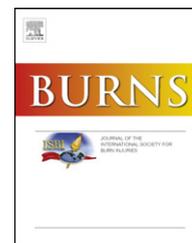


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Dried irradiated human amniotic membrane as a biological dressing for facial burns—A 7-year case series

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ABSTRACT

Background: Facial burns are common and have a significant impact on patient function and psychosocial well being. Human amnion has been used for many years as a temporary biological wound dressing in the management of partial thickness burns. The observed advantages of human amnion treatment include pain relief, ease of use, prevention of infection and acceleration of wound healing.

Objective: This study evaluated our 7 years of working with dried irradiated human amnion in the treatment of facial burns.

Method: A review of patients, treated with dried human amnion for facial burns between 2001 and 2008. Demographic details collected included age, gender, total facial surface area burned, type of burn and cause of injury. The effectiveness of the treatment was determined by wound infection rate, frequency of dressing reapplication, healing time and resulting scarring.

Results: Thirty-three patients with superficial partial thickness burn were identified (25 males, 8 females). The average age of the patients was 16.5 years (range: 8 months to 64 years). The causes included scalding ($n = 15$), contact burning ($n = 13$) and flash burning ($n = 5$). The mean percent total facial surface area burned was 2.7% (range: 0.5–8.5%). None of the patients developed facial wound infections. Eighty-five percent ($n = 28$) of the patients needed a single application of the dried amnion. The average healing time was 5.4 days (range: 2–14 days). Thirteen patients (39%) had burns confined to the facial area, of which three were discharged and treated as outpatients. Long-term follow up showed two hypopigmented scars, one hyperpigmented scar and one hypertrophic scar.

Conclusion: Superficial partial thickness facial burns can be effectively treated with dried irradiated human amnion membrane.

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1. Introduction

Facial burns are common in developing countries, varying from relatively minor insults to severe debilitating injuries. Their management remains one of the greatest challenges in burn care. They may disrupt the anatomical integrity, resulting in distortion of facial aesthetic subunits even in the absence of functional impairment [1]. Thus, the clinical, functional and aesthetic outcome of facial burns is critical to patient self-esteem and their ability to reintegrate into society.

The main goal in facial burn treatment should be restoration of normal facial features, with acceptable or good anatomical balance and symmetry as well as, dynamic facial expression [2]. However, standard guidelines for the treatment of facial burns have not been established. The management of facial burns may include operative and non-operative treatments or both, depending on the depth and extent of the burn. The face has a unique blood supply that supports accelerated wound healing. Hence, partial thickness burns of the face are typically treated

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conservatively with either topical medications or skin substitutes.

Historically, partial thickness burns were treated conservatively by debriding the blisters, daily washing, and the application of new bandages with topical medications two to four times each day. Partial thickness burns are very painful, as skin damage limited to the upper layer leaves the nerve endings exposed. Therefore, these procedures cause excruciating pain and anxiety in patients.

A number of occlusive dressings or skin substitutes have been developed in recent years with the aims of reducing pain, reducing fluid loss, providing better infection control, accelerating wound healing, reducing nursing load and economizing treatment. These skin substitutes allow re-epithelialization to occur underneath the dressing and eliminate the need for daily cleansing and frequent dressing changes. Hence, the stress for both patients and nurses is minimized [3].

Trials comparing the use of a skin substitute with topical medication on facial burns have shown that skin substitutes significantly improve the management and healing rate of partial thickness facial burns [4–6]. An increased healing rate is important, as a wound not healed by 10 days has an increased risk of scar formation [7]. The basis for the use of skin substitutes is that they create a moist environment [8], thereby reducing loss of water, protein and evaporative heat at the wound surface, as well as preventing bacterial contamination.

However, the varied contours and continued movement of the face has in the past limited the use of skin substitutes [9,10]. Thus, the standard care for facial burns remains an open technique using topical antibiotics. This in part is due to the financial cost and difficulty of using skin substitutes on a facial burns [9,11].

A variety of biological and bioengineered skin substitutes are available for the treatment of burns [12]. Autologous skin grafts have limited availability and are also associated with additional morbidity and scarring [13]. Bioengineered skin substitutes have the drawbacks of limited viability, limited quantity and high cost [14,15].

