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ORIGINAL ARTICLE

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Organotypical engineering of differentiated composite-skin equivalents of human keratinocytes in a collagen-GAG matrix (*INTEGRA Artificial Skin*) in a perfusion culture system

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Abstract *Background:* The production of autologous composite skin equivalents for the treatment of full-thickness skin defects in burns is time consuming and costly because of laboratory procedures which have to be performed manually. In the present study keratinocytes were seeded into *INTEGRA Artificial Skin* and placed in a perfusion culture system in order to evaluate the possibility of producing composite grafts in an automated system with the aim of establishing a cost-effective method of industrial production. *Methods:* Composite grafts of *INTEGRA* and human keratinocytes were raised in perfusion culture and grafted onto athymic mice to evaluate their potential to reconstitute a full-thickness skin substitute in vivo compared to grafts from standard stagnant cultures. *Results:* Cultured composites from perfusion cultures showed no significant histological differences compared to those from stagnant cultures; however, a tendency of improved cell growth and a more surface-oriented localization was observed. Cell proliferation and surface-bound differentiation were not impaired by the use of carbonate-independent buffering (HEPES), which is necessary for perfusion culture. The composite grafts from perfusion culture exhibited identical wound adherence and complete healing and histologically represented a multi-layered, keratinizing human epidermis. *Conclusion:* Engineering of differentiated composite skin equivalents is possible in a perfusion culture system, which offers technical and procedural and possibly even biological advantages compared to standard stagnant culture methods. The development of automated perfusion culture systems for the production of composite grafts in sizes required clinically (*scale-up*) will be the next step in the cost-effective engineering of large-scale composite skin equivalents.

Keywords Composite skin · Keratinocytes · *INTEGRA* Artificial Skin · Collagen–GAG matrix · Perfusion culture system

Introduction

The prompt closure of burn wounds is of major importance for the successful treatment of patients with major burn injuries. As a result of the accomplishments in modern intensive care medicine, prompt eschar excision and immediate wound closure can be safely carried out. With this procedure it has become possible for patients to survive even the most massive burns. However, prompt wound closure using autografts is seldom possible if over 50% of the total body surface area (TBSA) is covered with deep burns. In larger wound surfaces, however, a biocompatible skin replacement is essential for permanent wound closure. Today, the treatment of major burn wounds with autologous keratinocytes derived from a skin biopsy allows for extracted cells to expand by a

factor of 10,000. According to the clinical results found using autologous keratinocytes as a skin substitute, there has been some success in the treatment of burns larger than 70% TBSA. However, due to the lack of a dermal component and to the development of granulation tissue during the nearly 3 weeks needed to produce graftable keratinocyte sheets, the final keratinocyte take on the grafted surface is often insufficient. The skin coverage achieved is mechanically very fragile and tends to develop contractures due to granulation-mediated scar formation, so that this method alone is no longer considered to be sufficient [1,2]. Recent clinical experience has made it clear that an adequate and durable skin substitution which is functional can only be achieved by simultaneous application of dermal and epidermal structural elements. The artificial skin equivalent *INTEGRA Artificial Skin* has gained widespread use in the clinical treatment of full-thickness burn wounds [3,4]. An artificial skin substitute should be: free of bacteria, nontoxic, noninflammatory, nonantigenic while serving as a barrier against microorganisms, and vapor-permeable while contributing to temperature regulation. It must adhere to the wound bed and support local defense mechanisms and wound healing. It must be elastic, durable in the long term, and show a growth potential similar to human skin with a good aesthetic quality [1]. Several studies have addressed the issue of fragile keratinocyte sheets by cultivating keratinocytes in vitro and then grafting them onto dermal matrices [5, 6,7]. These methods, however, have proven to be of little clinical advantage, because the rate of cell expansion compared to keratinocyte sheets is lower, the time for the total cultivation process is prolonged, and the laboratory process of transferring vulnerable keratinocyte sheets onto a matrix is complicated and cannot be automated. These first composite grafts brought no real advantages in comparison to keratinocyte sheets and gained practically no clinical significance. Recently, successful clinical reports have been published, demonstrating the feasibility of secondary grafting of cultured epithelial autografts (CEA) onto excised burn wounds covered with *INTEGRA* [8,9]. Other groups have been able to show in a porcine model that keratinocytes extracted from a skin biopsy and seeded into a collagen–glycosaminoglycan matrix without any further cultivation processes reconstituted a porcine neoepidermis within a few weeks [10,11].

