The Euro Skin Bank: Development and Application of Glycerol-Preserved Allografts

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HISTORY

The Dutch Burns Foundation was established in 1971 as a national charity with the dual aim of promoting burn injury prevention and supporting research into treatment. As the value of human allografts in the treatment of severe burn injuries became apparent, this institution was encouraged to set up a centralized skin bank for the Netherlands. The arguments for a centralized facility included concentration of expertise, low investment and exploitation costs, and increased possibilities for research into wound coverage (R.P. Hermans, personal communication). The Dutch Burns Foundation established the Dutch National Skin Bank in 1976, and cryopreserved allografts became available for clinical use a year later.

Although initial experience was equivocal, the introduction of glycerol as a cryoprotectant greatly improved the quality of the allografts. The obvious importance of preservation techniques prompted study at the Red Cross Hospital, Beverwijk, into methods of further improving allograft quality.

After extensive preclinical and clinical trials were performed at the Red Cross Hospital, allografts preserved in 85% glycerol were introduced in 1984. Glycerol-preserved allografts (GPA) yielded clinical results consistently superior to those with cryopreserved allografts. In particular, GPA appeared to invoke a less vigorous rejection reaction. As a result of this experience the production of cryopreserved allografts was discontinued in 1985.

Since GPA was introduced, further studies have led to refinement and standardization of the preservation process. At the same time interest in GPA from burn centers outside the Netherlands has grown markedly. Increased demand for a limited commodity led the Dutch Burns Foundation to reorganize the skin bank as a semiautonomous, nonprofit subsidiary, and in 1993 the Dutch Skin Bank was relaunched as the Euro Skin Bank (ESB).

The ESB processes and delivers more than 1,000,000 cm^2 cadaver skin per year to countries in Europe and elsewhere. For several years supply has been limited by donor availability.

From the Beverwijk Burn Centre.
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ALLOGRAFT PROCUREMENT AND PROCESSING

Potential donors are notified by the Dutch tissue transplant organization Bio Implant Services, an affiliate of Eurotransplant, which coordinates transplant activities throughout Europe. The ESB complies with the European guidelines on donor selection and serologic testing, which are similar to those of the American Association of Tissue Banking. Pending European Union regulation of tissue banking, the ESB has voluntarily submitted to regular inspection by the Dutch Health Ministry to ensure that allograft processing meets the standards of good laboratory practice.

Glycerolization is uncomplicated. Harvested allograft is transported to the ESB laboratory in a solution of 50% glycerol in saline solution, to which penicillin (1 mol/L units) and streptomycin (1 gm) are added. The concentration of glycerol is increased in two steps to 85%, allograft being gently agitated for 3 hours at 33° C at each step. After glycerolization, the GPA are measured and trimmed before being rolled up and sealed in labeled plastic containers, which are stored at 4° C. The trimmings from each donor are stored in a separate container for bacteriologic testing. GPA are released for clinical use when serologic and bacterial results are negative. For transport, containers are packed in boxes and distributed by conventional postal and courier services.

CLINICAL USE OF GPA

GPA are similar to conventional cryopreserved allografts in clinical use as a temporary biologic cover for open wounds. The principal indications for GPA at the Red Cross Hospital are for scald burns and as an overlay for wide meshed autograft in extensive full-thickness burns. GPA are effective as a temporary cover for excised wounds for which autograft is unavailable. GPA are used occasionally in poorly vascularized wounds to improve the condition of the wound bed before autografting. The clinical use of GPA dermis in conjunction with cultured keratinocytes has been reported.[1]

The application of GPA as a biologic dressing for scald

burns has become the treatment of choice in our burn center. The allografts are easy to apply, provide immediate pain relief, and give excellent cosmetic results.[2] Kreis et al.[3,4] reported their experience with GPA as an overlay for widely expanded autografts either meshed 1:6 or expanded 1:9 with the micrograft technique described by Meek.[5,6] Results show consistent graft take, with most procedures resulting in >95% epithelialization at 6 weeks and a graft failure rate (<75% epithelialization) of less than 2%.

Although comparable published data are scarce, these experiences suggest that GPA perform at least as well as cryopreserved allografts in the clinical situations described.

ADVANTAGES OF GPA

The materials and equipment required for the manufacture of GPA are simple and relatively inexpensive, although processing is labor-intensive. GPA are stored in a domestic refrigerator and do not deteriorate appreciably at room temperature. Investment and exploitation costs are therefore relatively low; ease of handling confers practical advantages in transport and distribution. The uncomplicated preservation procedure is easily standardized, which guarantees consistent allograft quality.

The cells in glycerol-preserved cadaver skin are nonviable, although morphology is well preserved.[7] In standard light microscopy preparations no differences could be observed in structural integrity compared with fresh skin, although some shrinkage of keratinocytes is described. On electron microscopy cell shrinkage is more apparent, with the appearance of intracellular vacuoles, although intracellular organelles can still be identified. Matrix morphology appears to be intact.

Glycerol is a slow but effective bactericide; 97% of bacteriologic cultures from GPA are negative within 3 months. With the addition of antibiotics during harvesting, the number of positive cultures is reduced by 70%.[8] During 12 years of production of GPA only one donation has been discarded for persistently positive bacterial cultures.

