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ORIGINAL ARTICLE

Skingineering II: transplantation of large-scale laboratory-grown skin analogues in a new pig model

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Abstract
Background Tissue engineering of skin with near-normal anatomy is an intriguing novel strategy to attack the still unsolved problem of how to ideally cover massive full-thickness skin defects. After successful production of large, pig cell-derived skin analogues, we now aim at developing an appropriate large animal model for transplantation studies.
Materials and methods In four adult Swiss pigs, full-thickness skin defects, measuring 7.5 × 7.5 cm, were surgically created and then shielded against the surrounding skin by a new, self-designed silicone chamber. In two animals each, Integra dermal regeneration templates or cultured autologous skin analogues, respectively, were applied onto the wound bed. A sophisticated shock-absorbing dressing was applied for the ensuing 3 weeks. Results were documented photographically and histologically.
Results All animals survived uneventfully. Integra healed in perfectly, while the dermo-epidermal skin analogues showed complete take of the dermal compartment but spots of missing epidermis. The chamber proved effective in precluding ("false positive") healing from the wound edges and the special dressing efficiently kept the operation site intact and clean for the planned 3 weeks.
Conclusion We present a novel and valid pig model permitting both transplantation of large autologous, laboratory-engineered skin analogues and also keeping the site of intervention undisturbed for at least three postoperative weeks. Hence, the model will be used for experiments testing whether such large skin analogues can restore near-normal skin, particularly in the long term. If so, clinical application can be envisioned.

Keywords Tissue engineering · Skin culture · Pig model · Transplantation

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Introduction

In the preceding companion paper [1] ("Skingineering I", published in this issue) we have provided detailed information concerning the notorious clinical problems when large or massive full-thickness skin defects have to be covered. Moreover, we have outlined the rationale for tissue engineering of skin with near-normal architecture as a potential novel strategy to overcome the mentioned problem.

As a matter of fact, the successful transplantation of small human cell-derived skin analogues on athymic rats [2–4] represents an important step forward with regard to eventual clinical application of this technique, and it led us to design a further experimental series where autologous *large* scale transplantation of such constructs should be tested in a large animal model. In the aforementioned companion paper [1] we report on the engineering of pig cell-derived skin substitutes suitable for transplantation in a pig model.

The goal of the present study was to create such a pig model that allows standardised large-scale transplantation of laboratory-engineered autologous skin analogues. This model, if valid, would then be used for additional pre-clinical experiments looking in detail at various critical aspects including particularly functional and cosmetic long-term outcomes of the so reconstituted skin.

Materials and methods

Animals

Adolescent female Swiss pigs (Edelschwein), aged between 2 and 3 months, weighing between 30 and 35 kg were housed in a group of 4, received adequate quantities of pellet food, yoghurt, and they had access to water ad libitum. They were provided with natural day–night cycles. For the purpose of acclimatisation, housing began 2 weeks before the start of the experimental phase. Following skin biopsy, they were again housed in a group of 4 for another 3 weeks until the formal operative procedure. In order to minimise the risk of peer-inflicted damage to the operation site, they were thereafter housed individually in neighbouring stalls for the ensuing three postoperative weeks until they were killed (details see below).

Silicone chamber

In order to prevent any spontaneous healing processes from the wound edges, a silicone chamber was sutured to the wound edges after creation of the skin defect. This device was newly designed (by first author CS) and fabricated (by Carsten Linti, ITV Denkendorf, Germany; Fig. 1).

The transplantation chamber consists of two parts: the chamber, and the cover plate. Both parts are made from silicone rubber using a special cast procedure. The elastic chamber has hardness (shore A) of 80 after curing and removal from the mould. The cover plate is casted from a two-part silicone rubber that cures at room temperature. The flexible cover has hardness (shore A) of 25.

