Pediatr Surg Int (2013) 29:479–488
DOI 10.1007/s00383-013-3267-y

ORIGINAL ARTICLE

A new model for preclinical testing of dermal substitutes for human skin reconstruction

Fabienne Hartmann-Fritsch · Thomas Biedermann · Erik Braziulis · Martin Meuli · Ernst Reichmann

Accepted: 16 January 2013 / Published online: 1 February 2013

Abstract
Background Currently, acellular dermal substitutes used for skin reconstruction are usually covered with split-thickness skin grafts. The goal of this study was to develop an animal model in which such dermal substitutes can be tested under standardized conditions using a bioengineered dermo-epidermal skin graft for coverage.
Methods Bioengineered grafts consisting of collagen type I hydrogels with incorporated human fibroblasts and human keratinocytes seeded on these gels were produced. Two different dermal substitutes, namely Matriderm®, and an acellular collagen type I hydrogel, were applied onto full-thickness skin wounds created on the back of immunoincompetent rats. As control, no dermal substitute was used. As coverage for the dermal substitutes either the bioengineered grafts were used, or, as controls, human split-thickness skin or neonatal rat epidermis were used. Grafts were excised 21 days post-transplantation. Histology and immunofluorescence was performed to investigate survival, epidermis formation, and vascularization of the grafts.
Results The bioengineered grafts survived on all tested dermal substitutes. Epidermis formation and vascularization were comparable to the controls.
Conclusion We could successfully use human bioengineered grafts to test different dermal substitutes. This novel model can be used to investigate newly designed dermal substitutes in detail and in a standardized way.

Keywords Dermal substitutes · Matriderm · Tissue engineering · Split-thickness skin · Dermis · Epidermis · Skin reconstruction

Introduction

The today's gold standard to treat full-skin defects such as burns, giant nevi, or avulsion injuries is the transplantation of autologous split-thickness skin [1, 2]. Split-thickness skin can be applied directly on the wound bed, or it can be transplanted onto different acellular dermal substitutes such as Integra® (Integra) [1, 3–7] or Matriderm® (Matriderm) [8–13]. Dermal substitutes improve wound healing, impede scar contraction and the cosmetic outcome compared to transplanted split-thickness skin alone [1, 14, 15]. A newly designed dermal substitute needs to be tested preclinically for its biological behavior and compatibility with split-thickness skin. For most dermal substitutes, tests are performed in a pig animal model with porcine split-thickness skin [16–19].

The aim of this experimental study was to test whether a human bioengineered skin graft can be used to replace human split-thickness skin for the pre-clinical testing of acellular dermal substitutes used for skin reconstruction.

F. Hartmann-Fritsch and T. Biedermann contributed equally to this paper.

F. Hartmann-Fritsch · T. Biedermann · E. Braziulis · E. Reichmann
Tissue Biology Research Unit, Department of Surgery, University Children's Hospital Zurich, Zurich, Switzerland

M. Meuli (✉)
Department of Pediatric Surgery, University Children's Hospital Zurich, Steinwiesstrasse 75, 8032 Zurich, Switzerland
e-mail: martin.meuli@kispi.uzh.ch

Materials and methods

Cell isolation and culture

Human skin samples were obtained after informed consent from patients and/or parents; all described studies were approved by the Medical Ethical Committee of the Kanton Zurich. Human primary fibroblasts and keratinocytes were isolated as previously described [20].

Engineered dermal substitutes (acellular collagen hydrogels)

To engineer acellular dermal substitutes, a solution of bovine collagen type I was neutralized with a NaOH containing buffer [21] and poured into cell culture inserts with a pore size of 3 μm (BD Falcon, Basel, Switzerland). After incubation for 2 h at 37 °C, the acellular dermal substitutes were plastically compressed as previously described [22]. The acellular collagen hydrogels were incubated for 5 days in Rheinwald and Green medium (RGM) [20], before transplantation, with medium changes performed every 2–3 days.

Matriderm

Matriderm 1 mm (Dr. Suwelack Skin and Health Care AG, Billerbeck, Germany) was cut into round pieces of 26 mm diameter and incubated in RGM for 3 min immediately before transplantation.

