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## Effect of fresh human amniotic membrane dressing on graft take in patients with chronic burn wounds compared with conventional methods

Ali Akbar Mohammadi<sup>a,\*</sup>, Seyed Morteza Seyed Jafari<sup>a</sup>, Mandana Kiasat<sup>b</sup>,  
Ahmad Reza Tavakkolian<sup>b</sup>, Mohammad Taghi Imani<sup>a</sup>, Mehdi Ayaz<sup>b</sup>,  
Hamid Reza Tolide-ie<sup>c</sup>

<sup>a</sup> Shiraz Burn Research Centre, Division of Plastic and Reconstructive Surgery, Department of Surgery, Shiraz University of Medical Sciences, Iran

<sup>b</sup> Shiraz Burn Research Centre, Department of General Surgery, Shiraz University of Medical Sciences, Iran

<sup>c</sup> Faculty of Public Health, Gonabad University of Medical Sciences, Gonabad, Iran

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### ABSTRACT

**Background:** Burns are among the most devastating forms of injury. Nowadays the standard treatment for deep partial thickness and full-thickness burn is early excision and grafting, but this technique is not always feasible; and this leads to chronicity and microbial colonization of burn wounds. Interesting properties of human amniotic membrane made us use it in management of chronic infected burn wounds.

**Methods:** From January 2008 to September 2010, in a prospective clinical trial, 38 patients (76 limbs) with symmetric chronic burn wounds in both upper or lower limbs included in this study. Tissue cultures were taken from all the wounds. For the right, after debridement of granulation tissue and meshed split thickness skin grafting, the graft surfaces were covered with amniotic membrane dressing and in left limb wounds, after debridement, skin grafting was done in conventional method. 21 days later, the success rate of graft take was compared between two groups.

**Results:** The study group was composed of 76 limbs in 38 patients with mean age of  $27.18 \pm 6.38$  and burn in  $29.18 \pm 7.23$  TBSA%. The most common causes of the burn wounds chronicity in the selected patients was delayed admission due to poor compliance of the patients (44.8%). Staphylococcus was the most frequent isolate in wounds in our patients (62.85%). Mean graft take was observed in 90.13% of right upper limbs, and 67.36% of left upper limbs; which was significantly different ( $P < 0.001$ ).

**Conclusions:** Our study showed that human amniotic membrane dressing significantly increases the success rate of graft take in chronic wounds, and it can be recommended as an important dressing in chronic burn wounds management, due to interesting anti-microbial, and better graft take effects.

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\* Corresponding author. Tel.: +98 711 8219640x2; fax: +98 711 8217090.

E-mail addresses: [mohamadiaa@sums.ac.ir](mailto:mohamadiaa@sums.ac.ir), [sabetb@sums.ac.ir](mailto:sabetb@sums.ac.ir) (A.A. Mohammadi).

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## 1. Introduction

Burns are among the most common and devastating forms of injury in the world which consume large amounts of medical resources. Finding an appropriate material for dressing of burn wounds, remains a major concern, especially in extensive and deep skin defects, and wounds severely contaminated by microorganisms or poorly vascularized [1]. Nowadays the standard treatment for deep partial thickness and full-thickness burn is early excision and grafting [2,3]. However because of patients' general condition, limitation of autologous donor site in the patients with extensive burn wounds [3], poor equipments, and large number of admissions in some centres, this technique is not always possible; and this leads to high-incidence of chronic burn wounds specially in developing countries. The significant problem in delayed grafting technique is microbial colonization of the granulation tissue that reduces graft take, while increasing complications, mortalities, length of hospital stay, and cost [4]. The problem now arises is, how to go about applying skin graft on chronic-infected granulation tissue, which is controversial among the burn surgeons [5].

Availability, having all of the features of an ideal skin substitute [6], being economical, effective in superficial and limited size wounds, and antibacterial properties made us use human amniotic membrane in management of chronic infected burn wounds.

The present randomized clinical trial was to evaluate the effect of fresh amniotic membrane dressing on the graft take of chronic burn wounds comparing with conventional grafting methods.

## 2. Material and methods

From January 2008 to September 2010, in a prospective clinical trial, 38 patients (76 limbs) with chronic burn wounds (The wounds with more than two weeks after granulation tissue formation, shiny and slimy appearance according to the burn (Fig. 1) surgeon's diagnosis and infected due to positive wound tissue culture,  $\geq 10^5$  colony forming unit/gram of the tissue) in both limbs included in this study. All of the patients needed meshed split-thickness skin graft. Our exclusion criteria were age more than 60 and less than 16, serum albumin level less than 2.5, history of cardiovascular disease, renal failure, and diabetes mellitus. We received the approval local ethics committees (Shiraz University of Medical Sciences, Iran) and the patients provided informed consent.

