Wound Medicine 4 (2014) 1–4

Contents lists available at ScienceDirect

Wound Medicine

journal homepage: www.elsevier.com/locate/wndm

Dehydrated human amnion/chorion membrane allografts in patients with chronic diabetic foot ulcers: A long-term follow-up study

Charles M. Zelen [a,*], Thomas E. Serena [b], Donald E. Fetterolf [c]

[a] Professional Education and Research Institute, Inc., Roanoke, VA, United States
[b] SerenaGroup, Cambridge, MA, United States
[c] MiMedx Group, Inc., Department of Medical Affairs, Marietta, GA, United States

ARTICLE INFO

Article history:
Received 25 September 2013
Accepted 23 October 2013
Available online 19 November 2013

Keywords:
Amniotic membrane allograft
Diabetic ulcer
Wound care

ABSTRACT

Diabetic foot ulcers (DFU) are notoriously slow to heal and even in cases where primary healing is achieved ulcers frequently recur. An optimal treatment for DFU would be one that supports both rapid and long-term healing. Our purpose is to evaluate recurrence rates of DFU healed with use of dehydrated human amnion/chorion membrane (dHACM). Twenty-two patients with chronic DFU that healed with the use of dHACM were eligible for inclusion. All eligible patients had completed a single-center randomized clinical trial comparing rates of primary healing over a 12 week period with dHACM versus a standard regimen of care [20] (Zelen et al., 2013). Follow-up examinations were scheduled for 9–12 months after primary healing with dHACM. Subsequent evaluation of clinical records was made with IRB approval and patient consent. Eighteen of 22 eligible patients (81.8%) returned for follow-up examination. Mean wound size prior to treatment with dHACM was $3.1 \pm 3.8\ cm^2$, median $1.7\ cm^2$ (0.7, 13.5). Mean time to wound closure after dHACM initiation was 3.1 ± 2.8 weeks (median 2.0 weeks, range 1.0–9.0 weeks). At the 9–12 month follow-up visit 17 of 18 (94.4%) wounds treated with dHACM remained fully healed. These findings support the effectiveness of dHACM for treatment of DFU.

1. Introduction

Identification and implementation of the ideal treatment regimen for patients with diabetic foot ulcers is an increasingly common issue faced by clinicians worldwide. Due to factors such as peripheral vascular disease, neuropathy, and poor blood glucose control, diabetic ulcers are notoriously slow to heal. Underscoring the need for rapid healing, Lavery et al. reported that ulcer duration of greater than 30 days was independently associated with a 4.7-fold increase in infection, and that an infected foot ulcer increased the risk of hospitalization by nearly 56 times and risk for amputation by nearly 155 times [1]. Therapies that promote rapid and complete healing, thus reducing the risk for infection and amputation, can substantially improve quality of life while decreasing financial burdens to the individual and society overall [2].

Even in cases where primary healing occurs, studies have shown that despite ongoing intensive foot care, diabetic ulcers frequently recur – either in the same location or elsewhere on the foot [3,4]. Overall recurrence rates of 35–60% over 3 years, increasing to 70% over 5 years have been reported [5]. In one study of patients with recurrent diabetic foot ulcers, 34.4% of wounds recurred in the exact location as a previous ulcer, and almost 60% of recurrent ulcers appeared within 12 months of primary healing [6]. Indeed, it has been suggested that patients with a healed ulcer should not be referred to as cured, but rather as being in remission [7].

Natural human amniotic membrane has been used as a wound covering for over 100 years [8]. However, there are often issues relative to obtaining, preparing, and storing the tissue for use in clinical practice, as well as concern regarding the potential for infectious disease transmission. Recently, a system has been identified to gently process, sterilize and dry placental tissue obtained from screened and tested pregnant women scheduled to undergo Cesarean delivery. This proprietary process is used to create a dehydrated human amnion/chorion membrane (dHACM) allograft that can be stored at ambient temperature for up to 5 years (EpiFix® MiMedx Group, Inc., Marietta, GA) [9].

Comprising the innermost layer of the placenta, the amniotic membrane consists of a thin epithelial layer, a thick basement membrane and an avascular stroma [10]. These materials provide

Abbreviation: dHACM, dehydrated human amniotic/chorionic membrane.

* Corresponding author at: Professional Education and Research Institute, Inc., 222 Walnut Avenue, Roanoke, VA 24016, United States. Tel.: +1 540 344 3668; fax: +1 540 769 6381.
E-mail address: cmzelen@periedu.com (C.M. Zelen).

