INTERMINGLED SKIN GRAFTING: A VALID TRANSPLANTATION METHOD AT LOW COST

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SUMMARY. The almost forgotten method of intermingled skin grafting of allogeneic material with small autogeneic islets, once developed in the People's Republic of China, proves the feasibility of permanent healing of even extensive burn wounds, at low cost, and therefore an effective treatment possibility in poorer countries, as well as under conditions of a burn disaster. Intermingled skin grafting obtains a better elasticity of the reconditioned skin as elastic fibres of the allodermis survive, and this results in fewer contractures. From the cosmetic point of view the transplantation of autologous keratinocytes results in a better aesthetic homogeneous texture.

Introduction

Patients with extensive severe burn injuries present a high lethality rate. Although, due to advances in intensive care, it has been possible to extend the life of severely burned patients beyond the shock stage, many die from later complications.

The survival prognosis for patients with severe burn injuries depends on several risk factors. After direct appropriate first aid the main influencing factors are the size of the burned body surface and the age (children and older people have a higher lethality). Further factors are inhalation trauma, depth of burn, and sex (women are more endangered than men, because of their different skin structure), as well as cardiological, pulmonary, renal, and endocrine pre-diseases. Other risk factors are nicotine, alcohol, and drug abuse.

The only possibility to reduce the lethality of burn victims, or rather to optimize their survival prognosis, lies in an early and effective excision of the burned tissue and subsequent definitive coverage of the affected skin areas by auto-, allo-, or xenografts.

Autogeneic, allogeneic, or xenogeneic wound covering

To cover deep, partial-and full thickness burned skin areas auto-, allo-, or xenografts are considered, and already several of them have been combined to obtain an optimal definitive result. A temporary covering with biosynthetic materials alone *(Table I)* is still problematic, not permitted in every country, and cost-intensive, while the definitive coverage with split-thickness autograft skin, the "gold standard" in the treatment of burn injuries, lacks mechanical quality in third-degree burns and has limited capacity. In extensive burns (> 70% body surface), there is not enough autologous donor skin available to cover the burned

Table I - The various methods of wound covering by autogeneic, allogeneic, xenogeneic and biosynthetic materials and their possible applications

Autogeneic materials	Split-thickness autograft skin			Keratinocyte autografts
Form	*Sheet*	*Mesh-graft*	*Meek-graft*	Cultured sheet
Applications	Primarily face, cervix, hands, etc. in 2nd-degree burns	Major wounds in 2nd-3rd degree burns up to approx. 70% body surface		Partly 2nd- and 3rd-degree burns
Allo- and xenogeneic materials	Allograft	Xenograft (e.g. pig)	Amnion or alloderm	Keratinocyte allografts
Applications	Temporary or as matrix for neocorium in 3rd- degree burns	Temporary in 2nd- and 3rd-degree burns	Temporary in 2nd- or 3rd-degree burns	Temporary in 2nd-degree burns
Biosynthetic materials (examples)	Biobrane™, Epigard®, etc.(1)	TransCyte®(2)	AlloDerm®(3)	Integra®(4)
Applications	Temporary skin substitutes	Temporary matrix in 2nd- and 3rd- degree burns (USA)	Chorion substitute/matrix in 3rd-degree burns in combination with split-thickness or keratinocyte autograft sheets	

(1) [1,2] (2) LifeCell Corporation, USA.[3] (3) Integra Lifesciences Corporation, USA.[4] (4) Once Dermagraft, TC, Advanced Tissue Sciences Inc., USA.[5]

areas sufficiently by this standard treatment. But until now a permanent coverage has never been obtained successfully without some kind of autograft, because synthetic materials cannot assume the task of the physiological skin and allo- or xenograft skin will be rejected in the long term.

Auto- or allografts of cultured keratinocytes

As a possible solution to this problem in the treatment of extensive, severe burn wounds, the *in vitro* cultivation of autologous or allogeneic keratinocytes has been established over the past years.

