Original Article

Amniotic membrane in the treatment of leg ulcers

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ABSTRACT

Introduction: Amniotic membrane (AM), the most internal placental membrane, has unique properties including anti adhesive effects, bacteriostatic, wound protection and pain-reduction properties, as well as epithelialization initialization capacities. AM is widely available and less costly than other bioengineered skin substitutes. In a prospective study, we evaluated the safety, feasibility, and the effects on healing of AM graft in patients with chronic leg ulcers.

Material and methods: Amnion grafts were prepared from placentas harvested during cesarean section or normal delivery. 82 patients with leg ulcers randomly divided into two groups formed material for the study. In group I (study group n=42) the ulcers were covered with amnion graft and held in place with a secondary dressing. It was opened on 7th day. Group II (control group n=40) the ulcers were treated with EUSOL dressings daily for 7 days. Size of the ulcer, assessment of pain and epithelialization were assessed on 7th day and 15th day and at follow-up after 3 months.

Results: All grafted AM had adhered to the wound bed 7 days after being applied with a 100% engraftment rate. The percentage of granulation tissue increased signif cantly (from 18% on day 0 to 70% on day 15, p<0.0001), along with a signif cant decrease of f brinous slough (from 35% at day 0 to 14% at day 15, p<0.001). A signif cant clinical response occurred in 28 patients (66.6%) including complete healing (20%) in three during the 3-month follow-up period. The ulcer surface area decreased signif cantly from a mean value (+/- standard deviation) of 4.29 +/- 2.39 cm^2 at baseline to 2.81+/-2.02 cm^2 on day 30 (p<0.001). All patients experienced a signif cant reduction of ulcer related pain rapidly after AM transplantation. No adverse events were recorded.

Conclusion: AM transplantation seems to function as a safe substrate, promoting proper epithelialization while suppressing excessive f brosis. Advantages of biotherapy with AM are its easy availability and low-cost production, and that it can be applied as an ambulatory treatment without immobilization. AM transplantation may be considered as an alternative method for treating chronic leg ulcers.

Key words: Amniotic membrane, chronic ulcers, wound healing

Introduction

Human amniotic membrane (HAM) is the inner layer of the fetal membranes (the outer layer being formed by the chorion) and has been investigated as an alternative biomaterial for various purposes in reconstructive surgery and wound-healing research.

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In the treatment of burn wounds, it was used as a dressing for second and third degree burns, exhibiting superior qualities when compared with conventional dressing.[1,2] Amniotic membranes were used for the coverage of facial dermabrasion and as a cover for micro skin grafts.[3,4] Interest in HAM research and clinical investigations diminished as a consequence of the emerging awareness of AIDS and the consequent fear of virus transmission. New methods for the processing and long-term storage (cryo- preservation) of HAM were established, and its use in wound care and reconstructive surgery

was renewed. Transplantation to the eye seems to be possible because of the immune-privileged properties of HAM.[5,6].Amniotic membrane has been demonstrated to function as a tissue engineering scaffold material for corneal epithelium reconstruction by means of transplantation of epithelial cells on a lyophilized amniotic membrane (LAM).[7]AM is a natural scaffold, which is the supporting matrix upon which cells and tissues grow, and so it is considered as an important component of tissue repair with multiple clinical applications. Chronic leg ulcers are def ned as a defect in the skin, below the level of the knee and above the foot, persisting for 6 weeks or more.[8]We have used amniotic membrane as a dressing for chronic ulcers of the leg and compared with conventional dressings.

Material and methods

This study was performed to assess the use of amniotic membrane in the treatment of chronic non healing ulcers using AM from June 2010 to June 2015. Each patient signed an informed consent after accepting to be enrolled in the study. Ethical aspects whether substantial or procedural have been implicated in this study and approval was obtained.

82 patients with leg ulcers randomly divided, by chit method, into two groups formed material for the study. In group I (study group n=42) the ulcers were covered with amnion graft and held in place with a secondary dressing. It was opened on 7th day.

Group II (control group n=40) the ulcers were treated with EUSOL dressings daily for 7 days. Size of the ulcer, assessment of pain and epithelialization were assessed on 7th day and 15th day and followed up after 3 months.

