

Supplement 1: 1994

**Symposium on the use of
glycerol-preserved donor skin
(procedure devised by the
Euro Skin Bank)**



Organized by the Euro Skin Bank on February 25–26 1993
in Beverwijk, The Netherlands. For further information
contact: M. J. Hoekstra M.D., Euro Skin Bank, Velsersweg 12,
NL-1942 LD Beverwijk, The Netherlands. Telephone: + 31
2510 29311; Fax: + 31 2510 24408.

Edited by: Y. M. Barlow Ph.D. and A. C. J. de Backere M.D. at the
request of the organizers of the Symposium. Their help is
gratefully acknowledged.



Volume 20 Supplement 1 1994

Contents

- S3 Chairman's Overview**
- S4 Euro Skin Bank: large scale skin-banking in Europe based on glycerol-preservation of donor skin**
A. C. J. deBackere
- S10 Long term results of the treatment of scalds in children with glycerol-preserved allografts**
T. A. Brans, M. J. Hoekstra, A. F. P. M. Vloemans and R. W. Kries
- S14 Reduction in *Staphylococcus aureus* wound colonization using nasal mupirocin and selective decontamination of the digestive tract in extensive burns**
D. P. Mackie, W. A. J. van Hertum, Th. Schumburg, E. C. Kuijper, P. Knape and F. Massaro
- S19 Reconstruction of human skin from glycerol-preserved allodermis and cultured keratinocyte sheets**
I. McKay, B. Woodward, K. Wood, H. A. Navsaria, H. Hoekstra and C. Green
- S23 Cologne Burn Centre experience with glycerol-preserved allogeneic skin: Part I: Clinical experiences and histological findings (overgraft and sandwich technique)**
R. Horch, G. B. Stark, J. Kopp and G. Spilker
- S27 Use of glycerol preserved skin in plastic surgery**
R. Tjong Joe Wai
- S32 The use of glycerolized cadaver skin for the treatment of scalds in children**
R. Peeters, D. De Caluwe, C. Neetens and A. Hubens
- S34 Cologne Burn Centre experience with glycerol-preserved allogeneic skin: Part II: Combination with autologous cultured keratinocytes**
G. B. Stark and H. W. Kaiser
- S39 Expansion techniques for skin grafts: comparison between mesh and Meek island (sandwich-) grafts**
R. W. Kreis, D. P. Mackie, R. P. Hermans and A. F. P. M. Vloemans
- S43 History of the Euro Skin Bank: the innovation of preservation technologies**
M. J. Hoekstra, R. W. Kreis and J. S. du Pont
- S48 Histopathological evaluation of scalds and contact burns in the pig model**
T. A. Brans, R. P. Dutrieux, M. J. Hoekstra, R. W. Kreis and J. S. du Pont
- S52 Cultured epidermal autografts and allodermis combination for permanent burn wound coverage**
W. L. Hickerson, C. Compton, S. Fletchall and L. R. Smith
- S57 An animal model to study the significance of dermis for grafting cultured keratinocytes on full thickness wounds**
H. A. Navsaria, T. Kangesu, S. Manek, C. J. Green and I. M. Leigh
- S61 The use of glycerolized human allografts as temporary (and permanent) cover in adults and children**
J. Hussmann, R. C. Russell, J. O. Kucan, D. Hebebrand, T. Bradley and H. U. Steinan
- S67 What role does the extracellular matrix serve in skin grafting and wound healing?**
S. Mac Neil
- S71 The immunogenicity of glycerol-preserved donor skin**
R. Hettich, A. Ghofrani and B. Hafemann
- S77 Virucidal effect of glycerol as used in donor skin preservation**
J. van Baare, J. Buitenwerf, M. J. Hoekstra and J. S. du Pont



History of the Euro Skin Bank: the innovation of preservation technologies

M. J. Hoekstra^{1,2}, R. W. Kreis² and J. S. du Pont¹

¹Euro Skin Bank, Research Department and ²Rode Kruis Ziekenhuis, Burns Centre, Beverwijk, The Netherlands

