**Review Article**

Glycerolised Skin Allografts for Extensive Burns in Low- and Middle-income Countries

# Introduction

**Abstract**

**Introduction:** There has been a significant improvement in the outcome of treatment of large surface area burns in developed countries. A major contributory factor is an early excision and skin grafting of burn wounds. The initial coverage of large surface area deep burn wounds requires the use of temporary skin substitutes such as allografts due to limited skin autografts. Cadaveric skin allografts are the commonest source of skin allografts in use; however, there may be religious, cultural, cost, or other factors mitigating its availability and routine use in low- and middle-income countries (LMICs). Human skin allografts may be used fresh or stored in tissue banks to ensure its ready availability. The purpose of this review is to promote glycerolised skin allografts as a means of skin preservation in low-resource countries above other modalities cryopreservation due to its cost advantages and relative ease of operation. **Materials and Methods:** A literature search for articles related to human skin allograft use in burn care, skin banks, and glycerolised skin allografts in LMICs was done using PubMed, EMBASE, and Web of Science databases. The key words used were ‘allograft’ and ‘burn’ with a filter in the search for human studies. The relevant references in the articles obtained were also searched for and included in the review **Results:** Sixty- three journal articles were reviewed for contents in line with the objectives of this study. **Conclusion:** Glycerolised skin graft is a viable option for coverage of extensive burns in LMICs.

**Keywords:** *Allografts, income, low, middle, skin bank*

There has been a significant improvement in mortality from large surface area burns mainly in developed countries due to early burn wound excision and skin grafting being done in these countries.[1,2] The initial wound coverage of the large surface area burns will require a biological wound cover. Nevertheless, approximately 85% of major burns and 90% of fire-related deaths[3] occur in low- and middle-income countries (LMICs) settings which are largely lacking in facilities for skin banking. Child injury deaths from fire and flames are almost 11 times higher in low-income countries than in high-income countries (HICs).[3,4] The rate of child death from unintentional injuries of all kinds is over 10% in low-income countries.[3,4] The peculiarities of LMIC is that data are less accurate than that in HICs in general. The records in HIC are readily available, whereas those of LMICs are either not collected or only sparsely published.[4] The range of uncertainty surrounding stated estimates in South Asia and Sub-Saharan Africa is 10,000–14,000 deaths lower or higher.[4]

Burns in children under the age of 5 years are relatively higher in LMICs compared with HICs. Some LMICs such as India and Cote d’Ivoire report that approximately half of childhood burns was in infants.[5,6] It is reported that infants in Africa have three times the world average for that age group of fire- related burns.[7] In LMICs of the Americas, Europe, and the Eastern Mediterranean, fire-related burns are the leading causes of disability-adjusted life years (DALYs), which is the loss of the equivalent of 1 year of good health. In this population, more than 30% of the DALYs among men aged 15–44 years are due to death or disability from injury.[4]

Most of the studies reported in LMICs are hospital-based reports from burn centres or from hospitals in which burns are managed on special wards which may not be reflective of the community incidence of the injuries.[4] Though the reports from burn centres give valuable information about burns, especially major burns within a community, the prevalence of deformities, disabilities, and economic burden is better obtained in a comprehensive

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 community survey.[4] Burns was the second

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most common injury to children under 15 years of age in a rural community survey in Ethiopia. It was the leading cause of admission for injury to children’s hospitals in Ethiopia with an annual incidence of burns severe enough to restrict activity for a day or more being 80 per 1000 children.[8] Burns ranked third in outpatient visits to the hospitals.[9,10] Gupta *et al.*[11] in a review of 458 hospitals in 14 LMICs/MICs showed that many hospitals in LMICs are able to perform the initial burn management and resuscitation but are deficient in further burn management such as skin grafting and management of burn complications. Only Nigeria and Mongolia had the capacity to perform various categories of burn management investigation in their study.[11] Joseph *et al.*[12] also confirmed this observation among 32 LMICs of the capacity for their health facilities for acute burn management and deficiencies in further burn management.