Human bioengineered skin grafts (bioengineered grafts)

Human bioengineered skin grafts based on collagen type I hydrogels with incorporated human dermal fibroblasts and human keratinocytes seeded on these hydrogels were created as previously described [23]. Briefly, 10^5 human primary dermal fibroblasts were mixed with bovine collagen type I (BD Biosciences, Basel, Switzerland) and neutralized with a buffer containing NaOH. The solution was poured into cell culture inserts with a pore size of 3 μm (BD Falcon, Basel, Switzerland). After incubation of 2 h at 37 °C and 5 % CO_2, the hydrogels were plastically compressed [22]. The hydrogels were cultivated in DMEM supplemented with 10 % FCS, and media changes were performed every 2–3 days. 7.5×10^5 human primary keratinocytes were seeded onto the complete surface of the hydrogels 5 days thereafter. After 3 days of submerged cultivation in RGM, hydrogels were cultivated for additional 3 days at the air–liquid interface, before transplantation onto immuno-incompetent rats.

Human split-thickness skin

Human split-thickness skin (leftovers from split-thickness skin transplantations) was received from patients from the University Children's Hospital Zurich after informed consent from patients and/or parents. Split-thickness skin was stored in DMEM at 4 °C until transplantation.

Neonatal rat epidermis

Neonatal rat epidermis of appropriate size was obtained from full-thickness skin samples from newborn Wistar rats (University of Zurich animal breeding program). Full-thickness skin samples were treated overnight in dispase (BD Biosciences) diluted 1:1 in Hank's balanced salt solution (Invitrogen) containing 5 mg/ml gentamicin (Invitrogen) at 4 °C. To obtain an epidermis free of dermal components, the epidermis was peeled off the dermis immediately before transplantation.

Transplantation

All animal experiments were approved by the local Committee for Experimental Animal Research. The preparation of immuno-incompetent nu/nu rats (age 8–10 weeks, Harlan, Horst, The Netherlands) was performed as previously described [24]. Steel rings with a diameter for 26 mm were implanted into full-thickness skin defects on the back of the rats to prevent wound closure by ingrowth of rat skin into the wound area. Two different dermal substitutes, namely Matriderm and engineered acellular collagen hydrogels, were placed onto the wounds. The dermal substitutes were immediately covered with (a) human bioengineered graft, (b) human split-thickness skin, or, (c) neonatal rat epidermis. Controls were performed without any dermal substitute, where the coverage grafts were transplanted directly on the fascia (Fig. 1). Transplants were covered with a silicon foil (Silon-SES, BMS, Allentown, PA, USA). Sample sizes for each group are summarized in Table 1. Once a week, wound dressing changes and photographic documentations were performed. Animals were sacrificed 21 days post-transplantation. Grafts were excised, halved, and embedded in O.C.T compound (Tissue-Tek®, Sakura Finetek, Japan) or fixed in 4 % paraformaldehyde (Medite Medizintechnik AG, Nunningen, Switzerland) and then embedded in paraffin (McCormick, Richmond, USA).

Histology

For hematoxylin and eosin staining, 10 μm thick paraffin sections were cut and stainings were imaged by light microscopy [Nikon Eclipse TE2000-U inverted microscope