Concentrated glycerol has antiviral activity. Van Baare et al.[9] reported that at 37° C herpes simplex virus was inactivated by 85% glycerol within 6 hours, whereas polio virus was inactivated within 24 hours. At room temperature time to inactivation was increased to 8 days and 22 days, respectively, whereas at 4° C viral inactivation was poor.[9] In similar studies with intracellular viruses times to inactivation were moderately prolonged compared with the previous results.[10] Studies to investigate the effects of glycerol on strains of human immunodeficiency virus both in vitro and intracellularly are in progress (N. Vardaxis, personal communication).

These studies indicate that the antiviral properties of glycerol are principally dependent on glycerol concentration, temperature, and duration of exposure. Although some viruses may be inactivated by the current manufacturing process, further research must define the optimal processing conditions for virus elimination. Available data suggest that the possibility of virus transmission with GPA as now manufactured cannot be discounted. On the other hand, in 12 years' experience, in which more than 6,000,000 cm^2 GPA have been used clinically by burn centers in Europe, there have been no reports of suspected disease transmission.

Recent studies into the role of skin dendritic cells in skin immunology suggest a mechanism to explain the clear clinical impression that GPA is less immunogenic than cryopreserved skin. The known migratory characteristics of dendritic cells including Langerhans cells are absent in GPA, presumably because GPA cells are nonviable. Furthermore in contrast to fresh skin, homogenates of glycerol-preserved skin fail to invoke a response when incubated with allogeneic T cells; only when other antigen-presenting cells such as monocytes are added can an attenuated response be obtained.[11] Finally, in grafting studies in mice with fresh allografts allogeneic Langerhans cells have been detected in the regional lymph nodes of recipients within 48 hours, associated with vigorous T-cell replication.[12] Although these studies are incomplete, it is suggested that viable allogeneic dendritic cells are involved in the early host sensitization to allograft placement. Because cells in GPA are nonviable, the immunologic response is delayed until vascular ingrowth allows exposure to blood-borne antigen-presenting cells such as monocytes. Follow-up studies on a porcine model are in progress to elucidate this hypothesis.

CURRENT AND FUTURE ACTIVITIES

Demand for GPA produced by the ESB far exceeds supply. Faced with a finite donor pool within the Netherlands, the ESB has initiated cooperative projects to establish skin banks in other countries with the aim of increasing the availability of GPA, processed after a common protocol to ensure consistent quality. To date two burn centers in the Slovak Republic and two burn centers in the United Kingdom are participating in this initiative.

In addition to further research into the antiviral properties of glycerol and continuing studies into the characteristics of allograft immunology, the ESB has recently begun study of the potential clinical application of glycerol-preserved dermis as a permanent component in wound coverage techniques.

CONCLUSION

Extensive clinical experience spanning more than a decade in numerous burn centers suggests that GPA are effective as a temporary wound cover in burn injuries. As with all transplacement procedures with biologic materials, the possibility of disease transmission cannot be discounted, but evidence suggests that the risks involved in the use of GPA are extremely small.

Further progress with synthetic skin substitutes will eventually render the use of skin allografts in burn care redun-

dant. In the meantime the low costs of manufacture combined with ease of handling ensure that GPA continue to have a useful role in the clinical treatment of burn injuries.

REFERENCES

1. Schiozer WA, Hartinger A, Henkel V, Donnersmarck G, Muhlbauer W. Composite grafts of autogenic cultured epidermis and glycerol-preserved allogeneic dermis for definitive coverage of full-thickness burn wounds: case reports. Burns 1994;20:503-7.
2. Hermans MHE. Clinical experience with glycerol-preserved donor skin treatment in partial thickness burns. Burns 1989; 15:57-60.
3. Kreis RW, Vloemans AFPM, Hoekstra MJ, Mackie DP, Hermans RP. The use of non-viable glycerol-preserved cadaver skin combined with widely expanded autografts in the treatment of extensive third-degree burns. J. Trauma 1989;29:51-5.
4. Kreis RW, Mackie DP, Vloemans AFPM, Hoekstra MJ. Widely expanded postage stamp skin grafts using a modified Meek technique in combination with an allograft overlay. Burns 1993;19:142-5.
5. Meek CP. Extensive severe burn treated with enzymatic debridement and microdermagrafting. Am Surgeon 1963;29: 61-4.
6. Kreis RW, Mackie DP, Hermans RP, Vloemans AFPM. Expansion techniques for skin grafts: comparison between mesh and Meek island (sandwich-) grafts. Burns 1994;20: S39-42.
7. Richters CD, Hoekstra MJ, van Baare J, du Pont JS, Kamperdijk EWA. Morphology of glycerol-preserved human cadaver skin. Burns 1996;22:113-6.
8. de Backere ACJ. Euro Skin Bank: large scale skin-banking in Europe based on glycerol preservation of donor skin. Burns 1994;20:S4-9.
9. van Baare, J, Buitenwerf J, Hoekstra MJ, du Pont JS. Virucidal effect of glycerol as used in donor skin preservation. Burns 1994;20:S77-80.
10. Marshall L, Ghosh MM, Boyce SG, MacNeill S, Freedlander E, Kudesia G. Effects of glycerol on intracellular virus survival: implications for the clinical use of glycerol-preserved cadaver skin. Burns 1995;21:356-61.
11. Richters CD, Hoekstra MJ, van Baare J, du Pont JS, Kamperdijk EWA. Immunogenicity of glycerol-preserved human cadaver skin in vitro. J Burn Care Rehabil (in press).
12. Richters CD, van Pelt AM, van Geldrop E, et al. Migration of rat dendritic cells. J Leukocyte Biol 1996;60:317-22.