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The application of glycerol-preserved skin allograft in the treatment of burn injuries: An analysis based on indications

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ABSTRACT

Introduction: Glycerol-preserved skin allograft (GPA) plays a crucial role in the management of burns. Its indications include wound-bed preparation, definitive dressing and sandwich grafting technique.

Objective: We analysed the experience of using GPA and its efficacy in burn treatment in our burn centre.

Methods: All burns managed with GPA in our burn centre from October 2001 to May 2008 were analysed.

Results: Mean total body surface area (TBSA) of 43 consecutive cases was 28.7%. GPA adhered to the wound for an average of 8.4 days before rejection. The length of hospital stay of the survivors was 42.5 days. The autograft take after wound-bed preparation with GPA was 88.4%. For sandwich grafting technique, the autograft take was 74.4%. When GPA was applied for partial-thickness burn as definitive dressing, all patients achieved complete healing within an average of 19 days without further surgical intervention. Despite colonisation of burn wounds after application of skin allograft, the outcomes of autograft take and wound healing were not significantly different.

Conclusion: The selective and strategic use of the GPA in major burn patients ensures optimal benefits in the management of burns. It is versatile in various categories of burn wounds with minimal morbidity.

1. Introduction

Burn causes pathological flux of energy within a tissue, resulting in the disruption of functional integrity [1]. Regardless of the source of energy (thermal, chemical, electrical or radiation), the burn leads to a common pathway underlying the disruption of skin integrity. The skin is no longer able to function as a protective barrier to the environment. In addition to substantial pain and distress, the skin damage also causes exposure to infection, increased evaporative heat loss, as well as loss of body fluids, protein and electrolytes. For major burns, the extensive breach in the epithelial layer may cause systemic physiological derangements, including leakage of intravascular fluids and proteins into the interstitium, hypovolaemic shock and suppression of the immune system. Therefore, re-establishment of the skin barrier is crucial to normalise the victim's physiological state.

The approach to burn wound management varies according to total body surface area involved and the depth of burn. The superficial partial-thickness burn may heal without skin grafting, but it requires application of topical antimicrobial therapy, wound dressing or skin substitute in a moist wound-healing environment. The deep partial- and full-thickness burns, on the other hand, necessitate early tangential excision and autogenous skin grafting to decrease wound infection and mortality. The ultimate goal of burn treatment is to promote

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survival, rapid healing of the wounds, minimal scarring and abnormal pigmentation, with restored quality of life [2].

The use of cadaveric skin allografts as biological coverage or skin substitute in burn management dates back to World War II and is currently being practiced in many major burn centres all over the world [3–6]. Skin banks are also established to address the need for skin allografts in the respective centres. The benefits of using skin allograft in burns have been widely proven in the published literature [2,4,7].

There are two main types of skin allografts, cryopreserved allograft and glycerol-preserved allograft (GPA), which differ in the methods of processing and storage. The cryopreserved allograft was first introduced to treat burn victims in 1979. It is processed by a controlled freezing process (0.5–5 °C min^{-1}) and may be stored in liquid nitrogen at −196 °C or in a freezer at −80 °C. The GPA was introduced by the Euro Skin Bank in 1984; it is preserved in 85% glycerol and can be stored at +4 °C [8]. The process of glycerolisation destroys the vital structures, and, therefore, GPA is considered non-viable. Since glycerol preservation is simpler, more cost-effective and possesses antibacterial and antiviral properties as well as suppressed immunogenicity in allograft [3,8], GPA is more popular and commonly used in clinical practice [9]. To further increase the safety of GPA, it is recommended to expose the allograft to 98% glycerol for at least 4 weeks before clinical use [10].

The GPA was first introduced to the burn centre of the Hospital Universiti Sains Malaysia in 2001. The supply was imported from Euro Skin Bank, Beverwijk, The Netherlands. Lack of local supply and donors led us to use skin allografts in accordance with the particularities of our local set up in order to obtain its optimal benefits. The GPA was applied in our burn clinical practice for three major indications: (1) wound-bed preparation for severe burns before autografting, (2) definitive dressing for partial-thickness burn and (3) sandwich grafting technique of the widely meshed autografts. We analysed the experience of GPA application and its efficacy in treating our burned patients according to indications in our burn-care facility.