The patients selected in this study, have symmetrical burn on two upper or lower extremities. Before starting we decided to assign right limbs as intervention group and left limbs as control group in each patient. We considered all patients as their control group by comparing the right and left limbs of them to control different potential confounders. Although it was not a random allocation but prevented selection bias, since we determined the side of each intervention group before study, the surgeons were not permitted to select the side of intervention based on his decision. So the size and appearance of wound did not affect the selection of intervention.



**Fig. 1 – Old granulation tissue in a burn patient (these wounds are shiny and easily debrided with minimal digital pressure).**

At first, tissue cultures were taken from all the wounds to find the responsible pathogens and start appropriate antibiotic therapy. Debridement of granulation tissue was done with hand dermatome till removal of all slimy tissues and pinpoint bleeding appearance in depth of the wounds; then the wounds were covered with adrenaline (1/200,000) soaked gauze, transiently. Split-thickness skin were harvested with hand dermatome, and after meshing (1.5/1), grafted, and fixed with staples. Then a layer of amniotic membrane was applied on the grafted area. The amniotic membrane was covered with Vaseline gauzes and then dry gauzes as a dressing (Amniotic membrane group). In the left limb wounds (Control group) after debridement and skin grafting, Vaseline gauzes and then dry gauzes were used as dressing.

The grafted areas were dressed and splinted. The first post grafting dressing change was performed after five days which was followed by dressing change, every 2 days. The success rate of graft take was compared between two groups, after 21 days by the same surgeon using the formula:

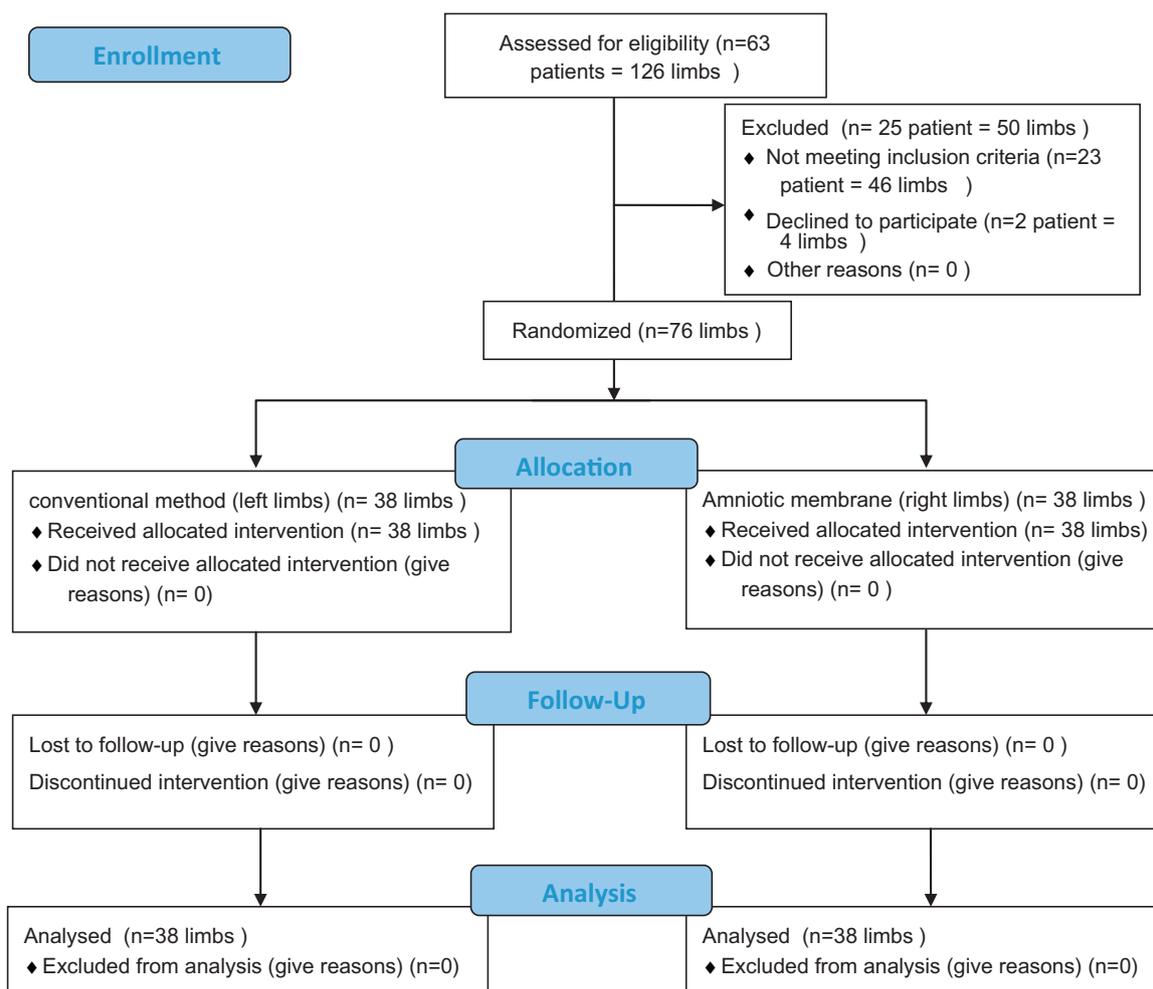
$$\frac{\text{graft takes surface area (cm}^2\text{)} \times 100\%}{\text{total grafted area (cm}^2\text{)}}$$