A skin bank facility is indispensable for burn care. In Holland, a skin bank was established in October 1976 on a national basis for economical reasons. Since 1981, overcoming early rejection by innovation of existing, and development of new, preservation technologies became the main scientific objective. The immunological benefits of glycerolization of skin became clear in 1983. In clinical practice, the use of glycerolized allograft in sandwich grafting changed this technique into a reliable procedure. Early rejection can still be a problem, especially in the non-immunocompromised patient. Future research projects are focused on immunomodulation of the skin by chemical, physical and immunological methods. Even the risk of HIV transmission is not now a drawback for the use of glycerolized skin.

Introduction

A skin bank was established under the patronage of the Dutch Burns Foundation on October 28th, 1976, and was set up on a national basis in order to supply (human) allograft cadaver skin to the three main burn care centres in The Netherlands – Beverwijk, Rotterdam and Groningen.

At that time, the development of artificial skin was promising and would become a reality in the near future. However, as we know now, it was still a wise decision to collect skin, which at first was preserved by deep-freeze techniques (Brown et al., 1953; Eade, 1958; MacMillan and Altemeier, 1962; Shick et al., 1969; Burleson and Eiseman, 1973; Robson and Krizek, 1973). DMSO was used as a cryoprotective agent but, after 2 years, it was replaced by glycerol in low concentrations (Polge et al., 1949; Lovelock, 1953; Lovelock and Bishop, 1959). The operating room was used initially, but soon the skin was harvested from post-mortem donors in the autopsy room.

Since 1977, allograft cadaver skin has been used to cover and protect delicate, widely meshed (1:6–1:9) autografts, preventing desiccation of the remaining vulnerable fatty tissue on the wound bed (Mowlem, 1952; Jackson, 1954; Sneep, 1959; De Groote, 1962; Alexander et al., 1981; Chih-Chun et al., 1982; Kreis et al., 1987).

In our hands, the outcome of these transplantation procedures was unpredictable and even after 1979, when the allograft top cover was meshed 1:1.5, the results were still controversial. Now, the original cryopreservation protocol is under discussion, since we realized at that time we did not have standard operating procedures according to the principles of Good Manufacturing Practice.

There was considerable debate about the issue of early

rejection of these grafts, and consideration was given to immunosuppression of the patients to prolong graft survival (Gillman et al., 1953; Burke et al., 1975). Regarding the papers of Israel Schechter (Weizman Institute, Rehovot, Israel) immunomodulation of the graft would be a better and simpler solution to overcome early rejection than immunosuppression of the patient (Schechter 1975, 1978). Based on the research programmes of Experimental Surgery (Prof. Dr P.J. Kloppe, Academic Medical Centre, Amsterdam) on glutaraldehyde-tanned dermal sheep skin collagen (Van Gulik, 1981), the Beverwijk research group became familiar with tanning technology. Glutaraldehyde is a well-known tanning agent for biomaterials, such as cardiac valves and umbilical vein grafts. Glutaraldehyde pretreated grafts are less antigenic and their influence on wound healing seems favourable (Thomas and Russell, 1974; Russell and Hopwood, 1976; Woodroof, 1978; Shoenfeld et al., 1980; Speer et al., 1980; Bajpai, 1983).

Glutaraldehyde

A research programme was organized by the members of the surgical team of the Burns Centre of the Red Cross Hospital, Beverwijk. Funds were donated from the Dutch Burns Foundation.

Using Schechter's protocol, allografts were processed for 20 min in concentrations of glutaraldehyde of 0.5 per cent or less. Lower concentrations could be favourable if the tissue became too stiff. However, treatment of the grafts with concentrations of glutaraldehyde below 0.3 per cent and shortening the processing times would lead to incomplete cross linking of the collagen of the dermal tissue. Shorter processing times would favour epithelial outgrowth underneath the allograft, but risk an early graft loss by rejection and desiccation. Longer processing times may lead to a durable wound coverage, but would delay epithelial outgrowth.

