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Safety and Efficacy of Amniotic Membrane Implant in Venous Leg Ulcers

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Abstract

Objective: To assess the safety and efficacy of amniotic membrane (AM) application in venous leg ulcers (VLU).

Methodology: Design: non-controlled pre-post clinical trial. A unique AM fragment was placed on the ulcer in strict sterility conditions. Main outcome measures: total healing rate, percentage of ulcer area reduction and diurnal and nocturnal pain assessment. Control visits: at baseline and 1, 2, 3, 4 and 8 weeks after treatment.

Results: Ten patients were recruited (79.6 years). 3 weeks after AM implant, pain had completely disappeared and ulcer area had reduced to nearly halved and, 8 weeks after AM implant, ulcer area was reduced in more than 80% and in two thirds of patients the ulcer was completely healed. No relevant side effects were observed.

Conclusions: The present study brings new evidence reinforcing the idea that AM dressing is a safety and effective alternative to heal VLU.

Keywords: Venous leg ulcers; Amniotic membrane; Ulcer healing; Pain; Effectiveness; Safety

Introduction

Venous leg ulcer (VLU) is a chronic condition with a prevalence rate of 3-5% and an annual incidence rate of 2-4 new cases/1000 inhabitants in the ≥ 60 year old population. Between 40 and 50% of these ulcers remain active for periods not less than six months and 10% of them can reach periods of five years. Moreover, once initially healed, a third of them recur in less than a twelve months period [1-3]. VLU also have a relevant impact on quality of life, especially because of pain [3,4]. Different approaches exist for the treatment of VLU including several medical products, but most of them have not demonstrated its effectiveness. Compression therapy is the central element of its management and the therapeutic option with better evidence on its effectiveness. However their results are usually poor because the difficulty of good adherence. Treatment of VLU remains a major clinical challenge because it's high prevalence, refractory nature, quality of life impact and economic consequences [2].

On the other hand, the amniotic membrane (AM), the most internal placental membrane, shows some physiological properties that make it a good candidate for the treatment of venous ulcers. AM does not express HLA-A,B,C and DR antigens, so it does not induce immunological reactions, and also has bacteriostatic properties, antiadhesive effects and the capacity to inhibit the metalloproteases in the biofilm of the ulcer provoking a rapid apoptosis of the inflammatory cells [5,6]. Some in vitro studies have shown antifibrotic capacity of AM in the process of transformation of fibroblasts into myofibroblasts [7,8]. Moreover, AM contains some angiogenic factors which contribute to faster granulation. AM is considered a biotherapeutic product and has been used as a substrate for epithelial growth in the management of ocular ulcers with positive results [9,10]. The evidence about its effectiveness in the treatment of VLU is growing and promising, but still scarce. The aim of this study was to assess the safety and efficacy of AM application in VLU in terms of total healing rate, reduction of the area of the ulcer and pain control.

Methodology

Design and population

A non-controlled pre-post clinical trial was conducted. Study population included subjects with VLU of primary or secondary origin. Candidates to participate were adults (≥ 18 years) suffering a VLU in a granulation stage (tissue stage I or II), with an area greater than 5 cm^2, and with more than 6 months of evolution. All participants must had to have the ability to understand and give, by writing, informed consent. Patients were excluded if lower limb ulcers were not of venous etiology (according to an Ankle/Arm index <0.75), in case of venous angiodysplasia, clinical signs of ulcer infection, present or past diagnosis of any neoplasm, treatment with chemotherapy and/or corticosteroids, severe liver disease, plasma levels of creatinine >1.90 mgr/dl or plasma albumin levels <2 g/l. Serological study for HIV, HBV and HCV was performed to ensure they were negative before inclusion. Patients were recruited in the Department of Angiology and Vascular Surgery of the Hospital of Mataró from March 2016 to April 2017. This was considered a pilot study to be perfomed in 10 patients. The study protocol was approved by the local ethical committee (CEIC 20/14) and by the General Direction of Health Regulation of the Catalan Government (Ref. 94698) once received the mandatory report of the Inter-territorial Health Council of the Ministry of Health of the Spanish Government. All participants gave their informed consent by writing before recruitment.