In order to gain a higher percentage of keratinocytes capable of division for the seeding of *INTEGRA* and to gain higher rates of cell expansion, as clinically required for burn patients, we recently published the results of a study [12] in which keratinocytes extracted from a human biopsy were first brought into a primary cultivation process, according to Rheinwald and Green [13]. As a result of this, the fraction of keratinocytes with the highest proliferative potential was selected and the total cell number could be increased for seeding the collagen–GAG matrix. We were able to show that these composite grafts were capable of reconstituting a differentiated, multi-layered, keratinizing human epidermis in the athymic mouse model.

As the production of large-scale composite skin grafts, as required for the coverage of major burn wounds, in standard culture dishes is labor intensive and costly, we investigated whether a perfusion culture system could offer a technical solution to these disadvantages. Moreover, perfusion culture systems are known to provide a possibility for optimizing the cellular environment by a continuous flow of fresh culture medium, which could potentially improve cellular growth and cellular differentiation under in vitro conditions [14, 15, 16]. In addition to that, we were interested in learning whether the perfusion culture method would also be able to generate composite grafts suitable for successful grafting. In order to evaluate this we modified the previously described experimental protocol and used the MINUTH Perfusion Culture System [14].

Materials and Methods

INTEGRA Artificial Skin

The process of producing *INTEGRA* has been described by Yannas and Burke [17]. For cell seeding, commercially available *INTEGRA* (Integra LifeSciences Corp., Plainsboro, USA) was used. After unpacking, *INTEGRA* (10×12.5 cm) was removed from the packing and left to drip dry without applying any mechanical force, such as squeezing or shaking. *INTEGRA* was then washed twice in 500 ml sterile 0.9% NaCl solution (Life Technologies, Karlsruhe, Germany) and incubated for 30 min in 100 ml Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Karlsruhe, Germany) which was changed twice. Next, the cells were incubated in 100 ml DMEM with 10% newborn calf serum (Life Technologies, Karlsruhe, FRG) for 2 h at 37°C and, finally, incubated in 100 ml keratinocyte cultivation medium according to Rheinwald and Green [13] for 12 h. Prior to seeding, the *INTEGRA* matrix was cut into pieces of 1.25×1.25 cm.

Cultivation of keratinocytes

Keratinocytes were extracted from a skin specimen of one female donor that had undergone breast reduction mammaplasty and was otherwise healthy. The patient had given informed consent. Cells were extracted by sequential trypsinization and were cultivated by the feeder-layer technique under differentiating conditions in DMEM/Ham's F12 medium (Life Technologies, Karlsruhe, FRG) with 10% fetal bovine serum and supplements (Sigma GmbH, Deisenhofen, FRG) as described by Rheinwald and Green [13]. The number of cells was increased in a primary cultivation process for 5 days to up to 60% confluence and the cells then stored in liquid nitrogen until further experimental use.