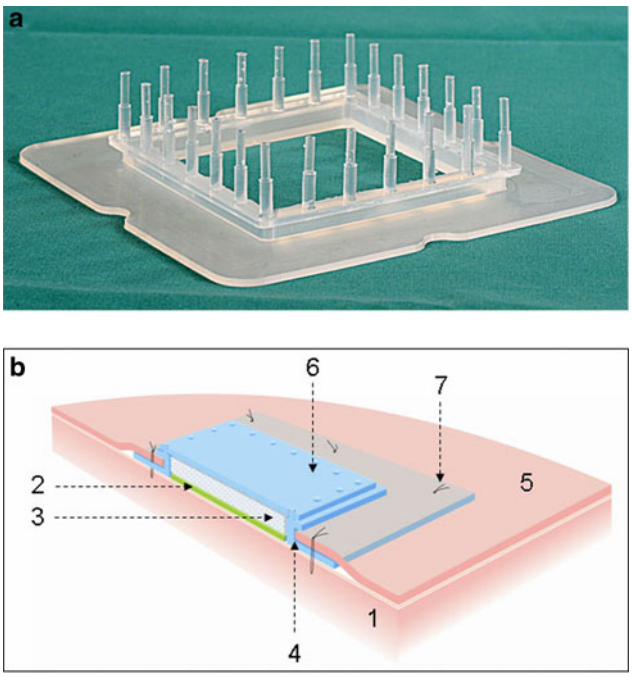


Fig. 1 Silicone chamber. **a** Silicone chamber designed to prevent spontaneous healing from the surrounding skin. The area inside the chamber measures 7 × 7 cm. **b** Schematic drawing showing the organisation of the wound area with the implanted silicone chamber: *1* muscle including overlying fascia, *2* skin analogue, *3* soft silver foam, *4* silicone chamber, *5* pig skin, *6* 2 mm thick silicone cover plate to close the chamber, *7* Fixation sutures

The moulded silicone rubber parts are biocompatible, biostable, and heat-resistant (200°C) and thus amenable to standard sterilisation processes, e.g. steam sterilisation.

Anaesthesia and pain management

The study protocol was approved by the local Committee for Experimental Animal Research (Kantonales Veterinäramt des Kantons Zürich, permission number 172/2009).

Prior to all surgical procedures, the pigs were fasted overnight to avoid vomiting and aspiration. At first, all animals were sedated by an intramuscular injection of a mixture of ketamine 20 mg/kg (Narketan® 10 ad us. vet., Vétoquinol AG, Switzerland), azaperone 1.25 mg/kg (Stresnil™ ad us. vet., Biokema SA, Switzerland) and atropine 0.03 mg/kg (Atropine 0.1%: Kantonsapotheke Zürich, Switzerland). A venous catheter was placed at the ear to apply propofol (20 mg i.v.) (Disoprivan® 1% Astra Zeneca, Switzerland) to facilitate intubation. Animals were then intubated with an endotracheal tube 8 mm in diameter (Aire Cuf™, Veterinary Endotracheal Tube, all Silicone, Bivona Inc., Indiana, USA) and artificial respiration was applied. Anaesthesia was maintained with 2.5% isoflurane (Attane™ isoflurane ad us. vet., Minrad Inc., New York,

USA) in oxygen (4.5 l/min). The operation field was shaved and the skin was disinfected.

Anaesthesia was terminated by leaving the animal on oxygen alone. When breathing normally, the animal was extubated. Intraoperative analgesia was provided by buprenorphine, 0.01 mg/kg (Temgesic®, Essex Chemie AG, Switzerland). Post-operative analgesia was provided by a transdermal fentanyl matrix patch (50 µg/h) (Durogesic® Matrix, Janssen-Cilag AG, Switzerland). Antimicrobial prophylaxis was intraoperatively provided by augmentin (600 mg i.v.) (Augmentin®, GlaxoSmithKline AG, Switzerland) and continued for 5 days (2 × 625 mg p.o.).

