Artificial skin and tissue regeneration
Lamme, E.N.

Citation for published version (APA):
Lamme, E. N. (1999). Artificial skin and tissue regeneration
Chapter 5

Allogeneic Fibroblasts in Dermal Substitution
Induce Inflammatory Responses and Interfere
with Dermal Tissue Regeneration

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Submitted for publication
Abstract

In the field of skin transplantation, dermal substitution with inclusion of connective-tissue synthesizing fibroblasts is a main objective. The use of allogeneic fibroblasts instead of autologous fibroblasts is more practical in the preparation of an "off-the-shelf" skin substitute. In this study, we compared different allogeneic fibroblast populations with autologous fibroblasts in dermal skin substitution.

Three allogeneic fibroblast populations were isolated from a female pig bred under SPF conditions (SPF fibroblasts), and from a female and a male pig (female and male fibroblasts) of the same breeding strain as two female acceptor pigs (autologous fibroblasts). The histocompatibility of the three donor pigs with the two acceptor pigs was tested with a Mixed Lymphocyte Reaction (MLR). Full-thickness wounds on 2 pigs were treated with dermal substitutes seeded with four different fibroblast populations (n=5) and transplanted in combination with split-skin mesh grafts. Six weeks after transplantation, wound contraction was measured by planimetry and wound cosmetic parameters were evaluated by two independent observers in a blinded fashion. At 2, 4 and 6 weeks biopsies were taken from the wounds. Histology was evaluated for the presence of inflammatory responses, the quality of dermal tissue regeneration, and the progress of ECM remodelling and collagen-bundle maturation.

The MLRs of both acceptor pigs showed the highest responses to peripheral blood mononuclear cells (PBMCs) of the SPF pig, and were low or negative to the PBMCs of the female and male donor pigs. Cosmetic appearance and wound contraction showed significantly better results for the wounds treated with autologous fibroblasts compared to wounds treated with SPF fibroblasts. The wound histology showed in all wounds treated with allogeneic fibroblasts an increased presence of inflammatory cells throughout the six weeks of the experiments. This inflammatory response was characterized by the presence of multiple foci with mixed lymphocytic and granulomatous inflammatory cells. In addition, the increased inflammatory responses retarded dermal tissue regeneration, remodelling of the ECM, and maturation of the newly formed collagen bundles. Six weeks after transplantation, the wounds treated with allogeneic fibroblast populations had dermal areas still granulating with thin collagen bundles organised parallel with the epidermis as in scar tissue. In contrast, the wounds treated with autologous fibroblasts showed a dermal tissue with mature collagen bundles organised randomly as in normal skin.

We concluded that for optimal restoration of dermal skin function without scar formation, the use of skin equivalents with autologous fibroblasts is to be preferred over skin equivalents with allogeneic fibroblasts.
Introduction

Ever since the first creation of a living-skin substitute by Bell et al. (1), much effort has been made to create an "off-the-shelf" skin replacement. The most practical choice would be the use of allogeneic cells. The advantages over the use of autologous cells are in eliminating patient donor sites, decreasing operating time, and avoiding a delay in treatment required for autologous cell culture and multiplication (2,3). During the last two decades, the attention was mainly focussed on cultured keratinocyte grafts and their application on burns and chronic ulcers (4). The results from these studies showed that keratinocyte allografts not only accelerate healing but also decrease the pain in both split-thickness skin graft donor sites and chronic ulcers (5,6). In addition, it became evident that allogeneic keratinocytes do not survive and are replaced by autologous keratinocytes (7,8). However, controversy still exists as to how long allogeneic keratinocytes survive (3). In immuno-competent animals, keratinocyte survival appeared to be relatively short (< 1 week) (9), whereas in patients with large burns a degree of immunosuppression exists which might favour prolongation of allogeneic cell survival (10,11). Furthermore, take and survival of allogeneic keratinocytes seem to be enhanced in the presence of dermis or a dermal equivalent (5,11,12). With respect to dermal substitution, it is commonly accepted that cultured keratinocyte grafts alone are insufficiently effective and that for adequate wound treatment both skin components, i.e. dermis and epidermis, are needed.