Seeding of the INTEGRA *matrix*

For seeding of the *INTEGRA* matrix keratinocytes were brought into a secondary cultivation phase, resuspended in keratinocyte medium, and distributed onto the matrix with a pipette. Cell density was

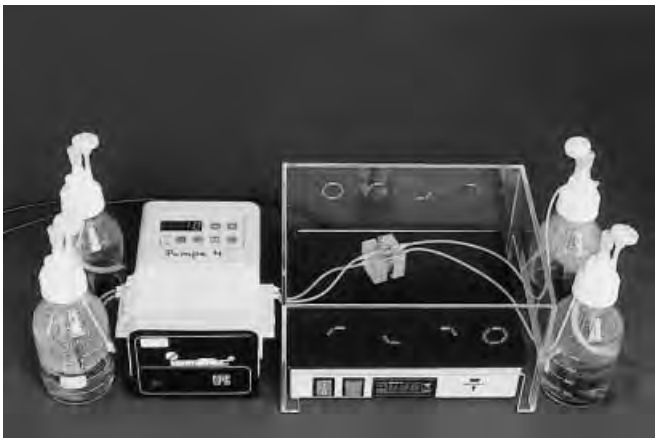

Fig. 1 MINUTH Perfusion Culture Container (MINUCELLS und MINUTISSUE Vertriebs-GmbH, Bad Abbach, Germany)

calculated to deliver $10^5/cm^2$ viable keratinocytes to the matrix in 0.5 ml/cm^2. The cells were integrated into the matrix by centrifugation at 500 rpm for 10 min. The inoculated matrices were then further cultivated in the MINUTH Perfusion Culture System.

MINUTH Perfusion Culture System

The MINUTH Perfusion Culture System [14] (MINUCELLS and MINUTISSUE Vertriebs-GmbH, Bad Abbach, Germany) is a closed culture system, providing a permanent perfusion with fresh culture medium (Fig. 1). Nutrition is thereby optimized while metabolic products are continuously eliminated from the cellular environment without fluid recycling, mimicking physiological conditions. For our experiment, a peristaltic pump permanently delivering pH-stabilized (25 mM HEPES) medium at 1 ml/h was connected to the culture container. A total number of six 2.25 cm^2 specimens of seeded composite grafts were submerged in one culture container. The system was then placed on a 37°C thermoplate, producing an optimal temperature environment. Under these conditions, composite grafts were cultured for 5 more days. Cultures were microscopically controlled daily and prior to grafting.

Grafting of composite-grafts

The animal experiments were performed according to the principles of laboratory animal care (NIH publication No. 86–23, revised 1985) and were approved by the animal welfare committee of the local state authorities (Bezirksregierung Hannover, Code 99/145) according to German law (§ 8 Abs. 1 TierSchG). Twenty athymic mice (Balb/c-nu/nu, male, mean weight 15 g) were grafted in a laminar-flow bench under sterile conditions. A group of ten mice received cell-seeded grafts, and ten mice received unseeded control grafts. Composite grafts as well as controls of unseeded *INTEGRA* were taken out of the cultivation chamber immediately prior to grafting and kept in culture medium on the sterile instrument table. For anesthesia, ketamine (0.001 mg/g) was administered subcutaneously. The backs of the mice were disinfected with a non-iodine-containing alcoholic skin disinfectant. Full-thickness skin defects of 2.25 cm^2 (1.5×1.5 cm) down to the muscle fascia, resecting the panniculus carnosus, were created with preparation scissors which corresponded to a defect size of about 10% TBSA. The grafts were brought to the wound, trimmed exactly to the wound size and fixed with multiple single stitches using a 6–0 nylon suture. The wound was covered with an occlusive dressing consisting of a sterile compress and Tegaderm (3 M Corp., St. Paul, USA). The dressing was finally secured with a self-adhesive tape, as these animals tend to gnaw off anything restricting their mobility. The dressing was removed on day 5 and the sutures were removed on day 10. The silastic sheet was peeled off on day 15. One of the 20 animals (unseeded control) died early on day 2 due to wound infection. A massive inflammatory reaction could be shown histologically with the immigration of neutrophilic granulocytes and necrosis of the inoculated keratinocytes. After sacrificing the remaining 19 animals, excision biopsies were harvested, including muscle fascia and native mouse skin at the biopsy edges, on day 5 (n=2 seeded/n=2 unseeded), day 10 (n=2/n=2), day 15 (n=2/n=2), 4 weeks (n=2/n=2), and 8 weeks (n=2/n=1) after grafting. Image analysis of wound size by photoplanimetric evaluation of the surface area on standardized photographs was carried out at 4 weeks and 8 weeks postgrafting before necropsy. Data for wound contraction were expressed as a mean percentage of the original wound size.