2. Patients and methods

This study included all burned patients who were admitted and treated in the burn centre of the Hospital Universiti Sains Malaysia and had been treated with a GPA from October 2001 to May 2008. The data were collected from patient records retrieved from the Medical Record Office and operative records. These patients were categorised based on their indication for GPA application. They were analysed in terms of their profile; total body surface area (TBSA) and depth of burn; operation; and outcomes such as percentage of autograft take, duration of wound healing, length of hospital stay and mortality rate. The statistical analyses were performed using SPSS for Windows package version 12.0.

Upon admission, the burn patients were stabilised haemodynamically with adequate fluid resuscitation according to the Parkland formula. Upon cleansing with antiseptic solution (chlorhexidine) under adequate analgesia, the TBSA of burn was assessed using the Lund and Browder chart. The burn-wound depth was assessed clinically and via laser Doppler imaging (LDI), followed by dressing with non-adherent dressing such as paraffin-impregnated dressing or lipidocolloid dressings. Early nutrition support was begun and intravenous albumin was given as necessary. The final depth of burn was evaluated again 48 h after injury with both clinical and LDI modalities. Subsequently, the decision regarding further surgical intervention or conservative measures was made. The deep partial-thickness and full-thickness burn wounds were covered with silver-impregnated dressings (e.g., Aquacel Ag® or Acticoat®) while waiting for the operative procedures.

2.1. GPA as a skin substitute in wound-bed preparation

In deep partial- or full-thickness burns, the burn eschar was tangentially excised until the appearance of viable tissue. GPA was applied to the burn wounds after excision to test the wound-bed readiness to take the skin graft. Secondary dressings that were applied above GPA consisted of paraffin-impregnated or lipidocolloid dressings, chlorhexidine-soaked gauze, dry gauze and crepe. If the outer dressing was soaked, it would be changed daily. The wounds were inspected every 3–5 days. If GPA adhered well to the wound bed, only the outer dressing was changed. If there were any signs of local wound infection, dressing would be changed to silver-impregnated dressings. The systemic antibiotic (cephalosporin groups) therapy was also started empirically and later adjusted based on the availability of the wound culture results.

When GPA adhered and was vascularised on the wound bed, with bleeding on removal, the wound bed was considered ready for autografting. If GPA did not adhere or was rejected, deep-wound swabs were taken for culture. This may be due to infection or a non-viable surface resulting from inadequate tangential excision. Further tangential excision was performed as needed and new GPA was applied. Subsequent autogenous graft take was documented.

2.2. Sandwich grafting technique

In the sandwich grafting technique, GPA was used to immediately overlay the widely meshed split-skin grafts, or 5 days after tangential excision in case of the Meek micrografts, when adherence to the wound bed allowed removal of the pre-folded gauze carriers. Secondary dressings over the GPA consisted of paraffin-impregnated, lipidocolloid or silver-impregnated dressings, followed by chlorhexidine-soaked gauze, dry gauze and crepe. The outer dressing was changed daily if soaked. The wounds and grafts were inspected every 3–5 days. If GPA did not adhere to the wound bed, it was re-applied. Deep-wound swabs were taken for culture when the GPA was sloughing or rejected. Local wound infections were treated with silver-impregnated dressings and systemic antibiotics as stated above. The percentages of autograft take and wound healing were assessed.

2.3. Definitive dressing in partial-thickness burn

At 48 h after injury when the assessment of depth of burn was verified superficial partial-thickness burn, the patients were treated conservatively with non-surgical measures. GPA was

