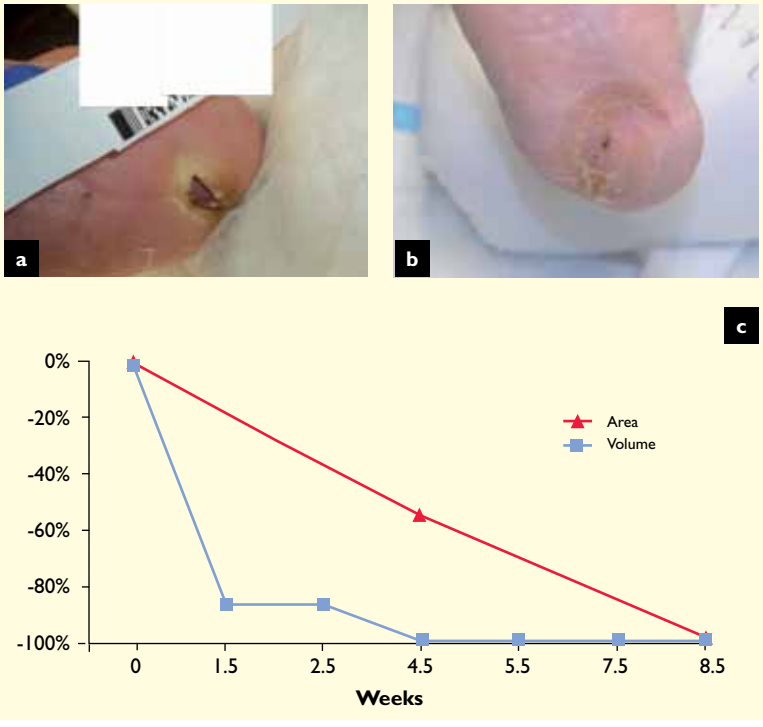


Fig 5. Case 5 wound located on inferior side of right heel. At initial presentation (a), at wound closure (b), and percentage reduction in wound area and volume (c)



Case 5

Patient 5 was a 64-year-old female with type 2 insulin-dependent diabetes, peripheral diabetic sensory neuropathy and hypertension. She also had a past surgical amputation of a left great toe due to a non-healing catagory II pressure ulcer. The patient was referred to our service after having a long course of IV antibiotics, surgical debridement of bone due to osteomyelitis, failure of a split-thickness skin graft, and failure of a skin substitute after 12 months of treatment.

The patient was initially treated with alginate, absorbent, and foam dressings for 4 weeks. After 4 weeks, we attempted healing the wound with dHACM alongside contact-casting. At the initial application of dHACM the wound was 1.5 x 0.7 x 0.7cm (Fig 5a). The patient had one application of dHACM a week for 4 weeks which was covered with a non-adherent dressing, an absorbent non-adherent dressing and a dry dressing (Table 1). The patient was contact-casted throughout her treatment due to her obesity and concerns that she would not adhere to the treatment. She was given diabetic molded shoes upon healing after 8.5 weeks (Fig 5b). A graph of the patient's healing time frame is provided in Fig 5c.

After the wound had healed, the patient was contact casted two more times in order to ensure that the wound would stand up to weight bearing without breaking down. The patient wore a reverse IPOs shoe for offloading until she could be transitioned to a diabetic shoe, in attempt to prevent recurrence of the ulcer.

Discussion

DFUs continue to plague both adherent and non-adherent diabetic patients. Uncontrolled blood sugars and a lack of personal skin hygiene only serve to further complicate the clinical picture. Therefore, while several treatment modalities exist for these types of wounds, non-adherent patients often require the combined treatments or a change from the initial treatment strategies.

The cases presented here show the use of a dHACM allograft as a viable treatment modality for DFUs. Our patients were able to achieve full wound closure in an average of 7.3 weeks, despite non-adherence in 3/5 cases. Not all of the patients used their offloading devices consistently, two of which had to be contact-casted to expedite healing. Another patient had a contact cast but kept getting it wet. Consequently, healing was delayed in these patients.

As the dHACM allograft combined with other treatment modalities were able to overcome non-adherence, we believe the allograft would be more effective in adherent patients.

Our results provide preliminary evidence that wound closure is attainable. They are unique in that

we show what can be expected with treatment in the real-life setting in patients with wound types and circumstances that would likely cause them to be excluded from randomised studies. Therefore, we believe that dHACM allograft products have a place in chronic DFU healing and should be considered when attempting to begin therapy or when patients are refractory to other standards of care.