In developing a dermal equivalent, there are several reasons to include fibroblasts. The presence of fibroblasts in dermal equivalents not only stimulates keratinocyte outgrowth, differentiation and basement membrane regeneration (13-15), but is also likely to accelerate dermal tissue regeneration (16,17). An important question remained to be answered: 'can allogeneic fibroblasts be used and are they as beneficial for wound healing as autologous fibroblasts?' This is also clinically important, especially since commercial skin equivalents containing allogeneic fibroblasts are being developed and emerge onto the market.

In the literature, the data are not conclusive regarding the immunogenicity of human fibroblasts (18-22), and whether or not they are able to persist in newly regenerated skin or are replaced by host cells without adverse effects on tissue regeneration (2). In most clinical studies it is difficult to evaluate the effects of allogeneic fibroblasts due to the small numbers of patients evaluated, their multiple wound variables, and the lack of histological data and controls. The purpose of this
study is therefore to compare the use of allogeneic fibroblasts populations to the use of autologous fibroblasts in dermal substitution using a porcine full-thickness wound model. For the allogeneic fibroblast populations we chose two populations of female, and male origin genetically closely related to the acceptor animals and one population of female origin (SPF) that was less related. The genetical relationship between donors and acceptors was investigated with Mixed Lymphocyte Reactions. In the wounds treated with dermal substitutes seeded with the different groups of fibroblasts, we investigated inflammatory reactions in the granulation tissue and the quality of the dermal tissue which was regenerated after six weeks.

Materials and methods

Mixed Lymphocyte Reaction (MLR)

Heparinized peripheral blood from the pigs, from which the different fibroblasts populations were isolated, was layered on a density gradient (Lymphoprep, Nycomed, Oslo, Norway) and centrifuged to remove erythrocytes and granulocytes. The interface was washed 3 times with PBS solution containing 1% BSA to remove thrombocytes. Since the time-point of isolation was different for each pig, peripheral blood mononuclear cells (PBMCs) were cryopreserved in FCS containing 10% DMSO in liquid nitrogen until use. To measure the MLR response, \(2 \times 10^4\) allogeneic PMBCs were incubated with \(2 \times 10^4\) autologous PBMCs for 6 days in round-bottom 96-wells plates. Cells were cultured in 200 µl DMEM media containing 10% FCS and 50 µM β-mercapto-ethanol. The numbers of proliferating cells were determined by adding 0.3 µCi \(^3\)H-thymidine (Amersham, Aylesbury, UK) to each well sixteen hours before harvesting. Incorporation was measured by liquid scintillation counting in average disintegrations per min (DPM). As controls, \(2 \times 10^4\) PMBCs of each animal were incubated separately to measure background proliferation.

Preparation of dermal substitute grafts

The allogeneic and autologous fibroblasts were isolated from skin biopsies taken from the backs of the pigs. Two allogeneic fibroblast populations were from a female
and male pig (female and male) of the same breeding strain as the acceptor animals from which the autologous fibroblasts were isolated. The third allogeneic fibroblast population was isolated from a female pig of a different breeding strain which was bred under SPF conditions (SPF fibroblasts). From all tissue biopsies epidermis was cutt off, dermal tissue was minced and digested for 1-2 hrs at 37°C in a phosphate buffer solution (PBS) containing 0.25% (w/w) collagenase A and dispase (Boehringer Mannheim, Mannheim, Germany) (1 ml/biopsy). The digest was sieved over a 70-μm cell strainer (Falcon, Becton Dickionson, Mountainview, AL), washed in culture media and subsequently cultured in DMEM supplemented with 10% (v/v) FCS, 1 mM L-glutamine and antibiotics (penicillin (100 IU/ml), streptomycin (100 mg/ml)) (all from Life Technologies, Breda, The Netherlands). Fibroblasts were passaged with PBS solution containing 0.25% trypsin and seeded in the dermal substitute the day before operation at an identical density (5x10^5 fibroblasts/cm^2) between passages 4 and 6. Dermal substitutes were seeded by inoculating the upper side of the pre-moistened matrices with culture medium containing the fibroblasts (1 ml/10 cm^2 of substitute). The seeding volume was absorbed by the substitute. The dermal substitute was a non-cross-linked native bovine collagen matrix (type I collagen from bovine skin) coated with a 3% (w/w) α-elastin hydrolysate (80% MW 60 kDa and 20% MW 200 kDa from bovine ligamentum nuchae) and was kindly provided by Dr. P. Tewes-Schwarzer (Dr Otto Suwelack Nachf. GmbH & Co., Billerbeck, Germany) (23). For each group extra substitutes were prepared which were fixed on the day of operation in 4% paraformaldehyde PBS solution for 16 h at RT.