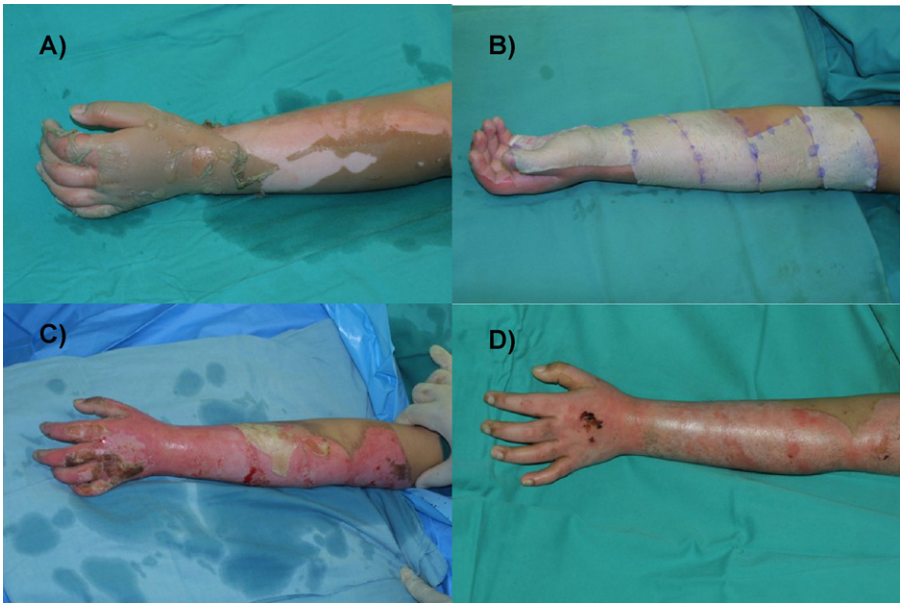

Fig. 1 – (A) A 5-year-old girl with flash burn due to hydrogen gas balloon explosion resulting in 14% partial-thickness burn on both upper limbs and face. (B) Sheets of GPA were applied over the left upper limb and secured with tissue glue. (C) After 10 days of GPA application, the GPA loosened and was removed with ease. The burn wound had achieved 97% epithelialisation. (D) By day 13 postburn, the wound had healed completely without limitation of joint movement.

applied to the wounds as definitive dressing, was secured with stapler or tissue glue and reinforced with absorptive secondary dressings. The wound inspection was performed every 5–7 days and only the secondary outer dressings were changed (Fig. 1).

3. Results

3.1. *Patient general data*

From October 2001 to May 2008, 477 burn victims received treatment from the burn centre at the Hospital Universiti Sains Malaysia. A total of 43 consecutive cases of burns were managed with the application of GPA (Table 1). There were 27 male and 16 female patients. Nineteen (44%) of them were paediatric patients less than 12 years old. The overall mean age was 23.3 years. Most patients (83.7%) sustained deep partial- or full-thickness burns. The mean TBSA of burn was 28.7 ± 18.5%, ranging from 3% to 70%. The burns were most commonly secondary to flame burn (55.8%), followed by hot water scalds (27.9%), chemical burn (2.3%), electrical burn (2.3%) and others (11.6%).

The mean and median times of admission after burn injury were day 2.5 and day 0, respectively. Twenty-eight

Table 1 – Profile of burn patients who received glycerol-preserved skin allograft from October 2001 until May 2008.

	Wound-bed preparation	Sandwich grafting technique	Definitive dressing	Overall
Number of patients	29	9	5	43
Gender				
Male	19	5	3	27
Female	10	4	2	16
Age in year [mean (range)]	28.0 (0.9–85.0)	16.3 (0.7–64.0)	8.2 (0.8–33.0)	23.3 (0.7–85.0)
Percentage of TBSA of Burn [mean ± S.D. (range)]	32.8 ± 19.9 (6.0–70.0)	26.0 ± 10.7 (16.0–45.0)	10.0 ± 6.4 (3.0–16.0)	28.7 ± 18.5 (3.0–70.0)
Depth of burn				
Superficial partial-thickness burn [N (%) of patients]	1 (3.4%)	1 (11.1%)	5 (100%)	7 (16.3%)
Deep partial-thickness burn [N (%) of patients]	14 (48.3%)	4 (44.4%)	0	18 (41.8%)
Full-thickness burn [N (%) of patients]	14 (48.3%)	4 (44.4%)	0	18 (41.8%)

Note: TBSA = total body surface area; N = number of patients; S.D. = standard deviation.