In their study of 1337 health facilities in 32 LMICs, only 379 health facilities (36.6%) had the capacity to do skin grafts. Of the facilities that could perform skin grafts, approximately half of the facilities had access to blood bank all the time. Relating these deficiencies in management to lack of capacity for wound coverage in patients with extensive deep burns, this means that about 18% of facilities in these countries had the potential for early coverage of deep burns.[12] The number of facilities in LMICs who have facilities for skin bank or use skin allografts is dismally low.[13]

Skin allografts and cadaveric and living donor allografts[14-16] provide biological wound cover with significant clinical benefits. The clinical uses of allografts include coverage of extensive full thickness wounds, meshed skin autografts,[17,18] and healing of partial thickness wounds. The benefits of the use of human skin allografts in wound coverage of burns have been well documented. These include reduction in healing time, reduction in length of stay, antimicrobial properties, and pain relief. There is a limit to the availability of cadaveric skin.[19-22] The recovering, processing, storage, and distribution of allografts for transplantation require skin banking.[23] Skin banks[24,25] are not readily available in most developing countries, though there is an obvious need for these.[26,27] Patients with extensive burns who require skin allografts will either have to travel to a high-income country for these services or sadly experience significant morbidity or mortality.

The aim of this paper is to review available literature on glycerolised allograft skin grafts and their use in LMICs to improve burn management outcomes and proffer suggestions for overcoming challenges to its use.

included in the review. The emphasis of the literature review is on the challenges to the human skin allograft usage in LMICs.

# Results

Sixty-three journal articles were reviewed for contents in line with the objectives of this study, and the results are presented in this section.

### Allograft skin procurement

*Sources of allograft skin*

Skin allografts are harvested from live donors or cadavers. The live donors may be family, friends, and well-wishers,[28] or it may be from body contour surgeries such as abdominoplasty or breast reduction surgeries.[29,30] The redundant normal skin from patients undergoing surgery for huge benign tumors such as huge subcutaneous lipoma may be a source. Skin allografts may also be obtained from the excess skin from a skin graft procedure or from an amputated extremity.[31]

Cadaveric skin is the most common source worldwide, but is not a ready source in most LMICs.

### Selection of donors

Potential donors for skin allografts are screened for transmissible diseases such as human immunodeficiency virus, types 1 and 2 (anti-HIV-1 and anti-HIV-2), hepatitis B surface antigen, hepatitis C virus (anti-HCV), syphilis, human T-lymphotropic virus I/II and CM for HIV-1/2, hepatitis B virus, and hepatitis C virus,[24,32] which are disease states that are contraindications to skin donation. Some skin banks restrict their age range of donors to 14–75 years of age,[33] whereas others have no age.[34] Potential donors who have extensive dermatitis, acute burn injuries, cutaneous malignancy, poor skin quality, or skin infections are excluded.[35]

### Harvest of skin allografts

Cadaveric human skin allografts should be procured within 24 h of death, within 12 h if the donor is refrigerated and within 15 h if donor is not refrigerated. It may be harvested as part of organ harvest procedure where it is usually done in an operating theatre or in the mortuary from consented relatives or donors. The post-mortem time before procurement has the singular greatest effect on the skin viability. The functional metabolic activity of the skin rapidly declines if the donor was not refrigerated within 18 h of death.[31,36] A radiometric viability assay may be used to test the viability of the cells on the dermal side of the skin. This is based on the conversion of 14C glucose into[14] CO by the dermal viable cells.[37] Skin

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# Materials and Methods

A literature search for articles relating to human skin allografts in extensive burn care, skin banking, and glycerolised skin allografts in LMICs was done using PubMed, EMBASE, Web of Science databases. The key words used were ‘allograft’ and ‘burn’ with a filter in the search for human studies. The relevant references in the articles obtained were also searched for and

is harvested from the posterior aspects of the body in order to preserve the appearance of the deceased for the family. It may be harvested from the back, buttocks, and posterior lower limbs.[38]

