The Use of Glycerol-Preserved Skin Allograft in Conjunction with Reconstructive and Flap Surgery: Seven Years of Experience

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ABSTRACT

Major reconstructive surgery may be extensive and prolonged, and it may cause edema and compromise the flap pedicle if closed under tension. Glycerol-preserved skin allograft (GPA) can provide a means for tension-free closure and temporary cover of the wound. Seven years of analysis on GPA used in conjunction with major reconstruction was undertaken to highlight its indications, results, and outcomes. Forty-seven patients were included, aged between 9 and 73 years. Majority of patients had reconstruction following tumor resection and trauma. The main indication for use of GPA was temporary, loose cover of the wound in 44% of cases; flap pedicle protection in 31% of cases; donor site wound cover in 10%; flap monitoring in one case; and management of flap-related complications in 6% of cases. Free flap reconstruction was performed in 72% of cases. In conclusion, GPA is a useful adjunct in reconstructive surgery. It can be used temporarily to allow tension-free wound closure, as well as to protect the flap pedicle until edema subsides and the pedicle becomes stable. This latter approach allows secondary wound closure and good esthetic outcome.

KEYWORDS: Glycerol-preserved allograft, skin transplantation, free flap, reconstructive surgery

Skin allograft has been used as a temporary wound cover for more than a century since first reported by Reverdin in 1869.[1] It is used mainly in burn victims with deficient donor sites for temporary wound closure after tangential excision, as an overlay of widely meshed autograft, and as a dermal substitute.[2] The skin allograft is also used outside the scope of burn surgery, but in these contexts it is not similarly popular. It can be used in the treatment of chronic ulcers, complex traumatic wounds, and virtually any wound that cannot be closed immediately for any reason.[3,4]

Skin allograft is available in several forms. Fresh skin allograft is believed to be the best option,[5] but resources are limited and it can be difficult to obtain. Cryopreserved allograft and glycerol-preserved allograft (GPA) are other forms of skin allograft that are harvested, processed, and stored. This makes them more readily available compared with fresh skin allografts.

The GPA was introduced by the Euro Skin Bank, Beverwijk, The Netherlands in 1984[3]; it is preserved in 85% glycerol and can be stored at +4°C.[1] On the other hand, the cryopreserved allograft was first introduced to

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treat burn patients in 1979.[6,7] It is processed by a controlled freezing process (0.5 to 5°C/min) and may be stored in liquid nitrogen at −196°C or in a freezer at −80°C.[1,5–7] Therefore, the cost of GPA production and storage is less expensive if compared with cryopreserved allograft.[5]

The glycerol used in the preservation process maintains the structural integrity but renders the allograft nonviable by destroying the vital structures of the cell and therefore suppresses the immunogenicity in the allograft.[1–3,6,7] The low antigenicity of the GPA results in less rejection, and repeated applications are possible without the occurrence of a second set of reaction.[3,6,8] In the cryopreserved skin allograft, the skin remains viable and therefore did not eliminate the antigenic properties.[1,6,8,9]

Though the risk of viral transmission via skin allograft from properly screened donors remains theoretical,[10] glycerol preservation is believed to reduce this risk.[6] Cryopreserved skin allograft that maintains the functional integrity of the allograft does not inactivate HIV.[11] The glycerol preservation process has been shown to reduce intracellular virus infectivity depending on glycerol concentration, time, and temperature.[5,11–13] This process is also shown not to elicit inflammation or toxicity to the tissue.[5]

We used GPAs that have been available through Euro Skin Banking since 2001. The main indications are for major burn injuries. At our institution, GPAs are also used in conjunction with major reconstruction, especially for free tissue transfer and for oncology and trauma cases requiring flap surgery.[14] Because our institution is the country's main referral center for oncology surgery and reconstructions, most cases are presented at advanced stages and require major resection and reconstruction. Prolonged surgery is involved, and usually there is associated tissue edema and swelling. This affects wound closure, especially if the flap pedicle is in an area where pressure or compression may occur if the wound is closed under tension. In this situation, GPA can be utilized as a temporary wound cover while waiting for the tissue swelling to subside. This process allows the pedicle or vascular anastomosis to become more stable. GPA can be removed later and the wound can be closed directly, partially, or with split-thickness skin graft. This will result in better esthetic outcomes and reduce the risk of the flap being compromised. The purpose of this study is to demonstrate how the GPA can be used in the reconstructive setting and its indications, results, and outcomes.

METHODS

This study included all cases admitted to our institution that had the application of GPA in conjunction with flap surgery during the period from 2001 to 2008. Data regarding patient demographics, diagnosis, indication, percentage of graft take, ultimate wound closure, and cover were collected from the relevant cases. The skin allografts were purchased from the Euro Skin Bank in the Netherlands. In general, GPAs were secured to the wound bed either by skin staplers or by sutures and occasionally with the aid of a "glue-stitch" (a topical skin adhesive: butyl-2-cyanoacrylate; Histoacryl®, B. Braun Aesculap, Tuttlingen, Germany). We used standard dressing with paraffin tulle, as well as moist and dry gauzes. Regular dressing changes were performed when necessary until definitive wound closure or cover could be provided.