Amnion and skin from cadaveric sources have proved to be an effective biological material for management of burn wounds [16–18]. Cadaver skin is not always an option due to infrastructure limitations, religion or poor social acceptance by society. Due to its greater availability and non-antigenic property, amnion has advantages over allograft skin and is therefore extensively used for the treatment of burns.

Few studies have reported the effectiveness of glycerol-preserved amnion for the treatment of burns [4,18]. Surprisingly, there have been no clinical trials to show the effects of dried irradiated amnion in facial burn wound healing. In this article, we present an evaluation our 7-year experience utilizing dried irradiated amnion for treatment of partial thickness facial burns.

2. Materials and methods

2.1. Patients

Patients who presented with partial thickness burns of the face at Hospital Universiti Sains Malaysia between 2001 and

2008 were enrolled in this study. The depth of the facial burns was assessed by a senior member of the staff prior to application of the dried irradiated amnion membrane. Demographic details collected included age, gender, total facial surface area burned, type of burn and cause of injury.

2.2. Procurement and preparation of dried irradiated amnion

Amniotic membranes are procured from placentae of mothers who have been antenatally screened for the possibility of blood-borne diseases such as HIV, HBsAg, HCV, syphilis, gonorrhoea, toxoplasmosis and cytomegalovirus. Membranes from placentae with intrapartum complications are discarded. Processing of amnion involves thorough washing with normal saline, soaking in 0.05% sodium hypochlorite solution for 30 min to 1 h, shaking several times with normal saline, drying, packing, and lastly gamma radiation at 25 kGy or lower according to the bioburden (Fig. 1).

2.3. Application of dried irradiated amnion

Twenty-nine patients (88%) presented within 6 h of acquiring their injuries. Assessment of burn depths and application of dried irradiated amnion sheets were performed within 1 h of arrival to the burn unit.

Visible large blisters were punctured and dead skin was removed. Wound swabs for culture and sensitivity were taken, followed by thorough and gentle cleaning of the wound with normal saline. Dried irradiated amnion sheets that were spread on a thin layer of sterile gauze were cut to size and applied onto the surface of the wound. No antimicrobial ointment or dressings were used on top of the amnion. These biological dressings were left in situ, until natural separation occurred. The dressings were inspected daily to monitor wound healing progress, as well as to identify problems such as infection, allergic reactions and displacement of dressing material. In cases where the dressings dislodged before wound epithelialization took place, a new dried irradiated amnion sheet was applied.

Patients with TBSA 2% or less, confined to the forehead, cheek or chin were treated on an outpatient basis. They were reviewed at our burn unit every 3 days, until the dried irradiated amnion sheets separate naturally and signs of epithelialization were seen. All patients were advised to apply sun protection lotion to their face and reviewed at the outpatient department within 2 weeks upon discharge. Further follow ups ranged from 1 to 4 months, depending on the severity of the injury.

2.4. Clinical observations

The effectiveness of the treatment was determined by

- Wound infection rate.
- Frequency of dressing reapplication.
- Healing time.
- Resulting scar.

Wound infection was diagnosed if the local wound demonstrated increased exudate and surrounding cellulitis