The ideal requirement for the allograft is that it remains in place throughout healing, while autologous epithelium regenerates and replaces the allograft tissue, thus closing the wound. It is necessary to wash the glutaraldehyde-prepared skin extensively with amino acids to neutralize the free aldehyde radicals, which have undesirable side-effects leading to disturbance of wound healing. Glycine 1.5 per cent, a common rinsing solution for cystoscopic procedures was used.

Table I. Results of pig experiments and transplant rejection

Type		Fresh/ control	Deep frozen	Glutaraldehyde 0.5%	Glycerol 0.5%	Glycerol 0.5%	Glycerol
N		10	4	6	4	10	10
First histological signs of rejection	*1	5.8	4	11	4	7.2	5.8
Infiltration leucocytes	*2	+++	+++	+++	+	+++ (6)/+ (4)	+(6)/+++ (4)
Sloughing of eschar	*3	4-7	4-7	18-21	18-21	18-21	18-21
Wound healing	*4	++	++	+	+	+(6)/-(4)	+++

*1 Observations were made on day 4, 7, 11, 14, 18, 21, 25, 32, 35

*2 + mild, located in upper part of dermis; ++ moderate, diffuse in dermis and wound bed; +++ violent, in dermis and wound bed

*3 Desiccation of eschar and replacement by granulation tissue and/or epithelium

*4 - no epithelialization, only contraction; + less epithelialization, mainly contraction; ++ moderate epithelialization, partly contraction; +++ good epithelialization, hardly any contraction

In an experimental pig study, concentrations of glutaraldehyde and time of treatment were optimized.

Pigs studies

After cardiectomy, skin was harvested from New Yorkshire pigs (Prof. Dr M.J. Janse, Academic Medical Centre, Amsterdam). Part of the skin was applied directly onto recipient pigs, and the remaining skin processed as follows:

Glutaraldehyde preservation Skin was impregnated with 0.5 per cent glutaraldehyde for 20 min at room temperature. The skin was subsequently washed three times in 1.5 per cent glycine for 15 min, and then stored at 4°C.

Cryopreservation Skin was deep frozen according to the method, at that time, of the Dutch National Skin Bank. Briefly, the skin was pretreated with 15 per cent glycerol (v/v) sealed in flat cryopreservation bags packed in polystyrene, frozen gradually to -80°C in a freezer and put into liquid nitrogen vapour for long-term storage (May, 1982).

Clinical practice has shown that human allograft cadaver skin also processed with 0.5 per cent glutaraldehyde and stored at 4°C led to early deterioration characterized by epidermolysis. Lyophilization of glutaraldehyde grafts appeared necessary to prevent this early hydrolytic deterioration.

Being familiar with Sørensen's experience, we did not consider freeze drying technology as a simple, inexpensive solution for storage of donor skin (Buchanan and Lehman, 1952; Chamblor and Sachs, 1969; Abbot and Sell, 1972; Sørensen and Jemec, 1973; Døssing and Sørensen, 1975; Friedlander, 1976). Billingham and Medawar's experiments (1952) using glycerol as the cryoprotective agent showed optimal distribution of residual water after treatment with high concentrations of glycerol. Later, Basile (1982) compared pig skin preserved by freeze drying with skin treated with glycerol in high concentrations. The skin was processed in pure 98 per cent glycerol and stored in 85 per cent glycerol to anticipate total dryness and brittleness (Meryman, 1966).

The results of the pig experiments are shown in Table I. We found that grafts treated with glutaraldehyde, including those pre- or post-treated with glycerol fulfilled the requirements for coverage of the wound bed quite well, but the outgrowth of autologous epithelium was poor compared to fresh and deep frozen grafts. Glycerolized skin permitted better wound healing (Figures 1a,b,c), and also showed a delayed sloughing of the eschar and a mild leucocytic infiltrate located high in the dermis (Figure 2). Slower

deterioration of the allograft and incorporation of dermal remnants were seen.