Keratinocytes are isolated from a small biopsy, and in 7-8 days a primary culture develops. After 10 days a complete sheet is cultured and can be applied to a gauze carrier.[6] The cultivation of such keratinocyte sheets sufficient, for a whole body surface takes between 3 and 4 weeks. During this period the wound bed must be kept low in germs and free of granulation tissue. Often cadaver allografts are used in preparation for this.[3]

Autologous keratinocyte sheets, the so-called cultured epithelial autografts (CEA),[7] are then applied to the excised wound bed. But CEA's fragile structure and its lack of dermal components remain poor in mechanical quality in deep partial- and full-thickness burns. After months and years a vesication or even a rejection is possible,[8-10] and finally controversial reports claim that less than 50% of autologous keratinocyte sheets heal. There are still problems with wound contractures, and the factor responsible is probably the abnormal structure of anchoring fibrils.[7,9]

Clinical results from keratinocyte autografting as well as allografting have also suggested a wound healing effect.[9] For example, keratinocyte allografts, derived from neonatal foreskin, have been applied to split-thickness donor sites, resulting in an accelerated re-epithelialization of the wound bed, which means a rapid reharvest, ready for recropping split-thickness autograft skin.[7]

This is possible due to the keratinocyte graft's production of extracellular matrix proteins, which can bind cytokines as well as having direct effects on keratinocyte differentiation and migration, and growth factors (including basic fibroblast growth factor) secreted by the allograft cells onto the wound. But this works only in superficial injuries. Wounds covered with keratinocytes alone remain fragile and generally do not result in acceptable long-term coverage.[7,9]

Intermingled skin transplants

In the 1970s an entirely new transplantation technique, developed in the People's Republic of China, was first brought to Germany, by B. Domres. It offered successful, life-saving treatment of patients with severe burn injuries covering more than 90% of the total body surface area. This stunning possibility was due to the fact that the new technique allowed excision of the main part of the burned skin very soon after the injury, immediately followed by a permanent coverage of the defects with a mixture of allogeneic corpse-skin and small autogeneic islands.

Between 1966 and 1980 this method was successfully used in 5262 cases of severe burn injuries, especially for large-area burns, when only a little of the patient's own skin was available for grafting.[11] Experiments with rats in 1988/89 by Kistler et al. showed the stages of healing of intermingled skin transplants at different time intervals.[11,12]

Methods

Immediately after the first shock stage the burn injuries receive a one-time topical treatment with 0.04 M cerium nitrate. Thereby a complexation and deactivation of the burn toxins is obtained and this permits a less hazardous choice of the optimal moment for the necessary gradual excision of the infected wound areas.

Subsequently these wounds are covered with freshly taken allograft skin, from which little squares of approximately 0.5 cm^2 are cut out, with a distance of 1-2 cm between them. One or two days after this transplantation the "holes" are filled with autologous split-thickness skin islands of the same size. Normally this can be done as a bedside operation without the necessity for further anaesthesia.[12] An alternative possibility to this would be to cut out the squares from the allograft skin in the second step, a few days after the transplantation, and replace them directly by the autologous split-thickness skin islands.

From these autogeneic islands, cells grow out radially and rapidly substitute the epidermal defects, covering the wound definitively with autogeneic epidermis, which is macroscopically obvious. Although during the first weeks the histological picture shows considerable infiltration with lymphocytes, this process of regeneration of the epidermis usually works without complications. Until the complete wound is successfully covered with autoepidermis all parts of the transplant, i.e. the autogeneic islands as well as the covered donor skin area, show considerable infiltration by lymphoid and polymorphonuclear cells. Then a decrease of the lymphocytic infiltration can be detected, while areas not yet covered with epidermis appear as massively infected wound ulcers.[11]

Nevertheless there is no obvious macroscopic rejection of the relatively large allogeneic parts, as would be expected for immunological reasons. Only the ectodermal and endodermal components of the allograft, i.e. epidermis, endothelium, and skin adnexa, are rejected over a period of 15-25 days, because of their significantly higher antigenicity, unlike that of the dermal connective tissue. The remnants of the allodermis seem to be gradually re-