Operation, grafting, and bandaging procedures

The protocol was approved by the University of Amsterdam Committee of Animal Welfare. Two female Yorkshire pigs (15 kg at arrival) were included in the study. Animal anaesthetics: before each surgical procedure, wound evaluation and bandage changing, the pigs were sedated by intramuscular injection with azaperon 4 mg/kg (Stressnill, Janssen-Cilag, Gent, Belgium). Complete anesthesia was induced with a face mask with 5% isoflurane and a 50:50 mixture of nitrous oxygen and oxygen (3-5 L/min). Anesthesia was maintained with 1%-2% isoflurane and the same air mixture. If necessary, post-operative pain was treated with a subcutaneous injection containing flunixine 50 mg/50 kg/day (Finadyne®, Schering-Plough, Segré, France).
Before the operation, in which the full-thickness wounds were created, 0.001 mg/kg atropine was included as additional sedative. After complete anesthesia was induced, animals were intubated and artificial respiration was applied. During the operation, vital functions were monitored and fluid loss was compensated by an intravenous infusion with Ringer's solution. This anaesthesia was antagonised with 0.005 mg/kg sufentanil (Sufenta®, Janssen-Cilag, Gent, Belgium) and post-operative analgesia was provided with 0.05 mg/kg buprenorphine (Temgesic®, Reckitt & Colman Products Ltd, Hull, UK), both administered intravenously.

One week before the operation, the hair was clipped from both dorsal flanks and the skin was disinfected with i.e. hibiscrub, 70% alcohol solution containing 0.6% chlorohexidin, and with 2% iodine solution. A grid was tattooed by cutting the skin with a scalpel till subepidermal depth and applying tattoo paste. On the day of the operation, the skin was disinfected as described above and full-thickness wounds (2.5 x 2.5 cm) were created on the back of the pigs using a dermatome. First split-skin mesh grafts (0.2 mm thick) were harvested from the wound sites, moistened in PBS, and expanded at a ratio of 1:3. The wounds were re-excised to the subcutaneous fat layer. The wounds were grafted with the fibroblast-seeded dermal substitutes and covered with the split-skin mesh grafts. Each treatment group of grafts was applied 5 times on both animals in a randomised fashion. The wounds were protected against dehydration and bacteria with a polyether urethane top layer (Exkin®, X-Flow, Twente, The Netherlands). Protection against mechanical trauma was achieved by wound coverage with one layer of hydrophilic gauzes (5x5 cm) fixed with adhesive tape, two layers of hydrophilic gauzes (20x20 cm) fixed with elastic adhesive tape from the back to the midriff/abdomen, and elastic stockings (Tubigrip). The pigs were housed individually and fed twice a day. At the moment of the operation the pigs weighed 30-35 kg and after 6 weeks 70-75 kg.

Macroscopical evaluations of wound healing

Wounds were evaluated weekly for 6 weeks. Wound contraction was followed by tracing the wound edges and the tattooed grid on transparent film. Wound contraction was measured by planimetry, expressed as percentage of reduction of original wound area, and was corrected for the locoregional growth of the animal. The final wound evaluation included a blinded score for three cosmetic wound parameters

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by two independent experienced observers. They scored on a 1-5 scale for wound colour (pink - purple/red), smoothness and skin level of epidermis, and wound suppleness or stiffness. The overall score ranged from 3 (normal skin) to 15 (excessive scarring) and was averaged for each group in both animals.