Operative procedures

Biopsy

Animals were anaesthetised as described above, placed in a left lateral position, and disinfected. A strip-shaped (1 × 10 cm) full-thickness skin biopsy was harvested from the right hemiabdomen, and the wound was sutured closed. The specimen was then processed as detailed in the twin paper [1].

Creation of full-thickness skin defect, chamber implantation, and transplantation of skin substitutes

Three weeks post biopsy, animals were again anaesthetised as described, placed in prone position, shaved, and disinfected. A square-shaped, 7.5 × 7.5 cm, full-thickness skin defect over the midline lumbar area was created. Particular care was taken to excise a tissue plate including the entire subcutaneous fat layer so as to ascertain all epithelial elements that could have contributed to later epithelialization were removed (Fig. 2a).

The silicone chamber was then implanted and fixed to the skin edges as outlined above (Fig. 2b; $n = 4$).

In two animals, the wound was covered with Integra dermal regeneration template (IDRT) in order to obtain immediate and reliable wound protection [5] a setting that allows studying the behaviour and the biologic effects of the chamber over time. In two other animals, a cultured skin substitute [1] was transplanted in order to phenomenologically test graft take (Fig. 2c). All wound sites were covered with a soft silver foam dressing. The chamber was closed with a 2-mm-thick silicone cover plate (Fig. 2d). With the purpose of protecting the operation sites in an optimal way against any sort of potential mechanical trauma over the ensuing 3 weeks, a multilayer shock absorber device was installed comprising elements attenuating direct as well as sheering forces (Fig. 3) and held in place by a customized garment (Fig. 4).

Postoperative management, euthanasia: harvesting and processing of samples

All animals were individually housed for 3 weeks postoperatively with weekly dressing changes and wound site documentations. Thereafter, they were killed by intravenous administration of pentobarbital (40–60 mg/kg i.v.,