Table 2 – Clinical data of burn patients treated with glycerol-preserved skin allograft for wound-bed preparation.

	Survivors	Mortality	Overall
Number (%) of patients	17 (58.6%)	12 (41.4%)	29
Percentage of TBSA of burn [mean ± S.D. (range)]	20.9 ± 9.1 (6.0–40.0)	49.6 ± 19.0 (15.0–70.0)	32.8 ± 19.9 (6.0–70.0)
Number of application of GPA per patient [mean ± S.D. (range)]	1.5 ± 0.7 (1–3)	1.9 ± 1.7 (1–6)	1.7 ± 1.2 (1–6)
Number of body regions involved per patient [mean ± S.D. (range)]	2.6 ± 1.0 (1–5)	2.9 ± 1.2 (1–4)	2.7 ± 1.1 (1–5)
Duration of GPA adherence to wound bed [mean ± S.D. (range)] (days)	8.1 ± 2.0 (5.0–13.0)	7.3 ± 2.0 (5.0–11.0)	7.9 ± 2.0 (5.0–13.0)
Application of GPA (number of patients)			
1 application	11	8	19
2 applications	4	2	6
3 applications	2	0	2
4 applications	0	0	0
5 applications	0	1	1
6 applications	0	1	1
Autografting following GPA (Number of patients)			
1 procedure	10	N/A	N/A
2 procedures	6	N/A	N/A
Defaulted	1	N/A	N/A
Percentage of autograft take after GPA [mean ± S.D.]	88.4 ± 13.6	N/A	N/A
Duration of complete wound healing [mean ± S.D. (range)] (days)	38.7 ± 18.0 (19 – 78)	N/A	N/A
Length of hospital stay [mean ± S.D. (range)] (days)	42.9 ± 20.2 (16 – 80)	N/A	N/A

Note: N/A = not applicable.

patients (65.1%) were admitted within 24 h of injury whereas another five patients (11.6%) were admitted on day 1 after injury. The rest of the patients (23.3%) were admitted from day 2 to 30 of injury mostly after initial resuscitation and treatment in other district or general hospitals before being referred to our institution for further wound management.

Two-thirds of these patients (29 patients) were indicated for wound-bed preparation. Nine patients (20.9%) were treated with GPA according to the sandwich technique to protect the meshed split-skin graft or the Meek micrograft. GPA was used as definitive dressing in the remaining five patients. GPA adhered to the wound bed for a mean of 8.4 ± 3.1 days before detachment. The length of hospital stay in the survivors was 42.5 days. The overall mortality rate was 30.2% (13 patients).

3.2. *GPA as skin substitute in wound-bed preparation*

A total of 46 sessions of GPA application were performed on 29 patients with mean TBSA of burn of 32.8 ± 19.9%, for the purpose of wound-bed preparation prior to autografting (Table 2). The duration of GPA treatment and adherence was 7.9 ± 2.0 days, ranging from 5 to 13 days. The majority of patients (65.5%) required only one application of GPA.

There were 22 autografting procedures performed at a mean of 8.9 ± 3.7 days (range: 4–18 days) following GPA application for wound-bed preparation in these survivors. Eleven patients were grafted with meshed grafts, three patients with Meek micrografts and two patients with both meshed and Meek grafts. The percentage of autograft take was 88.4 ± 13.6%. However, six patients (37.5%) required a second autografting due to graft failures. Complete wound healing was achieved

Table 3 – Clinical data of burn patients applied with glycerol-preserved skin allograft for sandwich grafting technique.