2213-9095
http://dx.doi.org/10.1016/j.wndm.2013.10.008

structural collagen and extracellular matrix, biologically active cells and a large number of important regenerative molecules [11,12]. Collagens type IV, V and VII provide an important substrate which is not only important for the structural integrity of the membrane but also facilitates wound healing and cellular ingrowth. Natural amniotic membrane has been shown to be antimicrobial, and can reduce pain, inflammation and scar tissue formation at the site of application; the membrane also contains essential growth factors and cytokines that may enhance the healing process [12–16].

Several case studies and clinical reports on the use of dHACM in various types of wounds are available [17–19]. In a recent randomized clinical trial of patients with chronic diabetic foot ulcers treated with biweekly application of dHACM versus a standard regimen of wound care, primary healing occurred in 92% of ulcers (12/13) treated with dHACM and only 8% of ulcers (1/12) receiving a standard protocol of wound care (moist wound therapy) in the 12 week study period [20]. Those patients that failed to heal in the randomized trial ($n = 11$) were subsequently treated with dHACM resulting in primary healing of 91% of diabetic ulcers (10/11) [21]. The purpose of the current investigation is to examine rates of ulcer recurrence within one year of treatment with dHACM.

2. Materials and methods

We conducted a follow-up study of patients previously enrolled in an IRB approved randomized clinical trial [20] comparing rates of primary healing with dHACM versus a standard protocol of wound care (moist wound therapy) over a 12 week period. Enrolled in the original study [20] were patients with type 1 or type 2 diabetes having a non-healing (minimum of 6 weeks duration) diabetic ulcer anywhere on the foot. Study inclusion/exclusion criteria and study procedures have been described in the previous publication [20]. Complete healing was defined as total epithelialization of the open area of the wound. Failure to heal was defined as less than 50% decrease in wound size after 6 weeks of study participation or not having completely healed within 12 weeks.

At completion of the clinical trial [20] those patients randomized to the standard care group who had not healed ($n = 11$) were then treated with biweekly application of dHACM [21].

Included in the present study are those patients from the initial randomized trial that were randomized to the dHACM treatment group and healed within 12 weeks and those that were randomized to standard treatment with moist wound care that subsequently received dHACM and healed after initial study completion. The study was conducted in a single center in Southwest Virginia under the direction of a senior clinician (CMZ) with expertise in diabetic foot care. Patients read and signed an IRB approved informed consent form prior to enrollment in the initial study and provided additional IRB approved consent for the current review of subsequent data and outcomes.

2.1. dHACM therapy

Prior to application of dHACM to the wound all necrotic tissue was removed using surgical debridement. The dHACM allograft was then covered with a non-adherent dressing (Adaptic®), followed by a moisture-retentive dressing (hydrogel) and a compression dressing. All wounds were offloaded using a removable cast walker (Active Offloading Walker; Darco of Huntington; West Virginia). Weekly clinic visits were required for wound assessment and dressing changes. All patients were seen by the investigator weekly for up to 12 weeks or until complete healing, whichever occurred first. During each weekly visit, ulcer cleansing with a sterile normal saline solution (rinsing, swabbing or irrigating), ulcer measurement with a graded centimeter ruler (length, width and depth) and a dressing change was conducted. When applicable, measurements were done after debridement. The wound area was calculated by multiplying the width and length measurements. An additional dHACM allograft was applied at week 2, week 4, week 6, week 8, and week 10 if the ulcer had not completely epithelialized. After healing was achieved patients were instructed on an intensive diabetic foot care routine and advised to wear diabetic shoes with insoles for ambulation. Strict blood glucose control was encouraged and monitored by each patient's private physician. Follow-up via clinic visits were encouraged and performed on a regular basis in majority of the patient population, in most instances every three to four months.

2.2. Study outcome

In this current study we sought to determine rates of ulcer recurrence within one year of primary healing with dHACM.

3. Results

Twenty-five patients were enrolled in the initial randomized trial. Of those 25, 13 were randomized to receive dHACM and 12 of 13 (92.3%) healed at a mean of 2.5 ± 1.9 weeks [20]. Of the 12 patients randomized to receive the standard regimen of care, one healed, and 11 exited the randomized study without healing and subsequently received dHACM [21]. Of these 11 patients, 10 (91%) healed completely within 9 weeks of starting treatment with dHACM (mean 4.2 ± 3.1 weeks) [21]. Twenty-two of 25 patients (88%) ultimately had complete healing of their diabetic foot ulcer after treatment with dHACM and were eligible for inclusion in the follow-up study. Four were lost to follow-up. Clinical characteristics of the 18 patients with follow-up data are presented in Table 1. Overall, at time of first dHACM allograft placement the foot ulcers had been present for a mean of 19.4 ± 13.6 weeks, median 14.0 weeks, range 6.0–54.0 weeks. Mean wound size at initiation of dHACM was 3.1 ± 3.8 cm^2.