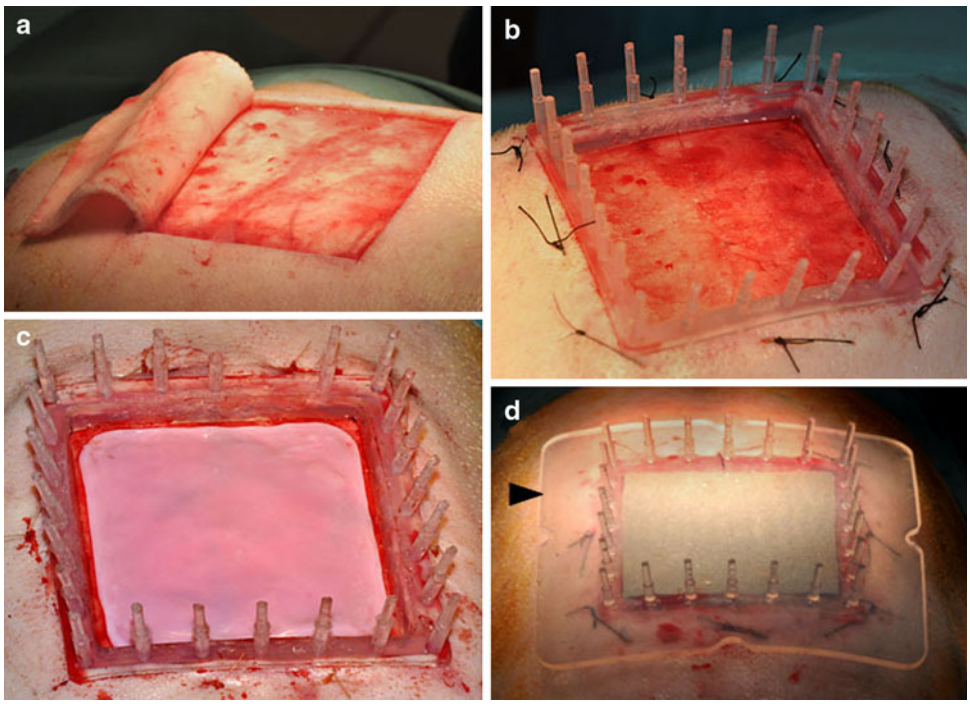


Fig. 2 Surgical procedure of skin substitute transplantation. **a** A full-thickness skin defect of 7.5 × 7.5 cm, located over the lumbar area is created. **b** The silicone chamber is implanted and secured by fixation sutures. **c** The cultured skin substitute is transplanted onto the full-thickness wound. **d** The wound is covered with soft silver foam (*grey*) and a 2-mm-thick silicone cover plate (*arrowhead*)

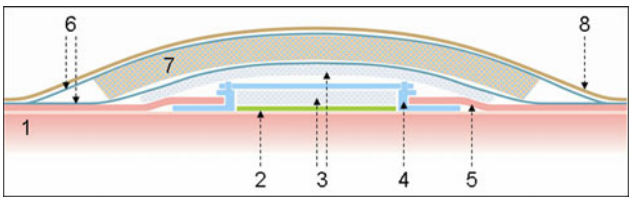
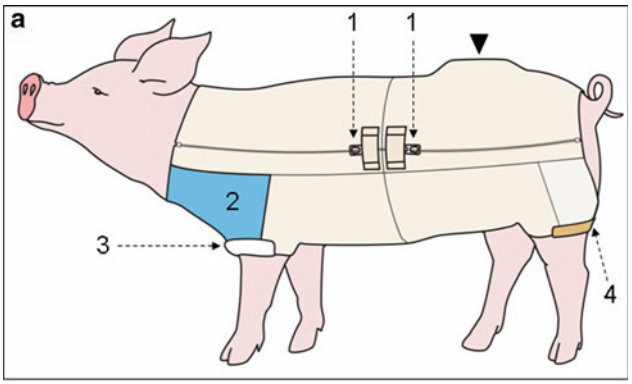


Fig. 3 Multilayered shock absorber device. Schematic drawing showing the architecture of the device: *1* muscle including overlying fascia, *2* skin analogue, *3* soft silver foam, *4* silicone chamber, *5* pig skin, *6* fixation tapes encasing the foam, *7* foam, *8* customized garment



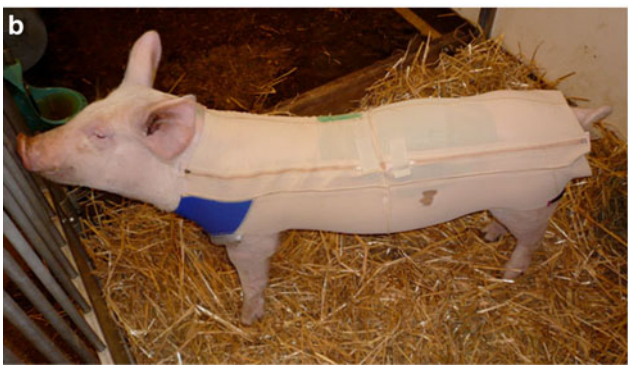


Fig. 4 Customized garment. **a** Schematic drawing of the customized garment for pigs, indicating the essential features: *1* two separate zippers, *2* neoprene insertion, *3* border of lambskin upholstery, *4* elastic fabric strip. *Arrowhead* indicates location of shock absorber device under garment. **b** Picture showing the "custom-made" garment on a pig

Esconarkon, ad us. vet., Streuli Pharma AG, Switzerland). After chamber removal, all wound sites were completely excised and processed for histology (H&E staining).

Results

All pigs survived all experimental procedures and the postoperative phase without any significant complications.

