Perspectives on burn scar evaluation and artificial skin

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Tissue engineering and skin substitution: a review

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Abstract  At present, much attention is focused on the development of artificial organs that replace destroyed or malfunctioning organs in human. The skin is no exception. Much effort has been undertaken, and costs been made, to develop skin substitutes that replace injured skin. Main areas of interest are the extensively burned patients who lack autologous donor skin and the venous or diabetic leg ulcers. Skin substitutes are not only claimed to replace lost tissue but they should improve the outcome of the current treatment modality for the full thickness wound: the autologous split skin graft. In this paper an outline is given of ongoing research and the results of clinical applied skin substitutes in acute burn wounds.
Introduction

Rheinwald and Green opened the way for epidermal substitutes in 1975 by developing a technique to culture keratinocytes that allowed clinical application\(^3\). Some years later, Yannas and Burke reported fundamental studies on the development of a biological dermal substitute derived from bovine collagen\(^4\). It has been hypothesised that the application of an artificial dermis results in the formation of a neodermis that has a structure comparable to normal dermis. These studies proclaimed the start of a new area in wound healing research, the area of tissue engineering as well as epidermal, dermal and full skin substitutes. In this paper an overview is given on the progress made by experimental and clinical studies on the different types of skin substitutes.

Epidermal substitutes

In 1975, a method became available for the use of enzymatic techniques to harvest cultured keratinocytes as intact sheets\(^1,2\). This technique allowed preparation of keratinocyte sheets for clinical use. Cultured epidermal autografts were considered beneficial for grafting of large burn wounds when donor sites were limited. Clinical studies demonstrated variable successes with the use of cultured epidermal autografts in burns. A major concern was the poor survival of the keratinocyte sheets\(^9,12\). A meta-analysis on the graft survival showed that the average survival was about sixty percent of the applied sheets\(^3\). The main causes of graft failure were wound infection and haemorrhage\(^9,11\). Other relative contraindications were grafting on granulation tissue and friction areas\(^9\). On the other hand, grafting on freshly excised wounds in an early stage showed improvement of the sheet survival\(^3\).

On the long-term, skin fragility and easy blistering were frequently encountered as result of a paucity of anchoring fibrils\(^14\)-\(^16\), probably because the anchoring fibrils take more than one year to be regenerated at a normal level\(^7\).
Although keratinocytes may be multiplied a 500-fold before the cells lose their proliferation potential, the time that is required for achieving a keratinocyte sheet for clinical application is disappointing. The critical delay of time is partially due to the necessity to form a multi-layered keratinocyte sheet and counteracts the widespread clinical use of cultured epidermal autografts. The development of biocompatible carriers for single-layered keratinocytes allowed grafting at earlier culturing stages. So far only preliminary clinical data have been published on this concept.

The chimeric epidermal substitutes, which are confluent sheets composed of allogeneic and autologous keratinocytes, may be considered for fast preparation of a cultured epithelium. Experimental studies suggested that the allogeneic/autologous cells can be applied in ratios like 20:1. Histopathological follow-up showed that only autologous epidermal cells remain in the regenerated epidermis after one to two months. At this moment no clinical trials have been published on the clinical application of the chimeric epidermal substitute.

Another solution to overcome the critical time period for culturing is to use lysates of cultured keratinocytes. Lysates seem to contain mitogenic activity for keratinocytes, endothelial cells, and fibroblasts and inhibited contraction by fibroblasts in collagen gels. In burn wounds, a gel with lysated keratinocytes appeared to have a stimulatory effect for epidermal repair comparable to that of cultured allogeneic keratinocytes.

Recently, Langerhans cells and melanocytes have been added to create a more complete epidermal substitute, but the clinical significance of adding these cells to the substitute remains to be determined.

We concluded that the clinical application of cultured keratinocytes for epidermal skin replacement seems beneficial in cases when conventional methods fail to supply sufficient skin covering. Moderate take results, wound covering delay, high costs, and skin instability on the long term are disadvantages of this technique that have important practical consequences.
Dermal substitutes

Basic physical requirements for dermal substitutes, were given by Yannas and Burke based on fundamental studies, and concern clinical features such as tear strength, elasticity, peel strength, handling and suturing characteristics. The ideal pore size between the fibres of the lattice to allow migration of the fibroblasts from the wound bed into the lattice was shown to be between 20 and 125 μm. Smaller pore sizes might limit cell migration, whereas larger pore sizes will not provide sufficient attachment area for the migrating cells. The biodegradation of the product should take place in about three to four weeks.