Eighteen patients returned to the clinic for follow up examination 9–12 months after healing (mean time from healing to follow-up was 11.2 months). At long term follow up 17 of the 18 ulcers remained healed (94.4%). The wound that reopened did so despite off loading with appropriate diabetic insoles and follow up podiatric care. Photos of wounds at time of initial application of dHACM, primary healing, and at follow-up are presented in Fig. 1.

Table 1
Clinical characteristics.

Variable	$N = 18$
Male gender (n/%)	5/27.8
Age (year)	58.8 ± 12.8
	58.5 (31, 80)
BMI (kg/m^2)	32.7 ± 6.7
	31.4 (23.1, 51.6)
Obese (>29.9 kg/m^2) (n/%)	10/55.5
Smoker (n/%)	3/16.7
Caucasian race (n/%)	15/83.3
Wound size (cm^2) at 1st application of dHACM	3.1 ± 3.8
	1.7 (0.7, 13.5)
Wound duration (weeks) at 1st application of dHACM	19.4 ± 13.6
	14.0 (6.0, 54.0)
Ulcer location (n/%)	
Forefoot or digital	9/50
Heel or midfoot	9/50
Time to healing with dHACM (weeks)	3.1 ± 2.8
	2.0 (1.0, 9.0)

Data presented as mean ± SD, median (min, max), or number (percent) as indicated.
BMI = body-mass-index.