The silicone chambers could be inserted into the wound and sewed in place according to the outlined specifications in all animals. Both IDRT and skin analogue placement onto the chamber-sheltered wound bed could be performed without any problem. Over the ensuing 3 weeks (formal dressing changes after 1 and 2 weeks), both the dressings and shock-absorber devices remained in place and intact. No adverse events, especially bleeding, infection, or traumatic damage, were observed. Macroscopically, the chamber did not cause any particular reaction within the surrounding tissues like, e.g. a pronounced inflammatory response or excessive granulation tissue production, nor did it exhibit any obvious signs of material damage like, e.g. deformation, frailty, altered consistency, surface erosion, rust, or colour change.

The IDRT implantation yielded a take of 100% after 1 week and the usually encountered peach colour after 3 weeks indicating correct ingrowth and vascularization in both animals [6]. No signs of infection and no obvious findings indicating any kind of unfavourable interaction of surrounding skin and/or IDRT with the chamber were identified (Fig. 5a, b). Histologically, the Integra demonstrated the expected and well-documented architecture of a neodermis 3 weeks post implantation [6, 7] (Fig. 5c).

Cultured dermo-epidermal skin substitutes showed a take (determined also 1 week after transplantation) of the dermal part of nearly 100%, whereas the epidermal coverage was estimated between 50 and 60% (Fig. 6a). Histologically (3 weeks post transplantation), the dermal compartment demonstrated the well-known features characterising a neodermis while the epidermis was well established and correctly stratified in some areas (Fig. 6b), whereas in other areas, it was missing.

Discussion

The study reported here provides compelling evidence that we have established a valid pig model that allows transplantation of large autologous skin analogues and also warrants the site of operation be kept undisturbed for at least 3 weeks after transplantation. The following pivotal profile properties of this large animal model deserve to be addressed in more detail.

First, the model permits transplantation of *sizeable* grafts measuring 7 × 7 cm. This size is suitable for large-scale transplantation as it equals for instance the usual size of commercially available cultured epidermal autografts for clinical use [8]. Also, it has similar dimensions as conventional split thickness skin grafts applied on human patients, especially on children [9].

Second, *autologous* transplantation as performed in this experiment parallels the future clinical situation where, obviously, only autologous skin analogue-coverage of full-thickness skin defects can potentially provide definitive repair in human patients.

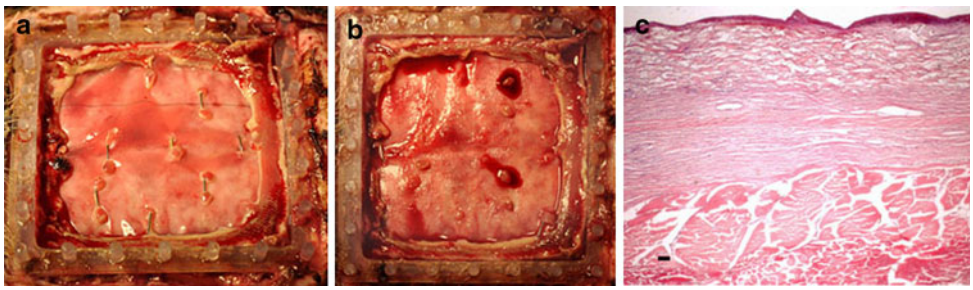


Fig. 5 Dermal substitute integra (IDRT) 3 weeks after transplantation. **a** Macroscopic view of the transplanted IDRT in place. **b** Macroscopic view of an IDRT-derived neodermis, silicone foil already removed. **c** Haematoxylin and Eosin staining of the excised IDRT (*scale bar* 100 μm)

