



Basic research

Dried gamma-irradiated amniotic membrane as dressing in burn wound care

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KEYWORDS

Amniotic membrane;
Air-dried;
Gamma-irradiated;
Storage;
Dressing;
Burn

Abstract *Rationale:* Dried amniotic membrane contains collagen matrix and key bioactive molecules like fibronectin, laminin, glycosaminoglycans and elastin. Fresh and cryopreserved human amniotic membrane has been widely explored as a biological dressing. However, fresh and cryopreserved amniotic membranes are not readily available or require special storage conditions. This investigation was aimed to study the functional and clinical efficacy of air-dried radiation sterilized amniotic membranes as dressing in burn wound care.

Methods: Amniotic membranes collected from placentae of screened donors were processed and sterilized by gamma radiation at 25 kGy. The fluid handling capacity, shelf life and clinical efficacy of air-dried gamma-irradiated amniotic membranes was evaluated.

Results: Fluid handling capacity of the air-dried irradiated amniotic membrane dressing was 3.79–4.2 g/10 cm² in 24 h. Infrared (IR) spectral scanning showed no degradation or change in the dried gamma-irradiated amniotic tissue after 2 and 5 years of storage. No effect of storage on the impermeability of the processed amniotic membranes to bacteria was observed. The dried gamma-irradiated amniotic membranes even after 5 years of storage provided an effective barrier to microbial penetration. The dried amniotic membranes were applied to 22 cases of scald, flame and electrical burns. Of the 22 patients studied, 19 patients had excellent results in the form of complete epithelialization of wound with an average healing time of 15–25 days.

Conclusion: IR studies and microbial permeability test indicate no qualitative changes in the material property of dried gamma-irradiated amniotic membranes after 2 and 5 years of storage. Air-dried amniotic membrane with the advantage of storage at room temperature as well as functional efficiency is an ideal dressing for burn wound care.

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Introduction

The role of skin substitutes in the treatment of burns is constantly evolving. The production and use of

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various types of skin substitutes has led to dramatic improvements in the odds of survival for severely burned patients [1]. Covering the burn wound with a skin substitute helps healing by providing a structural format to the underlying tissue. The general functions of skin dressing are protection of the wound, prevention of infection and promoting healing by providing an optimum microenvironment for healing. Wound desiccation is prevented and pain is decreased. As the dressing changes are performed less frequently, outpatient care is possible with a resultant decrease in both the length of hospital stay and the ultimate cost of burn care. Benefits of wound coverage with dressings for treatment of burns as an alternative method to the topical antimicrobial therapy are reported [2,3].

A variety of biological and bioengineered skin substitutes are available to address the need for burn wound coverage and tissue repair [4–6]. Despite improvements in cell culture techniques and developments in dermal matrices, tissue engineered skin substitutes have yet to achieve widespread use by clinicians. Currently, no engineered skin substitute can replace all of the functions of intact human skin [7]. Biological skin dressings because of their established clinical and commercial niche continue to be the therapeutic option of choice as compared to the bioengineered skin substitutes.

Amniotic membranes are among the most widely used biological dressing [8,9]. Amniotic membranes are obtained from the human placenta and possess most of the characteristics of an ideal skin substitute. It acts as an effective barrier, has good adherence to wound, is bacteriostatic and has no immunological reaction. Amniotic membranes maintain a physiologically moist microenvironment that promotes healing. Fresh amniotic membranes have a short life span as compared to the preserved membranes [9]. There is also possibility of bacterial, fungal or viral disease transmission of donor origin. The use of non-sterilized amniotic tissue is associated with the risk of infectious disease transmission [10]. Amniotic membranes are therefore processed and sterilized for long-term use. Gamma radiation is the most reliable and effective method for sterilization of tissue allografts [11,12]. Sterilization by gamma radiation has been found not to affect the clinical function of the amniotic membrane [13]. This is further supported by the work of Branski et al. [14] who have reported that sterilization with gamma radiation does not significantly affect the growth factor content in human amniotic membrane.