Histology and immunohistology

Both in vitro composite grafts and biopsies taken from the grafts were fixed in 10% buffered formaldehyde, embedded in paraffin, and cut by microtome to a thickness of 5 µm. Preparations were stained with hematoxylin and eosin. For immunohistological staining, paraffin-embedded slices were mounted on poly-L-lysin covered microscopic slides, deparaffinated with xylol, and incubated with anti-human keratin antibody (rat-anti-human-AE1/AE3; Dako Diagnostics GmbH, Hamburg, Germany) in order to demonstrate the human origin of the reconstituted neoepidermis.

Results

Using this method of inoculation, a reproducible seeding of keratinocytes could be achieved within the *INTEGRA* matrix. The vitality of the seeded keratinocytes was constantly more than 80% (trypan blue measurement). The distribution of the keratinocytes was relatively homogeneous; however, a significantly higher concentration was found underneath the silastic membrane. Histologically, keratinocytes were adherent to the matrix wall and displayed the morphological characteristics of proliferation and differentiation (Fig. 2). Scanning electron microscopy slides showed spherical keratinocytes 8–12 µm in size which were adherent to the matrix and had accumulated underneath the silastic membrane (Fig. 3).

All transplanted composite grafts were adherent to the wound bed throughout the 8 weeks of observation. Surgery was tolerated well by the animals. The surviving animals exhibited a natural behavior during the time of observation. After removal of the dressings, an increasing accumulation of keratin granula was macroscopically visible under the temporary *INTEGRA* silastic epidermis, with a concomitant loss of transparency. The unseeded control matrix, however, remained transparent. It was easy to remove the silastic sheet on day 15 without tearing the *INTEGRA* matrix off the wound bed. At this time, the presence of a transparent epidermal layer could be recognized macroscopically, which gradually lost its transparency in the fur-

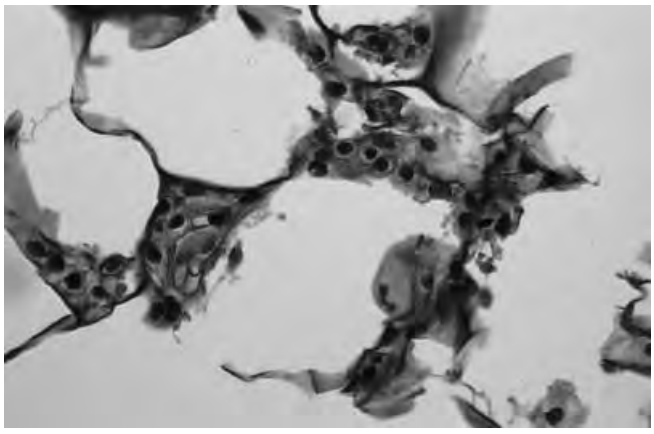

Fig. 2 In vitro seeded *INTEGRA* before grafting (hematoxylin and eosin, ×150). Proliferating keratinocytes, adherent to the *INTEGRA* matrix, forming "keratomorulae"

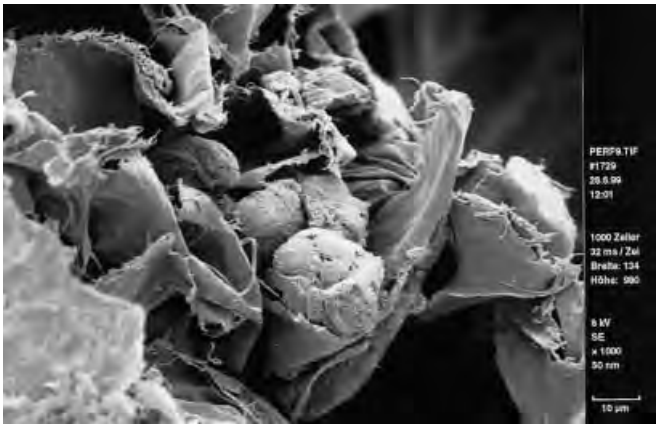

Fig. 3 In vitro seeded *INTEGRA* before grafting (scanning electron microscopy, ×1000). Spherically shaped keratinocytes adherent to the three-dimensional collagen–GAG matrix, average size 8–12 µm