The amniotic membranes were obtained from placenta during elective caesarean sections of mothers without sexually transmitted disease, endometritis, and premature rupture membranes, and with negative HIV, HCV, and HBS tests. After washing with normal saline, the separated amniotic membranes from placenta, were placed in a pot contain normal saline and 80 mg/L gentamicin, and stored in refrigerator at 4 °C.

**Table 1 – Causes of chronicity of the burn wounds.**

Causes of chronicity of the burn wounds	No. (%)
Delayed admission of the patients	17 (45%)
Infections	12 (32%)
Risk of general anaesthesia	7 (18%)
Shortage of donor site for skin graft	2 (5%)

## CONSORT 2010 Flow Diagram



Collected data were presented as mean and standard deviation (mean  $\pm$  S.D.). Statistical comparisons between groups were carried out by using SPSS 16.0 software using t-test. For the comparison differences were considered as statistically significant at  $P \leq 0.05$ . Normality of data was evaluated by one-sample Kolmogorov-Smirnov test. As the distribution of our data was skewed Wilcoxon signed ranks test was used to compare the graft take percent in two groups. Statistical analysis was carried out by using SPSS 16.0 software and  $P$ -value  $< 0.05$  was considered as statistically significant.

### 3. Results

The study group was composed of 76 limbs in 38 patients with mean age of  $27.18 \pm 6.38$  and burn in  $29.18 \pm 7.23$  TBSA%; the median time interval between burn trauma and surgery was 53 days in the patients (interquartile range: 46.5–60.25). The patients' limbs were divided into the amniotic membrane and control groups.

The most common causes of the burn wounds chronicity in the selected patients was delayed admission due to poor compliance of the patients (44.8%) (Table 1).

Staphylococcus was the most frequent isolate in wounds in our patients (62.85%) (Table 2).

Mean graft size was  $11.36 \pm 3.25\%$  in right limbs (amniotic membrane side) and  $10.75 \pm 2.94\%$  in left limbs. Mean graft take was  $90.13 \pm 5.13\%$  in right limbs (amniotic membrane side) and  $67.36 \pm 8.20\%$  in left limbs (Control side); which was significantly different ( $P < 0.001$ ) (Fig. 2). No adverse events were recorded from human amniotic membrane, and it was well tolerated and the surrounding skin did not show any irritative dermatitis in all patients.

### 4. Discussion

Burns are among the most dramatic trauma in world, and require immediate specialized care in order to minimize morbidity and mortality [7]. Nowadays early excision and grafting (E&G) is the standard treatment for deep partial thickness and full-thickness burn [2,3], but E&G is not feasible in many cases, specially in developing countries, which leads to chronicity of burn wounds.

Chronicity of the burn wounds is so frequent in some centres. The most common cause of burn wounds chronicity

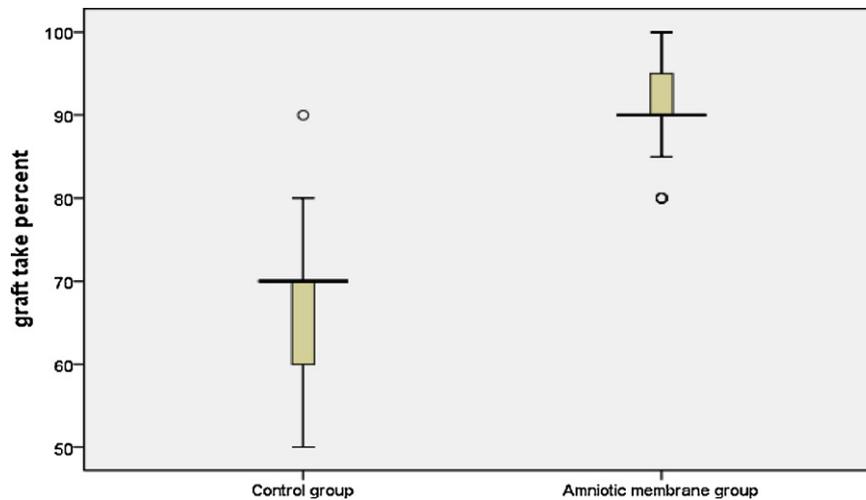


Fig. 2 – Graft take percent in amniotic membrane and control groups (data are presented as median and interquartile range).