Intervention and outcome measures

In all patients an AM fragment was implanted on the ulcer in strict sterility conditions. A unique AM fragment of 4.5 cm of diameter was administered to each patient. AM was preserved frozen and provided by the Tissue Bank of Barcelona (Ref BT7014). Once the AM was

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thawed by physiological saline at 37 degrees celsius for ten minutes, it was placed on the ulcer, and it was subsequently fixed by means of a secondary silicone dressing and hydrophilic gauze. The extremity was then bandaged by elastic bandage of 7 meters length and 10 centimeters width up to the level of the knee. This structure was only reviewed in the course of each control visit or in the presence of signs of inflammation or infection. All subjects were followed-up for 2 months with control visits at 3 days and at 1, 2, 3, 4 and 8 weeks after AM implantation. In each control visit, the perimeter of the ulcer was drawn on acetate paper to estimate ulcer area in mm^2 following the Kundin methodology. Healing rate in each control visit was calculated with the following formula: [(initial area–area at time i)/initial area]·100. Complete ulcer healing was considered when healing rate was 100% (ulcer area was 0). Ulcer pain during day and at night was assessed by a 0-10 cm visual analogue scale. Other study variables considered were age, sex, loneliness, educational level, co-morbidities, usual treatments, time of ulcer evolution, body mass index, primary or secondary etiology, serum creatinine and albumin levels, and incidence of any side effect such as inflammation, exudation or infection. All variables were assessed in all patients by the same experienced angiologist.

Analysis

All data were registered in an electronic database for posterior depuration and analysis. Numerical variables, including healing rates and pain, were described with means, medians, standard deviations and minimum and maximum values, while categorical ones were described by percentages. Time to total healing was calculated and the survival curve with ulcer was estimated using the Kaplan-Meier methodology. Comparison of survival curves according to sex, granulation stage (I or II), ulcer area (<or ≥ 1500 mm^2) or evolution time (≤ 18 or >18 months) were made using the log rank test. Comparisons of the area of the ulcer and pain between baseline visit (before intervention) and each control visit (1, 2, 3, 4 and 8 weeks after intervention) were made using the Wilcoxon ranks test. Statistical significance was established at a p value <0.05.

Results

Ten patients were recruited, eight women and two men, with an average age of 79.6 years (SD 15.4). All patients except one, who had an accidental fall and hip fracture, were followed during two months. Main characteristics of the study sample and the ulcer are described in Table 1. At baseline, mean area of the ulcer was 2.174,92 mms^2 (SD 1816, minimum 500 and maximum 5,964 mm^2). Table 2 shows the evolution of the area of the ulcer, the healing rate, the percentage of ulcers completely healed and diurnal and nocturnal pain during all study visits. It shows that, 3 weeks after AM implant, pain had completely disappeared and ulcer area had reduced to nearly halved, and 8 weeks after AM implant, ulcer area was reduced in more than 80%, and in two thirds of patients VLU was completely healed. Overall survival with VLU and survival curves according to etiology, grade and baseline ulcer size are presented in Figure 1. Regarding safety, one patient presented inflammation signs (dermatitis) a week after AM implant but they completely disappeared two weeks later. Four patients presented increased exudate after AM implant, which was resolved in a week. None of them presented clinical signs of ulcer infection. One patient suffered a femoral fracture during follow-up because an accidental fall, which was considered a serious adverse event not related with the study intervention. No other side effects were observed.

Discussion

The results of the present study indicate a positive effect of AM implant in the treatment of VLU. In a reduced number of patients with a VLU with a median time of evolution of 20 months, the AM implant achieved a complete ulcer healing in 50% of patients 4 weeks after treatment and in 66% of patients 8 weeks after treatment. These patients experienced a healing rate of 60% and 80% 4 and 8 weeks after treatment, respectively. Diurnal and nocturnal pain also improved, completely disappearing 3 weeks after AM implant. Moreover, study intervention showed to be safe and without serious adverse events.