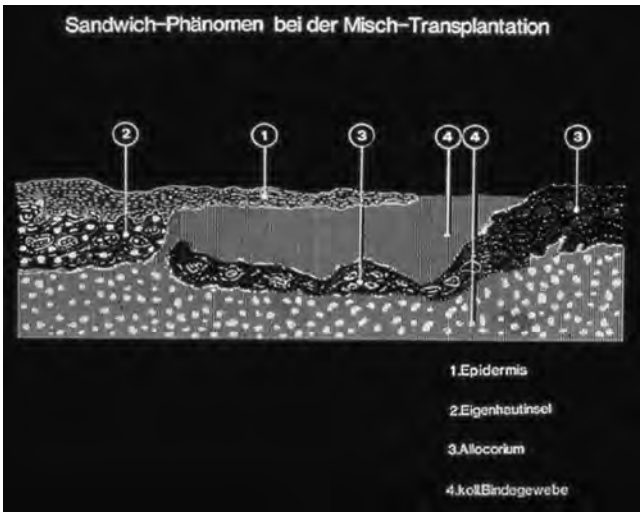

Fig. 1 - Schematic representation of the "sandwich phenomenon", incorporation of the allochorion: 1 = epidermis, 2 = autologous islands, 3 = allochorion, 4 = neochorion.

placed. (A general immunological rejection of the graft occurs in very few cases only.) This means that the allodermis obviously serves as a matrix for the outgrowing epithelial cells.

Experiments with tissue of rats of different sex have demonstrated that even after 111 days allogeneic cells remain in the tissue and that this allograft is interpersed with many elastic fibres, which demonstrates furthermore that intermingled skin transplants keep at least a part of their original elasticity.

After complete healing, the allograft is reduced to a relatively thin tissue layer, free of adnexa and embedded on both sides by connective tissue. The epidermis appears almost equally mid-high, basically smooth and with marginal hornification of the surface. The only conspicuous difference in comparison to normal epidermis is the lack of rete ridges and fewer adnexa. The Chinese called this typical of layering the "sandwich phenomenon" *(Fig. 1)*. It seems that the rejection of the allograft is avoided by the fast overgrowing of the autogeneic epidermis. A conclusion from this is that a temporal difference between the rejection of allodermis and epidermis can be taken as a main prerequisite for the successful treatment of severe burn wounds by using the intermingled skin transplant technique.

Histopathology

In an experiment with rats, biopsy specimens of 10 x 2 mm were excised on days 13, 20, 25, 34, 45, 100, and 111 post-operatively and fixed in 4% formaldehyde for 24 h. Sections of 5 µm were stained with haematoxylin and eosin, Masson/Goldner, or Van Giesen stains.[11]

Fig. 2 - A well-taken transplant with a dry wound bed.

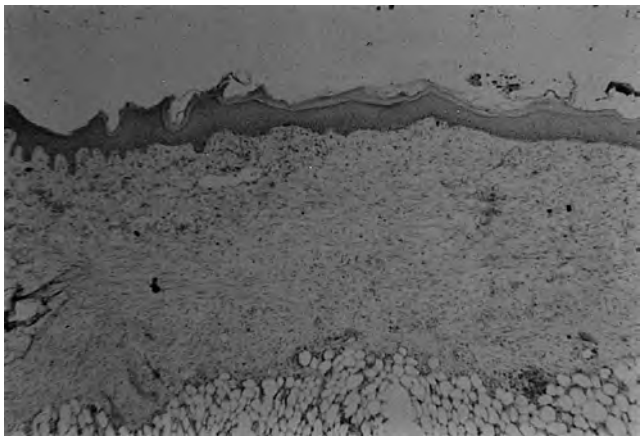

Fig. 3 - The allodermis, overgrown by autogeneic epidermis, after 29 days (E. v. G.). The autogeneic islands showed a typical skin structure with a smooth surface, rete ridges, and intact adnexa. The areas in between had a thin wave-like epidermal surface, while the allodermis underneath lacked all adnexa, appeared swollen and infiltrated, and was interpersed with many elastic fibres. The new parallel-striped collagenous connective tissue underneath was also infiltrated.

Five days after an extensive burn the animals` condition was very similar to that of severely burned patients. After removal of the eschar the wound bed was immediately covered with allograft split skin, including the autologous islands *(Fig. 2)*.