Randomised clinical trials have shown that dHACM allograft is both a clinical and cost-effective treatment for diabetic lower extremity ulcers. Zelen et al.[1] observed that after 4 weeks of first dHACM allograft application there were significant differences in mean wound reduction when treated with dHACM allograft versus standard care, 97.1 ± 7.0% and 32.0 ± 43.7% reduction ($p<0.001$), respectively. In our five-patient sample wounds reduced between 59% and 100% after approximately 4 weeks of treatment. We believe that this compares favourably with the results for our non-adherent population. Overall, healing rates after 4 and 6 weeks of treatment with dHACM allograft were 77% and 92% respectively, compared to 0% and 8.0% respectively with standard care ($p<0.001$).

In another randomised clinical trial, effectiveness and cost of the dHACM allograft and a living bilayer skin substitute, revealing a significantly quicker median healing time with the dHACM allograft (13 days) compared with the living bilayer skin substitute (49 days) with an 81.9% lower cost for graft material in the dHACM allograft group.[11]

Limitations and future studies

This study has several limitations. The number of non-adherent patients complicates the clinical picture when assessing product effectiveness and wound healing rates. It was a challenge to have each patient follow protocol and keep his or her contact casts from getting wet.

In addition, we did not implement consistent offloading protocols for all of our case studies. Only two patients were contact casted, while a third patient was allowed to opt out after receiving their first cast.

Standardised dressings were also not implemented for all patients. Patients received a combination of non-adherent, silver and absorbent dressing which were also changed as necessary. Therefore, a more consistent approach to dressings should be implemented in future studies.

Conclusion

Although this case study consisted of only five patients, it does not negate the fact that patients should be made aware of the potential wound complications of diabetes and educated on the healing process in order to facilitate patient adherence, and therefore, increased wound closure.

dHACM allograft products, such as EpiFix, which has a variety of applications in different wounds, are useful in treating special cases that are refractory to traditional management. These products should be considered in order to increase a physician's arsenal for the treatment of wounds. ■

References

1 Zelen, C.M., Serena, T.E., Denoziere, G,. Fetterolf, D.E. A prospective randomized comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. Int Wound J 2013; 10: 5, 502–507.

2 Sheikh, E.S., Sheikh, E.D., Fetterolf, D.E. Use of dehydrated human amniotic membrane allografts to promote healing in patients with refractory non healing wounds. Int Wound J 2014; 11: 6 711–717.

3 Turhan, V,. Mutluoglu, M,. Acar, A. et al. Increasing incidence of gram-negative organisms in bacterial agents isolated from diabetic foot ulcers. J Infect Dev Ctries 2013; 7: 10, 707–712.

4 Koob, T.J., Rennert, R., Zabek, N. et al. Biological properties of dehyrdrated human amnion/chorion composite graft: implications for chronic wound healing. Int Wound J 2013; 10: 5, 493–500.

5 John, T. Human amniotic membrane transplantation: past, present, and future. Opthalmol Clin North Am 2003; 16: 1, 43–65.

6 Willett, N.J., Thote, T., Lin, A.S. et al. Intra-articular injection of micronized dehydrated human amnion/chorion membrane attenuates osteoarthritis development. Arthritis Res Ther 2014; 16: 1, R47.

7 Tay, E., Utine, C.A., Akpek, E.K. Crescenteric amniotic membrane grafting in keratoprosthesis-associated corneal melt. Arch Ophthalmol 2010; 128: 6, 779–782.

8 Mehta M, Waner M, Fay A. Amniotic membrane grafting in the management of conjunctival vascular malformations. Ophthal Plast Reconstr Surg 2009; 25: 5, 371–375.

9 Maan, Z.N., Rennert, R.C., Koob, T.J. Cell recruitment by amnion chorion grafts promotes neovascularization. J Surg Res 2015; 193: 2, 953–962.

10 Serena, T.E., Carter, M.J., Le, L.T. et al. A multicenter, randomized, controlled clinical trial evaluating the use of dehydrated human amnion/chorion membrane allografts and multilayer compression therapy vs. multilayer compression therapy alone in the treatment of venous leg ulcers. Wound Repair Regen 2014; 22: 6, 688–693.

11 Zelen, C.M., Gould, L., Serena, T.E et al. A prospective, randomised, controlled, multi-centre comparative effectiveness study of healing using dehydrated human amnion/chorion membrane allograft, bioengineered skin substitute or standard of care for treatment of chronic lower extremity diabetic ulcers. Int Wound J 2014; Epub DOI: 10.1111/iwj.12395.

12 Sheenan, P., Jones, P. Caselli, A. et al. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. Diabetes Care 2003; 26: 6, 1879–1882.