RESULTS

In the 7-year period, 47 patients were included in the study. The age of the patients ranged from 9 to 73 years, with median and average age of 33 and 34 years, respectively. The male-to-female ratio was 1:1. The main diagnoses of the patients can be generally divided in four groups: reconstructive surgery for malignancy (55%), traumatic injury (31%), arteriovenous malformation (11%), and infective causes (2%).

The reconstructions were performed with pedicle flaps in 13 cases (27.6%) and free flaps in 34 cases (72.4%). The most common flaps used were the fibula osteocutaneous free flap (14 cases) and latissimus dorsi myocutaneous flap (free flap, 10 cases; pedicle flap, five cases). Other flaps were used much less frequently as shown in Table 1.

The indications for the use of GPA were temporary wound cover in 21 patients (44%), flap pedicle protection in 17 cases (36%), donor site cover in 5 patients (11%), management of flap-related complications in three cases (6%), and monitoring partially exteriorized free jejunal transfer for esophageal reconstruction in one case. The indications for its use are further elaborated in Table 2. In nine of the patients, GPAs were applied after emergency exploration of the flap for vascular and anastomosis-related problems.

The use of GPA as a temporary cover in this study was usually associated with prolonged surgery or extensive tumor resection that caused tissue edema and swelling. GPA was utilized as a prophylactic measure to achieve tension-free closure, hence preventing flap pedicle-related complications.

The total amount of GPA used in all cases was 6980 cm^2, and the amount used on the patients ranged from 50 to 475 cm^2 (median = 120 cm^2, average = 148 cm^2). The length of time for which the GPAs were left on the wound varied from 2 to 30 days (average = 15 days, median = 15 days). In most cases, the GPA was left in situ until edema and swelling subsided and until the anastomosis became stable in the case of free tissue transfer. There were three cases where the GPA was

Table 1 Type of Flaps Used for Reconstruction in Conjunction with GPA Application

Flap Type			No.
Free	Simple	LD myocutaneous flap	6
		Anterolateral thigh fasciocutaneous flap	4
		Gracilis myocutaneous flap	2
		Jejunal	1
		Mini-TRAM	1
		Radial forearm	1
	Neurotized	Motorized LD myocutaneous flap	2
	Composite	Fibula osteocutaneous flap	13
	Compound	LD and serratus anterior flaps	1
		Fibula osteocutaneous and soleus flaps	1
		Tensor fascia lata and vastus lateralis flaps	1
	Sequential	LD and lateral thoracic fasciocutaneous flaps	1
Subtotal			34
Pedicle	Simple	LD myocutaneous flap	5
		Temporalis fascia flap	3
		Pectoralis major myocutaneous flap	2
		Deltopectoral fasciocutaneous flap	1
		Gastrocnemius myocutaneous flap	1
		Leg—local fasciocutaneous	1
Subtotal			13
Grand total			47

GPA, glycerol-preserved skin allograft; LD, Latissimus dorsi; TRAM, transverse rectus abdominus myocutaneous.

removed before 1 week had passed. This was necessary in two patients for emergency exploration of the flap pedicle due to arterial thrombosis and bleeding from the anastomosis. In the other case, early wound cover with another flap was performed for definitive wound cover after skin paddle necrosis in the fibula osteocutaneous free flap, which had been debrided earlier.

In this series, the dressing was changed quite frequently in the first few days, either daily or on alternate days due to significant amounts of serosanguineous fluid discharge from the nearby opened drainage tube. Once the amount of fluid drainage had decreased, wound inspection and dressing changes became less frequent, extending to longer intervals of 3 to 5 days. The percentage of allograft "take" ranged from 0 to 100%. Good graft take was seen in 63.8% of cases, moderate in 23.4%, gradual sloughing off with time after prolonged application in 4.2% (two patients), and complete loss in 8.4% (four patients). Complete allograft failure was due to pseudomonas infection in one case. The other three cases were due to application on flaps that failed and became necrotic.

Final wound closure or covers achieved by direct closure were observed in 17% of cases, combination of partial closure and split-skin graft (SSG) in 17%, SSG alone in 51%, new flap in 8.5%, and no definitive cover in two cases. Among cases with no definitive cover, the wound was allowed to heal by secondary intention in one case, and the other case was due to mortality. In the cases where the wounds were partially or completely covered by SSG, we observed a reduction in the size of the wound and the amount of autograft used; better aesthetic results were seen with the GPA if compared with the larger amount of the autograft that needs to be used immediately in the first setting (Fig. 1).