Glycerol

Glycerol (glycerine)* was discovered by the Swedish chemist, K. W. Scheele in 1779. In 1823, Chevreul in France undertook an intensive study of Scheele's substance and obtained the first patent dealing with its manufacture. The very name, glycerine, is an adaptation of the Greek word "γλυκύς", meaning sweet, and was due to him. Glycerol, 85 per cent in aqueous solution, contains 83.5-88.5 v/v propane 1,2,3-triol. It is syrupy and slippery to touch, colourless, almost clear, odourless, sweet tasting, hygroscopic and miscible with water. Glycerol has developed from an insignificant curiosity into one of the world's most widely used chemicals. It is used extensively in pharmaceuticals as solvents, in creams, and as a humectant or lubricant in a multitude of products, such as gelatin capsules, ear drops, antibiotics and antiseptics, suppositories, elixirs and syrups. Glycerol is also a useful plasticizer in biomaterials, such as fibrin implants to make these materials soft, pliable and easy to use (Newman, 1968; Swinyard and Partak, 1985; Jungermann and Sontag, 1991).

According to Basile's, our own experiences and the experiences of others (Martin and Linwood, 1957; Barr and Linwood, 1957; Thomas, 1963; Burleson and Eiseman, 1972) with this material, its characteristics can be summarized as follows:

- Glycerol does not change the fundamental architecture of the skin: the ingrowth pattern on the wound bed is fully comparable to fresh autograft split skin. The primary adherence by fibrin bonding and secondary adherence as a result of fibrovascular ingrowth are the same.
- Glycerol is an effective antibacterial agent, when used as a storage medium, with slow but definitive action.
- Glycerol has interesting antiviral properties.
- Glycerol clearly diminishes antigenicity of tissues used in transplantation.

Glycerolized allografts

The glycerolization procedure was first implemented in 1983 and became the preservation technique of choice of the

*Glycerine ≡ glycerol 85 per cent.

Dutch National Skin Bank. It has been used in one form or another for the past 10 years.

Many burn centres became familiar with the existence of the Beverwijk Skin Bank and in 1992, the name was changed to the Euro Skin Bank because 70 per cent of all the skin processed was being delivered to more than 30 burn centres throughout Europe.

Between 1984 and 1991, a network of laboratories carrying out basic research was established to support scientific research on skin banking, transplantation procedures and wound healing. Despite the fact that the introduction of glycerol-preserved allografts seems to be a major improvement in skin transplantation technology, the antigenic properties can still cause unintended early rejection and desiccation of the grafts in non-extensive burns (Rappaport et al., 1964; Alsbjörn and Sørensen, 1985). Therefore, further suppression of antigenic properties by chemical, physical and immunological methods, without alteration of the original characteristics of the protein framework of the skin, will be a major goal of research programmes of the Euro Skin Bank.

Acknowledgements

Prof. Dr P. J. Kloppe, Department of Experimental Surgery, Academic Medical Centre, Amsterdam, The Netherlands and Prof. Dr Thea M. Vroom, Department of Pathology, University Hospital, Utrecht, The Netherlands are gratefully thanked for their scientific support and hospitality.