Amniotic membrane, with its unique properties of healing and promoting epithelialization has

been successfully used as a biological dressing. Dried amniotic membrane contains collagen matrix and key bioactive molecules like fibronectin, laminin, glycosaminoglycans and elastin [15]. However, most clinical experiences of human amniotic membrane have been with fresh or cryopreserved tissue. The present study was focused on the properties and clinical evaluation of air-dried gamma-irradiated amniotic membranes for the treatment of burn wounds.

Materials and methods

Processing of amniotic membrane

Amniotic membranes were isolated from the placentae of donors screened based on medical history and physical examination. The donors were tested for syphilis, hepatitis B virus, hepatitis C virus, and human immunodeficiency virus. Processing was done by washing successively the fresh amniotic membranes in sterile normal saline, 0.05% sodium hypochlorite solution and in distilled water. The clean amniotic membranes free of blood clots and debris were air-dried in a laminar flow cabinet to remove 95% of the moisture. Processed air-dried amniotic membranes were sterilized by exposure to 25 kGy of ^{60}Co gamma rays.

Fluid handling properties

The fluid absorbency, moisture vapour permeability, and fluid handling capacity of air-dried unirradiated and irradiated amniotic membranes were determined. The test fluid consisting of a solution of sodium/calcium chloride containing 142 mmol/L of sodium ions and 2.5 mmol/L of calcium ions, values typical of those found in serum and wound fluid was used [16]. For assessing the fluid absorbing capacity, samples of amniotic membrane dressing of known size and weight were placed into beakers of test solution. The dressings were removed from the solution at periodic intervals and gently blotted to remove excess liquid from the outer surface. The membranes were then reweighed. The total amount of fluid absorbed was calculated and expressed as g/10 cm². The change in the moisture vapour permeability of the dressings in the presence of test solution was determined as per the ASTM E96 method [17]. Samples of known weight cut from the dressing were fixed securely over the container containing the test solution. The containers were weighed and placed in an incubator at 37 °C. The containers were reweighed periodically. The loss

in weight due to passage of moisture vapour through the dressing was determined by the difference. The moisture vapour loss (MVL) was calculated as decrease in weight of the container per square meter area of dressing covering the container on 24-hour basis, using the formula $(G/t)/A$, where G is weight loss of the samples (g), t is test time (h), and A is effective membrane area (m^2). The fluid handling capacity (FHC; $g/10\text{ cm}^2$) of the dressing was calculated as the sum of the weight of test solution retained by the dressing and the weight of the fluid lost by transmission through the dressing as moisture vapour.

Storage studies

Shelf life of air-dried gamma-irradiated amniotic membranes was studied. Infrared (IR) spectra of the amniotic membranes stored for 2 and 5 years were recorded using FTIR Spectrometer Model Shimadzu 8101A. Spectral scanning was carried out in the range of $400\text{--}4600\text{ cm}^{-1}$ and % Transmission was measured. For comparison, infrared spectra of fresh unirradiated and irradiated samples were measured.

To evaluate any deterioration of the membrane during storage, the membranes were also tested for microbial impermeability. Impermeability of the amniotic membranes stored for 2 and 5 years was tested to various gram positive and gram negative bacteria. Five strains of bacteria – *Bacillus*, *Escherichia coli*, *Klebsiella*, *Pseudomonas* and *Staphylococcus* were used. Pieces of amniotic membranes were placed on Soyabean Casein Digest Agar plates. Suspensions of bacteria were prepared in sterile water and placed on the opposite surface of membranes and incubated [18]. Plates were checked after 24 h of incubation for growth and ability of the test organisms to migrate across the dressing surface.

Clinical studies

Twenty two patients with burn ulcers were included in the study. The study was approved by the ethics committee of SP Medical College Bikaner and informed consent was obtained from each patient prior to enrolment in the study. Distribution of the subjects according to the age and the extent of the injury are presented in Table 1. 64% subjects were male and 36% female. The healing effect of air-dried radiation processed amniotic membranes was studied on burn wounds comprising of flame, electric and scald burns. Efficacy was recorded as excellent for complete relief of symptoms and complete epithelialization,

Table 1 Number of patients in relation to age and extent of injury.