	Meshed skin graft	Meek micrograft	Overall
Number of patients	4	5	9
Number of applications of GPA per patient [mean ± S.D. (range)]	1.3 ± 0.5 (1.0–2.0)	1.8 ± 1.1 (1.0–3.0)	1.6 ± 0.9 (1.0–3.0)
Duration of GPA adherence to wound bed [mean ± S.D. (range)] (days)	14.8 ± 4.3 (10.0–20.0)	5.8 ± 1.6 (3.0–7.0)	9.8 ± 5.5 (3.0–20.0)
Percentage of autograft take [mean ± S.D. (range)]	92.5 ± 6.5 (85.0–100.0)	60.0 ± 37.9 (20.0–95.0)	74.4 ± 32.1 (20.0–100.0)
Healing of autografting (number of patients)			
Complete healing without re-grafting	3	1	4
One re-grafting	1	3	4
Two re-grafting	0	1	1
Duration of complete wound healing [mean ± S.D. (range)] (days)	35.0 ± 16.3 (22.0–58.0)	62.8 ± 25.3 (27.0–82.0)	48.9 ± 24.6 (22.0–82.0)
Length of hospital stay [mean ± S.D. (range)] (days)	55.5 ± 15.3 (38.0–74.0)	75.5 ± 43.5 (29.0–134.0)	65.5 ± 32.0 (29.0–134.0)
Mortality (Yes/No)	0/4	1/4	1/8

Table 4 – Positive versus negative wound culture following application of glycerol-preserved skin allograft.

	Wound-bed preparation	Sandwich grafting technique	Definitive dressing	Overall	Statistical Significance
Number (%) of patients					
Positive culture	23 (79.3%)	8 (88.9%)	1 (20.0%)	32 (74.4%)	
Negative culture	6 (20.7%)	1 (11.1%)	4 (80.0%)	11 (25.6%)	
Percentage of autograft take [mean ± S.D.]					
Positive culture	84.6 ± 20.1	76.9 ± 27.6	N/A	81.9 ± 22.7	$p = 0.808$*
Negative culture	87.5 ± 17.7	90.0 ± 0	N/A	88.3 ± 12.6	
Duration of GPA adherence to wound bed [mean ± S.D.] (days)					
Positive culture	7.7 ± 2.1	8.8 ± 3.9	4.0 ± 0	7.9 ± 2.8	$p = 0.009$*
Negative culture	8.8 ± 1.6	20.0 ± 0	12.0 ± 2.6	11.1 ± 4.1	
Duration of complete wound healing [mean ± S.D.] (days)					
Positive culture	34.0 ± 16.6	52.7 ± 23.9	23.0 ± 0	39.5 ± 20.6	$p = 0.123$*
Negative culture	40.0 ± 35.4	22.0 ± 0	17.0 ± 4.2	29.3 ± 25.4	

Note: N/A = not applicable.
* p-Value using Mann–Whitney test.

after 38.7 ± 18.0 days, ranging from 19 to 78 days. The length of hospital stay averaged 42.9 days.

The mortality rate in this group of patients was 41.1%. These patients sustained severe burns with mean TBSA of 49.6%. The most common causes of death were sepsis and acute respiratory distress syndrome. Two of these patients required five and six applications, respectively, of GPA due to extensive burns requiring multiple stages of operations in several body regions.

3.3. *Sandwich grafting technique*

A total of 14 sandwich grafting procedures were performed on 17 wounds in nine patients for whom TBSA of burns averaged 26.0%, ranging from 16% to 45% (Table 3). The split-skin grafts were principally meshed in 1:3 ratio, and the GPA was applied during the same procedure. The Meek micrograft had an expansion ratio of 1:3, 1:4 or 1:6 and was covered with GPA on day 5 after removal of the gauze carrier. The entire GPA was already meshed in a ratio of 1:1.5, as purchased from the Euro Skin bank.

The mean autograft take was 74.4%. There were four patients (44.4%) who had complete wound healing after the sandwich technique; another four patients required one repeat autografting. The average duration of healing was 48.9 days, and the mean length of hospital stay was 65.5 days.

Table 5 – Wound culture isolates of wound beds that received GPA.

	Number of patients	Percentage
Pseudomonas sp.	14	32.6%
Mixed growth	9	20.9%
Acinetobacter sp.	4	9.3%
Enterobacter	3	7.0%
MRSA	2	4.7%
No growth	11	25.6%

Note: MRSA = Methicillin resistant *Staphylococcus aureus*.

Eight patients recovered from their burns, and only one patient (11.1%) died due to sepsis.