ther course of observation. Even 8 weeks after grafting a clearly visible difference in color between native mouse skin and transplanted skin with its persisting residual transparency was evident. At this time all composite grafts displayed fine wrinkles and clinical signs of hyperkeratosis with shearing of keratinized skin, both of which are characteristic of human skin (Fig. 4). The completely integrated composite skin could be easily moved on the dorsal muscle fascia and lifted from the undersurface.

In the control grafts, no epidermal layer could be discerned macroscopically at the time of removal of the silastic sheet. Some 5–6 weeks after grafting, epithelialization from the wound margins was macroscopically completed. The unseeded *INTEGRA* control grafts were adherent and elastic; however, they displayed neither wrinkles nor hyperkeratosis. At 4 weeks postgrafting, the original wound size had contracted to an average of 17.7% in the seeded group and 22.4% in the unseeded

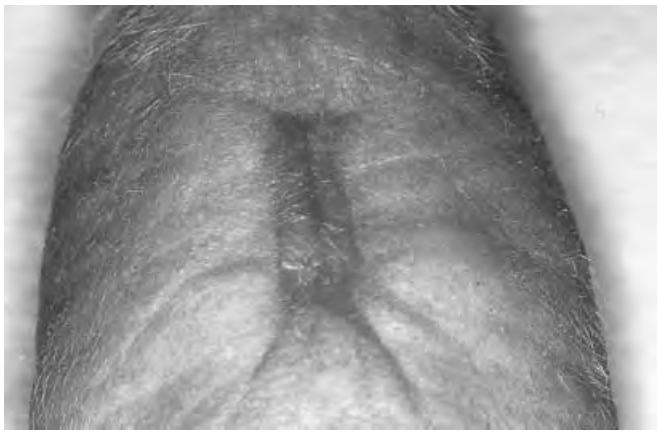

Fig. 4 Fully healed and epithelialized *INTEGRA* 8 weeks post-grafting, displaying a clearly visible difference in color to native mouse skin with persisting residual transparency and fine wrinkles with clinical signs of hyperkeratosis as a characteristic feature of human skin. Mean wound contraction 19.3%

control group. At 8 weeks post-grafting the original wound size had contracted to an average of 19.3% in the seeded group and 23.1% in the unseeded control group.

Description of histological findings in unseeded *INTEGRA*

In unseeded *INTEGRA* controls the matrix was only slightly infiltrated on day 5. Underneath the silastic membrane, very few infiltrated cells were present. The *INTEGRA*–wound bed interface displayed a layered cellular infiltrate consisting of equal amounts of macrophages and polymorphonuclear cells. On day 10, only few changes could be recognized, but an increase in infiltrate quantity was visible. The matrix was slightly more colonized by a mixed-cellular infiltrate with an increasing accumulation in the *INTEGRA*–wound bed interface. On day 15, the infiltrate had spread into the matrix and, in addition to the earlier findings of polymorphonuclear cells and macrophages, now contained fibroblasts and endothelial cells. Defined vessels could not be discerned, though. In the matrices examined, keratinocytes were not seen at this point in time. Eight weeks post grafting a complete native mouse epidermis had developed. At this point, the *INTEGRA* matrix was completely remodeled by native connective tissue.