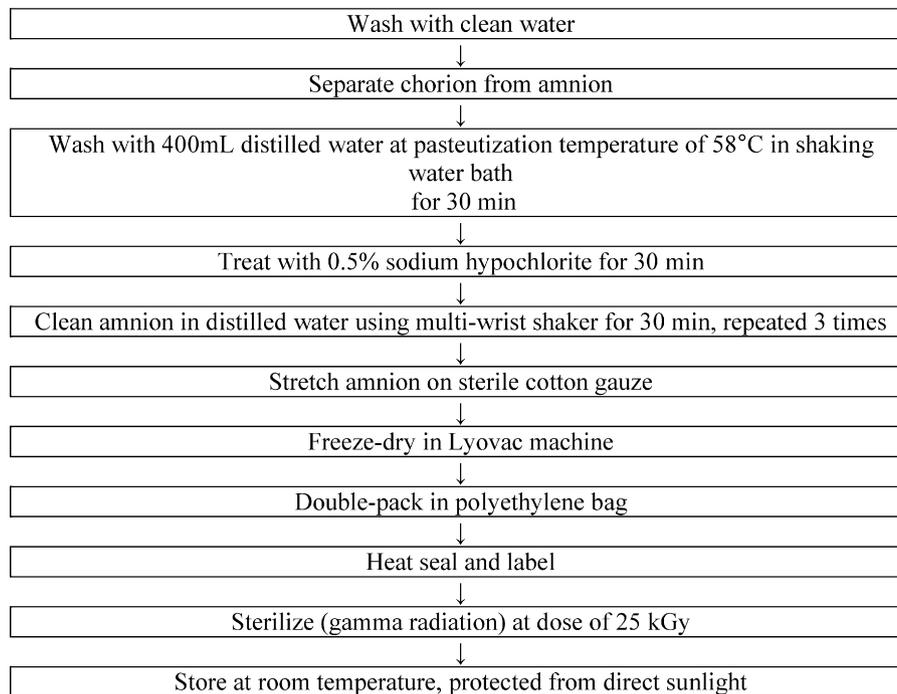


Fig. 1 – Summary of amnion processing.

[19]. Healing time was defined as the point when 95% of the wound re-epithelialized. The 95% value was chosen because a partial thickness burn is not uniform in depth. Some small areas often require extra time to heal beyond the majority of the wound. A study with patients exclusively suffering from facial burns would have been ideal in order to assess comfort and pain levels. However, exclusive partial thickness facial burns are very rare. Therefore, our primary outcomes were defined in terms of frequency of dressing change, wound infection rate, healing time and resulting scarring.

3. Results

3.1. Patient demographics

A total of 33 patients (25 males, 8 females) with superficial partial thickness burn of the face were treated with dried irradiated amnion between 2001 and 2008. The average age of the patients was 16.5 years (range: 8 months to 64 years). The causes included scalding ($n = 15$), contact burning ($n = 13$) and flash burning ($n = 5$). The mean percent total facial surface area burned was 2.7% (range: 0.5–8.5%) (Table 1). Four patients suffered inhalational injury and were thus intubated and nursed in the ICU.

3.2. Treatment and acute stay

No wound infection or excess exudate was noted that necessitated removal of the amnion sheets. Application of the dried irradiated amnion sheets was quick and easy. None of the patients complained of pain. The amnion dressings adhered well, and 85% ($n = 28$) of the patients needed only a single application of the dried amnion. In five patients, the

dressings were dislodged, resulting in reapplication with new dried irradiated amnion sheets. Average duration of treatment (healing time where 95% of the wound had re-epithelialized) was 5.4 days (range: 2–14 days) (Table 1). Thirteen patients (39%) had burns confined to the facial area; of these, three were discharged and treated on an outpatient basis. One patient succumbed to multiple organ dysfunction on day 8 post-burn. He suffered a total body surface area (TBSA) of 66%. Nonetheless, the facial wound healed on day 5.

3.3. Follow up

Patients were examined and photographs were taken during follow up visits at the outpatient department. Only one patient developed a hypertrophic scar. Two patients had hypopigmented scars and one had a hyperpigmented scar (Fig. 2). None of our patients applied sun protection lotion routinely. This could be due to the fact that having Fitzpatrick Classification Skin Type IV, Malaysians do not burn easily. Hence applying sun protection lotion is not a common practice.

4. Discussion

The use of amnion as biological dressings for burns dated back to 1910 [20]. It is an ideal dressing material as it alleviates pain, prevents infection [21–24], accelerates wound healing [3,23,25] and has good handling properties [26]. The advantages of being thin, adhesive, conformable and easily removable further idealize it for facial burns. As fresh amnion has a short shelf life and may not always be available when needed, various preservation methods have been introduced, i.e. cryopreservation in liquid nitrogen [27], preservation in silver

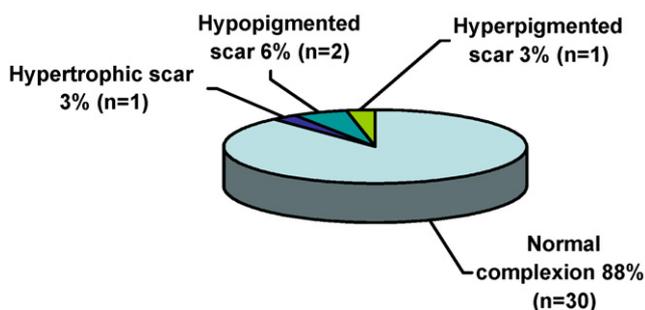
Table 1 – Results.