References

- Abbott W.M. and Sell K.W. (1972) Alteration of histocompatibility and species antigens in skin by freeze drying. *Surg. Forum*. **23**, 282.
- Alexander J.W., MacMillan B.G., Law E. et al. (1981) Treatment of severe burns with widely meshed skin autograft and meshed skin allograft overlay. *J. Trauma*. **21**, 422.
- Alsbjörn B.F. and Sørensen B. (1985) Grafting with epidermal Langerhans cell depressed cadaver skin. *Burns*. **11**, 259.
- Bajpai P.K. (1983) Antigenicity of glutaraldehyde-stabilized biological materials. In: Rubin L.R. *Biomaterials in reconstructive surgery*. C.V. Mosby Company, St. Louis, USA.
- Barr M. and Linwood F.T. (1957) Various sugars and polyols on the growth of micro-organisms. *J. Am. Pharm. Ass.* **46**, 219.
- Basile A.R.D. (1982) A comparative study of glycerinized and lyophilized porcine skin dressings for third degree burns. *Plast. Reconstr. Surg.* **69**, 969.
- Billingham R.E. and Medawar P.B. (1952) The freezing, drying and storage of mammalian skin. *J. Exp. Biol.* **29**, 454.
- Brown J.B., Fryer M.P., Randall P. et al. (1953) Postmortem homografts as 'biological dressing' for extensive burns and denuded areas. *Ann. Surg.* **138**, 618.
- Buchanan F.T. and Lehman E.D. (1952) An experimental study of preservation of skin grafts by the freeze-drying process. *Surg. Forum*. **3**, 637.
- Burke J.F., Quinby W.C., Bondoc C.C. et al. (1975) Immunosuppression and temporary skin transplantation in the treatment of massive third degree burns. *Ann. Surg.* **192**, 183.
- Burleson R. and Eiseman B. (1972) Nature of the bond between partial thickness skin and wound granulations. *Surgery*. **72**, 315.
- Chambler K. and Sachs A. (1969) The use of Etox lyophilized skin in burns. *Br. J. Plast. Surg.* **52**, 210.
- Chih-Chun Y., Tsi-Siang S. and Wei-Shai X. (1982) A Chinese concept of treatment of extensive third degree burns. *Plast. Reconstr. Surg.* **70**, 238.
- De Groote F. (1962) Uitgebreide verbrandingen behandeld met alternerende strips. *N. T. v. G.* **105**, 874.
- Dossing M. and Sørensen B. (1975) Freeze-dried and non-freeze-dried split-skin allografts on excised burns. *Burns*. **2**, 36.
- Eade G.G. (1958) The relationship between granulation tissue, bacteria and skin grafts in burned patients. *Plast. Reconstr. Surg.* **22**, 42.
- Friedlander G.E. (1976) The antigenicity of preserved allograft. In: Friedlander G.E. and Sell K.W. *Tissue banking for transplantation*. Grune and Stratton, New York, USA.
- Gillman T., Penn J., Brooks D. et al. (1953) Reactions of healing wounds and granulation tissue in man to auto-thiersch, auto-dermal and homodermal grafts. *Br. J. Plast. Surg.* **3**, 153.
- Gulik T.M. van (1981) *Processed sheep dermal collagen as a biomaterial*. Thesis. University of Amsterdam Rodopi, Amsterdam.
- Jackson D. (1954) A clinical study of the use of skin homografts for burns. *Br. J. Plast. Surg.* **7**, 26.
- Jungermann E. and Sonntag N.O.V. (1991) *Glycerine: a key cosmetic ingredient*. Marcel Dekker, New York.
- Kreis R.W., Vloemans A.F.P.M., Hoekstra M.J. et al. (1987) The use of non-viable glycerol-preserved cadaver skin combined with widely expanded autografts in the treatment of extensive third degree burns. *J. Trauma*. **29**, 51.
- Lovelock J.E. (1953) The mechanism of protective action of glycerol against haemolysis by freezing and thawing. *Biochem Biophys Acta* **11**, 28.
- Lovelock J.E. and Bishop M.W.H. (1959) Preventing of freezing damage to living cells by dimethyl sulphoxide. *Nature* **183**, 1394.
- MacMillan B.G. and Altemeier W.A. (1962) Homograft skin – a valuable adjunct to the treatment of thermal burns. *J. Trauma*. **2**, 130.
- Martin B. and Linwood F.T. (1957) A study of the inhibitory concentrations of glycerine-sorbitol and propylene-sorbitol combinations on the growth of microorganisms. *J. Am. Pharm. Ass.* **46**, 217.
- May S.R. (1982) Skin Banking and Preservation Proceedings. *Sixth ISBI Congress*. San Francisco, USA.
- Merryman H.T. (1966) Residual moisture. In: Merryman H.T. *Cryobiology*. p 636. Academic Press, London and New York.
- Mowlem R. (1952) Skin homografts. *Medicine Illustrated*. **6**, 552.
- Newman A.A. (1968) *Glycerol*. Morgan-Grampian, London.
- Polge C., Smith A.U. and Parkes A.S. (1949) Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature*. **164**, 666.
- Rappaport F.T., Converse J.M., Horn L. et al. (1964) Altered reactivity to skin homografts in severe thermal injury. *Ann. Surg.* **159**, 390.
- Robson M.C. and Krizek T.J. (1973) Predicting skin graft survival. *J. Trauma*. **13**, 213.
- Russell A.D. and Hopwood D. (1976) The biological uses and importance of glutaraldehyde. In: Ellis G.P. and West G.B. *Progress in medical chemistry*. Vol. 13. North-Holland Publishing Company, The Netherlands.
- Schechter I. (1975) Prolonged retention of glutaraldehyde-treated skin allografts and xenografts. *Ann. Surg.* **182**, 699.
- Schechter I. (1978) Increased resistance to infection of glutaraldehyde-treated skin grafts. *Br. J. Plast. Surg.* **31**, 229.
- Schoenfeld A., Ovadia J., Edelstein T. et al. (1980) The use of biological dressing in radical vulvectomy. *Eur. J. Obstet. Gynaec. Reprod. Biol.* **10**(6), 407.
- Shuck J.M., Pruitt B.A. and Moncrief J.A. (1969) Homograft skin for wound coverage. *Arch. Surg.* **98**, 472.