Age (years)	No. of patients	Extent of injury (TBSA burned)		
		<20%	20–50%	>50%
5–10	2	–	2	–
11–20	9	6	3	–
21–30	6	3	3	–
31–40	1	–	–	1
41–50	2	1	1	–
51–60	2	–	2	–

good for moderate relief of symptoms and epithelialization of >75% of burn area, fair for slight relief of symptoms and epithelialization of 25–75% of burn area and poor for no relief from symptoms and epithelialization of <25% of burn area.

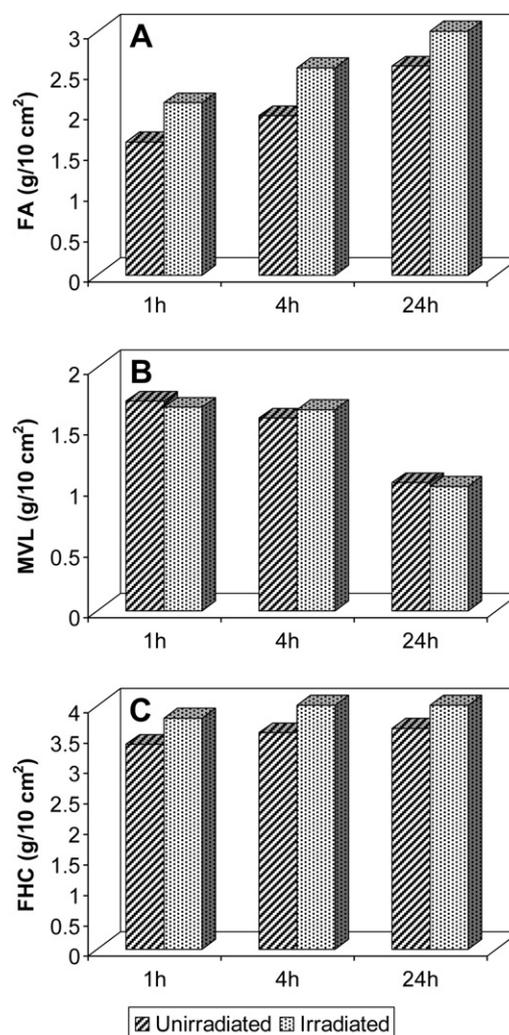


Figure 1 Fluid handling properties of unirradiated and irradiated amniotic membrane. (A) Fluid absorption (FA); (B) Moisture vapour loss (MVL) and (C) Fluid handling capacity (FHC).

Results

Fluid absorption and moisture vapour transmission rate of the amniotic membrane dressings was determined to assess the fluid handling capacity of the dressings. The fluid absorption (FA), moisture vapour loss (MVL) and fluid handling capacity (FHC) of the processed air-dried unirradiated and irradiated amniotic membranes is presented in Fig. 1. FHC was 3.36–3.62 g/10 cm² in 24 h for unirradiated and 3.79–4.2 g/10 cm² in 24 h for irradiated amniotic membranes.

Amniotic membrane samples including freshly processed, processed irradiated and stored samples after irradiation were subjected to IR studies for shelf life evaluation. The processed membranes stored for 2 and 5 years were characterized by FTIR. The qualitative natures of all the spectra are alike (Fig. 2). Amniotic membrane being a collagenous material showed the characteristic amide absorption bands at 1655 cm⁻¹ (amide I), 1551 cm⁻¹ (amide II) and 1239 cm⁻¹ (amide III). IR spectral scanning of stored amniotic membranes as compared to fresh unirradiated and irradiated amniotic membrane showed no degradation or change in the tissue on storage up to 5 years. Impermeability of the amniotic membrane to different bacterial strains – *Bacillus*, *E. coli*, *Klebsiella*, *Pseudomonas* and *Staphylococcus* was tested (Table 2). Amniotic membrane dressings were found to be impermeable to various bacilli and cocci strains. No effect of storage on the impermeability of the dried amniotic membranes to bacteria was observed. The amniotic membrane

dressing provided an effective barrier to microbial penetration up to 5 years of storage.