Demographic data with regard to age, sex, type and duration of ulcer and co-morbid were recorded. Inclusion criteria were presence of leg ulcers for more than 6 weeks with no improvement despite standard treatment and patients aged between 20 and 50 years.

In group I (n=48), Human AM was used as a dressing. It was prepared from placentae obtained from scheduled delivery by cesarean section or normal delivery following a non - complicated pregnancy (Fig.1 and 2). The AM was washed with physiological saline and cut into pieces of suitable size and was placed in contact with the ulcer and held in place with a secondary dressing after covering it with Vaseline gauze.(Figs. 3,4 and 5). The Preparation of the ulcers includes cleaning and mechanical debridement with a scalpel. The ulcers were observed after 48 hrs. If there is no infection or exudates, the dressing was reopened on 7th day. Size of the ulcer, assessment of pain and epithelialization were assessed on 7th day and 15th day.

Ulcer healing was assessed using the healed wound area. Healed wound area was calculated, in respect to the original wound area and the f nal wound area after 2 weeks and at the end of 3 months , by multiplying the length and breadth and expressed as square cms. Pain was assessed using a visual analog scale, where 0 represented no pain and 10 represented the worst pain. The data was analyzed using chi-square test.

Results

There were 62 venous ulcers, while 20 ulcers were traumatic ulcers. In group I, there were a total number of 42 leg ulcers and group II had 40. Age ranged from 26 to 43 years with a mean value 32.86±6.94. All patients of groups I and II were non diabetic, with no history of smoking or, hypertension.

A signif cant clinical response occurred in 28 patients (66.6%) including complete healing (20%) in three during the 3-month follow-up period. The ulcer surface area decreased signif cantly from a mean value (+/- standard deviation) of 4.29 +/- 2.39 cm^2 at baseline to 2.81+/-2.02 cm^2 on day 30 ($p<0.001$). All patients experienced a signif cant reduction of ulcer related pain rapidly after AM transplantation. No adverse events were recorded. In group II (control group), the ulcer area at the

Figure : 1
Separation of placental membranes

Figure : 2
Washing AM with normal saline

Figure : 3
AM applied over ulcer surface

Figure : 4
Vaseline gauze placed over AM

Figure : 5
Dressing with pads and bandage applied

Table 1. Treatment details in patients

Ulcer details	Group I (patients treated with A.M.)	Group II (patients treated with conventional treatment)
Chronicity of ulcer(range)	8-24 weeks	7-25 weeks
Chronicity of leg ulcer (mean+/_S.D.)	12.21±6.12 weeks	13±10.21 weeks
Ulcer surface area before treatment(mean+/-S.D.)	4.29±2.39 cm^2	4.8±0.65 cm^2
Ulcer surface area after treatment (mean+/-S.D.)	2.81±2.02 cm^2	2.12±0.23 cm^2

(p value <0.001, signif cant)

beginning of the study was 4.8±0.65 cm^2 (mean ± SD). The ulcer surface area decreased from a mean value (+/- standard deviation) of 4.29 +/- 2.39 cm^2 at base line to 2.12+/-0.23 cm^2 (Table 1), and ulcer f oor remained the same. Healthy granulations were present in two ulcers (18.2%) and absent in the rest of the ulcers. (81.8%). Table1 shows chronicity of leg ulcers, reduction in ulcer size with treatment. 40 patients had no pain in group I, while in conventional group, only 3 had no pain. (Table 2). AM graft was taken in 32 cases (70.7%), in 7 days following the graft application. In the other cases, the ulcers also showed complete healing on follow-up. Reduction in ulcer size shows signif cant difference between group I (control group) in comparison to group II (P<0.001) in which AM was used.

Table 2. Assessment of pain in patients

Pain level	Group I No	Group II No
None	40	3
Mild	6	13
Moderate	2	20
Severe	0	6

(Group I- patients treated with AM,

Group II patients treated with Eusol dressings)

Discussion

Davis in 1910 reported the f rst use of amniotic membrane in 1910 as a skin substitute.[9] Due to lack of practical methods of preparation, sterilization and storage, its use was limited till Dino et al.[10] demonstrated that amniotic membrane from routine deliveries could be sterilized and kept for six weeks at 4°C and safely used on acute second

degree burns and on skin donor sites. This led to successful use of AM for healing of all kinds of skin lesions.