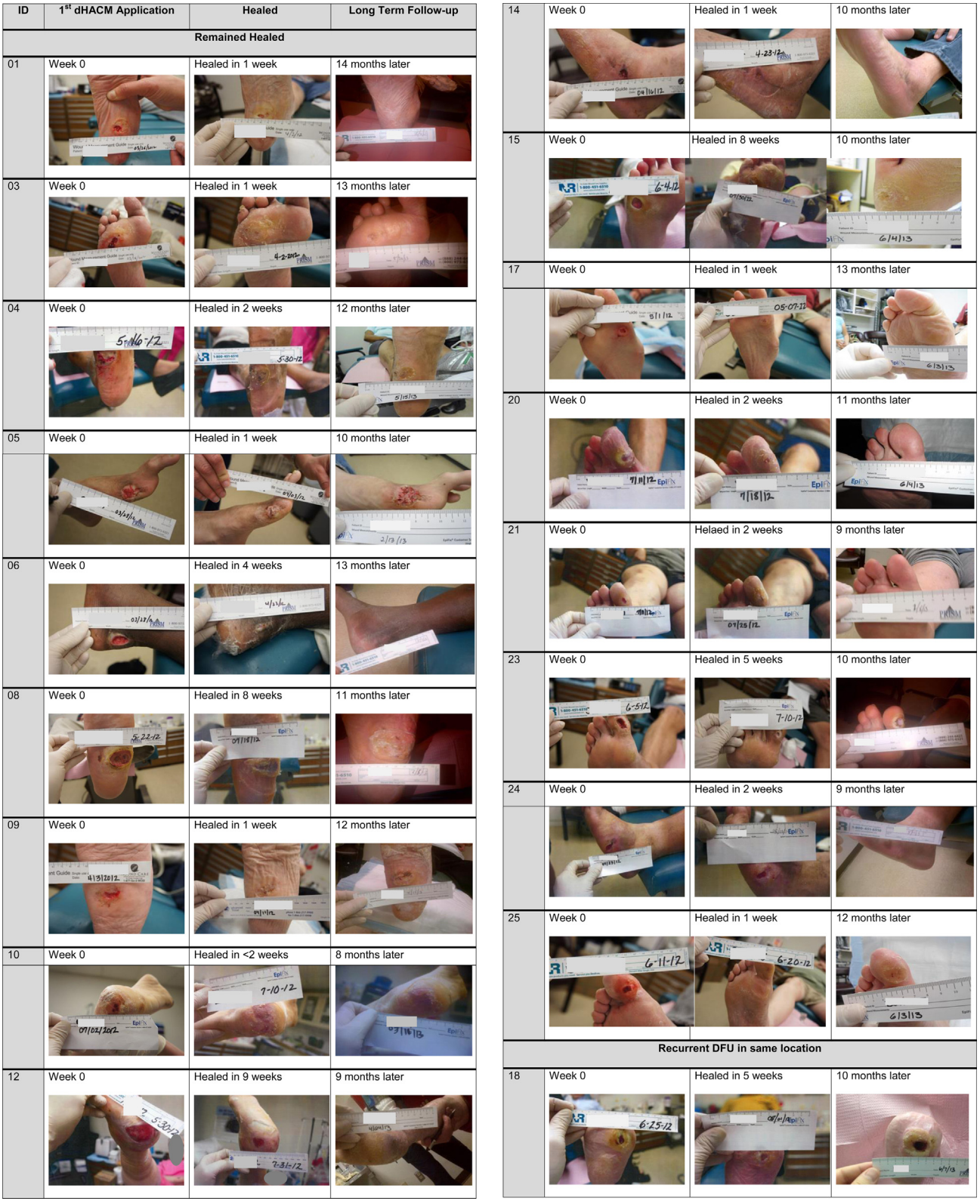

Fig. 1. Images of wounds at 1st dHACM application, when healed, and at long term follow-up visit.

4. Discussion

In the present study of 18 patients with chronic diabetic foot ulcers that healed within 12 weeks after treatment with dHACM, all but one treated ulcer (94.4%) remained healed almost one year later. These results support the results of the original clinical trial [20] and the second crossover study [21] showing that dHACM is an effective treatment for closure of diabetic foot ulcers.

The pathogenesis of foot ulcers is often complex and their management is difficult. Knowledge of new techniques, technology and products can allow the clinician to excel in their effort to provide optimal care and promote positive outcomes for these

challenging patients. Advanced therapies such as dHACM, bioengineered skin substitutes and wound care technologies such as the topical gel becaplermin (Regranex®, Systagenix Wound Management) and living skin equivalents Apligraf® (Organogenesis) and Dermagraft® (Shire Regenerative Medicine) have been shown to accelerate the healing process in many patients, yet there is no perfect treatment for all patients in all situations [22].

Treatments which can accelerate the healing process, thus reducing the risk for infection, are highly desirable in the management of diabetic ulcers, although it is also important to assess if healing is maintained over a longer period of time. Apelqvist et al. [5] reported an ulcer recurrence rate of 34%, 61% and 70% at 1, 3 and 5 years after primary healing respectively. In another study of patients with recurrent diabetic foot ulcers, it was reported that 34.4% of ulcers recurred in the location of a previous ulcer, and recurrence occurred within 12 months of primary healing in almost 60% of patients [6]. In the present study of patients treated with dHACM only 1 of 18 patients (5.5%) experienced a recurrent diabetic foot ulcer.

A proprietary process of advanced tissue stabilization and preservation has allowed for widespread use of human amniotic membrane in the form of a dHACM allograft [9]. The dehydrated allograft is operationally efficient as it can be transported and stored at ambient temperature up to 5 years, minimizing the need for complex policies for receiving and storing certain graft materials that may require subzero refrigeration, or may have a short storage life that often leads to wasted product. Handling characteristics of the dHACM material minimizes time for application which improves clinically efficiency. The dHACM is provided in a number of different sizes, minimizing waste when used on ulcers of various sizes and at various stages in the healing process.

Limitations of the current study are inherent to those of a retrospective study design and small sample size. Four patients were lost to follow-up and we are unaware of the status of their wound. Larger studies are needed to confirm our findings.

5. Conclusion

Prior studies have shown that dHACM can promote rapid healing of diabetic ulcers with a 97.1 ± 7.0% reduction in wound size within 4 weeks of first allograft application [20]. Long term follow-up of wounds treated with dHACM showed that 94.4% of these wounds remained closed. These results illustrate that dHACM promotes both rapid and sustained healing. dHACM appears to be a viable treatment option for the management of recalcitrant diabetic foot ulcers.

Declaration of interest

The author C.M.Z. reports no conflict of interest. T.E.S. is an advisor for MiMedx. D.E.F. is employed by MiMedx as Chief Medical Officer. This study was sponsored and funded by MiMedx, Marietta, GA. The Professional Education and Research Institute received research funds to conduct the trial.

Funding source

The study was sponsored and funded by MiMedx. Staff employed by the study sponsor assisted investigators with data analysis.

