Amniotic membrane grafting in patients with epidermolysis bullosa with chronic wounds

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Background: Severe forms of epidermolysis bullosa (EB) are characterized by chronic, nonhealing wounds.

Objective: We sought to evaluate the usefulness of amniotic membranes in patients with EB.

Methods: A retrospective chart review of patients with EB who were treated with amniotic membranes (two patients, 8 applications) was conducted. The primary outcome measure was number of days to complete healing, and the secondary outcome measures were a qualitative wound score, a visual analog scale score, and potential adverse effects.

Results: The number of days to detect a significant clinical response, defined as greater than 50% improvement, was 40.3 ± 21.2 days. The median qualitative wound score was 2 (range 0-5). The mean visual analog scale score at last follow-up was 31.4 ± 26.8. No adverse events were noted.

Limitations: Retrospective design, healing assessed by comparing photographs, and partial grafting of some wounds were limitations.

Conclusion: This proof-of-concept study revealed the potential usefulness of amniotic membrane grafting in promoting healing of chronic wounds in patients with EB (J Am Acad Dermatol 2010;62:1038-44.)

Key words: amniotic membrane; biological dressing; chronic wounds; epidermolysis bullosa.

Epidermolysis bullosa (EB) refers to a group of inherited bullous disorders, characterized by fragility of the skin and mucous membranes, and blister formation in response to minor friction or trauma. Persistent skin damage adversely affects the patient's quality of life. In the absence of a cure, the current therapeutic goal is the prevention and healing of chronic wounds. In patients with EB, chronic inflammation and bacterial infection are two of the factors that interfere with proper wound healing. The characteristics of an ideal dressing for EB are to control moisture balance, be nonadherent and atraumatic, reduce pain, allow epithelialization, promote healing, be widely available, and be inexpensive.[1]

Abbreviations used:

AM:	amniotic membrane
EB:	epidermolysis bullosa
RDEB:	recessive dystrophic epidermolysis bullosa
VAS:	visual analog scale

The amniotic membrane (AM) has many natural biological properties that prevent scarring, reduce inflammation, stop the formation of blood vessels, minimize infection, and promote wound healing.[2] There are several successful reports of AMs used in patients with extensive burns and venous ulcers where it was demonstrated to be safe, easy to use, and extremely beneficial in allowing fast re-epithelialization of denuded skin.[2-10]

The objective of this study was to evaluate the usefulness of AMs in treating chronic wounds in patients with EB.

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METHODS

Patients and setting

A retrospective chart review of patients with EB, who were treated with AMs, was conducted at our hospital from November 2007 to July 2008. The study received approval from our research ethics board. The grafting was performed in chronic wounds, defined as present for more than 3 months, which failed to heal despite topical and systemic therapy. One wound has previously failed artificial grafting with Apligraf (Organogenesis Inc, Canton, MA).

CAPSULE SUMMARY

- Patients with epidermolysis bullosa have chronic, nonhealing wounds.
- Amniotic membranes are biological dressings that improve wound healing.
- In this retrospective study we proved the concept that amniotic membrane may be beneficial in patients with epidermolysis bullosa with chronic wounds.
- Further prospective studies are needed.

AM grafting

AMs were recovered from placentas that would normally get discarded from cesarean sections. The donors were extensively screened, at the time of delivery and 6 months after, for infectious organisms, such as HIV, hepatitis B and C, human T-lymphotropic virus 1 and 2, herpesvirus 1 and 2, cytomegalovirus, toxoplasma, and syphilis, and the seasonal West Nile virus. In addition, the AMs were tested for bacteria. AMs were kept at −80°C until they were clinically applied. The membranes were supplied from Comprehensive Tissue Centre Capital Health (Edmonton, Alberta, Canada).