Twelve days after this transplantation, most of the necrotic alloepidermis was rejected, the histological examination showing an intense infiltration of lymphoid cells and polymorphonuclear leucocytes. By now the skin adnexa had disappeared completely, while the dermis surface was infiltrated with granulocytes. The autogeneic islands, clearly distinct from the surroundings, were also considerably infiltrated by lymphatic plasma cells, while the col-

Fig. 4 - Section showing three-layered structure of the dermis with (bottom of figure) the autoepidermis, the extensive proliferation of connective tissue between the epidermis and the allodermis with clumped elastic fibres (E. v. G.).

lagen fibres in the region were swollen, and partially elastic fibres could be identified. Beneath the islands parallel-striped collagenous connective tissue was visible.

By day 20 even the adnexa could be seen on the autologous islands and it was microscopically obvious that epithelialization proceeded radially from them. The allodermis, without epidermis, appeared to be ulcerated and covered with fibrin.

The autogeneic islands had an atypical skin structure with a smooth surface, rete ridges, and intact adnexa. The epithelialized areas in between showed a thin wave-like epidermal surface with only shallow rete ridges. The allodermis underneath lacked all adnexa and appeared swollen, considerably infiltrated, and interspersed with many elastic fibres. It was placed on top of fresh collagenous connective tissue, which was also considerably infiltrated *(Fig. 3).*

Areas that were not covered with epidermis showed differing layers depending on their distance from the autologous islands. Proximal regions consisted of three layers, i.e. young connective tissue with a layer of allodermis in between.

Fig. 5 - Human intermingled skin 2 years after transplantation (phase contrast). Depending on its distance from the autogeneic islands, the allograft is directly covered with autogeneic epithelium. The remaining allochorion is clearly visible as a bright area in between an expanded layer of neochorion. The new epidermis (without rete ridges) is located at the upper left side. On the right the autochorion is shown as an expanded bright layer.

On the other hand, in the more distal regions, the allodermis was ulcerated with massive granulocyte infiltrations which increased in number towards the surface.

On the day the wound bed was almost completely covered with epidermis, the different tissue layers of the allotransplant were clearly visible and scarcely infiltrated by lymphocytes; there was extensive proliferation of connective tissue between the epidermis and the allodermis with clumped elastic fibres (E v. G.). The epidermis seemed to be nearly normal, although the autoepidermis of the islands contained relatively large swollen fibres *(Fig. 4).*

The allodermis was almost overgrown by autoepidermis. Elastic fibres appeared where autodermis covered the allodermis. No scar formation took place between the autoepidermis and the allodermis. The histological results were the same after 111 days. As a result the allodermis was surrounded by collagenous connective tissue and the allografts were directly covered with autogeneic epithelium *(Fig. 5).*

The demonstration of the presence of elastic fibres in the allodermis also shows that skin transplanted in this way keeps at least part of its basic elasticity *(Fig. 6).*

Case report

In 1982, during a military conflict in the Laghman province of Afghanistan, a 21-year-old male sustained severe burn injuries as a result of a blast in a petrol tank. Immediately taken away from the battlefield, he received the treatment available in that environment for a period of

Fig. 6 - The intermingled skin graft obtains part of its basic elasticity from the elastic fibres of the allodermis that survive.

Fig. 7 - After debridement of the patient's left knee joint, a Fixateur was installed to stabilize it.

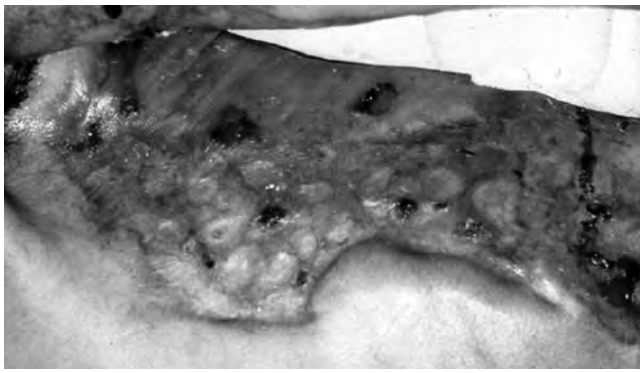

Fig. 8 - Allograft skin with autologous split-thickness skin islands, taken from the patient's scalp.

6 months. After this he was brought across the border to the Afghan Surgical Hospital in Peshawar, Pakistan.