It has long been believed that AM had remarkable therapeutic potential in ulcer healing, but only in the last few years scientific evidence supporting the rationale for its use and its safety and effectiveness has begun to appear [11]. The results of the present study agree with this incipient evidence about the effect of AM graft in the treatment of ulcers. While there is abundant scientific evidence about the effect of AM implant for the treatment of corneal ulcers [9-13], clinical evidence about its effect in the treatment of VLU is still relatively scarce. Few non controlled trials in a limited number of patients have evaluated the effect of AM grafting in VLU refractory to usual treatments. Mermet et al. published a pilot study in 15 patients showing a significant

	N (%)
Sex (female)	8 (80%)
Family support: · loneliness · live with couple	 1 (10%) 9 (90%)
Educational level: · Primary school · Secondary or superior	 8 (80%) 2 (20%)
Treatment with NSAI	3 (30%)
Etiology: · Primary VLU · Secondary VLU	 9 (90%) 1 (10%)
Ulcer location: · External face · Internal face · Dorsal face	 5 (50%) 4 (40%) 1 (10%)
	Mean (SD)
Age	76.1 (15.4)
BMI	29.1 (7.7)
Creatinine	1.05 (0.5)
Albumin	4.2 (0.26)
Time of ulcer evolution	52.8 (105.2)

Table 1: Description of main sample characteristics.

	Baseline visit	1 week after	2 weeks after	3 weeks after	4 weeks after	8 weeks after
Mean (SD) ulcer area (mm^2)	2175 (1817)	1470 (1501)*	1596 (1795)*	1445 (1837)*	1243 (1731)*	278 (424)*
Median ulcer area (mm^2)	1365	1092*	1047*	867*	402*	0*
Healing rate	---	29.2 (29.3)	37.6 (30.8)	46.6 (38.2)	61.8 (41.7)	84.3 (26.5)
% of complete healing	---	0%	0%	20%	50%	66,7%
Diurnal pain (VAS)	1.6 (1.8)	0.4 (0.97)	0*	0*	0	0
Nocturnal pain (VAS)	2.9 (3.5)	1.0 (2.0)*	0.2 (0.63)	0*	0*	0*

*Statistically different in comparison to the baseline value (p<0.05) (Wilcoxon ranks test).

Table 2: Evolution of main outcome measures.