The AM application was done in our outpatient dermatology clinic. The skin was cleansed with normal saline and the AMs (supplied in approximate 3- × 3-cm pieces) were applied on the affected areas after thawing at room temperature for 5 minutes. No suturing was required. To improve adhesiveness, we used blow by sterile air at 42°C for 5 minutes provided by a Bair Hugger Patient Warming System (Arizant Healthcare Inc, Eden Prairie, MN). A silicone dressing was applied to prevent movement and suprainfection. Patients were instructed not to remove dressings for 7 days. At the 7-day mark the dressing was replaced and topical antibiotics were prescribed to reduce the risk of suprainfection. In addition, all patients were instructed to receive oral broad-spectrum antibiotics for 2 weeks before and 2 weeks after grafting to reduce the risk of infection.

Outcome measures

Photographs of wounds were taken before and after each application and were used to compare results with baseline photographs. The scoring was done by a single investigator by comparing photographs at each follow-up visit against baseline. The primary outcome measure was number of days to complete healing, and the secondary outcome measures were a qualitative wound score, a visual analog scale (VAS) score, and potential adverse events. The qualitative wound score assessed the degree of redness (0 = none, 1 = pink, and 2 = beefy red); exudates (0 = none, 1 = mild, and 2 = moderate/severe); odor (0 = no and 1 = yes); and size (0 = smaller, 1 = same, and 2 = bigger). The extent of the wound at follow-up was compared with baseline with a 100-mm VAS where 0 represented no healing and 100 represented complete healing. For analysis purposes, scores of 0 to 24 represented no or minimal improvement, 25 to 49 represented mild improvement, 50 to 74 represented significant improvement, and 75 to 100 represented complete healing.

Statistics

Descriptive statistics were calculated. Mean, median, SD, minimum, and maximum were determined for continuous variables. Number and percentage of patients were determined for discrete variables.

RESULTS

Patient characteristics

The characteristics of the two patients, both with recessive dystrophic-type EB (RDEB), are summarized in Table I. Patient 1 was an 18-year-old man who had chronic wounds on his left buttock, right upper aspect of his chest, another chronic wound in his left buttock which extended to the back of thigh, right front aspect of his trunk, left front aspect of his trunk, and upper aspect of his back. Patient 2 was a 16-year-old girl who had chronic wounds on the front and back of her left hand, and front upper aspect of chest. In both patients, the grafted wounds were present for at least 3 months (up to 2 years). The characteristics of the AM grafts are shown in Table II. The mean total surface area of AM used was $72.00 \pm 24.05\ cm^2$. The patients were prescribed oral broad-spectrum antibiotics, such as moxifloxacin/oxofloxacin to reduce infection. Topical treatments included silver sulfadiazine or mupirocin with silicone dressings.

Table I. Patient characteristics

	Patient 1	Patient 2
Age, y	18	16
Sex	Male	Female
Weight, kg	59.7	25.8
Height, m	1.69	1.29
BMI, kg/m^2	20.9	15.5
BMI, percentile	43	6
Last hemoglobin, g/L	106	98
Last albumin, g/L	34	21.1
Type of EB	RDEB	RDEB

BMI, Body mass index; *EB*, epidermolysis bullosa; *RDEB*, recessive dystrophic epidermolysis bullosa.

Table II. Amniotic membrane graft characteristics

Patient, application	No. of AMs used	Approximate surface area, cm^2	Wound location
P_1A_1	10	90	Left buttock
P_1A_2	4	36	Right upper aspect of chest
P_1A_3	12	108	Left buttock and back of thigh
P_1A_4	7	63	Right front aspect of trunk
P_1A_5	5	45	Left front aspect of trunk
P_1A_6	8	72	Upper aspect of back
P_2A_1	8	72	Front and back of left hand
P_2A_2	10	90	Front upper aspect of chest

A, Application; *AM*, amniotic membrane; *P*, patient.

Qualitative analysis

In patient 1, application 1 (P_1A_1), granulation tissue appeared with some yellow discharge and odor by day 5. The patient reported marked alleviation of pain. Epithelialization began at 12 days and there was complete healing with no new development of blisters (Fig 1). In the second application, P_1A_2, patient reported itching in the grafted area with discharge on day 5. The wound size decreased and granulation tissue appeared by day 28. Improvement was detected on day 10 with appearance of pink granulation tissue and alleviation of pain in the third application, P_1A_3. In the fourth application, P_1A_4, by day 14, there was a reduction in wound size and improvement in appearance of wound despite excess discharge and moderate odor. No pain associated with dressing changes was reported. Similar findings were reported in the fifth application, P_1A_5. In the sixth application, P_1A_6, by day 62, large eroded angulated plaques resulted in difficulty sleeping because of pain. There was no change in size.