DISCUSSION

The use of skin allograft as a biological dressing has long been recognized to have multiple advantages.[1–6,15] The main advantages of skin allograft include a physiological barrier that decreases the loss of water, electrolytes, proteins, and heat from the wound bed. Skin allograft also creates a mechanical barrier that reduces microbial contamination, hence reducing the chance of wound infection.[5] Based on these properties, skin allograft can prevent drying of the wound and also prevent wound bed deterioration.[3] The ability of skin allograft to adhere well to the wound bed has been an important clinical indication of the readiness of the wound bed to receive skin autograft, which is usually referred to as a "take-test."[16] Another clinical observation that suggests that the bed is suitable for autograft is whether removal of the skin allograft causes bleeding.[3] Applying a skin allograft to the wound is also believed to better prepare the wound bed for the skin autograft.[3] It is also reported that the application of skin allograft in burn victims provides an early reduction of pain, prevents deepening of the wound, and also lowers the occurrence of hypertrophic scarring.[6,15]

Table 2 Indications for Skin Allograft Application in Conjunction with Reconstructive Surgery

Indications	No. of Patients (%)
Temporary wound coverage	21 (44.6%)
• Overcame flap/surrounding tissue edema due to prolonged surgery. The application of skin allograft allowed edema to subside and enabled secondary wound closure.	
• Complete flap (especially the tip and distal portion) survival ensured by avoiding early tension in the critical area. The concerned area(s) were left loose, and the exposed area(s) were covered with skin allograft until the flap circulation improved within a few days/week. Secondary suturing was performed at a later stage.	
Flap pedicle protection	17 (36.1%)
• Used at initial surgery where the flap inset caused stretching/tension, kinking, or compression of the flap pedicle. The skin allograft was used to bridge the wound gap and cover vital structures such as the neurovascular pedicle, tendon, or bone. This allowed the flap circulation and perfusion to stabilize prior to secondary reinsetting of the flap.	
• Used in emergency surgery to salvage the flap (i.e., in the case of vascular thrombosis/pedicle kinking), as the flap tissue became swollen/edematous and flap dimensions had been altered from the previously planned defect.	
Donor site wound management	5 (11%)
• Used as a dressing to allow edema to subside and to allow deep cavity/undermined wounds (muscles, etc.) to adhere, granulate, and become shallower, which produced better wound bed for skin graft take.	
• Wound closed later by secondary suturing or less skin autograft.	
• Used as a wound cover in a delayed/staged procedure (e.g., it covered the deltopectoral flap donor wound until the next stage where part of the flap was returned back to donor defect).	
Flap-related wound complications	3 (6.4%)
• Covered the exposed wound (recipient bed or flap edges), preventing desiccation or further flap necrosis in the case of failing flap/postdebridement.	
• Used temporarily to resurface the wound bed in cases of complete flap failure.	
Flap monitoring	1 (2.1%)
• Used in muscle-only free tissue transfer for flap cover in the case of exteriorized free jejunal transfer. After the flap perfusion had stabilized, the skin allograft was removed and flap reinset or definitive cover was placed (e.g., secondary suturing or skin autograft).	

As explained earlier, GPAs have low antigenicity, which results in decreased incidence of rejection. Repeated applications are possible without development of subsequent reaction.[3] The ability to enhance revascularization is perceived as one of the most important features of GPAs. The exact mechanism for its angiogenic stimulus and induction of the development of granulation tissue is unknown.[3] One possible explanation may be due to the presence of cytokines at the cut surface of grafts.[10]

The risks of transmissible diseases in skin allograft are the same as in other tissue transplantation. Therefore, proper patient selection and screening is of utmost importance. However, the way GPAs are processed reduces the chances of virus infectivity, in particular, HIV transmission as described earlier.[5,11–13]

The advantages of skin allograft can be further expanded from our 7 years' experience with the use of GPA in conjunction with reconstructive and flap surgery. The availability of GPA is a substantial asset in the surgical armamentarium of a reconstructive surgeon. The technique is usually useful in a long surgery when major resection or reconstruction is undertaken and the associated tissue swelling and edema compromises tension-free wound closure. This process in turn may affect the flap pedicle and hence the vascularity or perfusion of the wound. The use of GPA in relation to flap reconstructive surgery allows more time for edema and swelling to subside, resulting in direct or partial closure of the wound with better aesthetic results. In the instance where the flap pedicle may be compromised, GPA application allows tension-free closure of the wound, therefore preventing pedicle compression. The advantage of GPAs in this situation is that they can be safely applied directly on the vascular pedicle (Fig. 2), which cannot be achieved by any other type of dressing.