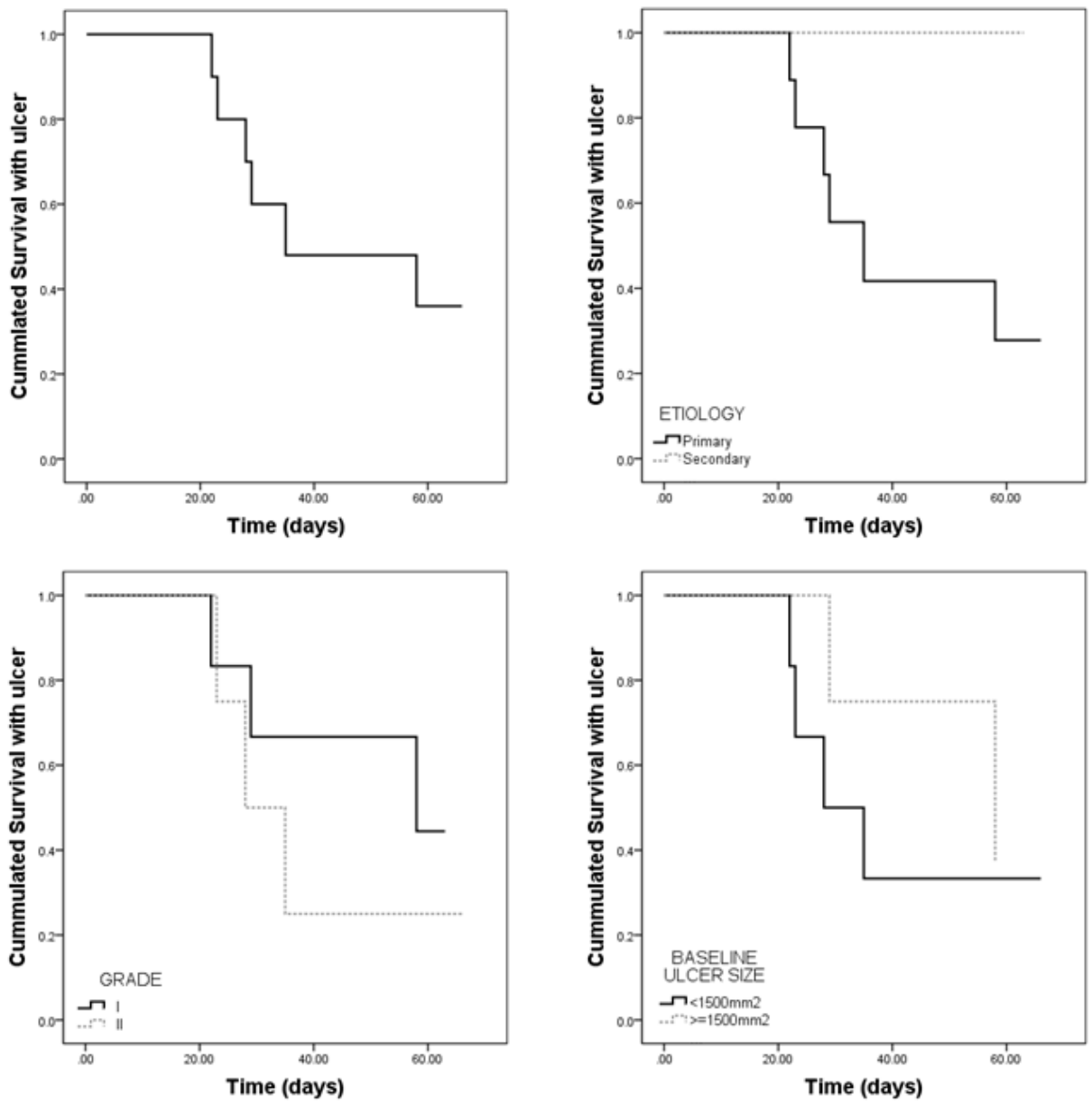

Figure 1: Overall survival with ulcer and survival with ulcer according to ulcer etiology, grade and size.

increase in granulation tissue, a significant reduction in ulcer-related pain, a 36% healing rate and a complete healing in 20% of cases at 3 months of follow up without adverse events [14]. Gutierrez-Moreno et al. published another prospective study with 4 ulcers (in 3 patients) and reported that one out of 4 ulcers had completely healed and the other three showed a 50% healing rate, pain was reduced in 86% and no side effects were observed at 8 weeks of follow-up [5]. Werber et al. performed a prospective study of 20 leg wounds treated with cryopreserved amniotic membrane and fluid allograft and showed that 18 of them (90%) healed during the 12-weeks observation period [15]. Barr describes a series of 7 patients with refractory leg wounds in which dehydrated amniotic membrane allograft (DAMA) was used, and reported that complete wound healing was observed in 6 (85%) patients, with an average time to closure of 7.9 weeks [16]. Lintzeris et al. reported a retrospective case series of 8 patients also treated with DAMA and reported that all wounds were healed in an average time of 5.7 weeks without adverse events [17]. Francis et al. published preliminary results of a prospective study conducted in 40 patients in which a single AM transfer was done and also concluded that this treatment is safe, useful, inexpensive, well-accepted by patients and with great potential in healing resistant VLU [18]. A non-randomized controlled study comparing DAMA with conservative care showed a significant difference in time to healing favoring graft treatment, suggesting that DAMA may accelerate ulcer healing [19]. On the other hand, there are also some randomized controlled trials assessing AM implant effectiveness in VLU healing. Hanumanthappa et al. published the first randomized clinical trial in two hundred middle age (mean age 45 years) Indian patients with VLU. It shows a significantly improved

epithelialisation rate at 21 days in the intervention group in comparison to the control group (80% vs 40%, respectively) and concludes that AM ulcer dressing is superior to conventional dressing in the management of varicose ulcers [20]. Serena et al. enrolled 84 subjects in a randomized controlled trial comparing DAMA and compression therapy versus compression therapy alone, and observed a significant improvement in healing rate at 4 weeks in the experimental group in comparison to the control group [21]. In another randomized controlled trial, ElHeneidy et al. observed a complete healing in all 14 ulcers in the intervention group (33 days in mean), while in the control group with conventional ulcer dressing all ulcers showed no reduction in their size [6]. Finally, a recent multicentre controlled trial evaluating DAMA in venous leg ulcers randomly assigned 109 patients to receive DAMA plus compression therapy or compression therapy alone and observed that complete ulcer healing was achieved in 60% in the intervention group and 35% in the control at 12 weeks [22]. All these studies report significant advantage of AM dressing, with complete ulcer healing in a very relevant percentage of patients in few weeks, which agree with the results of our study.