Acknowledgments

The authors would like to thank Guilhem Denoziere and Niki Istwan from MiMedx for their technical help and administrative assistance as well as Morgan Stepanek, BS and Lynne Baker RN, the clinical research coordinator and research nurse respectively, from the Professional Education and Research Institute for their clinical and administrative support.

References

[1] Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, Lipsky BA. Risk factors for foot infections in individuals with diabetes. Diabetes Care 2006;29:1288–93.
[2] Albert S. Cost-effective management of recalcitrant diabetic foot ulcers. Clin Podiatr Med Surg 2002;19:483–91.
[3] Ghanassia E, Villon L, Thuan Dit Dieudonné JF, Boegner C, Avignon A, Sultan A. Long-term outcome and disability of diabetic patients hospitalized for diabetic foot ulcers: a 6.5-year follow-up study. Diabetes Care 2008;31:1288–92. http://dx.doi.org/10.2337/dc07-2145 [Epub 2008 Apr 4].
[4] Dubský M, Jirkovská A, Bem R, Fejfarová V, Skibová J, Schaper NC, Lipsky BA. Risk factors for recurrence of diabetic foot ulcers: prospective follow-up analysis of a Eurodiale subgroup. Int Wound J)2012;(June). http://dx.doi.org/10.1111/j.1742-481X.2012.01022.x [Epub ahead of print].
[5] Apelqvist J, Larsson J, Agardh CD. Long-term prognosis for diabetic patients with foot ulcers. J Intern Med 1993;233:485–91.
[6] Galea AM, Springett K, Bungay H, Clift S, Fava S, Cachia M, Incidence and location of diabetic foot ulcer recurrence. 2012. http://www.woundsinternational.com/casereports/incidence-and-location-of-diabetic-foot-ulcer-recurrence [accessed 06.05.13]
[7] Armstrong DG, Mills JL. Toward a change in syntax in diabetic foot care: prevention equals remission. J Am Podiatr Med Assoc 2013;103:161–2.
[8] John T. Human amniotic membrane transplantation: past, present, and future. Ophthalmol Clin North Am 2003;16:43–65.
[9] Fetterolf DE, Snyder RJ. Scientific and clinical support for the use of dehydrated amniotic membrane in wound management. Wounds 2012;24:299–307.
[10] Schmidt W. The amniotic fluid compartment: the fetal habitat. Berlin: Springer Verlag; 1992. p. 1–98.
[11] Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. Dynamic reciprocity in the wound microenvironment. Wound Repair Regen 2011;19:134–48.
[12] Solomon A, Evangelista M, Soncini M. Human term placenta as a therapeutic agent: from the first clinical applications to future perspectives. In: Berven E, editor. Human placenta: structure and development. Nova Science Publishers; 2010. p. 1–48.
[13] Mermet I, Pottier N, Sainthillier JM, Malugani C, Cairey-Remonnay S, Maddens S, et al. Use of amniotic membrane transplantation in the treatment of venous leg ulcers. Wound Repair Regen 2007;15:459–64.
[14] Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. Cornea 2000;19:348–52.
[15] King AE, Paltoo A, Kelly RW, Sallenave JM, Bocking AD, Challis JR. Expression of natural antimicrobials by human placenta and fetal membranes. Placenta 2007;28:161–9.
[16] Tseng SC, Li DQ, Ma X. Suppression of transforming growth factor-beta isoforms, TGF-beta receptor type II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. J Cell Physiol 1999;179:325–35.
[17] Forbes J, Fetterolf D. Dehydrated amniotic membrane allografts for the treatment of chronic wounds: a case series. J Wound Care 2012;21:290–6.
[18] Sheikh E, Sheikh E, Fetterolf DE. Use of dehydrated human amniotic membrane (dHAM) allografts to promote healing in patients with refractory non-healing wounds. Int Wound J)2013;(February). http://dx.doi.org/10.1111/iwj.12035 [Epub ahead of print].
[19] Shah A. Using amniotic membrane allografts in the treatment of neuropathic foot ulcers. J Am Podiatr Med Assoc 2014 (in press).
[20] Zelen CM, Serena TE, Denoziere G, Fetterolf DE. A prospective randomised comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. Int Wound J 2013;10:502–7. http://dx.doi.org/10.1111/iwj.12097 [Epub 2013 Jun 7].
[21] Zelen CM. An evaluation of healing with the use of dehydrated human amniotic membrane allografts in patients with chronic diabetic foot ulcers. J Wound Care 2013;22:347–51.
[22] Shores JT, Gabriel A, Gupta S. Skin substitutes and alternatives: a review. Adv Skin Wound Care 2007;20:493–508.