Effect of amniotic membrane on graft take in extremity burns

Ali Akbar Mohammadi a, Hamed Ghoddusi Johari b,*, Shima Eskandari c

a Shiraz Burn Research Center, General Surgery Department, Division of Plastic and Reconstructive Surgery, Shiraz University of Medical Sciences, Shiraz, Iran
b Trauma Research Center, General Surgery Department, Shiraz University of Medical Sciences, Shiraz, Iran
c Trauma Research Center, General Surgery Department, Shiraz University of Medical Sciences, Shiraz, Iran

A R T I C L E   I N F O

Article history:
Accepted 22 January 2013

Keywords:
Amniotic membrane
Burn
Skin-graft fixation

A B S T R A C T

Background: Several studies have shown that the application of amniotic membrane as a biological dressing in the management of burns is accompanied by rapid re-epithelialisation and healing as it diminishes the oozing of plasma, bacterial count and fluid, protein and heat loss. This study evaluates the effect of amniotic membrane on graft take in split-thickness skin graft of extremity burns.

Methods: From October 2008 to January 2010, in a prospective clinical trial, 54 patients (108 limbs) with second and third degree burns, covering 4–15% of total body surface area (TBSA), were included in this study. All patients needed split-thickness skin grafts for burn-wound coverage. Selected patients had symmetric burns on two (upper or lower) extremities. Then in every patient, the extremities were randomly divided into two groups: in one limb, the skin graft was traditionally fixed with skin staples (control group) and in the other limb the skin graft was covered with an amniotic membrane (amnion group). Therefore, in every patient the graft was covered with an amniotic membrane in one extremity and fixed with skin staples in the other extremity. Finally, the duration and success rate of complete graft take was compared between the two groups.

Results: The study group was composed of 108 limbs in 54 patients (27 males and 27 females) with a mean age of 23.54 ± 4.9 years and burn 9.03 ± 2.69% TBSA. The mechanism of burn was flame (63%), scald (18.5%) and flash (18.5%). The rate of complete graft take was 96.76% and 88.79% in the amnion group and in the control group, respectively. The mean duration of graft take was 6.98 ± 1.35 days in the amnion group and 13.9 ± 1.66 days in the control group. This difference was statistically significant (P < 0.001).

Conclusions: Our results show that although the amniotic membrane has no negative impact on graft take, it significantly reduces the duration of complete graft take, which is very important for both the patient and the health-care system.

© 2013 Elsevier Ltd and ISBI. All rights reserved.

1. Introduction

The revascularisation of a skin graft depends on the immobilisation of the graft on the wound bed. In cases in which circular dressings are needed, the graft may be displaced under the dressing, leading to partial graft loss [1]. For this reason, several methods of graft fixation have been described. A common technique is to use stitches [2], but this is time consuming and the stitches need to be removed after

* Corresponding author. Tel.: +98 917 713 1640; fax: +98 711 2330724. E-mail address: ghoddusib@yahoo.com (H.G. Johari).
0305-4179/$36.00 © 2013 Elsevier Ltd and ISBI. All rights reserved.
http://dx.doi.org/10.1016/j.burns.2013.01.017
Table 1 - A consort diagram to assess for bias in enrollment.

<table>
<thead>
<tr>
<th>Enrollment</th>
<th>Control Group [n=54 (54 limbs)]</th>
<th>Randomized and Enrolled in the study [n=54 (108 limbs)]</th>
<th>Amnion Group [n=54 (54 limbs)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessed for eligibility (n=90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Excluded (n= 36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not meeting inclusion criteria (n=36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Follow-Up</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lost to follow-up (n=0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Analysed (n=54)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Materials and methods

From October 2008 to January 2010, in a prospective clinical trial, 54 patients (108 limbs) with second- and third-degree burns, covering 4–15% of total body surface area (TBSA) were included in this study in our burn centre. All patients needed split-thickness skin grafts for burn-wound coverage. Our excluding criteria were wound infection, age more than 60 years and history of cardiac disease, renal failure, diabetes mellitus and any other severe, underlying metabolic disorder. We received the approval of Shiraz University of Medical Sciences Ethical Committee. All the patients (or their parents) signed an informed consent.

We selected patients who had symmetric burns on two (upper or lower) extremities. Then in every patient, the extremities were randomly divided into two groups: in one limb the skin graft was traditionally fixed with skin staples (control group) and in the other limb the skin graft was covered with an amniotic membrane (amnion group) (Table 1). All procedures were performed by an experienced burn surgeon using a (1.5/1) meshed graft.

In the amnion group the graft was placed on the wound bed, the amniotic membrane wrapped around the grafted extremity and the dressing applied. The membrane adheres to itself when wrapped around the extremity, and there is no need to use any stitch or staple.

With meticulous dissection during a preparation procedure, amniotic membrane pieces as large as 10–20 x 30–50 cm are retrievable. In children and in the upper extremity of adults, it is very easy to wrap the extremity with an amniotic membrane. In the lower extremity, usually it is possible to do the same. However, even in situations where the size of a single membrane is not enough, it is possible to use two or three pieces of amniotic membrane to cover the skin graft (Fig. 1).
We have a tissue bank at our burn centre for amniotic membranes retrieved from the elective caesarean sections of mothers without any sexually transmitted disease, endometritis, premature rupture of the membranes or meconium-stained amniotic fluid. The amnion is carefully separated from the chorion and placenta and washed thoroughly with normal saline until a whitish, smooth, transparent layer remains, which is then stored in normal saline–gentamicin solution at 4 °C. If human immunodeficiency virus (HIV), HBS, hepatitis C virus (HCV) and Venereal Disease Research Laboratory (VDRL) tests are negative for both mother and umbilical cord, the amniotic membrane will be used. Routine cultures are obtained from stored membranes to rule out bacterial contamination [5].