Different materials have been used as base component of a dermal substitute such as collagen, synthetic materials and human cadaveric donor dermis. The seeding of autologous or allogeneic fibroblasts on an acellular lattice has been performed to create a living dermal substitute. Both allogeneic and autologous fibroblasts have been beneficial with respect to macroscopic and histological results in different in vitro and animal studies. In the case of allogeneic cells, rejection might be expected due to immune reactivity of the recipient. Although allogeneic fibroblasts induced no immunogenic reactions in the host, they may accelerate second-set rejection. The major drawback of autologous fibroblasts is the delay in grafting that is caused by the time required to culture sufficient cells.

Also endothelial cells have been used for the preparation of a skin equivalent with a capillary network to improve graft revascularization. The clinical significance of such promising adjustments is yet unclear.

Collagen lattice

Collagen is the most prevalent protein of normal human dermis, and provides a unique combination of strength and flexibility to the dermis. The choice for collagen seems therefore logical, also because of its abundance in nature and its low antigenicity. Products have been added for further improvement of wound healing parameters like glycosaminoglycans (GAG) chondroitin-6-sulphate, hyaluronic
Based on their inventory studies, Yannas and Burke created a highly porous acellular lattice of bovine collagen combined with GAG derived from shark cartilage. The addition of this GAG, chondroitin 6-sulfate, was based on a study in which chondroitin 6-sulfate provided more elasticity, delay of biodegradation, a more open pore structure, and no inflammation during fibroblast ingrowth. In vitro studies could not confirm the positive effect of chondroitin 6-sulfate, dermatan sulphate, and hyaluronic acid. In animal studies, the addition of elastin components to the collagen lattice resulted in a reduced cellular influx, a decreased number of myofibroblasts and more randomly orientated collagen bundles resembling normal skin. Native collagen seemed to be superior to reconstituted collagen, since native collagen degraded less rapidly than reconstituted collagen. Reconstituted collagen was degraded within seven days, whereas a native collagen matrix was totally absorbed within six weeks.

Integra (Lifesciences Corp., Plainsboro, NJ) is the bilayered membrane based on the concept of Yannas and Burke. The dermal layer consists of a combination of bovine collagen and chondroitin 6-sulfate covered by a disposable ‘epidermal’ silicone layer. The silicone sheet acts as a barrier against bacteria, controls water evaporation, and provides mechanical support. The bilayered membrane is applied during the first operation after preparation of the wound bed. The silicone layer is removed after an average period of two to three weeks and replaced by a split skin graft. In the first clinical study, a good neodermis was provided resembling the normal dermis. The bovine collagen was not detectable after seven weeks, indicating an effective biodegradation. In a multicenter trial, Integra was applied in 139 wounds of 106 patients. Twenty-six patients were evaluated for more than one year and showed less hypertrophic scarring with comparable appearance and function compared to the control site. Histopathological studies on biopsies taken from 131 patients treated with Integra demonstrated good wound repair with a minimum of scarring. No significant immunological problems were found after the application of Integra in patients with extensive thermal injuries.

Suzuki published comparable data with an Integra-like skin substitute in small clinical settings. According to the authors, the skin sub-
stitute resulted in good take results, showed low antigenicity, and the silicone layer was easily removed during the second operation because of a high biodegradation rate of the lattice. Experimental studies on full-thickness wounds in pigs showed good dermal regeneration and a reduction of wound contraction for a bovine collagen/ elastin dermal substitute seeded with fibroblasts. The quality of dermal regeneration was significantly improved for wounds treated with dermal substitutes with high numbers of autologous fibroblasts (500,000 versus 100,000 cell per square cm).