The healing effect of AM implant can be explained by different factors. First, AM provides a physical wound protection and an appropriate moist environmental favoring epithelialization [23]. Secondly, as previously mentioned, it does not express antigens of histocompatibility avoiding rejection, and provides an anti-inflammatory environmental helping healing [24]. Thirdly, AM structure shows anti-adhesive effects and bacteriostatic properties, reducing the risk of infection [25], which is a cause of ulcer chronification. Finally, AM contains angiogenic factors which contribute to faster granulation, and it is believed that it also contains other growth factors that could stimulate cell proliferation. In the present study, as well as in the other mentioned studies that have assessed AM effect in VLU, AM dressing has shown a significant improvement in ulcer-related pain. This effect may be secondary to a better ulcer hydration, to the anti-inflammatory action of AM or to the healing effect itself, because of a reduction of the ulcer area and, consequently, the number of sensory endings exposed.

The present study presents some limitations. The first one refers to the non-controlled design and the lack of a control group. As VLU is a chronic condition and all patients presented an ulcer with, at minimum, 6 months of evolution, a before and after comparison was considered an appropriate exploratory design in which every subject act as its own control. However, we are the opinion that further well-powered randomized controlled trials are still required to control for possible "spontaneous" healing or ulcer improvements. A second weakness is the limited sample size, which was enough to detect significant differences in ulcer area and pain during follow-up but does not allow subgroup and multivariate analyses, and could compromise the representativeness of the sample. Finally, the non-blinded assessment of ulcer area could introduce certain bias, although we consider this outcome measure a quite objective one.

There is a lack of an effective treatment for VLU. Apart from compression therapy, the rest of more than hundred therapeutic strategies available are considered sanitary products, and have demonstrated safety but not efficacy. In fact, it is usual in VLU patients to be treated with several different dressings in few months. There is a lack of well designed studies comparing the effect of such strategies especially in comparison to compression therapy, considered the gold standard, and there is also an urgent need to explore for new therapeutic strategies to treat such a devasting clinical condition in terms of pain, quality of life and economical costs. AM dressing has a great potential in treating VLU and has shown positive and promising results. The results of the present study brings new evidence about a clinically relevant healing effect of AM and reinforce the idea that AM dressing is a safety and effective alternative to heal venous leg ulcers.

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Conflict of Interest Disclosure

All authors declare that they have no conflict of interest in relation to the present study.