**Histological analysis of wound healing**

After 2 and 4 weeks, a 4 mm punch biopsy was taken from each wound and after 6 weeks a cross biopsy from the middle of the wounds was taken (0.5 x 3-4 cm). The biopsies were fixed in 4% formaldehyde PBS solution for at least 16 h at RT, and subsequently embedded in paraffin according to standard procedures. Hematoxylin and eosine (H&E) stains were used to visualize cell infiltration and dermal architecture. In each biopsy, the degree of inflammatory response was scored on a 0 - 5 scale (0 a few lymphocytes/granulocytes - 5 high numbers of inflammatory cells and presence of localised foci of mixed lymphocytic and granulomatous responses).

Six weeks post-wounding, the dermal architecture was analysed for the presence of non-remodelled amorphous ECM, cellularity, and collagen-bundle orientation (random, as in normal skin or parallel with the epidermis as in scar tissue). The maturation of collagen fibers was investigated with polarized light in H&E stained wound sections.

**Statistical analysis**

In each animal, five wound per treatment group were used (randomly assigned to different wound locations). The results were analysed for significant differences with a paired Student's t-test. A p-value below 0.05 (two-sided) was considered to be statistical significant. The cosmetic appearance scores were evaluated using the non-parametric Wilcoxon signed rank test.

**Results**

**Mixed Lymphocyte Reactions (MLR)**

In Figure 1, the MLR responses are shown of the PBMCs of the acceptor pigs (A and B) to the different allogeneic PBMC populations. The SPF pig, which was genetically the least related to the acceptor pigs, evoked the highest response, i.e. 4-6
times higher than background values. The MLR responses to the female and male PBMCs were relatively low: only the MLR with PBMCs of pig A and the male pig induced some response, almost 3 times higher than background values. The figure shows a representative example of 3 independent experiments and the mean values (n=4) were corrected for the sum of the background counts of the two corresponding single PBMC populations (<1500 DPM).

![Mixed Leukocyte Reaction](image)

**Figure 1.** Mixed Lymphocyte Reaction responses of PBMCs of pigs A and B to PMBCs of the allogeneic pigs, female, male and SPF in DPM. The data shown are a representative example of 3 independent experiments and the mean values (n=4) were corrected for the sum of the background counts of the two corresponding single PBMC populations which was never higher than 1500 DPM.

**Wound cosmetic parameters and contraction six weeks post-wounding**

The three cosmetic parameters scored were wound colour, epidermal skin level and smoothness, and elasticity/pliability of the regenerated skin. In Figure 2 the cosmetic appearance of the wound is shown for each treatment group 6 weeks post-wounding. The wounds treated with the dermal substitute seeded with autologous fibroblasts (A) showed the strongest resemblance to normal skin, whereas the wounds treated with the dermal substitutes seeded with allogeneic fibroblasts populations of female, male and SPF origins (B-D) were of mixed red/pink colour and had an uneven skin level with inflexible fibrotic wound areas. The wound treated with the substitute seeded with SPF fibroblasts had contracted the most. The overall scores for the cosmetic appearance for each treatment group in both pigs is shown in Figure 3A. In both animals, the substitutes seeded with SPF fibroblasts significantly worsened the cosmetic outcome of wound healing. In Figure 3B, the average wound contraction of each treatment group on both pigs is shown. In pig A the substitutes seeded with male and SPF fibroblasts, and in pig B the substitutes seeded with SPF fibroblasts induced significantly more wound contraction than did treatment with the dermal substitute seeded with autologous fibroblasts.
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Figure 2. Cosmetic appearance of wounds 6 weeks after grafting. The wound treated with a substitute seeded with autologous fibroblasts (A) resembled normal skin the most. The wounds of the other groups, female (B), male (C) and SPF fibroblasts (D), still had red and fibrotic wound areas. The wound treated with the substitute seeded with SPF fibroblasts had contracted the most.