Patients	n	%
Male	25	76
Female	8	24
Total	33	100
Mean age	16.5 years (range: 8 months to 64 years)	
Depth	Superficial partial thickness burn (n = 33)	
Total facial burn surface area	2.7% (range: 0.5–8.5%)	
Causes of facial burn		
Scalding	15	46
Contact burning	13	39
Flash burning	5	15
Amnion application		
Single	28	85
Twice	5	15
Average healing time	5.4 days (range: 2–14 days)	
Infection	None	
Resultant scar		
Normal	29	88
Hyperpigmented	1	3
Hypopigmented	2	6
Hypertrophic	1	3

nitrate [28], storage in antibiotics solution, glycerol-preserved sheets [29], dried sheets [30] and gamma-irradiated sheets (Figs. 3–5).

None of our patients with burns confined to the facial area (n = 13) complained of pain following application of the dried irradiated amnion. The children remained calm and analgesic requirements were minimal. Ravishanker et al. [18] compared the use of amnion and traditional closed dressings for burns, and reported that 80% of the patients treated with closed dressings complained of pain and discomfort and needed some form of sedation, especially during the changing of dressings.

Wound re-epithelialization occurred within 2–14 days (average 5.4 days). In contrast, a study by Branski et al. [4] revealed that the average healing rate for facial burns treated with topical antimicrobial ointment was 8 days. Meanwhile, Ravishanker et al. [18], who used glycerol-preserved amniotic membranes, reported a healing rate of 7–10 days for superficial burns and within 20 days for partial thickness burns. As the wound healed, the amnion dressing peeled off naturally,

**Fig. 2 – Resulting scar.**

leaving soft and supple skin. In addition to its physical properties of reducing water and heat loss [31], Kim et al. [32] suggested that the mechanism responsible for the observed rapid healing was inhibition of proteinase activity. Reduction of polymorphonuclear leukocyte infiltration results in a reduced inflammatory response.

None of the partial thickness burns treated became infected or developed any kind of hypersensitivity to the dried irradiated amnion dressings. It has been reported that human amniotic epithelial cells do not express HLA-A, -B, -C, and DR antigens or beta 2-microglobulin on their surfaces, thus contributing to the lower inflammatory response to and relatively delayed rejection of this type of biological dressing [33].

Two patients had hypopigmented scars and one had a hyperpigmented scar. These patients had burns confined to the face, hence we were not able to compare the healing nature with covered areas and not exposed to the scorching equatorial sun. Only one of our patients developed hypertrophic scarring. Reports on the prevalence of hypertrophic scarring following treatment with biological dressings are not yet available. This patient also developed hypertrophic scar to the anterior trunk. As the trunk area was not treated with dried irradiated amnion, this could possibly imply that scar hypertrophy is a genetic variable and relatively unaffected by the nature of dressing.

In all cases, the amnion dressings adhered well to the wound surfaces, and needed no extra dressings to help them remain in situ. Eighty-three percent of our patients required only a single amnion application. The dried amnion sheet dressings are thin, pliable and conform well to the wound surface. Application to the wound surfaces was easy, simple and quick as the amniotic membranes were supported with cotton gauze (Fig. 3). No special equipment or skills were required. This reduces not only patient discomfort, but nursing time as well. In contrast, glycerol-preserved membranes had to be spread over the wound surface with the help of plain dissecting forceps. All air bubbles and excess fluid had to be smoothed out to ensure good contact. Ravishanker et al. [18] reported that it takes approximately 1 h for the glycerol-preserved amniotic membrane to dry following application to the wound.

Procurement and storage of amniotic membranes is easy and inexpensive [4,34]. Amnion is “freely” available from the operation theatres. The decreased frequency of dressing changes also contributes to cost reduction. The dried amnion sheets are available for use of the shelf and have a long shelf life.

Several studies have reported the superiority of amnion over topical antibiotic treatment. Ghalambor et al. [35] compared the effectiveness of fresh amniotic membranes with the classical method of antibiotic ointment (nitrofurazone) and gauze bandaging in burned patients. Two cases in the amniotic membrane group and 17 cases in the control classical group developed infections. It was thus concluded that amnion dressing significantly reduced the rate of infection, required less frequent dressing changes, and lowered the costs for both hospitals and patients.