Another benefit of GPA use in flap-related surgery is that in the event the flap needs to be explored for vascular or anastomosis-related complications, the patient's own skin autograft will not be jeopardized or wasted should it be used in the first instance instead of skin allograft. GPA can also be used as a temporary cover in case of partial flap failure or necrosis until the

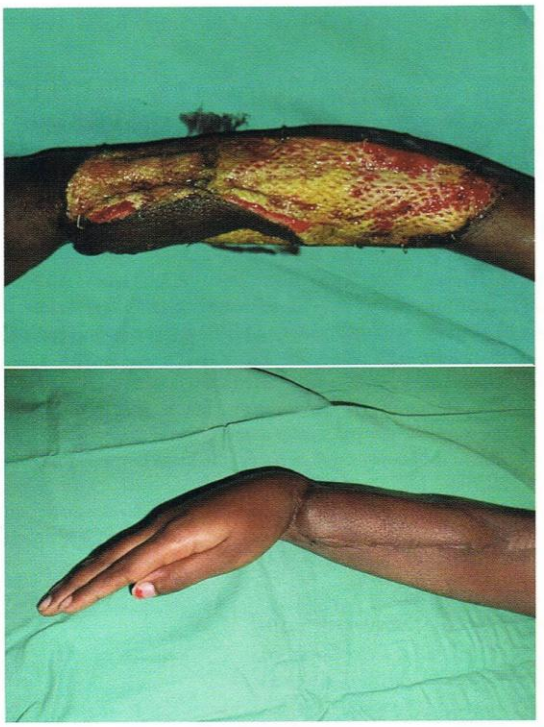

Figure 1 A 9-year-old girl who had Ewing's sarcoma excised and reconstructed with fibula osteocutaneous free flap, complicated with venous thrombosis. Emergency exploration was performed. Postexploration, the flap edges were not reapproximated because of excessive tension secondary to tissue edema, which may compromise the pedicle. (Top) Glycerol-preserved skin allograft covering the wound on day 10 postsurgery just prior to removal and secondary suturing after the swelling had subsided. (Bottom) Six weeks postsuturing. Skin flap settling well with good aesthetic outcome for the patient.

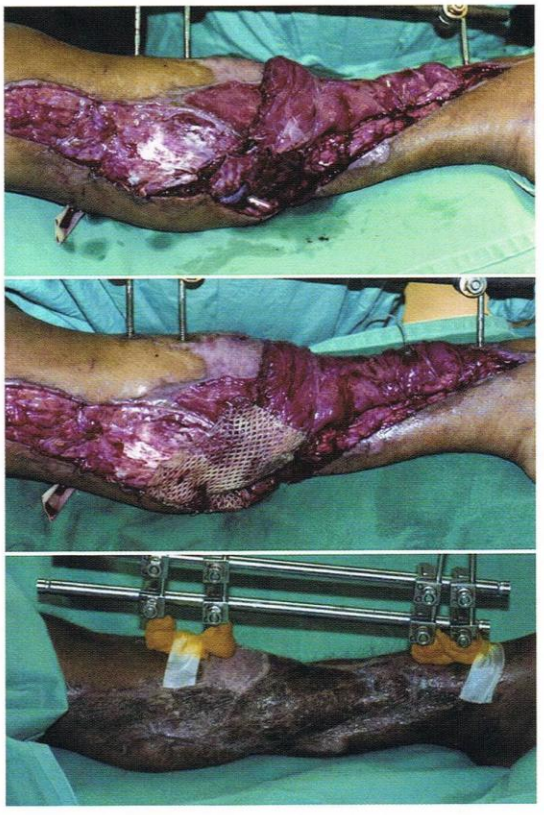

Figure 2 A 20-year-old male with traumatic soft-tissue loss and segmental tibia loss over the distal aspect of the left leg. First stage of soft tissue transfer with gracilis free flap and arteriovenous loop. (Top) Gracilis flap inset and anastomosis completed with exposed pedicle (arrow). (Middle) Intraoperative picture showing glycerol-preserved skin allograft used to cover the exposed pedicle. (Bottom) Postoperative picture showing healed wound after split-skin graft application for final wound cover and pedicle covered by mobilizing adjacent skin after edema had subsided.

devitalized areas are well demarcated. The skin allograft can then be removed and covered with another flap or with SSG.

The use of GPA in free flap surgery also provides temporary cover for flap-monitoring purposes, for example, in the free jejunal transfer or in muscle-only transfer. GPAs provide a good biological dressing material to cover the flap until the anastomosis becomes stable and the flap can be buried or covered with SSG. We also used GPA for temporary coverage of the donor site in some cases where generalized swelling and edema developed after prolonged surgery. The GPA can later be removed and the wound can be partially closed with less area requiring autograft.

In general, the GPA can be used safely without any major complications when used as an adjunct to reconstructive and flap surgery. In conclusion, based on our experience in this series, we propose a new indication for the use of GPA as an adjunct to reconstructive flap surgery, because it provides a host of benefits and advantages.

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