In patient 2, application 1 there was some pink granulation tissue with excessive discharge and some bleeding on day 7. Despite instruction the dressing was changed after 2 days. There was more pink granulation tissue and areas of skin re-epithelialization by day 14. No pain was reported by the patient. In the second application, islets of new skin within the wound bed and spontaneous re-epithelialization was detected on day 7. Despite increased discharge, the appearance of the wound was much improved. By day 18, there was minimal improvement in the extent. By day 49, almost complete healing of grafted area was reported (Fig 2).

Quantitative analysis

The mean number of days to detect a significant clinical response, defined as at least 50 on the VAS, was 31.4 ± 26.8. The median qualitative wound score at last follow-up was 2 (Table III) (range 0-5). The mean score of the VAS at last follow-up was 47 ± 32.3 (Table III). Based on the VAS, there was complete healing in one application (12.5%), significant improvement in 3 applications (37.5%), mild improvement in two applications (25%), and minimal improvement in two applications (25%) at the last follow-up. There were no reported adverse events or side effects. There was no reblistering noted in any of the grafted areas. The applications were followed up for a mean of 40.3 ± 21.2 days.

DISCUSSION

Skin fragility is inherent to all types of EB; however, clinically there are wide differences in the wound healing even within the same type/subtype of EB. Patients with RDEB are particularly at risk for having chronic wounds that take months to years to heal or that may never heal. Potential explanations are: poor nutritional status and chronic losses that lead to low hemoglobin and albumin levels, systemic and local inflammation, multibacterial critical colonization and infection preventing proper re-epithelization, exuberant granulation tissue as a result of inflammation and bacterial microflora, and further skin breakdown at the same site because of the patient's underlying genetic defect.[1] Most patients with RDEB have a significant portion of their bodies covered with wounds that "stuck" in an inflammatory phase. Current management strategies used to improve healing (dressings, topical antibiotics and bactericidals, systemic anti-inflammatory antibiotics) are limited in their success rate.[1] Preliminary data suggest that artificial skin grafting may improve wound healing in this population.[11] However,