3.4. *Definitive dressing in partial-thickness burn*

There were five patients with superficial partial-thickness burn (average 10% TBSA) treated with GPA as definitive dressing. Four patients achieved complete healing at an average of 19 days without further surgical intervention, whereas one patient was lost to follow-up. The GPA adhered to the wound bed for an average of 10 days before detachment. The mean length of hospital stay was 16.6 days. There was no mortality in this group of patients.

3.5. *Wound swab culture results following application of GPA*

Wound swab cultures from burn wounds, when the GPA rejected, were positive for bacterial growth in 32 patients (74.4%) among the overall patient population. The positive culture results were not significantly different between those admitted within 24 h of injury and those admitted after 24 h of injury (71.4% vs. 80.0%; p-value was 0.719). When GPA was used for wound-bed preparation and sandwich grafting, the cultures were positive in 79.3% and 88.9% cases, respectively. Instead, only one patient was positive for culture when GPA was applied as definitive dressing (Table 4). The most common organisms isolated were *Pseudomonas* sp., followed by mixed growth, *Acinetobacter* sp., *Enterobacter* and methicillin-resistant *Staphylococcus aureus* (Table 5).

Overall, the duration of GPA adherence to the wound bed was shorter (7.9 days) when the wound cultures were positive as compared to negative culture (11.1 days). This difference was statistically significant ($p = 0.009$). The percentage of autograft take was lower when wound cultures were positive (81.9% vs. 88.3%). Complete wound healing was achieved later if the wound cultures were positive (39.5 days vs. 29.3 days). However, the differences in percentage of autograft take and duration of complete healing were not statistically significant.

4. Discussion

The properties of an ideal burn dressing can be summarised in four P's: protection, proteolytic effect, promotion of healing and pain relieving [2]. Cadaveric skin allograft possesses several key characteristics of an ideal wound dressing, including good adherence to the wound bed, water vapour transport, antimicrobial characteristics, low toxicity and antigenicity, ease of application and removal, a long shelf-life and minimal storage requirements [6,11]. There are other benefits of skin allograft application, such as decreased loss of water, electrolyte and protein. Skin allograft application also reduces pain and thus allows exercise and ambulation, and it decreases the incidence of contractures [12]. With these features, human cadaveric skin allografts still play a crucial role in the treatment of burn, despite emergence in the market of a wide variety of skin substitutes and biological dressings. The principal indications of skin allograft in burn management include: (1) temporary cover on freshly excised wounds; (2) sandwich grafting technique to overlay widely expanded autografts and (3) its use to improve the quality of the wound bed prior to autografting [3,6,9]. Skin allograft is also used as interim covering following burn-scar release [3].

Before the availability of GPA in our centre, we generally used conventional dressing for coverage of burn wounds in patients. These dressings consisted of daily paraffin-impregnated dressing or silver sulphadiazine cream, gauze and crepe. We encountered difficulties of providing early complete tangential excision and skin grafting in severe burns. With the limitation of donor skin, the freshly excised burn wounds were grafted with widely meshed split-skin grafting. The graft take was not remarkable in this group of patients. In addition, the staged tangential excision and coverage with the conventional dressings exposing the wounds for long period might be complicated by burn-wound infection and sepsis. The graft take on the wound beds that were not optimised and prepared might end up with poor graft take. Furthermore, an increased loss of proteins, fluids and electrolytes from the burn wounds that were covered with conventional dressings was noted. Haemodynamic stability and control of infection were not easily achieved, especially in those of extreme ages (very old or very young). Frequent changes of conventional dressings were required, resulting in distress. With these problems on hand, we decided to apply GPA in the management of burn patients in selected cases in our centre since 2001. This strategy has been proven to have positive impact on the outcome in our patients.

Due to the high cost and limited availability of GPA at our centre, our approach was to select patients according to indications. The major-burn patients with majority deep partial- to full-thickness burns and limited availability of autograft donor sites represented the priority in our patient selection. This is reflected in our patient population with average $28.7 \pm 18.5\%$ TBSA of burn and deep partial- or full-thickness in depth in 83.6% of cases. Extreme age group patients, either children or the elderly, whose lower ability to withstand the severe metabolic stress of burn subjected them to higher mortality and morbidity, also required management with GPA as the best available option of skin substitutes. With application of GPA, we ensured that these groups of patients were optimised for the best chances of autograft take on the first attempt. Otherwise, autograft failure may lead to the grave consequences of repeated autografting with further waste of the donor autograft, wound infection, sepsis or even mortality. GPA is also considered in patients in whom frequent dressing changes are to be avoided, especially among the paediatric age group. To our knowledge, analysis of these strategic approaches has not been reported previously.