Description of histological findings in seeded *INTEGRA*

The seeded *INTEGRA* matrix on day 5 was colonized by equally distributed, viable keratinocytes. Keratinocyte accumulations (*keratomorula*) underneath the superficial silastic layer had opened onto the surface (*keratinocyte bubbling*). Apart from the keratinocytes, the matrix showed almost no infiltrate. This infiltrate was, as found

Table 1 Relative cell abundance, intramatrix location and order of appearance (– no cells, ± low cell number, + medium cell number, ++ high cell number). **PMN*=polymorphonuclear cells

	Day 5				Day 10				Day 15			
	PMN*	Macrophages	Fibroblasts	Keratinocytes	PMN	Macrophages	Fibroblasts	Keratinocytes	PMN	Macrophages	Fibroblasts	Keratinocytes
Unseeded *INTEGRA*												
Infiltrate total	±	±	–	–	+	+	–	–	++	++	++	–
Sub-silicone *INTEGRA*	–	–	–	–	–	–	–	–	–	–	–	–
INTEGRA–matrix	–	–	–	–	–	–	–	–	++	++	++	–
Transition zone *INTEGRA*–wound bed	±	±	–	–	+	+	–	–	++	++	++	–
Seeded *INTEGRA*												
Infiltrate total	±	+	–	++	+	+	++	Epidermis	+	+	++	Epidermis
Sub-silicone *INTEGRA*	–	–	–	++	–	–	+	++	–	–	+	++
INTEGRA–matrix	–	+	+	++	–	++	++	–	–	++	++	+
Transition zone *INTEGRA*–wound bed	±	+	+	–	–	+	+	–	–	+	+	–

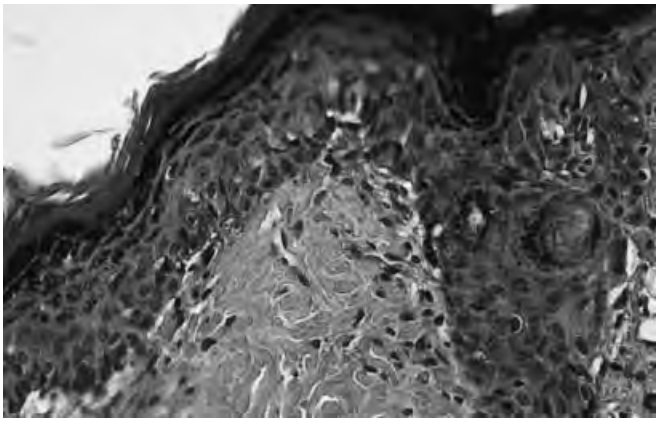

Fig. 5 Seeded *INTEGRA* on day 10 postgrafting (hematoxylin and eosin, ×150). Formation of multi-layered epithelium of surface-bound migrating keratinocyte formations ("keratomorulae"), opening up to the surface ("keratinocyte bubbling")

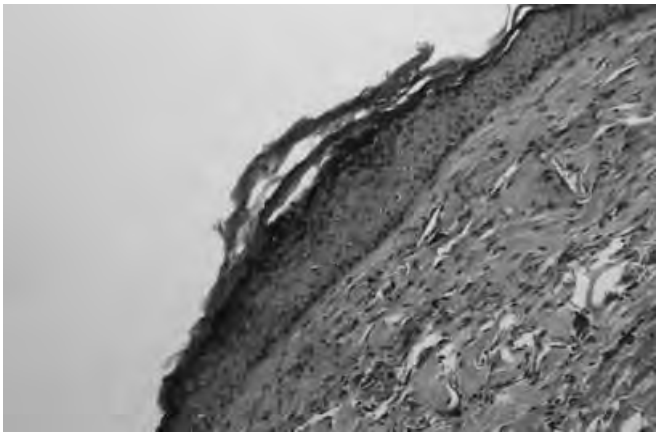

Fig. 6 Seeded *INTEGRA* 4 weeks postgrafting (hematoxylin and eosin, ×150). Completely developed multi-layered, stratified, squamous human epidermis, with former matrix displaying no cellular infiltrate. Keratinocyte cysts have completely disappeared, and the basal, granular, and horny layers can be clearly distinguished. Mean epithelial thickness: 157.8 µm