Dried amniotic membranes were applied to 22 cases of flame, electric and scald burns (Table 3). Out of 22 patients, 19 patients had excellent results with complete epithelialization of wound. 2 patients with flame burn had good results with >75% epithelialization. Slight relief of symptoms and epithelialization of 25–75% of burn area was observed in one case. The time taken for epithelialization ranged from 15 to 32 days.

Discussion

Skin plays a crucial role in the sustenance of life through the regulation of water and electrolyte balance, thermoregulation and by acting as a barrier to external noxious agents including microorganisms. When this barrier is disrupted due to burns, these functions are no longer adequately performed. It is therefore vital to restore its integrity as soon as possible. Biological, synthetic as well as tissue engineered skin substitutes are used to cover the burn wound and help healing by providing a structural format to the underlying tissue. A burn wound dressing needs to provide some basic physical and biological function during its residence in the wound bed. Fluid handling capacity has important implications for the ability of the dressing to cope with exudates production in vivo. Fluid handling characteristics of burn dressing contribute in creation of the microenvironment conducive to the healing of wounds. The fluid handling capacity of the processed

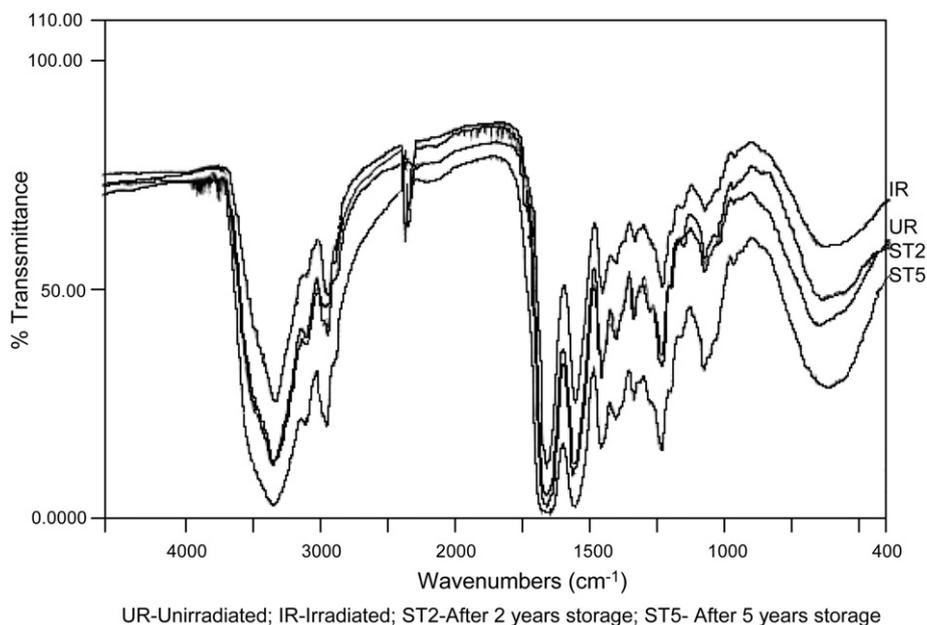


Figure 2 Infrared spectra of amniotic membranes after different storage period.

Table 2 Effect of storage on microbial impermeability of amniotic membrane.