Table 2 – Wound culture report.

Wound culture report	No. (%)
Staphylococcus	25 (63%)
Pseudomonas	7 (16%)
Kelebseilla	4 (11%)
Entrobactor	2 (5%)
Two colonies (pseudomonas and staphylococcus)	2 (5%)

in our burn centre is delayed admission due to poor compliance of the patients (44.8%). The other factors can make the wounds chronic can be mentioned as; infections, risk of general anaesthesia, shortage of donor site for skin graft, poor equipment and peri-operative care systems, and large number of admissions in crowded centres.

A significant problem in delayed graft technique is microbial colonization of the granulation tissue [4]. Thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humoral immune responses were observed in the burn patients, and the burn wound surface (specially in deep partial-thickness and in all full-thickness burns) is a protein-rich environment consisting of avascular necrotic tissue (eschar) that provides a favourable niche for microbial colonization and proliferation [7].

Microbial colonization of the granulation tissue reduces graft take, while increasing complications, mortalities, hospital stay, and cost [4]. These made chronic granulation tissue management, a controversial concern among the burn surgeons [5].

As results show in this study, the most frequent isolate in bacterial wound colonization was staphylococcus followed by pseudomonas that is similar to some other studies [8]. However, it may differ many factors such as burned TBSA% inpatient or outpatient management comorbid diseases and time interval between burn and wound culture [5]. Nowadays management of the chronic burn wounds is not definite, so we designed our study to evaluate the effect of human amniotic membrane on chronic burn wounds management.

Human amniotic membrane, a thin semi transparent tissue forming the innermost layer of the foetal membrane [1,9] was introduced as an effective dressing in 1910 by John Staig Davis [10].

Despite of low popularity of human amniotic membrane as a dressing in developed countries; availability, lack of cost, simple sterilization and storage process made amniotic membrane as a popular and accepted dressing in superficial and limited burn wounds [11,12], and as skin graft fixator [13].

Laboratory evaluations have showed that the basement membrane zone of this interesting membrane, which resembles human skin both morphologically and ultra-structurally, shares major basement membrane components with human skin [14].

Due to low immunogenic characteristics, amniotic membrane seems to be suitable tissue for transplantation; rarely clinical signs of acute rejection have not been observed when HAM has been transplanted into volunteers [1,15,16].

By using this membrane less frequent local wound infections and sepsis were noticed [2]. Providing a cover, the amniotic membrane protects the wounds from the environment [13], and can prevent or at least decrease burn wound s colonization [17,18]. Bacteriostatical effect on gram positive of progesterone and bactericidal role of lysozyme content of amniotic membrane can be known as human amniotic membrane antibacterial properties [2,15]. The other materials such as Allantion and urea extracted by them and some immunoglobulins are effective in wound infections prevention [16].

By use of human amniotic membrane the patient requires less blood, and albumin transfusion [2,19].

Amniotic dressing was accompanied by much less pain in burn patients because of less frequents dressing changes and less inflammatory response to HAM; because of not expressing of HLA-A, B, C, DR or beta-2 microglobulin on its epithelial [2,19].

Recent studies have shown that HAM is accompanied by rapid re-epithelialization and promotion of wound healing and granulation tissue development, by inhibition of leucocyte protease activity – which reduces polymorphonuclear leucocytes infiltration – and angiogenesis stimulation [12,13,15,17,20,21].

In previous studies the amniotic membrane has been recommended as dressing for superficial and limited burn

wounds or as a skin graft fixator, and there is no reported prospective study evaluating the effect of the human amniotic membrane on the infected chronic burn wounds management.

Potential of disease (such as HIV, HBV, and HCV) transmission and bad smell due to constitutional character of some amniotic membrane should be mentioned as the main problems of HAM [2,10].

In the present study we evaluated the human amniotic membrane as a dressing on skin graft take of old infected wounds and compared the effect of this method on graft take with conventional skin grafting method.

## 5. Conclusion

Our study showed that human amniotic membrane dressing significantly increases the success rate of graft take in old-infected wounds, and beside all the previous effects, human amniotic membrane can be recommended as an interesting dressing in old infected burn wounds management, due to its magical anti-microbial, and better graft take effects.

## Conflict of interest

None declared.

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