HUMAN AMNIOTIC MEMBRANE AS A VERSATILE BIOLOGICAL DRESSING A PRELIMINARY REPORT

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Abstract
The subject of human amniotic membrane as a biological dressing is reviewed and experience of four wounds, three from acute burns and one from trauma is presented. Freeze dried amniotic membrane was used as the material for application next to the wound after the usual surgical cleansing. This process was repeated daily or on alternate days depending on the individual response. Beneficial effects noted included reduction of pain, promotion of healthy granulation and increased tendency of graft take in acute burns. In the chronic post-traumatic ulcer the treatment was not as effective in reduction of bacterial counts as a topical antibiotic (soframycin) used in an adjacent area of the same wound. Its trial as a biological dressing in burns merits consideration in view of this preliminary trial (JPMA 37:290, 1987).

INTRODUCTION
Human amniotic membrane has been used off and on for many years for the purpose of providing epithelial cover in various cutaneous and mucous membrane defects. The following are some of the more common settings involving its use. The objective in each of these has been to spare a donor site.
1. As a wound cover in massive acute burns where autograft skin is not available.
2. To provide a temporary surface lining in cavities where the surgeon feels that epithelial growth from margins or depth of the defect will ultimately complete the resurfacing. Cavities of neovaginoplasty and orbital extention and buccal lining deficiencies after prophylactic or therapeutic shave or peel are examples of such uses.
3. To cover chronic ulcers where previous skin grafts have repeatedly been lost. Amniotic membrane being expendable, even two or three times a day changes have been utilized.

The advantages claimed have been 1,2,4-7
1. Reduction of pain.
2. Reduction of bacterial count.
3. An effective vapour barrier.
4. Durable cover of raw surfaces.
A trial of the freeze-dried irradiated amniotic membrane was undertaken. This paper presents the experience of this study.

MATERIAL AND METHODS
Four cases of skin loss, three from acute burns and one from trauma, currently on the Plastic Surgery and Burns Service of Civil Hospital, Karachi, were selected for a preliminary study of the efficacy or otherwise of freeze-dried irradiated amniotic membrane. This preparation was processed according to the following routine:
Fresh placentae were procured from labour room operation theatre of Department of Obstetrics,
Liaquat Medical College, Hyderabad, and placed in polyethylene jars containing sterile normal saline. Donors had to have no history of jaundice, attacks of malaria or venereal diseases. Placentae were transported to Atomic Energy Medical Centre, Jamshoro, where these were processed. Amniotic membrane was separated, cleansed and washed with several changes of sterile normal saline to remove blood etc. under clean hygienic conditions. The cleansed membrane was cut in pieces approximately 10cmx12.5cm in size, and spread on sterile pieces of surgical gauze. The amniotic membrane was then freeze-dried at a temperature of -60 degrees C under vaccum (at atmospheric pressure of \(10^{-2}\) mbar) for 48 hours. Two pieces of freeze-dried amniotic membrane each were then double sealed in clean polythene envelopes and labelled for identification with Batch Number and date of processing. 200 envelopes were packed in card board boxes and sent to Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad, for radiation sterilization. The sealed boxes were irradiated with gamma rays to 2.5 mega rads (25 K Gray) in a batch type cobalt-60 irradiator available at PINSTECH. Ten of the samples from each 200 were taken at random and subjected to bacteriological cultures both for aerobic and anaerobic organisms.

Wounds were washed with Betadine scrub and dead tissue debrided with forceps and scissors followed by a rinse with normal saline. The desired pieces of amniotic membrane were removed from packages aseptically, soaked in saline for 2-3 minutes, and then applied to the raw areas. This was followed by a dressing consisting of sterile gauze, cotton and bandage. The same process was repeated daily or on alternate days using new amniotic membranes each time. At the time of dressing change the following observations were made:

1. Complaint of pain in the wound or any other subjective symptoms.
3. Quality of granulation tissue.
4. Epithelial growth from edges of the wound.
5. Graft take.

When a wound was judged to be clinically ready for skin grafting the same was performed in the operating room. Daily dressings were done thereafter and the status of the grafts assessed.