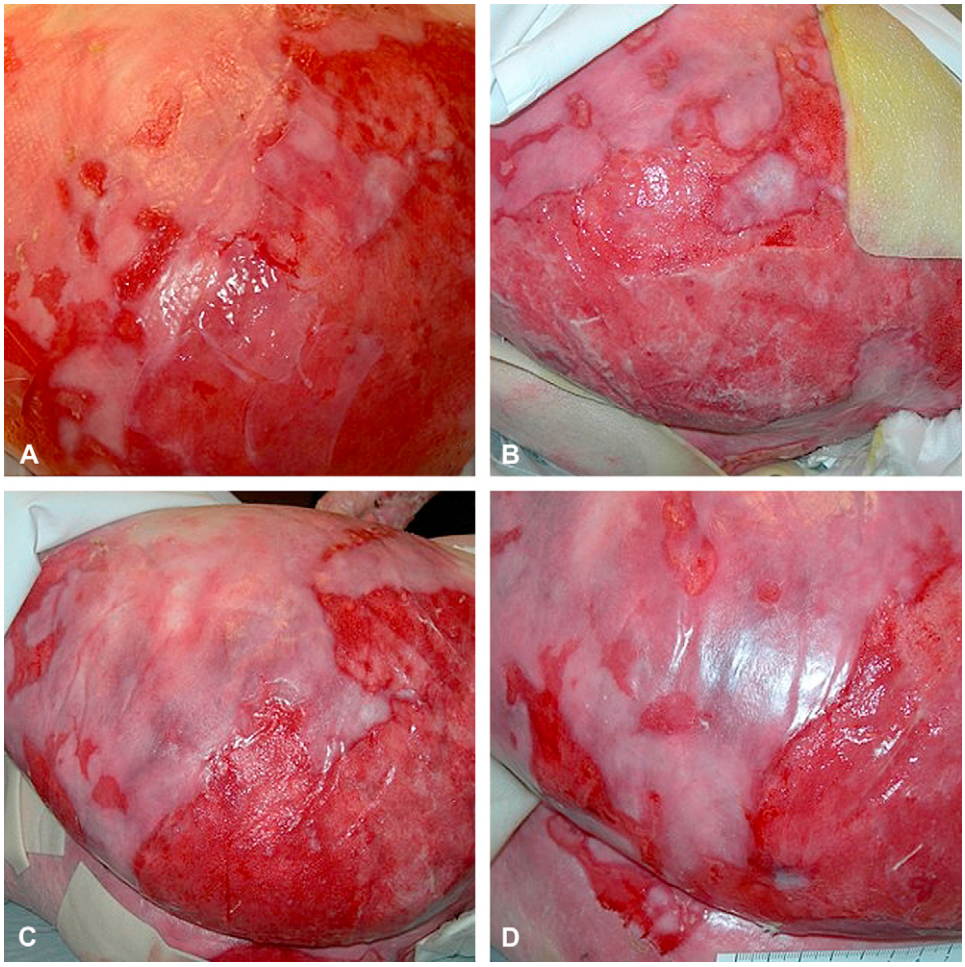

Fig 1. Chronic wound on left buttock in patient 1, application 1. At day 0 (**A**). Granulation tissue appeared by day 5 (**B**). Epithelialization began at 12 days (**C**). There was complete healing with no new development of blisters at 28 days (**D**).

availability and prohibitive costs are significant limitations to their widespread use.

To our knowledge, there are no retrospective studies evaluating the potential use of AM in EB. Although small in size, our proof-of-concept study revealed the potential efficacy of AM grafting in promoting healing in patients with EB and chronic, nonhealing wounds.

The AM has many qualities of an ideal biological dressing. It spontaneously re-epithelializes denuded skin and alleviates pain. The AM has many qualities of an ideal biological dressing that will address the poor healing seen in some patients with EB. The AM has documented anti-inflammatory (by down-regulation of transforming growth factor-β expression and suppression of proinflammatory cytokines),[12,13] antiangiogenic (both through decreased expression of angiogenic factors and enhanced production of antiangiogenic factors),[14] and antifibrotic[12,13] properties, which are beneficial for wound healing. It has been noted that AM application promotes re-epithelization by inducing keratinocyte proliferation and differentiation through a variety of growth factors.[15] Because of its low immunogenicity, rejection associated with clinical use of fresh AM is extremely rare.[16] Because of its antibacterial properties and good wound adherence, the AM serves as an effective barrier to the external environment.[16-18] Moreover, the AM reduces heat, fluid, and protein losses, yet allows appropriate moisture to the wound.[19] Owing to its thin, lightweight, and elastic material, the AM promotes mobility in patients.[20] In addition, pain has been reported as being substantially diminished after AM application.[21,22]

The use of AMs in the treatment of chronic ulcers is well established in the literature.[23-29] In a pilot prospective study using AM grafting for venous leg ulcers, Mermet et al[30] reported a significant increase in granulation tissue (from 16% ± 24% at day 0 to 56% ± 33% at day 30) and a decrease in fibrinous