**Synthetic lattice**

Synthetic materials are used in surgery for a long time. The most commonly used materials are degradable sutures. More recently, different synthetic materials have been developed as dermal substitutes and tested in different *in vitro* and *in vivo* studies as skin replacements, such as Vicryl (polyglactin acid mesh), Dexon (polyglycolic acid mesh), and Polyactive. Polyactive (IsoTis BV, Biltoven, the Netherlands) is an example of a biodegradable synthetic bilayered polymeric matrix made of a polyether/polyester co-polymer. The top layer is dense, whereas the under layer is porous. The material was biocompatible, showing only mild cellular reaction in a histological study in goats. Another animal study showed positive effects of the fibroblasts with respect to a higher collagen deposition and a slower degradation time for the cell seeded versus the acellular substitute.

Dermagraft (Advanced Tissue Sciences, La Jolla, CA, USA) is a Vicryl mesh impregnated with viable, allogeneic human neonatal foreskin fibroblasts. A clinical study showed no immunologic reaction against the allogeneic fibroblasts. New elastin was not detected after one year. In a mouse model with controlled bacterial contamination Dermagraft did not increase the occurrence of graft loss compared to the control site. Dermagraft treatment was assessed to be cost-effective compared to standard treatments for diabetic foot ulcers.

**Allogeneic cadaveric donor skin**

Human donor skin is theoretically the perfect dermal substitute as it provides a natural three dimensional collagen structure and a
natural basement membrane complex that is known to play an important role in the process of normal wound healing. The antigenicity of the cryopreserved donor skin has been attenuated by cryopreservation. Moreover, noncellular components of the cadaveric dermis have been shown to be relatively nonimmunogenic. In addition, decellularised allogeneic donor skin gave better results compared to the cellular allogeneic donor skin. In a human and porcine wound model, the allograft supported fibroblast ingrowth, neovascularization, and epithelialisation and resulted in good wound healing without evidence of inflammatory reaction and immunogenic response. The potential danger of transmittance of human immunodeficiency virus, other contagious diseases, and immunological rejection, however, limits the possibilities for using cadaveric donor skin.

AlloDerm (LifeCell Corp., Branchburn, NJ) is an acellular dermal substitute processed from cryopreserved human cadaver skin. Cells of the epidermis and dermis are removed, leaving the remaining tissue intact. In a multicenter trial (43 patients) equal take rates were found comparing the AlloDerm split-skin graft application versus the standard split-skin graft application. Thinner mesh grafts applied on AlloDerm resulted in better take rates, probably because of a lower metabolic demand and shorter nutrient distance. In the long term, less scarring and contracture was demonstrated. In an animal model, a poor survival of thick split skin grafts was shown and thin split skin graft were necessary to obtain acceptable take-rates in one stage grafting together with the acellular allogeneic dermal substitute underneath the split skin graft.

In conclusion, acellular collagenous, synthetic or allograft dermal substitutes can be easily stored and used as an off-the-shelf product. Although experimental work shows promising results, more clinical research is mandatory to establish clinical effectiveness. Some procedures still require an autologous split skin graft for epidermal coverage. However, if the epidermal layer has to be provided by means of cell culturing, the costs will increase considerably.

A cellular dermal substitute can be created by impregnating autologous or allogeneic fibroblasts into the dermal substitute. Fibroblasts
have shown to improve dermal repair in animal models but the clinical effectiveness needs to be determined to date.

**Complete skin substitutes**

When replacements of both epidermis and dermis were developed, both techniques were combined to design a complete skin substitute. A full skin equivalent may be the answer for the lack of donor sites in the extensively burned patient. Moreover, the dermal component overcomes the technical problem of the delivery of cultured keratinocyte alone, as they are difficult to handle. Different dermal substitutes have been tested that served as a carrier for keratinocytes. Keratinocytes adhered better to Dermagraft than to AlloDerm, however allogeneic dermal substitutes gave a more natural formation of rete ridges compared to a collagen based dermal substitute. Others used two collagen layers, the lower layer contained large pore sizes and was seeded with fibroblasts whereas the upper collagen contained smaller pore sizes for keratinocytes. The application of fibroblasts in the dermal layer was found to promote epidermal growth by remodelling collagen fibres and by secreting growth factors. Vice versa, keratinocytes have been shown to stimulate elastin formation in the dermal layer within 2 months.