Human skin allografts may be harvested as a split-thickness skin graft usually with the use of a dermatome; this may then be meshed or be preserved as sheet grafts. Meshing of the

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skin graft does not adversely affect the survival of the graft, although the mechanical action of meshing may cause physical injury to the tissues. Meshing does not also increase the rate of bacterial contamination at the time of skin banking. It does not adversely affect the banked skin viability.[37] Human skin allografts may also be harvested as full-thickness skin grafts using a scalpel blade if the source of the allografts is from body contour surgeries or redundant skin in huge benign tumours. Split-thickness skin graft harvesting with the use of dermatomes following the removal of the panniculus in the operating room has also been described.[39] Skin excised during panniculectomy is attached to a rectangular Lucite plastic covered with layers of sterile drapes to make the tissues taut for even cuts. Split-thickness skin harvest using a dermatome at 0.38 mm (0.015inch) depth can then be obtained.[36]

### Mitigating factors

There are factors mitigating against the use of skin allograft procurement in LMCs. These obstacles include legislation on skin organ transplant, cultural or religious restrictions to the harvesting of allografts, economic considerations, and logistics or institution constraints.

In most LMICs, there is no legislation on organ transplant which places the risk of abuse of skin organ donation or litigation on the harvesting team. The laws that relate to cadaveric skin allografts in Hong Kong are modelled on the English law, which is expressed in the Human Organ Transplants Act 1989. This law prohibits ‘the commercial dealing in organs and restricts the transplanting of organs between unrelated persons’.[40] In France, all use of products from the human body for therapeutic ends are regulated by the following three laws called ‘Lois de bioéthique’: ‘Law no. 98-535 of July 1, 1998 on the reinforcement of sanitary surveillance and the control of sanitary security for products for human use’; ‘Law no. 94-654 of 29th February, 1994 on the donation and use of elements of the human body’; and Law no. 96-452 of 2 May 28, 1996’.[40] LMICs who do not have existing legislation will need to provide a legal framework for allograft skin transplants to avoid untoward consequences to practitioners who will engage in its use, possibly modelling their laws after the English or French laws.

Harvesting cadaveric skin may also be a challenge in some LMICs due to religious and cultural bias. Although there is sparsity of literature on the sociocultural attitude of people in LMICs to human skin allografts, Ogunlaja *et al.*[41] observed sociocultural impediments to the use of amniotic membrane for medical reasons, which could mirror that of allograft skin. In a survey of 216 pregnant women receiving antenatal care in hospitals, the fear of using the placenta for money rituals, fear of endangering the baby’s life, ignorance of placenta donation, fear of using the placenta to change the child’s destiny, belief that it is a part of the baby, their culture forbids it, husband’s non-consent, their religion forbids it, and it was not acceptable to the mother formed part of the reasons for failure to donate their placenta for medical reasons. Their

level of education as well as religion of the mothers was not significantly contributory to their decisions. However, education of the mothers on the medical benefits of the placenta donation resulted in significantly more respondents willing to donate their placenta, 25.5% compared with the initial 9.3% (*P* < 0.001).[41]

The challenges are reported in Nepal with human skin allografts, where mortality for burn patients with total body surface area (TBSA) over 40% was almost 100% of included limitation of donors.[42] This has alluded to various factors such as the fact that it was against the general Hindu belief which values the wholeness of the body after death, the need to have the family consent even if the patient gave consent, also people prefer to die at home than in the hospital, making procurement more challenging.