Figure 3. Overall cosmetic scores (A) and average wound contraction (B) of the different treatments six weeks after grafting. The X-axis indicated the seeded fibroblast populations. The SPF fibroblasts significantly worsened the cosmetic outcome of wound healing compared to autologous fibroblasts (* p<0.05, Wilcoxon signed rank test). The SPF fibroblasts induced in both pigs significantly more wound contraction compared to treatment with autologous fibroblasts. The male fibroblasts did the same in pig A (* p<0.05, paired t-test).
Histological observations and inflammatory responses during healing

In Figure 4, a representative example is shown of the granulation tissue of each group 2 weeks post-wounding. In Figure 4A, C, E, and G an overview is given of the dermal area (in between epidermis and subcutaneous fat), whereas Figure 4B, D, F and H are enlargements of insets to illustrate the cellular composition of the granulation tissue. The granulation tissue of the wounds treated with substitutes seeded with autologous fibroblasts (Fig. 4A and B) show the presence of some diffusely distributed inflammatory cells, which is a typical image for normal healing wounds. In contrast, the wounds treated with substitutes seeded with female fibroblasts (Fig. 4C and D), male fibroblasts (Fig. 4E and F) and SPF fibroblasts (Fig. 4G and H) showed a marked increase in the numbers of inflammatory cells together with the presence of localised mixed lymphocytic and granulomatous responses. These inflammatory foci were never observed in the wounds treated with substitutes seeded with autologous fibroblasts.

![Figure 4](image-url)

Figure 4. Granulation tissue of the wounds 2 weeks after grafting. The wounds treated with substitutes seeded with autologous fibroblasts (A, B) resembled a normal healing wound with the presence of few granulocytes and lymphocytes. In contrast, the 'allogeneic' wounds with female (C, D), male (E, F), and SPF fibroblasts (G, H), showed an increased numbers of inflammatory cells and the presence of mixed granulomatous and lymphocytic inflammatory foci. The increased inflammation also appeared to over-activate the keratinocytes in the epidermis, as evidenced by increased thickness of epidermis and large rete ridges. A, C, E and G: original magnification 20x, B, D, F and H: 5x enlargements of insets.
Furthermore, in the wounds treated with substitutes seeded with allogeneic fibroblasts the epidermis appeared to be overstimulated, was thicker, and had larger rete-ridges when compared to wounds treated with autologous fibroblasts seeded substitutes. In time, the granulation tissue matured progressively and the numbers of lymphocytes and granulocytes diminished. In Figure 5, the scores for the intensity of the inflammatory responses in time are shown for both pigs A and B. The number of inflammatory cells in the wounds treated with substitutes seeded with allogeneic fibroblasts never subsided to the level of the wounds treated with substitutes seeded with autologous fibroblasts.

![Inflammatory cells in granulation tissue](image)

**Figure 5.** Relative scores for the degree of inflammation in the granulation tissue of each treatment in time. In wounds treated with substitutes seeded with autologous fibroblasts far fewer inflammatory cells were observed than in the wounds treated with substitutes seeded with allogeneic fibroblast populations. After 6 weeks, the 'autologous' wounds had similar numbers of inflammatory cells as normal skin, whereas in the 'allogeneic' wounds the numbers were still increased.

**Dermal tissue regeneration six weeks post-wounding**

In the wounds treated with autologous fibroblasts, the regenerated dermal tissue showed randomly organized collagen bundles as in normal skin after six weeks (Fig. 6A and B). Moreover, all collagen bundles turned out to be of a mature nature under polarized light (Fig. 6C). In contrast, in the wounds treated with allogeneic fibroblasts the scar tissue contained areas which were still granulating (fibroblasts...
origins: female Fig. 6D-F, male Fig. 6G-I, and SPF fibroblasts Fig. 6J-L). These areas contained higher cell numbers than the wounds treated with substitutes seeded with autologous fibroblasts which are illustrated in Fig. 6B, E, H and K (higher magnification of insets). The collagen bundles in these areas were still of an immature nature (Fig. 6F, I and L) and had a tendency to be organised parallel with the epidermis, which is typical for scar-tissue formation. Furthermore, wounds treated with allogeneic fibroblasts still contained inflammatory cell foci (arrowheads in Fig. 6D, G and J).


