Correspondence

**Viability of cadaver skin grafts stored in skin bank at two different temperatures**

Sir,

In India, about 5 to 7 million burn cases occur every year and a great number of them can be salvaged if provided with a wound cover in time[1]. Homografts limit infection, reduce protein and electrolyte loss and alleviate pain[1]. The use of skin allografts is desirable in the treatment of patients with large wounds due to thermal injury. Hence the idea of preserving skin was conceived several years ago which led to development of skin banks. Skin Bank in L.T.M. Medical College & Hospital, Mumbai, was started in the year 2000 and is the first Skin Bank with the capability of harvesting cadaveric skin grafts in our country. Freezing of skin has been tried earlier and the necessary pre-requisites include treatment of skin with glycerol or dimethyl sulphoxide (DMSO)[2]. Short-term storage in liquid nutrient medium is clinically desirable for certain length of time. Hence refrigeration using nutrient media becomes essential to maintain the viability[3].

A study was conducted at Skin Bank, L.T.M. Medical College, Mumbai, to evaluate the effect on viability of skin allografts on long-term storage with only phosphate buffer saline (PBS) at 4°C and PBS with 15 per cent glycerol at -70°C. In a preliminary study in our hospital (unpublished), we studied PBS and RPMI-1640 as preservation media, and both gave comparable results. So, PBS was used in this study, as it is cost-effective than RPMI-1640.

A total of 33 split-thickness skin grafts obtained from cadaver donors after written consent from their relatives, were studied from July 2002 to December 2006, after approval from the Institutional Ethics Committee. Exclusion criteria were cadaver with systemic sepsis, skin malignancy or skin diseases, and positive serology for anti-HIV, HBsAg, anti-HCV and VDRL test. Three donors were excluded from the study – 1 positive for HIV antibodies, 1 positive for HBsAg and 1 for HCV antibodies. Therefore viability test was performed on 30 cadaver skin grafts.

Split-thickness grafts 1/100th inch were obtained within 24 h of death by carefully preparing the donor area with 1 per cent povidone-iodine and spirit. Skin grafts were assayed for viability by measuring tetrazolium reductase activity as described by Hershey et al[4]. Skin pieces (1-2 cm²) were incubated with 2 ml each of 1.5 per cent (w/v) 2,3,5-triphenyl tetrazolium chloride, A.R. (TTC) (Hi-Media, Mumbai, India) and 3 per cent (w/v) sodium succinate (Hi-Media, Mumbai, India) in vacuum for 1 h at 37°C. Vacuum was created using McIntosh Filldes’ anaerobic jar without the catalyst. The skin pieces were then blotted dry and red coloured formazan extracted using ethylene glycol monomethyl ether (Merck, Mumbai, India). The optical density (OD) was measured using colorimeter (HANS 102 Model, Kanad Vidyut, Pune, India) at 490 nm. Distilled water was used as negative control. Skin grafts showing an OD value of >0.2 were considered viable[4]. All viable cadaver skin grafts were preserved using PBS with antibiotics at 4°C or PBS with antibiotics and 15 per cent glycerol (Merck, Mumbai, India) at -70°C. Antibiotics used were crystalline penicillin 1 lakh units/100 ml (Alembic, Baroda, India) and gentamicin 80 mg/100 ml (Nicholas, MP, India). Viability of the skin grafts from both the preservation media was checked once every week up to 1 month.

At procurement, 22 out of 30 cadaver skin grafts were viable (73.3%) and eight were non-viable (26.7%). All viable grafts retained viability at -70°C in PBS with 15 per cent glycerol at the time of receiving (0 h), after 24 h, after 1, 2 and 3 wk, and after one month. The 22 viable skin grafts stored in PBS at 4°C were viable at the time of receiving (0 h) and after 24 h, but only 6 (27.3%) were viable after 1 wk and none after 2 wk.
Viability is an important parameter during low temperature storage, as cells deteriorate when removed from their physiological state. Various media like RPMI-1640, saline, Eagle’s MEM with L-glutamine, PBS, Ringer’s solution, Ham’s F-12 and DMEM, etc., have been used in the past as preservation media. Culture medium is considered a better medium than saline, as it is rich in nutrients such as inorganic salts, amino acids and vitamins that are necessary for graft viability. These media prevent qualitative changes in the graft.

Zhu showed that 90.3 per cent of the viability was retained in PBS at one week which gradually decreased after 2 weeks. Thus, for long-term storage of skin or any other tissue, cryopreservation is desirable. Use of cryoprotective agent (CPA) is essential to protect the cells from mechanical injury. The CPAs act by partially binding water. Many workers have used 15 per cent glycerol as a cryoprotectant, while others have used DMSO. However, DMSO is more toxic than glycerol. We used 15 per cent glycerol in this study, as it does not have any toxic side effects and penetrates the cells slowly.

Bravo and colleagues found that human cadaver skin grafts after procurement and just prior to further tissue bank processing exhibit approximately 60 per cent of the metabolic activity found in fresh skin samples obtained from living surgical donors. They also showed that cryopreserved skin retained higher viability as compared to skin stored at 4°C, which is also shown in the present study. Skin cryopreserved with DMSO retained higher viability than glycerol. Using 15 per cent glycerol in this study, we found 100 per cent viability even after one month of storage. Konstantinou et al showed that cryopreserved split-thickness skin retained 64.6 per cent viability when stored in MEM with 15 per cent glycerol as compared to 100 per cent viability of fresh skin.

For low temperature storage, many workers have used liquid nitrogen at -196°C since electrically driven freezers are prone to failures. In the present study, we used -70°C ultra low temperature deep freezer and we did not encounter any failure.

To determine the effectiveness of any cryopreservation procedure, it is important to select appropriate viability assay. There are several techniques to determine the viability of tissues such as dye-exclusion test with trypan blue, DNA synthesis using H-thymidine, glucose and oxygen consumption assays, production of lactate, nuclear magnetic resonance (NMR) spectroscopy, etc. The method used in the present study did not require expensive chemicals or infrastructure to carry out the test. The McIntosh Fildes’ jar was used to create vacuum, but nitrogen gas was not used to replace the vacuum in the jar. Yet the formation of red coloured formazan could be seen. Hence it can be used in small laboratories to carry out routine viability test which is simple, easy to perform and can yield quantitative data.

Skin can also be stored as non-viable wound cover using 98 per cent glycerol as preservation medium as done in Euro Skin Bank started in 1976 by Hermans and colleagues. Besides, it also has antibacterial and antiviral properties and maintains the integrity of skin structure. Therefore, 8 grafts which were non-viable, were preserved in 98 per cent glycerol and used as wound cover. The British Association of Tissue Banks (BATB) guidelines suggest the storage of skin after 6 months as non-viable graft in 98 per cent glycerol, but it is difficult to rehydrate and is inferior for transplantation purposes.

In conclusion, our study showed that long-term storage of cadaver skin grafts can be effectively done at -70°C. The concentration of glycerol is important because at higher concentration, the skin loses its viability. The findings also indicate that PBS with 15 per cent glycerol appears to be a suitable preservation medium for storage of cadaver skin grafts.

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