Amnion is a thin, tough, transparent membrane. It is about 10-15 micrometer thick. and is made up of two membranes, the inner amniotic membrane and the outer chorion. Chorionic side of the membrane is rougher and mucinous. Amnion can easily be separated from chorion and placenta as far as the umbilical cord. Once separated, the amnion is found to be smooth and shining and much tougher and more elastic and easier to clean than the thicker chorion, which does not strip from the placenta. Chronic leg ulcers are def ned as a defect in the skin, below the level of the knee and above the foot, persisting for 6 weeks or more. A previous study found that 60%–80% of chronic leg ulcers had a venous component, 10%–30% was associated with arterial insuff ciency, and other factors included diabetes mellitus and rheumatoid disease. Arterial and venous insuff ciency combined in 10%–20% of cases. Chronic leg ulcers often heal poorly if there is no revascularization. Different lines of treatment are based on optimized local wound care: cleansing, debridement and dressings, compression therapy, and skin grafting.[11]

AM graft can be used as placental tissues contain a large quantity of growth factors and it down regulates transforming growth factor TGF)-β and its receptor expression by f broblasts and in doing so it reduces the risk of f brosis. Suppression of transforming growth factor-beta isoforms, TGF-beta receptor type II, and myof broblast differentiation in cultured human corneal and limbal f broblasts by amniotic membrane matrix. Therefore, an AM scaffold can modulate the healing of a wound by promoting tissue reconstruction rather than promoting scar tissue formation.[12]

AM is a natural scaffold, which is the supporting matrix upon which cells and tissues grow. In addition, the AM has other biological properties important for tissue repair, including anti-inf ammatory, antimicrobial, antif brosis, antiscarring, and low immunogenicity. AM may thus be regarded as a bio-therapeutic product composed of a single layer of epithelial cells that lie on a basement membrane and of a non-vascular collagenous stroma. These three components give AM its benef cial properties, including anti adhesive effects, bacteriostatic properties, wound protection, pain reduction, and epithelialization effects. The most striking effect noted by Faulk et al[13] using amniotic membrane on chronic leg ulcer was the development of new vessels which they thought was due to some angiogenic factors acting on capillary endothelium Burgos[14] conf rmed the presence of angiogenic and mitogenic factors in amniotic membrane and held them responsible for producing healing in the wounds. Freeze dried (lyophilized) amniotic membrane was used by Unger[15] on split skin graft donor sites who found it to be equivalent to an ordinary dressing and fail to notice any enhanced rate of healing. Freeze dried irradiated membrane is also used as described above, but before application it is soaked in sterile saline for 1-2 min.[16]

The difference in potential of stimulating neo-vascularization and re-epithelization of fresh and lyophilized membrane is still controversial. Freeze dried amniotic membrane with freeze dried dermo-epidermal graft and concluded that chorion-amnion grafts were as effective as dermo-epidermal grafts.[17,18] We have used fresh amniotic membrane obtained from normal delivery or Caesarean section with good results.

Mermet et al [19] in a prospective pilot study, in which they evaluated the safety, feasibility, and the effects on healing of AM graft in 15 patients with chronic venous leg ulcers. The percentage of granulation tissue increased signif cantly (from 17% on day 0 to 69% on day 14, P=0.0001), along with a signif cant decrease of f brous slough (from 36% at day 0 to 16% at day 14, P,0.001). There was signif cant reduction in ulcer size and pain level as well. Also, Alsina- Gibert and Fauste[8] performed AM transplantation for four refractory vascular ulcers.

Complete wound re-epithelialization was achieved for one ulcer by week 8; in the other three cases, there was a 50% reduction in size compared to baseline. At week 16, the mean reduction in wound size for the four ulcers was 81.93%. The corresponding reduction in pain intensity was 86.6%. No adverse effects were observed. Similar results were obtained in a study by El Heneidy et al.[20] In this study, we have successfully used AM for healing of chronic ulcers as compared to conventional therapy.

Conclusion

AM graft can be a choice for wound healing. In addition to being an excellent scaffold, it has unique biological properties that are important for tissue repair, including anti- inf ammatory, antimicrobial, antif brosis, anti-scarring, reasonable cost and low immunogenicity.

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