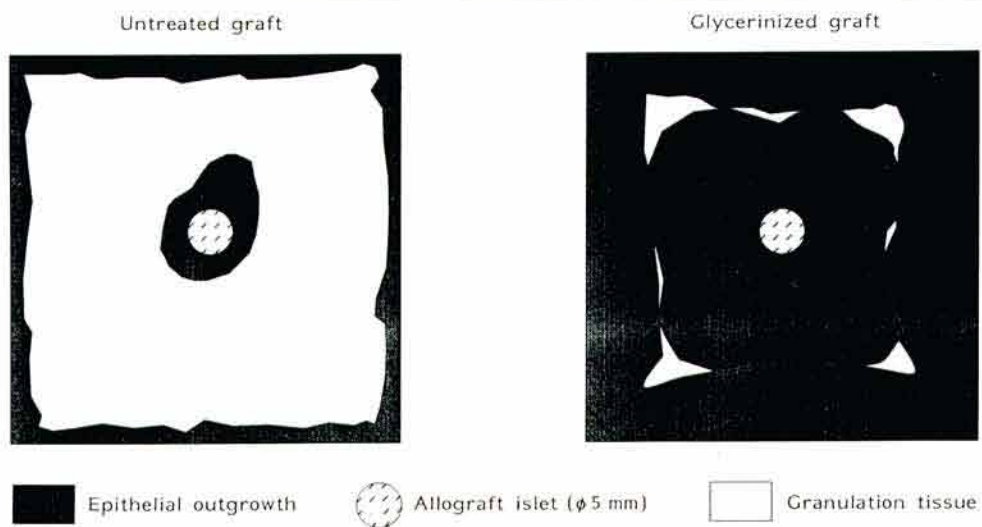
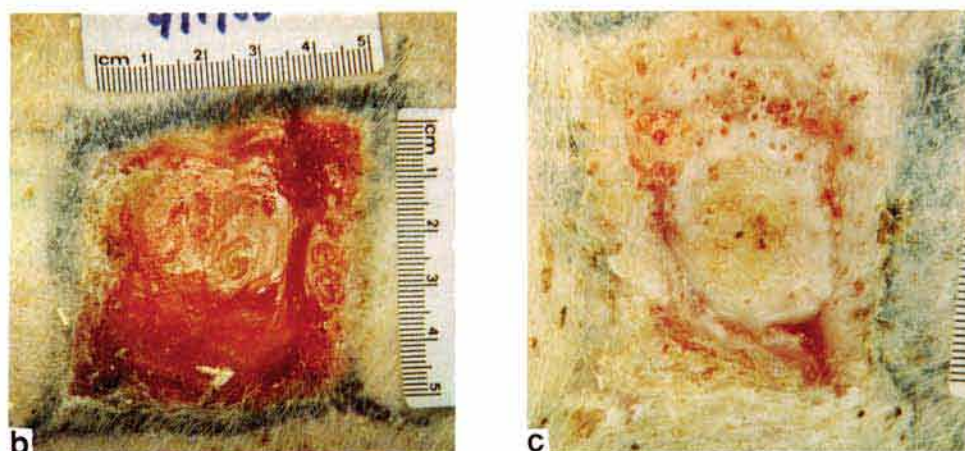
**a**