Economic considerations are also a factor. In Nepal, the start- up cost of equipment used in setting up their first skin bank in collaboration with international collaborators was $18,219, and the estimated cost for operations in the first year for 10 donors was $35,000 and between $10,000 and $15,000 for subsequent years.[43] Reports from LMICs with skin allograft programs put the initial cost ranging from $100,000 to $400,000.[44] The capital funds were from governments, international non- governmental organisations, private foundations, and the International Atomic Energy Agency (IAEA). IAEA spent

$6.31million between 1980 and 2000 to establish and support radiation and tissue banking in 16 countries in Asia and Pacific regions.[44]

Logistics and institutional constraints reflect in the trainings required to start and maintain a successful skin allograft program. The training courses cover strategies in public and professional awareness, radiation sterilisation methodology, and quality assurance in tissue banking, to mention a few. The readiness of the host institution for the skin allograft program, challenges such as maintaining trained personnel, and hands- on training programs were also found to be expensive and time-intensive.[44]

### Allograft skin preservation: glycerol preserved vs. other types of skin storage methods

The glycerol-preserved cadaveric allograft (GPA) was introduced by the Euro Skin Bank in 1984. The skin is preserved in 85% glycerol and stored at +4°C.[45] Glycerolised-preserved skin is termed non-viable because vital structures of the skin are destroyed in the process of glycerolisation. This method of preservation is however simpler and more cost-effective than the other methods such as cryopreservation. It also possesses antibacterial and antiviral properties in addition to suppressing the immunogenicity of the allograft.[23,46] These properties have made it an allograft preservation of choice in clinical practice in developed countries[47] and make it an attractive method of choice for preservation of allografts in LMICs. Cryopreservation is mainly used in the USA, whereas glycerol preservation is more commonly used in most Western European Burn centres.[48,49]

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Marshall *et al.* recommended that the allograft be exposed to 98% glycerol at 20°C for at least 4 weeks before clinical use. This is at variance with protocols using 85% glycerol, the argument being persistence of intracellular viruses (herpes simplex 1 and polioviruses) in 85% concentration. Skin allograft in 98% glycerol is difficult to handle; hence, glycerol 85% is preferred which is also safe, especially in countries with low incidences of HIV and hepatitis B and C. Glycerol 85% at 4°C is preferred in most skin banks.[50]

### Modalities of storage

1. *Refrigeration*: Fresh allograft skin is the preferred biologic dressing for the temporary coverage of excised extensive full-thickness burn wounds due to its more rapid adherence and rapid vascularisation. The skin is typically stored at 4°C in tissue culture medium with or without antibiotics. Refrigeration slows the metabolic rate of the viable cells, and nutrient tissue culture medium supports cellular metabolic activity. Recent studies suggest that skin viability can be maintained for up to 2 weeks at 4°C if the nutrient medium is changed every 3 days.[19,51] The major shortcoming of this storage method is the limited time that viability can be maintained. It has been common practice to cryopreserve the skin within 5–7 days of refrigeration.
2. *Cryopreservation*: Harvested skin allograft is cryopreserved using dimethylsulphoxide (Me2SO) or 20% glycerol. The allograft is first incubated for 20 min at 4°C in a medium

containing Me2SO or for 40 min at room temperature (22°C) in a medium containing glycerol. The incubation time is to allow for the cryoprotectants to equilibrate within the tissues. Glycerol penetrates cells more slowly, hence the longer incubation time. The lower temperature of 4°C for Me2SO is to minimise potential toxic effects. The tissues are then cooled at a constant cooling rate of −1°C/min to −70 to −100°C prior to placement in either a mechanical freezer or liquid nitrogen. The cryopreserved skin may then be transferred into a liquid nitrogen freezer. Skin stored in a mechanical freezer (−70 to −100°C) can be maintained for 3–6 months, whereas storage in liquid nitrogen (−150 to −196°C) has been shown to maintain viability for up to 10 years.[52]