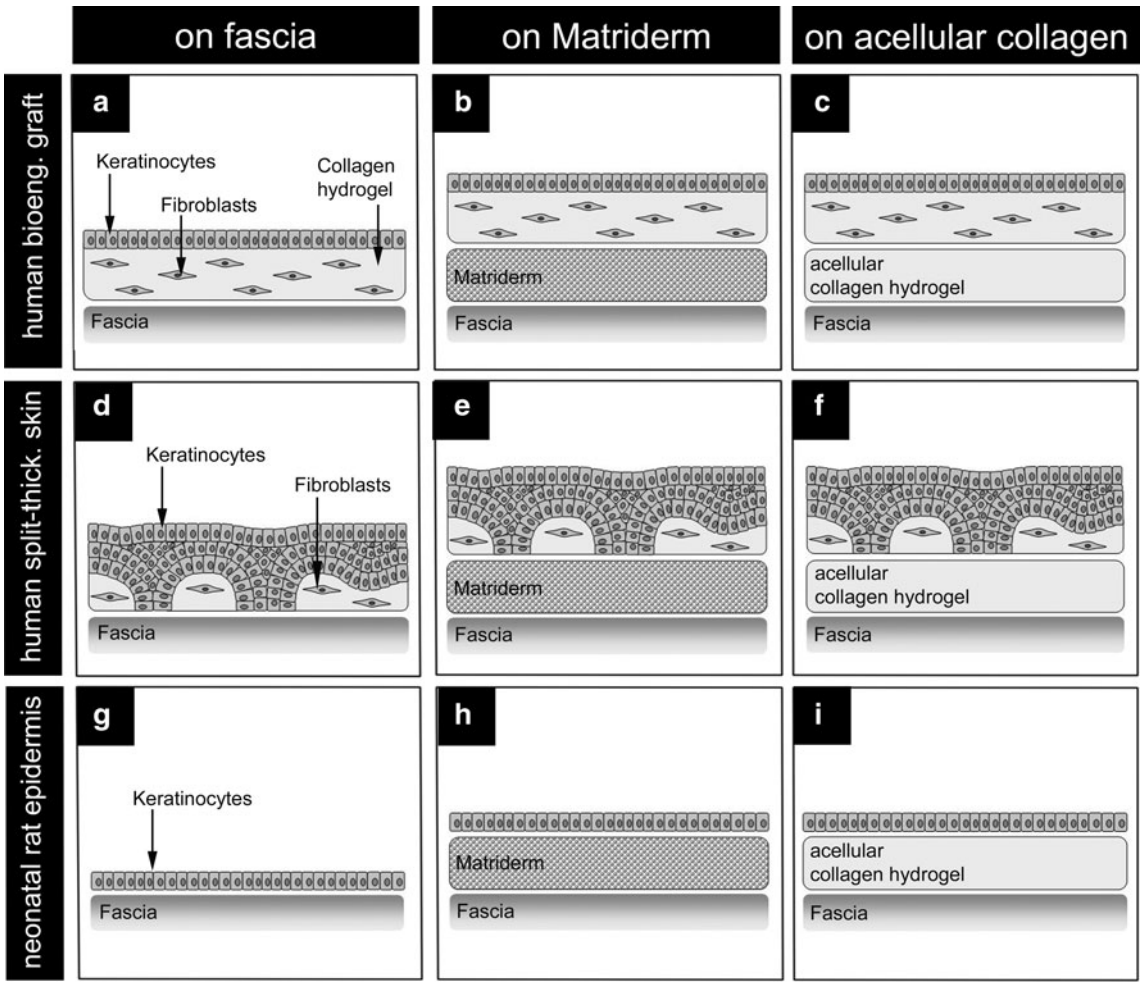

Fig. 1 Schematic overview on the different combinations of dermal substitutes and coverage used in this study. Human bioengineered graft transplanted onto the fascia (**a**), on Matriderm (**b**), or, on engineered acellular collagen hydrogel (**c**). Human split-thickness skin transplanted onto the fascia (**d**), on Matriderm (**e**), or, on engineered acellular collagen hydrogel (**f**). Neonatal rat epidermis transplanted onto the fascia (**g**), on Matriderm (**h**), or, on engineered acellular collagen hydrogel (**i**)

Table 1 Summary of the sample sizes for each group

	On fascia	On Matriderm	On acellular collagen
Human bioengineered graft	10	3	4
Human split-thickness skin	1	1	1
Neonatal rat epidermis	4	4	4

connected with a DXM1200F digital camera (Nikon AG, Egg, Switzerland)].

For immunofluorescence stainings, O.C.T embedded tissue was frozen at −20 °C, and sectioned at 10 µm. Permeabilization was performed in ice-cold acetone for 5 min, thereafter sections were air-dried, and washed three times in phosphate buffered saline (PBS). After blocking with PBS containing 2 % bovine serum albumin (Sigma, Buchs, Switzerland) for 30 min at room temperature, sections were incubated with the pre-labeled antibodies for 1 h at room temperature. Sections were washed three times with PBS, then nuclei were stained with 1 µg/ml Hoechst 33341 (Sigma, Buchs, Switzerland) in PBS for 5 min at room temperature. Sections were washed two times with PBS, and finally mounted with Dako fluorescent mounting solution (Dako, Baar, Switzerland).

Antibodies

The following antibodies were used: K1 (clone LHK1, 1:200, Novus Biologicals, Littleton, USA); Lam5α3 (clone P3H9-2, 1:100, Santa Cruz, Labforce AG, Nunningen, Switzerland); CD31 (clone TDL-3A12, 1:50, BD Biosciences Pharmingen, Basel, Switzerland); CD90 (clone AS02, 1:100, Dianova, Hamburg, Germany).