In our centre, GPA is applied to cover the partial-thickness burn as definitive biological dressing until the underlying burn wounds have epithelialised. The dressing changes only involve outer secondary dressing without causing unnecessary trauma and pain. These painless and easy dressing changes have been noted in earlier studies [3]. Our observations showed that this benefit is particularly important in children with burns in order to avoid the physical and psychological impact associated with frequent and painful dressing changes. None of our patients required further surgical intervention (autografting) when covered with GPA as definitive dressing. This result is much better than those obtained by Vloemans et al. (15% out of 57 treatments) [13], Peeters et al. (31% out of 84 patients) [14] and Brans et al. (53% out of 45 patients) [15]. Unnecessary tangential excision with sacrifice of healthy dermal tissue layer and blood loss was avoided in these partial-thickness burns. The preserved dermal layer, even if it is thin, is crucial for wound healing with minimal hypertrophic scarring, as observed in our patients. However, the small number of patients and patient selection bias in our study should be considered. As local factors such as desiccation, oedema, infection and hypoxia may cause secondary deepening of a burn wound [16,17], the protective barrier effects of GPA prevent progression of superficial partial-thickness burn to deep partial-thickness or full-thickness burn. Epithelialisation of the underlying wound bed promoted by GPA could be another contributing factor.

Early tangential excision and grafting of burn wounds are the primary treatment in deep partial- and full-thickness burn. However, there are situations that restrict immediate autografting after tangential excision, such as difficulty in diagnosing burn depth (e.g., in chemical and electrical burn), limited donor sites in severe burns and poor general condition of patients, that preclude the harvesting of autograft in the same operation. The freshly excised burn wound could be optimised and conditioned to prepare for subsequent autografting by application of skin allograft. This is in accordance with the concept of wound-bed preparation, as defined by Falanga, with the overall goal of optimising the wound bed and ensuring unimpeded wound repair [18]. The mechanisms of skin allograft in wound-bed preparation could be attributed to the increase in vascularity, including promoting angiogenesis with enhanced capillary in-growth on the wound bed [2,6]. A viable skin allograft can re-vascularise by inosculation just like autologous split-skin graft. In addition, skin allograft can provide growth factors and essential cytokines while creating chemotaxis and proliferation at the wound beds. When GPA was used to condition the burn wound bed after tangential excision in burn patients in our study, a satisfactory autograft take of 88.4% was achieved. Only one repeated autografting was required in 37.5% of the patients.

Meek micrografting and widely meshed skin graft are employed for extensive, deep partial- or full-thickness burns with limited availability of donor site of autogenous skin. Sandwich grafting technique denotes overlay of the autografts with the skin allograft. The allograft prevents desiccation of the wound bed in the interstices of widely expanded autografts and also reduces bacterial colonisation [6]. Epithelialisation of the wound bed also has been accelerated when GPA is applied [19]. We observed that the adherence of allograft also protected the autografts from shear, thus improving the chances of take. In our study, complete healing without re-grafting was achieved in 44.4% of patients. This result is better than that from the study by Vloemans et al. (23.5%) [3]. The mean autograft take was 74.4%. All our patients had complete wound epithelialisation by 7 weeks. The mean duration of hospital stay of 65.5 days was comparable to that in other studies (56–72 days) [3,19]. In the study conducted by Dhennin, in 29 severe burn patients who were treated with GPA as sandwich graft, the mean wound closure rate was 88%, and 89% of the operations resulted in wound healing without need for a second procedure [5].