On admission his survival prognosis was very low. He had full-thickness burn wounds of nearly 50% of body surface, with discharge of foul-smelling, greenish pus. His muscles were considerably wasted and atrophied and he was unable to extend either knee.

He then received the classical treatment for burn injuries: he got an isolated room, was kept as antiseptic as possible, and his wounds were exposed to air and dressed as and when indicated. Sufficient rations of fluids, local and general antibiotics, pain killers, and other appropriate drugs were given to him, as also as ten pints of blood. In a second treatment series by a plastic surgeon at the Government Leady Reaning Hospital, Peshawar, the burn wounds were off and on dressed and skin grafts were done according to a gradual and successive schedule. During this period almost 20 pints of blood were transfused, and he received antibiotics, analgesics, etc. But regretfully all attempts were in vain. Most of the grafts were rejected and the general condition deteriorated.

Then, in 1983/1984, he was transferred for treatment to the University Hospital in Tübingen, Germany. The third-degree burn wounds were still unhealed and infected with an extent of 40-50% of the patient's body surface. His left knee joint was bent in contracture due to deep burn wounds. The patient also had extreme cachexia with hookworm infestation, urinary bladder stones, and ongoing diarrhoea, as also a right eardrum perforation.

After a high-caloric infusion pre-treatment and normalization of the metabolic situation, as well as the restoration of normal bowel function under Vermox therapy, the patient was able to receive debridement of the left knee joint and stabilization with an external fixator *(Fig. 7)*. Following this, the first skin transplantation started. For this, in preparation for an intermingled skin grafting, the patient's burn wounds were covered with fresh allograft skin, from which little squares of approximately 0.5 cm^2 were cut out, according to the above-described technique.

Three days later, after starting revitalization of the allografts, the patient received the second skin transplantation. The cut-out square holes of the allograft skin were covered with autologous split-thickness skin islands of the same size, taken from the patient's scalp.

Although the patient's general condition was still not optimal, the operation was successful up to that point *(Fig. 8)*.

Because of the persisting infection a small part of the transplants did not take and was covered by a third skin transplantation one month after the second one. During this, autologous split-thickness skin islands were taken once again from the patient's scalp to cover the counterpart cut-out areas of allograft skin.

About 15 months after admission the patient was able to walk independently, only with one stick. Today he lives in Pakistan, is married, has two children, and works as a tailor.

Discussion and conclusion

In comparison with the keratinocyte method, the most frequently used treatment for extensive severe burn injuries during the past years, the intermingled skin transplant technique offers several advantages.

The cultivation of autologous keratinocyte sheets needs at least three weeks. During this time the wound bed must be kept low in germs and free of granulation tissue, which means high costs of personnel and materials. The cultivation of the keratinocyte sheets costs about 500 US dollars per 25 cm^2 (the total body surface measures about 20,000 cm^2). Also, the further rejection of a temporary cover means an additional burden for the patient.

In the best case the sheets should be applied to the wound bed preferably freshly excised, as this supports the grafts better than granulating tissue. This best case is given by the use of intermingled skin grafting, which is immediately available. Its cost is less than 10% of the cultured keratinocyte procedure.

Furthermore, intermingled skin grafting obtains better effects in elasticity of the reconditioned skin, as the elastic fibres of the allodermis survive, resulting in fewer contractures. From a cosmetic point of view alone, the difference of the tissue is visually better with this method, while the treatment with autologous keratinocytes until now affords better aesthetic results owing to its homogeneous texture. Intermingled skin grafting is a successful and significant low-cost method for subsequent definitive coverage of extensive severe burn injuries.

RÉSUMÉ. La méthode presque oubliée de la greffe cutanée mixte de matériel allogène avec des îlots autogènes, développée il y a quelques années dans la République Populaire de Chine, démontre la possibilité réelle d'obtenir la guérison permanente de brûlures même très étendues avec des coûts limités, ce qui offre des occasions favorables de traitement dans les pays plus pauvres, comme aussi dans les conditions qui se produisent après un désastre par feu. La greffe cutanée mixte mène à une élasticité supérieure de la peau reconditionnée puisque les fibres élastiques de l'alloderme survivent, ce qui provoque une fréquence moins élevée de contractures. Du point de vue cosmétique la transplantation des kératinocytes autologues produit une texture homogène meilleure.

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