Discussion

Previously, we demonstrated that a dermal substitute in combination with split-skin mesh grafts is capable of improving dermal tissue regeneration compared to split-skin mesh-graft treatment alone (23, 24). Since this dermal substitute consisted of native non-cross-linked collagen and elastin, the bio-absorption of the material occurred without significant inflammatory reactions (24, 25). Furthermore, the addition of autologous fibroblasts to the dermal substitute not only showed survival and proliferation of these fibroblasts after implantation, but also an additional improvement in dermal tissue regeneration (17, 26). The use of autologous fibroblasts in the clinic implies a delay of treatment, for which the use of allogeneic fibroblasts could be advantageous. The present study was designed to establish whether or not allogeneic fibroblast populations were able to stimulate dermal tissue regeneration without inducing adverse inflammatory reactions.

Dermal substitutes were implanted within 24 hours after seeding of the fibroblast populations to avoid differences in cell density in the substitute which could occur with culture of substitutes in vitro. The SPF allogeneic fibroblast population was the least related to the acceptor pigs, which was also reflected in the highest MLR response in both acceptor pigs. The response with PBMCs from the other two allogeneic female and male pigs was comparable to background levels; only in pig A the PBMCs of the male pig did induce some response. Despite these negative MLR responses, immunological reactions were observed in the granulation tissue of wounds treated with these fibroblasts. This is not surprising since it is known that even after HLA matching and negative MLR responses, allogeneic skin grafts were ultimately rejected (27). In our study, allograft rejection was never observed. However, the observed inflammatory and immunological responses retarded dermal tissue regeneration and appeared to induce more scar tissue. In addition, SPF fibroblasts

< Figure 6. Regeneration of dermal tissue of the wounds treated with substitutes seeded with autologous (A-C), female (D-F), male (G-I), and SPF fibroblasts (J-L) six weeks after grafting. Figures A, D, G, and J give an overview of the dermal tissue; B, E, H, and K are enlargements of insets illustrated in A, D, G, and J, respectively; and C, F, I and L are images of the regenerated tissue under polarized light which highlights matured collagen. The regenerated tissue of the 'autologous' wounds resembles normal skin, whereas the 'allogeneic' wounds still had granulating areas in the middle of the regenerated tissue with increased cell numbers and non-remodelled ECM with immature collagen bundles. In addition, the 'allogeneic' wounds still contained mixed granulomatous and lymphocytic inflammatory foci (arrow heads). A, D, G and J, original magnification 18x; B, E, H, and K 120x, and C, F, I and L 15x.
induced significantly more wound contraction and significantly impaired the wounds' cosmetic appearance compared to autologous fibroblasts. For the female and male fibroblast populations the average contraction was higher in both pigs compared to that of the autologous fibroblasts, but only the contraction induced by male fibroblasts in pig A proved to be significantly higher. In addition, the contraction data correlated well with the levels of the MLR responses found in both pigs.

In a similar porcine wound model, Reagan et al. (18) compared the use of acellular to cellular allogeneic dermal grafts. They found that the cellular dermal grafts elicited a drastic inflammatory response which seemed to be directed against epithelial follicular elements but not against fibroblasts or extracellular matrix elements. This inflammatory response also increased wound contraction and worsened wound cosmetic outcomes and, as was found in our study, these grafts did not show signs of rejections. In the literature, rejection reactions after renal transplantation have been reported to increase the presence of myofibroblasts and tissue fibrosis (28). Since myofibroblasts mediate wound contraction (29), it is feasible that the observed inflammatory reactions induced fibroblast differentiation into myofibroblasts or prolonged the presence of myofibroblasts. This could explain the increased wound contraction and scar formation we found in the wounds treated with the 'allogeneic' substitutes.