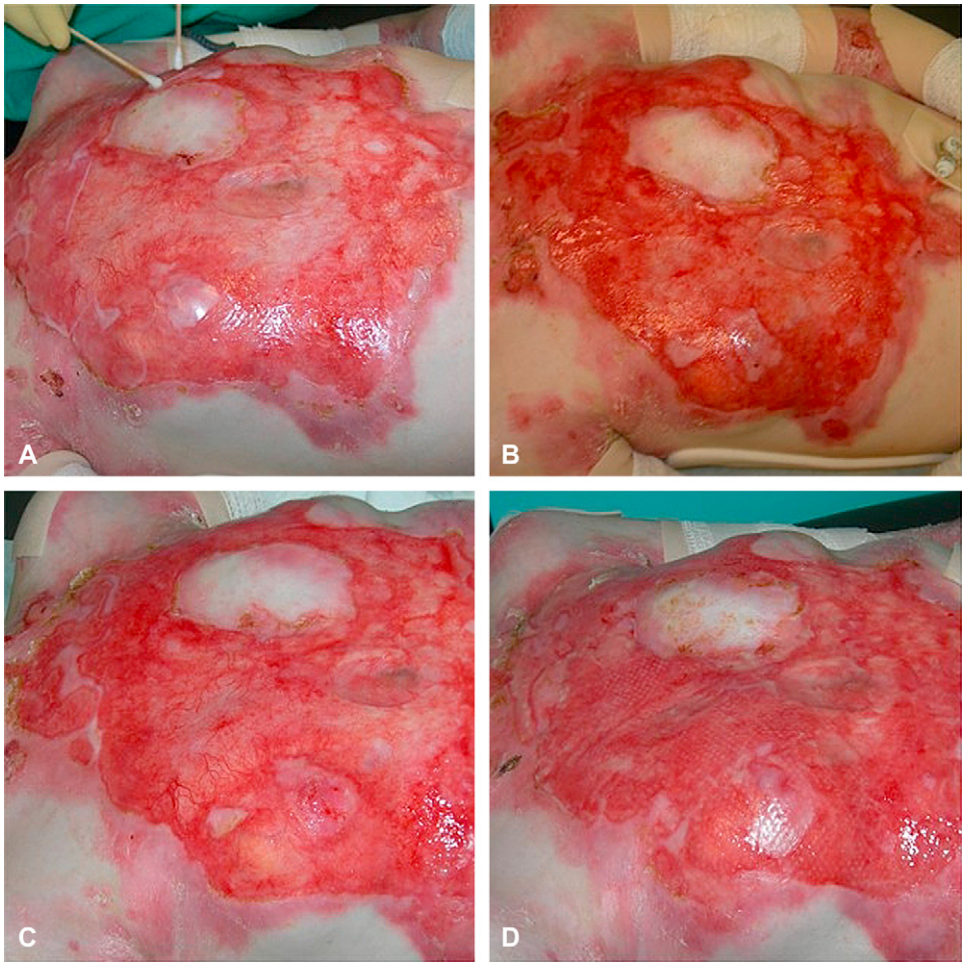

Fig 2. Chronic wound on front upper aspect of chest in patient 2, application 2. At day 0 (**A**). Islets of new skin within wound bed and spontaneous re-epithelialization was detected on day 7. Despite increased discharge, wound appearance was much improved (**B**). By day 18, there was more improvement in extent (**C**). By day 49, almost complete healing of grafted area was seen (**D**).

slough (from 36% at day 0 to 16% at day 14). A significant clinical response was observed in 12 patients (80%), including complete healing in 3 patients (20%), during the 3-month follow-up period. In addition, advantages such as the absence of side effects, low-cost production, and ambulatory application were noted.[30] In our study, a significant clinical response, defined as a score of at least a 50 on the VAS, was observed in 4 of 8 applications (50%). Based on the VAS, there was complete healing in one application (12.5%), significant improvement in 3 applications (37.5%), mild improvement in two applications (25%), and minimal improvement in two applications (25%) at the last follow-up. Our findings suggest that AMs are beneficial in terms of promoting wound healing.

In the literature there are relatively few accounts of the use of AM in EB. Martinez Pardo et al[21] examined the clinical application of AMs with prior wound debridement in a patient with dystrophic EB. Spontaneous epithelialization occurred in a week, and pain and immobility improved in a few hours. Hasegawa et al[22] evaluated the efficacy of AMs in the treatment of nonhealing ulcers in 3 patients with RDEB. Significant pain relief was noted in all 3 cases. Healthy granulation tissue appeared in 3 days, and complete re-epithelialization was noted within 2 to 10 weeks. Our qualitative results were comparable. In our study, healthy granulation tissue first appeared at days 5 to 28, and re-epithelialization first appeared at days 7 to 14. However, in our study complete re-epithelialization was not observed in all applications. Hasegawa et al[22] repeated the AM preparation and application procedure once a week for 10 weeks as a way of enhancing the chances of re-epithelization.