**RESULTS**

**Case 1:** 20 years old male with 22% deep burn of six days duration involving the right upper limb, right hemithorax and hemiabdomen, was transferred from elsewhere. Amniotic membranes were applied for 15 days (daily change of dressing), beginning on day 12 post bum.
- Pain was reduced right after the first dressing.
- Soakage of dressing diminished after 8th day of treatment.
- Granulation tissue appeared 4-6 days post application and was exuberant at day 10 of treatment.
- Epithelization from edges was not detectable.

Split skin grafting of the wound was done on days 21 and 26 to separate areas of the wound. Two postage stamp sized grafts from the second session took, but all the others were rejected.

**Case 2:** 37 years old male with 16% burn 5 days old; 9% bum on right upper limb was taken as a study area. Of this 9%, 4% located on the forearm was superficial. Duration of application of membrane was 11 days beginning on day 5 post burn.
- Pain was markedly relieved.
- Surface exudate changed from frank purulent to serosanguinous.
- Granulation was seen on day 4 and was healthy and marked on day 10.
- On 8th day, the 4% area on the forearm was completely epithelialized.

On 15th day postburn, split thickness skin grafting was done and the grafts took uneventfully.

**Case 3:** 15 years old female with 22% burn of 12 days duration; 9% of the burn, which was deep and
involved the whole right upper limb, was selected as study part. Membranes were applied for 16 days, beginning on 12 day postburn, with alternate day changes.
- Pain was markedly relieved.
- Improvement in quality of surface exudate was noted within 6 days.
- Pinkish healthy granulation tissue was marked at day 10.
- No epithelization was seen.
Skin grafting was done on day 17 and all grafts took.

Case 4: 22 years old male with traumatic nonhealing ulcer of the dorsum of right foot of 6 months duration. The ulcer was divided into two zones by a bridge of intact skin. The proximal portion (77cm²) was treated with soframycin impregnated gauze (sorfatulle) and distal portion (100cm²) was treated with membrane. Membranes were applied for 14 days.
- Pain in the area of membrane was relieved more as compared to sofrarvmcin treated area.
Dressing soaking of both the areas reduced within 4 days.
- Granulation tissue appeared within 6 days in membrane treated area and on day 8 in soframycin treated area; on day 10 both areas appeared similar.
- Epithelization from the margins marked in sofraniycin treated area only.
Skin grafting was done at the end of study but graft did not take on either area. Surface cultures became negative on day 12 in soframycin treated area (Staph. Aureus was cultured from the admission swab). However, the membrane treated area developed Proteus growth by day 10 while retaining the original Staph. Aureus.

DISCUSSION
Relief of pain was the most significant benefit from the use of freeze-dried amniotic membrane and was admitted voluntarily by all patients. This phenomenon has been observed by Others 1,4,6-7 and appears to relate to adherence of the membrane to a de-epithelized surface, thus obtruding exposed nerve endings in the wound.
Change in the character of the exudate and the appearance of granulations tended to be for the better in acute burns, but no benefit was noticed in the one chronic post traumatic case. In fact the membrane appears to have actually made the treated part (as opposed to the antibiotic and gauze dressed part) worse by permitting new bacterial colonisation. It is quite possible that this biological material provided a real nutrient for the growth of mixed flora.
Membrane itself could not have been the source of the new organism (Proteus) because it was radiation iritized and each batch had been tested for sterility. Similar occurrences have been observed occasionally with skin grafts in sujacute and chronic burns. Application of new autografts to residual infected raw areas may not only result in complete lysis of the fresh grafts but may also lead to loss of already established grafts.
Promotion of epithelization seems to relate to the overall beneficial effect on wound healing. Reduction of the exudate and pus and appearance of healthy granulations with the use of amniotic membrane not surprisingly promoted epithelial growth from the edges.
Improved graft -take was noticed in all three acute burns (some graft loss in case No.1 appears to be due to hasty application, i.e. before the wound was ready to accept the graft). This fact alone, if borne out by our future proposed study, will be a significant advantage of amniotic membrane treatment. Skin grafts are always at a premium in acute burns and if amniotic membrane can promote early preparation of the wound for grafting and also help improve graft-take, it would have more than fulfilled its role as a physiological dressing.
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