References

1. Verdú J, Marinel Lo J, Armans E, Carreño P, March JR, et al. (2009) Conferencia Nacional de Consenso sobre ulceras de la extremidad inferior. Conuei Barcelona Edika Med edit.
2. Marinel J, Roura Lo (2010) Concepto, clasificación epidemiología de las úlceras de extremidad inferior. Ulceras de la extremidad inferior. Glosa SL edic.
3. Posnett J, Gottrup F, Lundgrenn H (2009) The resource of wounds on health-care providers in Europe. J Wound Care 18: 154-161.
4. Persoon A, Heinen MM, Van der Vleuten CJ (2004) Leg Ulcers: a review of the impact on daily life. J Clin Nurs 13: 341-354.
5. Gutiérrez-Moreno S, Alsina-Gibert M, Sampietro-Colom L, Pedregosa-Fauste S, Ayala-Blanco P (2011) Estudio coste-beneficio del trasplante de membrana amniótica para úlceras venosas de extremidades inferiores refractarias a tratamiento convencional. Actas Dermo-Sifiliogr 102: 284-288.
6. El Heneidy H, Omran E, Halwagy A, Al-Inany H, Al-Ansary M, et al. (2016) Amniotic membrane can be a valid source for wound healing. Int J Womens Health 8: 225-223.
7. Lee SB, Li DQ, Tan DT (2000) Supression of TGF-beta signaling in both normal conjuntival fibroblast and ptegiryal boody fibroblast by amniotic membrana. Curr Eye Res 20: 325-334.
8. Singer AJ, Clark RA (1999) Coutaneous wound healing. N Engl J Med 2: 738-746.
9. Galber B, Lohmann CP (2000) Hypopyon after repeated transplantation of human amniotic membrane onto the corneal surface. Ophthalmology 107: 1344-1346.
10. Gris O, López-Navidad A, Caballero F, del Campo Z, Adán A (2003) Amniotic membrane transplantation for ocular surface pathology: long-term results. Transplant Proc 35: 2031-2035.
11. Zelen CM, Snyder RJ, Serena TE, Li WW (2015) The use of human amnion/chorion membrane in the clinical setting for lower extremity repair: a review. Clin Podiatr Med Surg 32: 135-146.
12. Shields CL, Shields JA, Armstrong T (2001) Management of conjuntival and corneal melanoma with surgical excision, amniotic membrane allograft and topical chemotherapy. Am J Ophthalmol 132: 576-578.
13. Rejzek A, Weyer F, Eichberger R, Geghart W (2001) Physical changes of amniotic membranes through glycerolization for the use as an epidermal substitute. Light and electron microscopic studies. Cell Tissue Bank 2: 95-102.
14. Mermet I, Pottier N, Sainthillier JM, Malugani C, Cairey-Remonnay S, et al. (2007) Use of amniotic membrane transplantation in the treatment of venous leg ulcers. Wound Rep Reg 15: 459-464.
15. Werber B, Martin E (2013) A prospective study of 20 foot and ankle wounds treated with cryopreserved amniotic membrane and fluid allograft. J Foot Ankle Surg 52: 615-621.
16. Barr SM (2016) Dehydrated Amniotic Membrane Allograft for Treatment of Chronic Leg Ulcers in Patients with Multiple Comorbidities: A Case Series. J Am Coll Clin Wound Spec 6: 38-45.
17. Lintzeris D, Yarrow K, Johnson L, White A, Hampton A, et al. (2015) Use of a Dehydrated Amniotic Membrane Allograft on Lower Extremity Ulcers in Patients with Challenging Wounds: A Retrospective Case Series. Ostomy Wound Manage 61: 30-36.

18. Francis J, Shajimon CR, Bhat AK, Kanakambaran B (2015) Use of Amnion Transfer in Resistant Nonhealing Venous Leg Ulcers. Indian J Surg 77: 457-462.

19. Miranda EP, Friedman A (2016) Dehydrated Human Amnion/Chorion Grafts May Accelerate the Healing of Ulcers on Free Flaps in Patients With Venous Insufficiency and/or Lymphedema. Eplasty 16: e26.

20. Hanumanthappa MB, Gopinathan S, Guruprasad RD (2012) Amniotic membrane dressig versus conventional dressing in lower limb varicose ulcera: A prospective comparative study. Int J Biol Med Res 3: 1616-1620.

21. Serena TE, Yaakov R, DiMarco D, Le L, Taffe E, et al. (2015) Dehydrated human amnion/chorion membrane treatment of venous leg ulcers: correlation between 4-week and 24-week outcomes. J Wound Care 24: 530-534.

22. Bianchi C, Cazzell S, Vayser D, Reyzelman AM, Dosluoglu H, et al. (2017) A multicentre randomised controlled trial evaluating the efficacy of dehydrated human amnion/chorion membrane (EpiFix®) allograft for the treatment of venous leg ulcers. Int Wound J 15: 114-122.

23. Ravishanker R, Bath AS, Roy R (2003) "Amnion Bank"--the use of long term glycerol preserved amniotic membranes in the management of superficial and superficial partial thickness burns. Burns 29: 369-374.

24. Gomes JA, Romano A, Santos MS, Dua HS (2005) Amniotic membrane use in ophthalmology. Curr Opin Ophthalmol 16: 233-240.

25. Quinby WC, Hoover HC, Scheflan M (1982) Clinical trials of amniotic membranes in burn wound care. Plast Reconstr Surg 70: 711-717.