Table III. Comparison of last follow-up versus baseline

Patient, application	Day of follow-up	QWS score	VAS score
P_1A_1	0	2	*
	5	2	31
	12	1	60
	28	0	100
P_1A_2	0	3	*
	5	3	0
	12	2	7
	28	2	1
P_1A_3	0	3	*
	5	1	9
	10	3	0
	17	1	35
	38	2	68
P_1A_4	0	2	*
	14	2	15
	27	1	41
	62	4	39
P_1A_5	0	2	*
	14	0	20
	27	1	42
	62	1	55
P_1A_6	0	4	*
	14	3	17
	27	4	0
	62	3	8
P_1A_1	0	3	*
	7	5	0
	14	1	20
	46	1	42
	73	1	42
P_1A_2	0	2	*
	7	1	28
	18	2	63
	49	1	74

A, Application; *P*, patient; QWS, qualitative wound score; *VAS*, visual analog scale.
QWS gave score from 0 (best result) to 7 (poor result). QWS assessed degree of redness (0 = none, 1 = pink, and 2 = beefy red); exudates (0 = none, 1 = mild, and 2 = moderate/severe); odor (0 = no and 1= yes); and size (0 = smaller, 1 = same, and 2 = bigger). VAS gave score from 0 (no healing) to 100 (complete healing), where scores of 0-24 represented no or minimal improvement, 25-49 represented mild improvement, 50-74 represented significant improvement, and 75-100 represented complete healing.
*Baseline.

The decrease in pain was noted in all applications immediately after AM grafting with a significant impact on the quality of life. The analgesic mechanism is not well understood; however, it probably relates to the limited contact between the wound bed and external environment with covering of sensitive nerve terminals. In addition, AM use decreased the need for frequent dressing changes that is typically associated with pain.[31]

The current management strategies of the chronic wounds in EB involve application of nonstick, preferably silicone-based, dressings. Anecdotally, although their application provides a moist environment conducive to wound healing and prevents further bacterial contamination, it is rarely associated with a hastened healing process. Although desirable, a comparison between AM grafting and standard dressings cannot be performed given the lack of such studies in the EB population.

The only formal studies in EB were performed using tissue-engineered skin grafts. Apligraf (Organogenesis Inc) has been used in chronic wounds of patients with EB, and although no adverse events were seen, 7 of the 9 chronic wounds treated were still open at week 18.[32] Another bilayer dressing that resembles normal-appearing skin, OrCel (Forticell Bioscience Inc, New York, NY; previously termed "composite cultured skin"), has also been used in EB. No statistically significant difference was observed with respect to the time to wound healing after 3 treatments when OrCel (Forticell Bioscience Inc) was compared with its collagen sponge component alone (without the epidermal keratinocytes or dermal fibroblasts) and to standard care.[33]

Because of its retrospective nature, there were several limitations to this study. It was difficult to discriminate the extent of healing of wounds that were partially grafted. In addition, nongrafted areas may have contaminated and interfered with healing in grafted areas. Although treatment recommendations were meant to minimize the risk of infection, sometimes patients were noncompliant to these recommendations. Also, the number of and time interval between follow-up visits differed between patients, as these were scheduled to coincide with clinic visits.

Despite its limitations, this study expands the limited therapeutic repertoire available to patients with EB with chronic nonhealing ulcers. The AM offers many advantages over traditional dressings (increased healing rates, decreased bacterial contamination, decreased need for repeated dressing changes, decreased pain) and potentially can be of unlimited supply. The safety concerns are very minimal because of stringent manufacturing protocols and infectious screening. As the demand increases, the costs will decrease. Currently, AMs are produced for ophthalmic uses; hence, the standard 3- × 3-cm size. Larger sizes will likely increase the ease of application and decrease the chances of cross-contamination in partially grafted wounds. Larger prospective studies are needed, as are studies comparing AM with other biologic or tissue-engineered dressings.

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