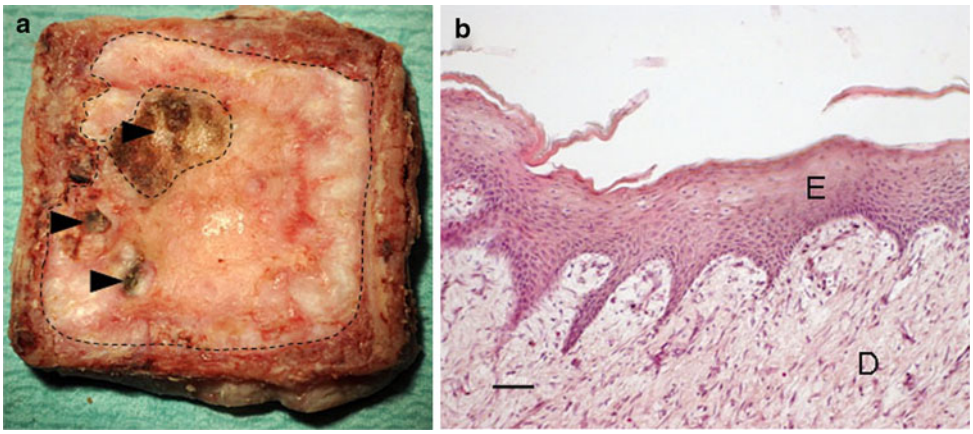


Fig. 6 Laboratory-grown skin substitute 3 weeks after transplantation. **a** Macroscopic view of the excised skin substitute. *Dotted line* indicates epithelialized area. *Arrowheads* indicate locations of previously taken punch biopsies. **b** Haematoxylin and Eosin staining of the excised skin substitute with close to normal epidermis (E) and dermis (D) (*scale bar* 50 μm)

Third, the silicone chamber insertion was done for scientific reasons alone: we wanted, particularly in view of the future experiments, to explicitly and reliably shield the area of interest from any (even partial) *false-positive results* through healing from the wound edges. Overall, the chamber seems to be a valid device in that its handling proved to be easy, and it granted the wanted strict compartmentalisation of the operation field from the surrounding skin. Moreover, there were no macroscopically detectable adverse effects, in particular impaired healing, coming from the chamber itself. Of note, IDRT healed in promptly and uneventfully as was to be expected, while the much more delicately engineered skin analogues healed with spotty epidermal deficits. The random pattern epidermal lack, however, speaks against a chamber-associated problem as then, the non-epithelialized areas would most likely lay adjacent to the chamber. Yet, the graft healing observed must not be over-interpreted in any direction. The goal of this experiment was the establishment of a large animal/large graft transplantation model, not the in-depth investigation of results after grafting. The latter will be looked at specifically in a next series of formal experiments.

It is noteworthy that our chamber is by far the *largest and first rectangular* such device ever published. All other chambers used for similar purposes were round in shaped and had a maximum diameter or 4 cm [10–12]. In terms of an outlook, we figure that the size of this chamber can easily be enlarged, or, alternatively, two or more adjacent chambers could be jointly used in the same animal if large enough.

Fourth, an obligatory requirement of the model is *maximum graft protection for the first postoperative weeks*, when the graft is most vulnerable, and when the animal is presumably most annoyed and therefore prone to get rid of the irritation in any way possible. Therefore, we chose the back as operation site since the animal cannot reach this area with mouth or extremities. Also, rubbing is, even though not entirely impossible, rather challenging. Clearly, adequate pain management and individual housing, the combination of a closed-up chamber, and a sophisticated overlying shock-absorbing dressing proved effective in all animals alike. This aspect cannot be overemphasised since all envisioned future experiments rely on long-term observation (up to 6–12 months), so grafts must definitely survive the initial phase until, when stable enough, they can eventually be left without dressing.

Finally, pig skin is the *closest relative* with regard to human skin in terms of anatomy and physiology [13, 14]. Therefore, a pig model as the one presented here appears to be the most appropriate choice for a preclinical setting in view of future skin analogue transplantations in human patients.

In conclusion, we devised a practicable new pig model allowing transplantation of large autologous, laboratory-engineered skin analogues and that, importantly, allows

keeping the site of intervention clean and intact for the first three postoperative weeks. This model appears suitable to reliably conduct those experiments that will investigate in detail whether large tissue-engineered skin analogues can consistently reconstitute near-normal skin also in the long run. If so, this strategy may eventually be introduced into clinical practice.

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