Figure 1. **a**, Difference of outgrowth between an intermingled split skin autograft island of 5 mm on a wound bed of 5 × 5 cm **b**, covered with an untreated allograft and **c**, a glycerolized allograft, 4–5 weeks postgrafting.

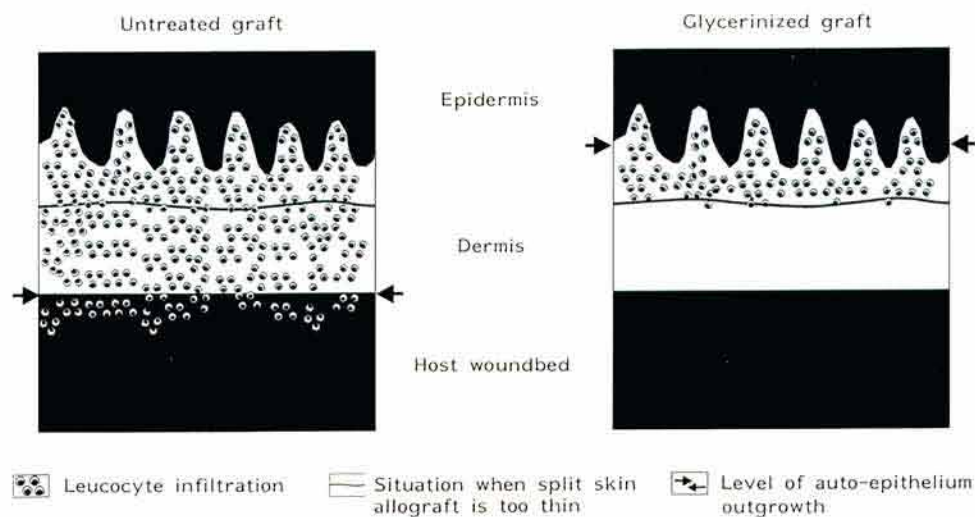


Figure 2. Difference in leucocyte infiltration into the allograft and wound bed.

- Sneep A.J. (1959) The significance of skin homografts in the treatment of deep burns. *Arch. Surg. Neerl.* **11**, 368.
- Sørensen B. and Jemec B. (1973) Freeze drying of skin. *Scand. J. Plast. Reconstr. Surg.* **7**, 35.
- Speer D.P., Chvapil M., Eskelson C.D. et al. (1980) Biological effect of residual glutaraldehyde in glutaraldehyde tanned collagen biomaterials. *J. Biom. Mat. Res.* **14**, 753.
- Swinyard E.A. and Partak M.A. (1985) Glycerine. In: Goodman and Gillman. *Locally acting drugs* (Section X, Chapter 40) Surface-acting drugs. 7th ed.
- Thomas R.P. (1963) Glycerine. *Arch. Ophthalmol.* **70**, 625.
- Thomas S. and Russell A.D. (1974) Studies on the mechanism of the sporicidal action of glutaraldehyde. *J. Ap. Bact.* **37**, 83.
- Woodroof E.A. (1978) Use of glutaraldehyde and formaldehyde to process tissue heart valves. *J. Bioeng* **2**, 1.

Correspondence should be addressed to: Dr M. J. Hoekstra, Euro Skin Bank, Research Department, Velsersweg 12, NL-1942 LD Beverwijk, The Netherlands.