It is generally believed that skin allograft possesses the antimicrobial properties of controlling bacterial invasion and proliferation in the wound beds by acting as a mechanical barrier and through glycerol antimicrobial effects [2,6,12]. However, this is in contrary to our finding of positive culture results in 74.4% patients when the GPA was sloughy or rejected, especially our observations of *Pseudomonas* sp. and other Gram-negative bacteria. These positive cultures were independent of timing of early or late admission of our patients. The problem of burn wound bed colonisation with *Pseudomonas* bacteria was also noted by Blome-Eberwein et al. in their study, but the incidence was not stated [8]. Another quantitative microbiological analysis on burn wound beds beneath adherent allograft in 21 patients by Greenleaf et al. revealed 21% infected wound beds ($>10^5$ colonies per gram) and 30% colonised wounds ($<10^5$ colonies per gram) [20]. To prevent the potential of superimposed infection in selected cases, we applied a secondary antiseptic-soaked dressing (e.g., chlorhexidine) or silver-impregnated dressing (e.g., Aquacel Ag® or Acticoat®) over the skin allograft. The significance of this approach will be further investigated in future randomised controlled studies.

Our results show that despite the presence of bacterial colonisation in the wound bed following application of GPA, there is no statistically significant difference between the wound culture-positive and -negative patients in the outcomes of percentage of autograft take and duration of complete wound healing. These results indirectly showed that the bacterial proliferation in the wound bed was just colonisation and did not progress to clinically significant wound infection that adversely affected the autograft take and prolonged wound healing.

Once adhered to the burn wound beds, skin allograft may exhibit features of re-vascularisation as part of the process of 'take'. The incorporation is at the level of dermal collagenous matrix [21]. Clinically, the peeling off or non-adherence of skin allograft to the wound bed is judged clinically as a sign of rejection apart from its dry, sloughy or necrotic appearance, although no tissue biopsy is taken for confirmation of rejection in histology. The skin allograft is known to be more susceptible to rejection than other tissue and organ allograft due to the skin's unique intrinsic immunological features, including high concentrations of Langerhan's and other dendritic cells as antigen-presenting cells, and extracellular matrix glycoproteins that position the T cells for activation and effector functions [22]. In attempts to use the skin allograft as a longer term substitute for the patient's skin, various methods have been developed to prevent or delay rejection of allograft, either by modifying the recipient immune response, or by reducing the allograft antigenicity [23,24].

Detachment of GPA was also observed when the underlying burn wounds had re-epithelialised. Similar to the observation by Blome-Eberwein et al. [8] the superimposed infection was also noted to accelerate the detachment of GPA. This is supported by our findings that the positive culture did shorten the duration for which GPA adhered to the wound bed: 11.1 versus 7.9 days ($p = 0.009$).

Overall, the duration of GPA adherence to the wound bed in our study of 8.4 days is comparable to the study by Vloemans et al. (8 days) [3]. Burd et al. noted that the GPA adhered between 1 and 2 weeks when applied to six children with partial-thickness burns [21]. When compared with other clinical studies using cryopreserved skin allograft, the duration of GPA adherence is not markedly different. Eldad et al. had demonstrated that cryopreserved skin allograft adhered for an average of 11 days to the partial-thickness burn wounds of 12 patients after mechanical debridement without surgical excision [25]. See et al. observed that cryopreserved skin allograft adhered well to clean and debrided wounds for 4–7 days when applied to 17 severe burn patients [26]. Our result did not support previous study [27,28] that claimed GPA to be less antigenic with decreased rejection reaction than cryopreserved skin allograft. However, a randomised comparative study is needed to further quantify the difference. Our experience found that GPA is easy to handle and store, as widely recognised in other centres [3,8,29]. These advantages made it more preferable than cryopreserved skin allograft in our centre.

5. Conclusion

With unique biological characteristics, GPA is applied in our centre for wound-bed preparation before autografting, as definitive dressing for partial-thickness burn and sandwich grafting technique. The selective and strategic usage of the skin allograft in major burn patients, especially those of extreme ages, ensures optimal benefits in the management of burns. Colonisation of burn wounds after application of skin allograft does not significantly affect the outcomes of autograft take and wound healing. The versatility of GPA for usage in various categories of burn wounds proved to be effective with minimal morbidity.

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