In both groups in the fifth postoperative day the dressings were removed, the wounds irrigated with normal saline, the skin graft covered with sterile Vaseline gauze and the dressings applied again. This dressing change continued every other day until complete graft take was achieved. The success rate of graft take was compared between two groups, after 21 days, by the same surgeon using the formula:

\[
\text{graft-take area (cm}^2\text{)} \div \text{total grafted area (cm}^2\text{)}
\]

Finally the duration and success rate of complete graft take were compared between the two groups.

The collected data were presented as mean and standard deviation (mean ± S.D.). Normality of data was evaluated by the one-sample Kolmogorov–Smirnov test. As the distribution of our data was skewed, the Wilcoxon signed-rank test was used to compare the graft take percent in two groups. Statistical analysis was carried out by using Statistical Package for the Social Sciences (SPSS) 16.0 software and \(P < 0.05\) was considered statistically significant.

3. Results

The study group was composed of 108 limbs in 54 patients (27 males and 27 females) with a mean age of 23.54 ± 4.9 years and 9.03 ± 2.69% TBSA burn. The mechanism of burn was flame (63%), scald (18.5%) and flash (18.5%). The patients divided into two groups: 54 limbs in the amnion group and 54 limbs in the control group. The rate of complete graft take was 96.76% and 88.79% in the amnion group and in the control group, respectively. The mean duration of graft take was 6.98 ± 1.35 days in the amnion group and 13.9 ± 1.66 days in the control group. This difference was statistically significant (\(P < 0.001\)).

4. Discussion

Using the amniotic membrane as a skin-graft fixator is convenient for both the patient and the surgeon [5]. This is particularly appropriate in children or burns involving the extremities. The graft is placed on the wound bed, the amniotic membrane wrapped around the grafted extremity and the dressing is applied. The membrane adheres to itself when wrapped around the extremity, and there are no stitches or staples to be removed in future. This is very helpful for the patients as removal of the staples is stressful, painful and even frightening for them, particularly for the children.

Previously in a preliminary study, we used this technique in 40 cases and we did not observe any complications such as graft loss, infection or rejection [5]. However, our study was not well designed because the cases were selected randomly and we did not have any control group. Hence, we decided to perform the current study in highly selected cases. In other words, we selected the patients who had symmetric burns on two (upper or lower) extremities. Then in every patient, the extremities were randomly divided into two groups: in one limb the skin graft was traditionally fixed with skin staples (control group) and in the other limb the skin graft was fixed and covered with amniotic membrane (amnion group). By this method, the procedures were performed in the same patient and the physiological status of the patients was equal for both groups.

Several studies have shown that the application of the amniotic membrane as a biological dressing in the management of burns is accompanied by rapid re-epithelialisation and healing [6–10]. The amniotic membrane has been found to be an effective biological dressing for burns, as it diminishes the oozing of plasma [7], bacterial count [8] and fluid, protein and heat loss [9].

Amnions can be readily obtained from caesarean deliveries after screening for viral diseases. They have been used to cover clean, partial-thickness wounds and donor sites and applied as a temporary dressing for freshly excised burns; it has advantages such as pain relief, prevention of infection, maintenance of a moist environment to promote healing, good adherence to wounds and simple handling [10–15]. Laboratory investigations have revealed that the basement membrane (BM) of amnions shares major BM components with human skin, and the BM zone resembles human skin both morphologically and ultrastructurally [16]. The epithelial side of denuded amnions has been shown to support the proliferation, spreading and differentiation of corneal, mucous and bronchial epithelial cells [17–19]. Furthermore, the stroma of amnions can serve as a dermal matrix in which...
fibroblasts show good adherence and proliferation [20,21]. Limited immunogenicity, along with anti-inflammatory effects and other characteristics, make it a suitable transplantation material for ocular surface reconstruction, and the immune privilege of the amnion makes it a biological immune barrier for xenotransplantation [22–24]. Moreover, amnion procurement and processing are easy and the amnion can be sterilised and preserved at low cost for long periods without obvious architectural changes [25–27].

Further, multiple studies have shown that human amniotic membrane is accompanied by rapid re-epithelialisation and promotion of wound healing and granulation-tissue development by inhibition of leucocyte protease activity – which reduces polymorphonuclear leucocyte infiltration – and angiogenesis stimulation [28–31].

The most important concern regarding the use of the amniotic membrane is the potential of disease transmission, which can be prevented by screening tools. However, we could not find any reported disease transmission via the amniotic membrane in the literature after using screening tests. The only problem with the use of the amniotic membrane is its malodour for some patients [10]. However, it did not impact the course of treatment in our patients.

As our results show, in the amnion group we had 96.76% complete graft take, which is an excellent result. In addition, by using the amniotic membrane, the duration of graft take shortens dramatically, a finding which is very important for both the patient and the health-care system. However, before recommending it as a routine procedure in burn centres, further clinical trials should be done.

A remaining and important question is: does the amniotic membrane, as a skin-graft fixator, have any effect on the degree of pruritus and hypertrophic-scar formation or not? To answer this question we are following up our cases.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Acknowledgement**

We would like to thank Department of Surgery, Trauma Research Center, Shiraz Burn Research Center, and Ghotbedin Burn Hospital personnel for their cooperation in this work. It should be mentioned that, this survey is based on the thesis of Dr. Hamed Ghodossi Johari for finishing general surgery residency, in Shiraz University of Medical Sciences.

**References**