in unseeded matrices, concentrated in the *INTEGRA*–wound bed interface. In contrast, the infiltrate displayed very few polymorphonuclear cells but a well-balanced portion of macrophages and fibroblasts. On day 10 the *INTEGRA* surface was covered by a closed human epidermis, showing more *keratomorulae* with visible centripetally oriented keratinization migrate and open onto the matrix surface (Fig. 5). In the *INTEGRA* matrix fibers which were visible, fibroblasts predominated. An *INTEGRA*–wound bed interface was no longer discernible and a beginning neovascularization and an equal-balanced cellular infiltrate of fibroblasts and macrophages were displayed. The relative cell abundance, intra-matrix location, and order of appearance are summarized in Table 1. Immunohistological staining with anti-human-cytokeratin antibodies (rat-anti-human-AE1/AE3; Dako Diagnostics GmbH, Hamburg, FRG) on day 15 showed a multi-layered differentiated epidermis of human origin. Four weeks postgrafting, a completely developed multi-layered, stratified, squamous human epidermis is present with the former matrix displaying no cellular infiltrate (Fig. 6). Keratinocyte cysts have completely disappeared. Within the epidermal layer, the basal, granular, and cornified layers can be clearly distinguished. At this time the mean epithelial thickness of the reconstituted human epidermis was measured to be 157.8 µm whereas epithelialized control grafts with native mouse epidermis stemming from the wound margins showed a mean epithelial thickness of 59.7 µm.

Discussion

This experiment demonstrated that a perfusion culture system is suitable for the production of composite grafts of *INTEGRA Artificial Skin* and human keratinocytes. These composite grafts raised in perfusion culture were able to reconstitute an organized human epidermis within 10 days in the athymic mouse model, a biological phenomenon previously found in stagnant cultures [12].

As soon as 5 days after application to the wound the *INTEGRA* matrix was completely interspersed with proliferating and migrating keratinocytes. The inflammatory response seemed distinctly reduced as the slight infiltrate in the *INTEGRA*–wound bed interface contained very few polymorphonuclear cells but a significant number of reorganizing fibroblasts. On day 10 the surface of the keratinocyte seeded *INTEGRA* was covered by an intact epidermal layer of human keratinocytes and the *INTEGRA*–wound bed interface was already vascularized and no longer clearly discernible. In mice grafted with unseeded *INTEGRA* signs of delayed wound healing characterized by a high content of wound debriding polymorphonuclear neutrophils and a late appearance of reorganizing fibroblasts were observed. A defined neovascularization could not be recognized in a comparable period of time even though endothelial cells were visible. Obviously, keratinocytes not only stimulate reepithelialization but also seem to speed up the wound healing process as a whole. This is documented by the appearance of fibroblasts and the formation of neovascularization at an early stage.

Earlier clinical trials with *INTEGRA* as a dermal substitute have shown that a vascularized neodermis can develop within 2–3 weeks [3,4]. At present the treatment of full-thickness burn wounds with *INTEGRA* involves removing the silastic sheet 2–3 weeks after application or at a time when there is a sufficient donor area to harvest an ultrathin epidermal graft. Grafting of the ultrathin autologous split-thickness skin provides definitive surgical wound closure. Prerequisite for this procedure is a sufficient source of harvestable split-thickness skin. The problem of an insufficient source of harvestable skin for secondary grafting can be circumvented by simultaneously culturing autologous keratinocyte sheets (CEA) during the incorporation of *INTEGRA* [8,9] as the two biological processes require about the same amount of time.