For immunofluorescence stainings, primary antibodies were pre-labeled with either Alexa488 or Alexa555 conjugated polyclonal goat F(ab′)2 fragments, according to the manufacturer's instructions (Zenon Mouse IgG Labelling Kit, Molecular Probes, Invitrogen, Basel, Switzerland).

Fluorescence microscopy

The immunofluorescence stainings were analyzed with a Nikon Eclipse TE2000-U inverted microscope, equipped with Hoechst, FITC and TRITC filter sets and connected with a DXM1200F digital camera (Nikon AG, Egg, Switzerland).

Results

Macroscopic appearance of epidermis on transplanted grafts

Immediately after transplantation, the human bioengineered grafts macroscopically appeared similar in all groups (transplanted either directly on the fascia, on Matriderm, or, on an engineered acellular collagen hydrogel) (Fig. 2a–c). At the time point of transplantation, human split-thickness skin macroscopically looked similar in all three groups, the underlying dermal substitute was not visible through the split-thickness skin (Fig. 2d–f). At the

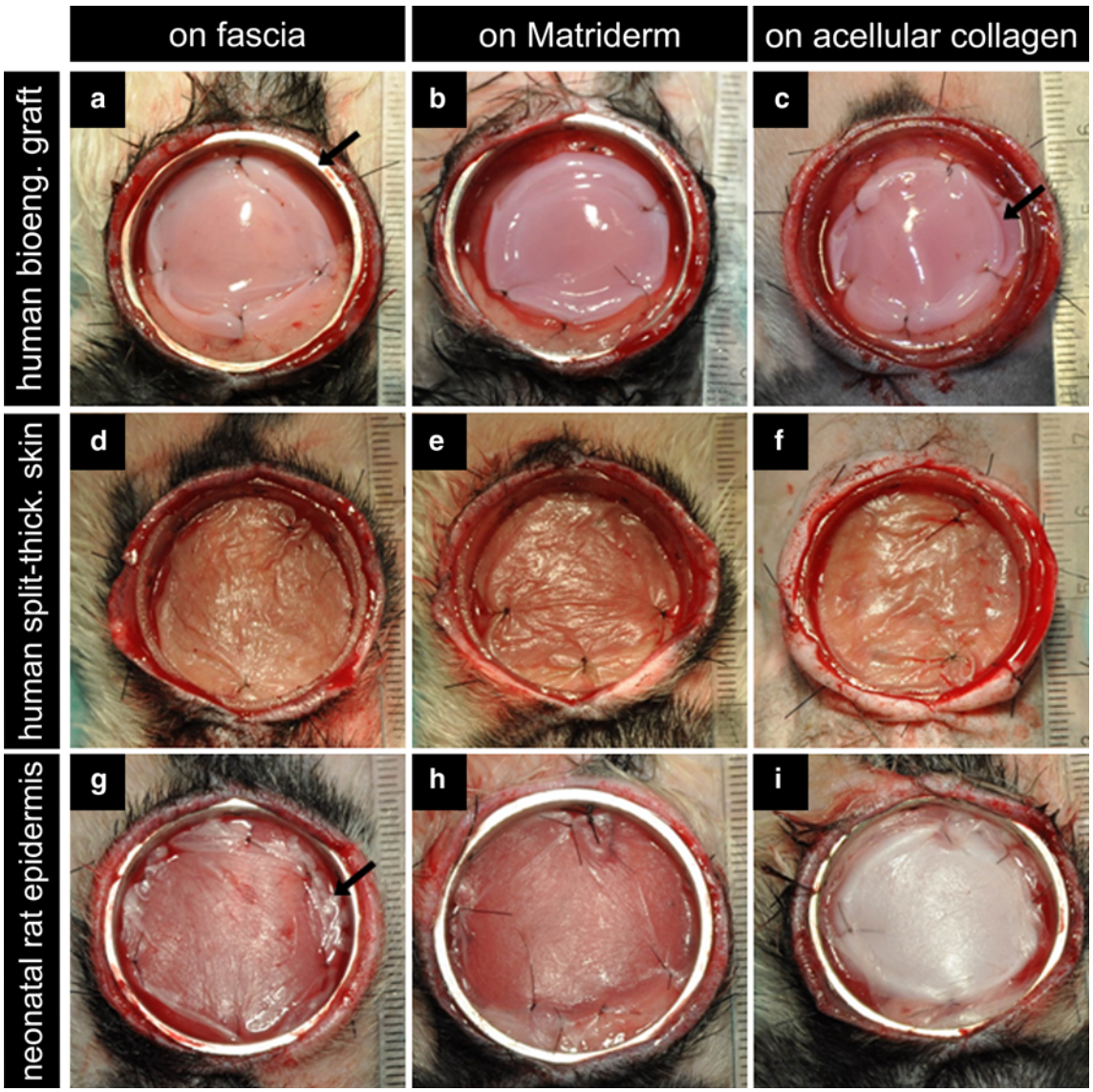

Fig. 2 Macroscopic view of grafts at the time point of transplantation. **a–c** Human bioengineered graft appearance is similar in all three groups. The underlying engineered acellular collagen hydrogel is detectable in **c** (*arrow*). **d–f** Human split-thickness skin shows similar appearance in all three groups. **g–h** Neonatal rat epidermis looks similar when transplanted onto fascia and Matriderm. **i** Neonatal rat epidermis looks *pale* when transplanted onto engineered acellular collagen hydrogels. Diameter of the ring (*arrow* in **a**) 26 mm (**a–i**)

time point of transplantation, neonatal rat epidermis appeared very thin and transparent (Fig. 2g–i) and the underlying dermal substitute was visible. Matriderm seemed red and dull, as a result from its incubation in RGM prior to transplantation (Fig. 2h). The engineered acellular collagen hydrogel shined white through the neonatal rat epidermis (Fig. 2i).

21 days post-transplantation, a complete take of the bioengineered grafts in all three groups was observed (Fig. 3a–c). Grafts showed an epithelialized surface and seemed well integrated into the rat tissue. 21 days post-transplantation, the split-thickness skins seemed well integrated into the rat tissue and in all three groups good take was observed with epidermis covering the complete surface (Fig. 3d–f). 21 days post-transplantation, neonatal rat epidermis transplanted directly onto the fascia (Fig. 3g) macroscopically showed a better take rate compared to neonatal rat epidermis transplanted on Matriderm (Fig. 3h) and on engineered acellular collagen gel (Fig. 3i).

Histological analysis of grafts excised 21 days post transplantation

Grafts excised 21 days post-transplantation were sectioned and stained with hematoxylin and eosin. Human bioengineered grafts showed a stratified epidermis of 6–9 layers