The first clinical trial showing the efficacy and safety of amnion in the treatment of pediatric partial thickness facial



Fig. 3 – Dried irradiated amnion sheet packed in a transparent plastic bag, minimizing storage space and distribution cost. Amnion spread on a thin layer of cotton gauze, allowing easy handling and quick application onto wound.



Fig. 4 – 17-Year-old male with 4% superficial partial thickness facial burn following a scald injury. Dried irradiated amnion applied after wound debridement. Complete wound healing evident on day 7 after-burn.



Fig. 5 – 44-Year-old woman with 4% partial thickness burn after a house fire. She was intubated for inhalational injury. Complete wound healing evident on day 7 post-burn. Follow up at 2 months after-injury shows complete healing with no evidence of hypertrophic scar.

Table 2 – Advantages of using dried irradiated amnion sheets over glycerol-preserved amnion.

Dried irradiated amnion sheets can be used directly after removal from packaging. In contrast, glycerol-preserved amnion needs to be rinsed prior to application.
Dried irradiated amnion is stretched over sterile cotton gauze, hence application to the wound is easy and quick. Glycerol-preserved amnion has to be spread over the wound surface with the help of plain dissecting forceps, hence the application is painful.
Dried irradiated amnion sheets are packed in transparent plastic bags, and are thus easily distributed. Glycerol-preserved amnion is stored in foil pouches or bottles/containers.
Dried irradiated amnion sheets have a long shelf life of 5 years, and are stored at room temperature.
Amniotic membranes irradiated at the recommended dose of 25 kGy are free of microbial contamination.

burns was recently described by Branski et al. [4] at the Shriners Hospital for Children in Galveston, Texas. Patients were randomized to receive fresh frozen amnion and antimicrobial ointment ($n = 53$) or antimicrobial ointment only (1% Nystatin, 2% Polymyxin and Bacitracin) ($n = 49$), and monitored for wound healing. Reapplications of facial dressings were significantly shorter in the amnion group (0.5 ± 2 versus 6 ± 3 in the control group). However, time to healing, length of stay and the development of hypertrophic scarring were not different between the groups. This study indicated that amnion is safe and has advantages in wound coverage for second-degree facial burns, especially among the pediatric patient population.

In contrast, our study utilized dried irradiated amnion sheets without any topical antimicrobial application. There were no significant difference with regards to healing time, infection rate and reapplication rate for the amnion dressing (Figs. 4 and 5). Only one (3%) of our patients developed a hypertrophic scar, whereas Branski reported nine (17%) such cases following treatment with fresh frozen amnion.

We demonstrated that dried amnion sheets fulfill the qualities of an ideal biological dressing. However, amnion also has disadvantages. An amicable understanding and agreement between the obstetrics and burn units is essential as screening for hepatitis, syphilis and HIV need to be performed on the donors. Although the processing of amniotic membranes is carried out under aseptic conditions, the possibility of microbial contamination cannot be excluded [36]. Amniotic membranes irradiated at the recommended dose of 25 kGy have a high sterility assurance level and are microbiologically safe for clinical use [37]. However, gamma irradiation requires specialized and expensive equipment. Although amniotic dressings have a slightly unpleasant odour, no major concern among our patients was reported.

Singh et al. [37] compared the effectiveness of air-dried, gamma-radiated amniotic membranes and glycerol-preserved amniotic membranes. There were no significant differences in the rate of infection, rate of healing or development of hypertrophic scar between the two different types of amniotic biological dressings. Hence, it was concluded that gamma radiation does not adversely affect the clinical efficacy of the amniotic membranes.

5. Conclusion

To date, the effects of dried irradiated amnion in facial burn wound healing have not been reported. This study demonstrated that dried irradiated amnion as a biological dressing

was safe, simple and effective for promoting wound healing and preventing wound infection, especially in the paediatric patient population. Although there was no significant difference in clinical effectiveness between dried irradiated amnion sheets and glycerol-preserved amnion, the former has many advantages (Table 2). It is accepted that irradiation is the most suitable method for the sterilization of tissue allografts.

In developing countries where cultural and financial constraints limit the use of cadaveric skin, xenografts or bioengineered skin substitutes, human amnion is an excellent alternative as it is readily available at a low cost.

Conflict of interest statement

None of the authors have any financial and personal relationships with other people or organisations that could inappropriately create a conflict of interest with any information presented in the manuscript.

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