Bacteria	Type	Impermeability	
		After 2 years	After 5 years
<i>Bacillus</i>	Gram Positive, Rod	+	+
<i>Escherichia coli</i>	Gram Negative, Rod	+	+
<i>Klebsiella</i>	Gram Negative, Rod	+	+
<i>Pseudomonas</i>	Gram Negative, Rod	+	+
<i>Staphylococcus</i>	Gram Positive, Cocci	+	+

+ = Impermeable; - = Permeable.

air-dried irradiated amniotic membranes was 3.79–4.2 g/10 cm² in 24 h. The values obtained for the processed amniotic membrane are in agreement with those reported for the evaporative water loss from burns in the order of 5 g/10 cm²/24 h. Thomas and Loveless [19] have reported FHC of 12 different hydrocolloid dressings from <1 to 6 g/10 cm² in 24 h. A burn wound dressing must also constitute a barrier against the external contaminating agents. No effect of storage on the impermeability of the processed amniotic membranes to bacteria was observed. The dried gamma-irradiated amniotic membranes even after 5 years of storage provided an effective barrier to microbial penetration.

Biomedical applications of infrared spectroscopy to diagnose tissues are reported [20]. It has prospects in various fields of biodiagnostics to detect and characterize diseases, tumors and other pathologies. Jackson et al. [21] have reported infrared spectroscopy for the analysis of body fluids and tissues both in vitro and in vivo. Fourier transform infrared spectroscopy has also been used as a method for evaluation of the collagen dressings [22,23]. In the present study, FTIR has

been employed as a characterization technique to evaluate chemical changes in the amniotic tissue on storage. Overall qualitative spectra of the amniotic membranes on storage were similar. No shift in amide I, amide II and/or amide III peak were observed. The degradation or deterioration of amniotic membrane on storage would tend to produce the relative variation in IR absorption troughs. This kind of addendum is absent in the samples after storage of 2 and 5 years. The results are supported by the work of Rosales et al. [24] who have reported that gamma-irradiated human skin allograft can be stored for as long as 24 months at room temperature.

The application of dried gamma-irradiated amniotic membrane on burn wound favoured epithelialization and 86.4% ($n = 22$) patients had excellent results in the form of complete epithelialization of wound with an average healing time of 15–25 days. Amniotic membranes are reported to be useful as temporary biological dressings for the treatment of superficial and partial-thickness burns [25]. Ease of availability, negligible cost and facilitated wound healing makes this temporary biological dressing generally superior to either cadaver skin allograft or pig skin xenograft. The epithelium in human amniotic membrane provides good protection from evaporative loss, as well as barrier function, whereas the fibronectin and collagen matrix provide some dermal function. Amniotic membranes used for temporary coverage of burn wounds exert both mechanical and physiological effects by protecting the wound, maintaining microbial control and hastening wound maturation. A variety of wound dressings are being developed for satisfying the ideal characteristics of a skin dressing. Control of evaporative water loss and prevention of bacterial infection are the most important properties required for a successful burn covering. The results of the present study show that the amniotic membrane dressings have the desired properties of a successful burn wound

Table 3 Epithelialization and dressing duration with amniotic membrane in relation to type and extent of injury.

Type of injury	% TBSA	Number of cases				Dressing duration	
		Total	Epithelialization				
			100%	>75%	25–75%		<25%
Flame Burn	<20%	3	3	–	–	–	15–22 days
	20–50%	6	4	1	1	–	15–32 days
	>50%	1	–	1	–	–	25 days
Electric Burn	<20%	4	4	–	–	–	21–24 days
	20–25%	2	2	–	–	–	15–22 days
Scald Burn	<20%	3	3	–	–	–	15 days
	20–25%	3	3	–	–	–	21–22 days

dressing such as fluid handling properties, microbial impermeability and shelf life.

Conclusions

Amniotic membrane has properties that are helpful in wound healing and has been widely used as a skin substitute. A number of methods to process and preserve amniotic membrane have been used. However, fresh and cryopreserved amniotic membranes are not readily available or require special storage conditions. Infrared spectroscopy studies and microbial permeability test indicate no qualitative changes in the material property of dried gamma-irradiated amniotic membranes after 2 and 5 years of storage. Dried amniotic membrane with the advantage of storage at room temperature as well as functional efficiency is an ideal dressing for burn wound care.

Conflict of interest

None.

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