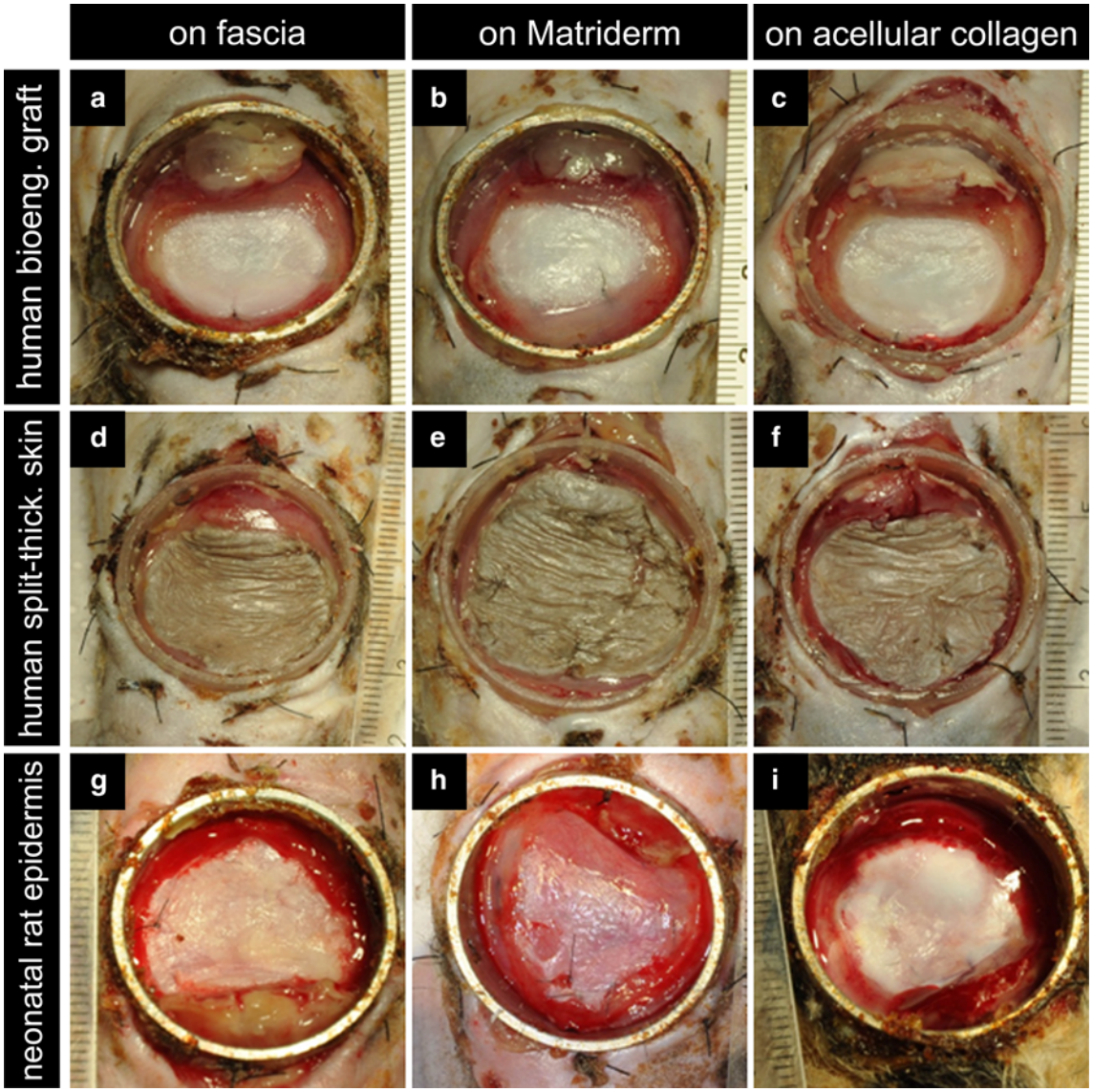

Fig. 3 Macroscopic view of grafts 21 days post-transplantation. **a–c** Human bioengineered grafts show complete take in all three groups. Grafts look almost identical in all three groups. **d–f** Human split-thickness skin shows complete take in all three groups. Grafts look almost identical in all three groups. **g–i** Neonatal rat epidermis shows only partial take that is similar in all three groups. The surviving epidermal islets look very fragile and do not seem to be firmly attached to the wound bed

that was similar in all three groups (Fig. 4a–c). Remnants of Matriderm could be detected between the bioengineered graft and the underlying rat tissue (Fig. 4b). The bioengineered graft could be distinguished from the engineered acellular collagen hydrogel, which was partially populated by rat cells (Fig. 4c).

Human split-thickness skin showed similar appearance in all three groups, with a multi-layered epidermis, prominent rete ridges, and a very prominent stratum corneum (Fig. 4d–f).

Transplantation of neonatal rat epidermis was partially successful when transplanted directly on the fascia, where a 2–3 layered epidermis could be detected (Fig. 4g). When transplanted on Matriderm (Fig. 4h) or on engineered acellular collagen gel (Fig. 4i), the epidermis did not survive, and only some single epidermal cells could be detected. The Matriderm material could not be clearly distinguished from the underlying rat tissue (Fig. 4h). The engineered acellular collagen hydrogel was clearly