Apligraf® (Organogenesis Inc., Canton, MA, USA), formerly known as Graftskin, is a commercially available bilayered cultured full-skin replacement of a bovine type I collagen lattice with cultured human dermal fibroblasts seeded with allogeneic human keratinocytes obtained from human neonatal foreskin. Studies on immunodeficient athymic nude mice demonstrated a continuous basement membrane in two weeks and tightly packed collagen fibres thirty days post-wounding. Apligraf® was tested on fifteen patients with small acute surgical wounds after removal of skin cancer. A good cosmetic appearance was obtained, although the authors noticed marked wound contraction. No clinical symptoms of rejection were seen, and studies for toxicity were negative. Treatment with Apligraf in combination with compression therapy was beneficial for venous ulcers in comparison to compression therapy alone.
in a large multicentre trail, moreover, no rejection or sensitisation was noticed. In a publication of a large study on acute non complicated excisional wounds (n=107), the authors state that tissue therapy may be safe and useful. However, the survival of Apligraf after one month was poor. In a multicenter-randomised trial, a combination of Apligraf and split skin graft was tested versus a split skin graft treatment alone in forty burn patients. Sixteen patients completed the follow up of two years. The sites treated with Apligraf over the split skin graft showed a significant increase of Vancouver Scar Scale score suggesting an improvement of functional and cosmetic outcome.

Compton et al. gave an extensive outline of the temporal sequences of the formation of a new epidermis and dermis after the application of a collagen-GAG based (Integra-like) dermal layer seeded with autologous keratinocytes in an animal study. The epidermis reached confluence within nineteen days post-grafting and formed a fully differentiated, normally oriented epidermis with rete ridges. Simultaneously, a neodermal connective tissue matrix was formed beneath the newly formed epidermis. The skin substitute was completely dissolved mainly as result of a foreign-body giant cell reaction that peaked ten days after grafting. Nevertheless, no acute inflammation or evidence of immune stimulation was observed. Clinical application of the skin substitute with a collagen-GAG based dermal layer showed no difference in the qualitative outcome after one year compared to the split skin graft in a study with seventeen patients. Less scarring was noted at the test site, and pigmentation was better; however, more regrafting was needed. Antibodies against the bovine collagen were not found.

In three extensively burned patients a cultured autologous epidermal substitute was combined in a two step procedure with Integra that was applied during the first operation. The combination of the skin substitute and Integra lead to a functionally stable and cosmetically acceptable wound closure. Further studies are required to know whether such approaches reduce time to definitive closure of burn wounds and whether they improve long term functional and cosmetic outcome.
Allograft cadaveric donor skin was shown to be a good dermal substitute with natural collagen, elastin, and basement membrane structures. Cuono et al. reported clinical cases on successful skin resurfacing with an allogeneic cryopreserved dermis engrafted with cultured epidermal autografts. The antigenicity of the allograft was attenuated by cryopreservation and by removing the allogeneic epidermis.

A skin substitute consisting of xenogeneic dermal substitute from porcine origin together with autologous keratinocytes was applied in an experimental rat model. The three dimensional collagen scaffold of the original xenogeneic dermal layer was mostly replaced after six weeks, without inducing a severe inflammatory response. In contrast to collagen fibres, original elastic fibres were still observed after twenty weeks. This finding corresponded to another histopathological study where elastin could still be detected after two years in the area treated with allograft but not in the control area.

**Conclusion**

Tissue engineering and skin substitutes might be the future solution for complicated wound healing, especially in complicated cases like burns when donor sites are lacking. At present, many practical problems are encountered that have to be dealt with, such as the survival of full skin substitutes even in uncomplicated wounds like acute excisional wounds.

Much research has been done on different types of skin substitutes, with not seldomly promising results. On the other hand, it has been stated that ‘no studies have convincingly demonstrated savings of total hospitalisation costs by use of cultured skin substitutes of any kind’, nevertheless we feel that it is much to early to come to definitive conclusions. The lack of statistical evidence may be due to the absence of clinical effectiveness of skin substitutes. However we criticise the approach of most studies that have been performed to show clinical effectiveness of skin substitutes as most studies have small sample sizes and lack an objective evaluation of the study outcome by reliable and valid scar assessment tools. Large sized con-
trolled clinical trials with objective scar evaluation are mandatory to show whether skin substitutes are the treatment modalities of future wound healing.

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