1. *Lyophilisation*: Skin can also be lyophilised by freeze-drying or incubation in glycerol. Lyophilisation removes free water from the tissues to the external environment, which is different from deep freezing that turns water to ice crystals.[53] Lyophilisation reduces the immunogenicity of allograft skin without interfering with its beneficial properties. It involves removal of water from the skin by sublimation. A vacuum is applied to the tissue and it condenses the removed water molecules downstream. Drying is done to prevent degradation reactions, and till it is less than 5% residual water (gravimetric measurement). This can be done using a freeze dryer (Christ Alpha 2-4, Germany).[54]
2. *Glycerol preservation*: This entails rinsing the allograft with glycerol solutions in concentrations increasing from 50%,

70% then to 85%. For each concentration, the allograft skin is agitated at 33°C for 3 h. GPAs are then stored at 2–8°C. Various antibiotics such as ceftazidime (500 mg/100 mL) and gentamycin (80 mg/100 mL) could be added to the glycerol solution. GPA storage is about 2 years.

### Application of glycerol preserved in burn care

GPA has been reported to have the advantage of being superior to cryopreserved allograft in the sandwich grafting technique in major full thickness burns.[42] There is more reliable take and outgrowth of the autograft due to moderate rejection of the allograft skin and moderate inflammatory reaction in the wound bed. This is due to reduced immunogenicity of GPA.[55] GPA is also reported to be as good as cryopreserved allograft in the treatment of partial thickness burns.[56] Beverwijk Burn Centre uses the sandwich grafting procedure[16] as the treatment of choice for patients with extensive burns. GPA is used on the excised burn wounds and granulating burn wounds, which have been widely meshed autografted.[23] The GPA firmly attaches to the wound bed on vascular contact; however, there is slow rejection of the allograft and limited inflammation of the wound. This mitigation of the immunogenic reaction in the wound allows the meshed autograft to close the wounds. It has been reported that the sandwich technique results in better take of the meshed autograft than when using meshed autograft without allograft coverage.[23] The Meek technique is, however, reported to be more efficient for enlargement of skin graft and was preferable for patients with very extensive burns, elderly patients, and those in poor general clinical condition. GPA was also used for partial thickness burns as a biological dressing.[23,24] This will reduce the burn pain and limit change of dressing to the secondary dressings.[57] GPA can be used to test the wound bed readiness of an excised deep partial-thickness or full-thickness burn wound to take a skin graft.[16] GPA is beneficial as a skin substitute for children and the elderly who have a higher mortality and morbidity from major burns due to their lower ability to withstand the severe metabolic stress of burns. Another benefit of the use of GPA is to avoid frequent change of dressings, especially in the paediatric age group. This reduces the physical and psychological impact of frequent painful dressings in children. GPA serves as a protective barrier in partial-thickness burns to prevent it from deepening due to infection, hypoxia, or oedema.

Before glycerol, human skin allograft is used clinically, and it is important to remove the glycerol from the skin. This is done by washing the skin repeatedly in normal saline. This is done for at least 30–60 min.[58] If the glycerol is not removed and the skin has open wounds, it may result in high systemic concentrations. This may lead to myonecrosis at high doses. The muscle breakdown products may lead to renal failure and may result in death.[58]

### Skin banking in LMICs

Skin banking is an expensive undertaking and not readily available in poor-resource countries. There is no known skin

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bank in East and West Africa.[59] This coupled with high cost of commercial artificial skin products has denied poor patients access to skin cover for those who need it most. Though there are challenges in procuring cadaveric skin allografts in LMICs, living skin allografts can be used as an option. The abundance of donors and minimal pre-operative preparation are ideal for the poor countries. This has been used successfully in some countries with good results, although with very small number of patients reported.[28] Acceptability, however, has been retarded by cultural beliefs and fear of contacting diseases.[60]

Saidi[61] in Kenya reported that five patients underwent live skin allografting for those with deep burns of more than 40% of the burn surface area. The skin donors were patients’ mothers and siblings.