In most studies fibroblasts were included in a dermal equivalent for their positive effects on epidermal regeneration. In the present study, differences in outgrowth or closure of the meshed split-skin graft interstices could not be observed, since the wounds completely epithelialized in between the evaluation time points 1 and 2 weeks post-wounding. Hansbrough et al. (30) showed that allogeneic fibroblasts allowed rapid epidermal regeneration of meshed epidermal interstices xenografted to nude mice in a vicryl mesh dermal substitute. Treating burn patients with this 'allogeneic' dermal substitute (Dermagraft®), they did not observe evidence of immunological rejection towards the allogeneic fibroblasts or to the vicryl fibres 14 days after grafting (31). However, in their histological analysis the authors mentioned that there were minimal signs of inflammatory reactions: giant cells were observed and vicryl fibres were extruded from the wound surface. Unfortunately, the study design allowed only comparison to treatment with split-skin mesh grafts and not to a dermal substitute with autologous fibroblasts. The skin equivalent, Graftskin®, contains both male allogeneic fibroblasts and keratinocytes and is nowadays called Apligraf®. This
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A substitute has been applied on patients with surgical wounds and chronic ulcers without clinical signs of rejection (21,32,33). The immunocompatibility is claimed to be based on several factors: the lack of antigen-presenting cells bearing MHC class-II molecules, the cytokine environment which down-regulates unprimed T-cells, and the limited access of host immune cells to graft cells. Apligraf® is a modification of the organotypic collagen gel construct originally described by Bell et al. (1). Bell also reported that these grafts populated with allogeneic fibroblasts were accepted across the barriers of histocompatibility (19,20), but persistence of the allogeneic fibroblasts was only investigated by karyotyping of allogeneic cells in isogeneic transplanted rats. Furthermore, this added confusion to this research field, since the majority of the evidence seemed to be in favour of non-survival of cultured allografted cells, especially for keratinocytes (3). More recently, Otto et al. (11) also detected male fibroblasts and keratinocytes in a female patient up to 2.5 years after grafting with a more sophisticated molecular detection technique. Although the quality of the regenerated dermis was not investigated, the long survival of male fibroblasts and keratinocytes seems to indicate that in these types of skin equivalent allografted cells may survive longer than expected.

The basis for transplantation rejection is the expression of MHC-I and MHC-II molecules. If CD8+ T cells interact with MHC-II molecules, this immediately elicits a cytotoxic response (34). On resting fibroblasts, the expression of MHC-I is low and MHC-II not detectable. After fibroblast activation, the MHC-I levels are up-regulated and in chronic inflammatory loci the expression of MHC-II on fibroblasts has been reported (35,36), especially in the presence of IFN-g (37). In vitro, IFN-g induced MHC expression on fibroblasts did not seem to be able to stimulate or only moderately stimulate unprimed allogeneic T-cell or lymphocyte responses (38,39). However, when mixed with primed T cells, the allogeneic fibroblasts did induce T cell proliferation (40). In addition, T cell-fibroblast interactions (both autologous and allogeneic) increased the secretion of inflammatory cytokines IL-1, IL-2 and TNF-a (41). In wound healing the latter are able to contribute to increased inflammation and tissue fibrosis (42). The culture conditions of cells also play a role in the expression of MHC molecules. The coculture of keratinocytes with 3T3-fibroblasts induced expression of both MHC-I and II on these fibroblasts (43) and the 3T3 fibroblasts were able to sensitize a graft recipient for accelerated second-set rejection (44). The data presented in our present study also seem to support the fact that allogeneic fibroblasts are
recognized by lymphocytes and provoke inflammatory responses. We believe, therefore, that for true skin substitution the use of autologous fibroblasts in a dermal equivalent is to be favoured rather than that of allogeneic fibroblasts. Nevertheless, when they are used as a temporary cover to stimulate wound healing, allogeneic fibroblasts might play a role in triggering the immune system. This can be especially interesting in chronic wounds in which the immune status is often impaired.

In conclusion, for optimal restoration of dermal skin function without scar formation, the use of skin equivalents with autologous fibroblasts is to be preferred to the use of skin equivalents with allogeneic fibroblasts.

Acknowledgements
The authors thank Ms. G.E.E van Noppen for critical reading of the manuscript, and the colleagues of the GDIA institute for their excellent animal anesthetics and capable animal care. This study was supported by Smith and Nephew Co.(York, UK).

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