The processes described above, with reconstitution of an intact epidermal layer in vivo using an in vitro seeded *INTEGRA*, renders secondary ultrathin, split-thickness skin or keratinocyte grafting unnecessary. When *INTEGRA* is seeded with keratinocytes in vitro a self-epidermis can form in a single step in vivo. By using such a single-step wound coverage procedure valuable time is gained clinically and a second surgical procedure can be avoided. The laboratory procedures for the production of keratinocyte-seeded *INTEGRA* in a perfusion culture model are less time consuming and more cost-effective than the production of keratinocyte sheets. This is due to the relatively short cultivation period in vitro after cell isolation and seeding until the composite grafts reach a state of proliferation and differentiation which is suitable for grafting. In addition to this, composite grafts, in contrast to keratinocyte sheets, can be applied at a subconfluent stage, suggesting that keratinocytes may exhibit a higher proliferative activity when grafted at a subconfluent state. This might be explained by the lower degree of contact inhibition because cell-to-cell contacts are not fully developed. Moreover, adhesion-dependent cells require a three-dimensional, biological or biocompatible matrix, allowing for a spatial orientation and separation as well as physiologic population densities of cells during the cultivation process (*organotypical culture*) in order to fabricate graftable three-dimensional organ equivalents. However, in commonly used culture devices (flasks, dishes) a controlled cellular environment cannot be maintained. In particular, when using matrices, cells are subjected to gas and nutritional gradients in their microenvironment, which practically remain unchanged when culture media are changed. In perfusion culture systems with a constant, defined media flow such gradients do not develop, which may potentially help to overcome these problems [18, 19, 20,21].

The histomorphologically described process of the development of circularly arranged proliferating and differentiating keratinocytes (*keratomorula*) with migration directed towards the surface (*keratinocyte bubbling*) and the development of a multi-layered, keratinizing epidermis have not been shown in any of the other wound healing processes known to us today. The gas permeability of the silastic membrane (*air–liquid interface*) and chemotactic signals in the wound itself presumably play a distinct role in this migration and differentiation process. Experimental data from in vitro cellular experiments were able to demonstrate the importance of oxygen for the growth and differentiation of human keratinocyte cultures [20, 22,23].

It is remarkable though that the dynamic process we have described takes place as early as in the phase of imbibition, thus at a time when vascularization is not yet established. The reconstitution of a neoepidermis seems to detach mechanical bindings between the matrix and the silastic membrane so that this physical barrier can be removed easily after 2 weeks.

Calculating a seeding density in the matrix of 10^5 keratinocytes/cm^2 and a realistic extraction rate of around 2×10^6 keratinocytes/cm^2 from a human skin biopsy, the nominal expansion without a primary cultivation process would be 20-fold. In this calculation, one has to consider the very low grow-on-rate of primary cells of only 1%–15%, which reduces the real expansion rate to a maximum of threefold. If a period of cell cultivation and expansion is allowed before seeding as we performed, real expansion rates of 20- to 200-fold can be accomplished, which are clinically necessary for the coverage of extensive burn wounds. Another advantage of

this cultivation process is that a higher percentage of cells capable of multiplication than of uncultivated keratinocytes are being inoculated in the matrix, which results in a faster reconstitution of an intact neoepidermis, making even lower seeding densities possible.

Conclusion

We were able to show that keratinocytes seeded into a porous dermal matrix can be grown in pH-stabilized media in a semi-automated perfusion culture system, such that manually performed and repeated laboratory procedures during the cultivation process can be reduced. Looking ahead, we believe that early seeding of a matrix with keratinocytes which have undergone a primary cultivation process followed by an *intramatrix* cultivation phase in automated perfusion culture systems until cell (sub-) confluence and until proof of a surface-bound differentiation represents the future in developing composite skin substitutes. By this method, both a high surface expansion rate and mature composite grafts capable of reconstituting a neoepidermis within a very short time in vivo can be obtained. These factors are essential in the treatment of critically burned patients. In order to establish such grafts, further technical developmental steps in engineering large-scale organotypical perfusion culture containers (air–liquid interface cultures) need to be undertaken by the industry, so that the cultivation processes can be upscaled and automated.

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