In Egypt, the option of harvesting allografts from living donors undergoing body contouring surgeries was explored. In Al- Azhar University, three body contouring procedures were selected as their source of harvesting GPA, which include abdominoplasty, large breast reduction, and vertical thigh lift. The skin was harvested as a full-thickness skin graft using a scalpel blade after the excess tissue in the procedure was excised. Reasons for harvesting the allograft as full-thickness graft include the advantage of increased dermal thickness in the allograft for better vascular growth and the difficulty of using a dermatome to harvest skin from the excised flap. The skin was preserved in glycerol and kept in a refrigerator for storage before use. In their report,[42] they were able to harvest 22,000 cm2 of skin in 1 year (1830 cm per month). This was better than Villalba *et al.*,[30] who also reported harvesting skin allografts from body contouring surgery from abdominoplasty and mammoplasty with skin harvest of 1030 cm per month.[42] This is comparable to some local skin bank reports. Ralston reported 80,000 cm2 of skin in 5 years[42] in Sheffield Skin Bank and Torrero reported 95,000 cm2 of skin in 5 years in Barakaldo, Spain. The disadvantage is the small quantity of skin harvest per procedure, 0.06 m2 per donor compared with

0.4 m2 per cadaveric donor.[30] For cadaveric donor harvest, about 18% of the body surface area which is approximately 3,500 cm2 of skin can be harvested.[24]

The first cadaveric donor skin allograft in India was established on April 24, 2000. Within the first 10 years, there were 249 donors and 151 recipient burn patients. The procurement was mostly done within 4 h of death, and majority of the procurement was done at home. The method of preservation was changed from cryopreservation to glycerol preservation in 2007 because of the difficulty in sustaining the cost of preservation.[43] After 7 years of commencement of skin banking in India, the method of allograft preservation was changed from cryopreservation to glycerol preservation because of difficulties in maintaining and repairing the ultra- cool refrigerator, inability to sustain uninterrupted power supply, and the fact that glycerol preservation was cheaper and cost-effective.[43]

Cai *et al.*[62] reported the establishment of the first skin bank in Nepal, an LMIC in South Asia in 2017, and the successful use of allografts on five patients with extensive burns using glycerol-preserved skin allograft. Although there was no skin allograft donor within the first year of the setup of the Nepal Skin Bank, public educational awareness of the benefits of cadaveric skin in patients with extensive burns resulted in 4 donors out of 200 donor pledges which was used to treat six patients with extensive burns TBSA over 20%. Four out of these patients survived and have become community advocates for skin allograft donation. An important point in their experience is the need to also educate the family about skin donation as the family can override the consent of the patient after demise.[62]

The estimated cost of 1 cm2 of GPA in a skin bank is $0.55,[63] whereas in the Euro Skin Bank, it is €0.91.[58] This makes it a favourable option of preservation in LMICs.

Public educational awareness in the community in India was said to be responsible for the number of donors and the fact that majority of the procurement was done at home.[43]

# Conclusion

Glycerolised skin banking is the option to sustain skin banking for LMICs. Michael *et al.*[26] in Nigeria emphasised the importance of continuous medical education on skin donation and skin banking among health professionals to improve the attitude of doctors and nurses towards the use of skin allografts.

Though religious and to a lesser extent cultural restrictions to human skin allograft harvest and usage may be prevalent, providing education on the benefits of the medical usage of the skin allografts would result in increased yields of skin allograft procurement and its usage in burn care.[41]

The Indian experience[43] can be used as a guide in formulating a plan for sustained skin banking for the purpose of burn care and chronic wound care. The plans can be modified to the regions or countries involved. There is need for improved funding for health which will make the facilities for the utilisation of GPA available in health facilities in LMICs. Glycerolised skin banking should be used in LMICs as part of the protocol in the care of patients with burns, especially major burns. This will significantly improve on the mortality figures in major burns in the LMICs.

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### Conflicts of interest

There are no conflicts of interest.

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