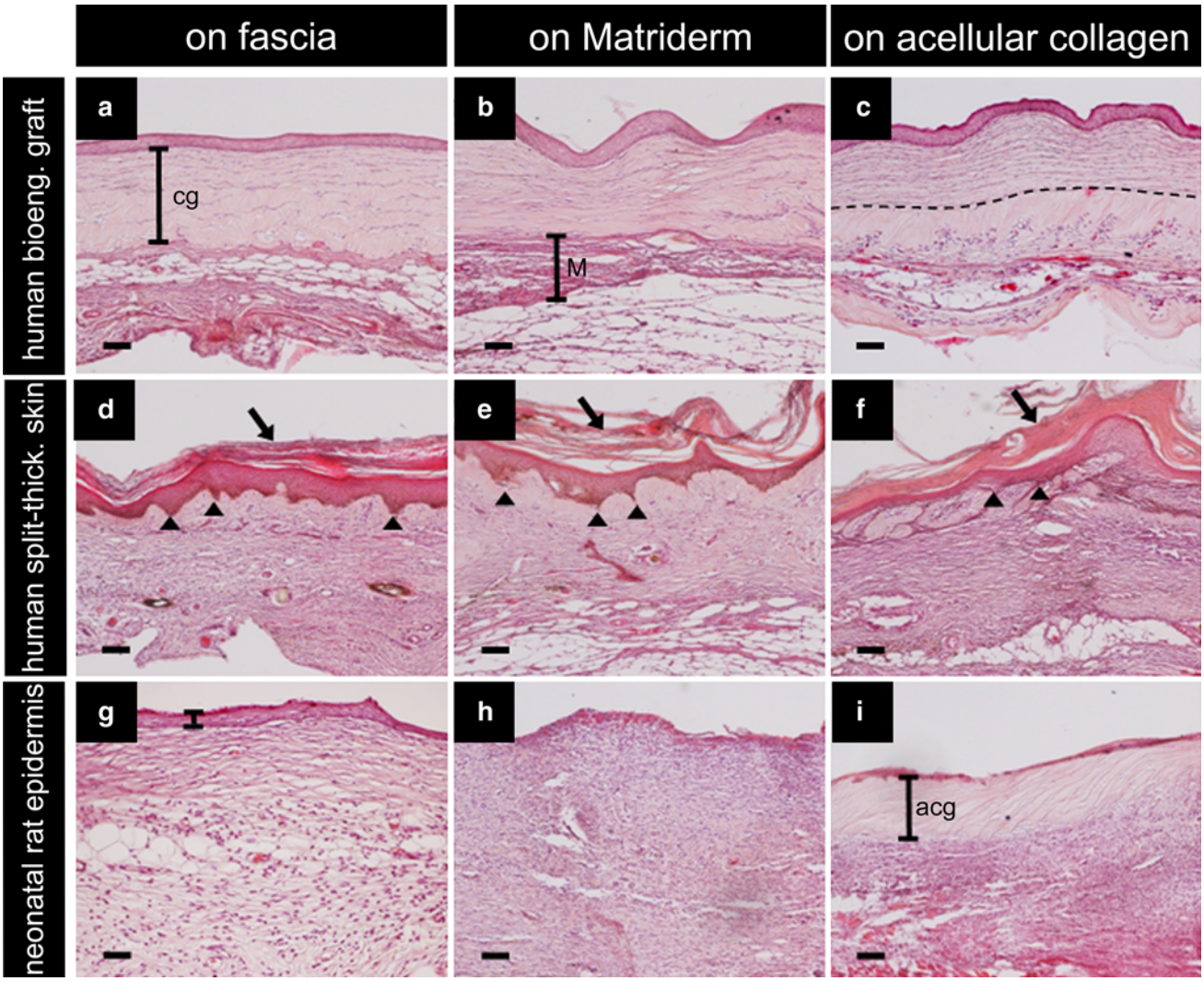

Fig. 4 Histological evaluation of grafts (hematoxylin and eosin staining) excised 21 days post-transplantation. **a** Human bioengineered grafts transplanted onto the fascia show 6–9 keratinocyte layers and the dermal part of the bioengineered graft can be clearly distinguished from the underlying tissue (*cg* collagen gel). **b** Human bioengineered grafts transplanted on Matriderm show similar appearance as in **a**. Remnants of Matriderm are detectable between the bioengineered graft and the underlying rat tissue (*M* Matriderm). **c** Human bioengineered grafts transplanted on acellular collagen show similar appearance as in **a**. The border between graft and engineered acellular collagen hydrogel can be discerned (*dotted line*). **d–f** Human split-thickness skin looks similar in all three groups. The normally configured epidermis shows a stratum corneum (*arrows*) and rete ridges (*arrow heads*). **g** Neonatal rat epidermis transplanted onto the fascia is very thin and unstratified (*black line*). **h** Neonatal rat epidermis transplanted onto Matriderm does not survive, no epidermis is detectable. **i** Neonatal rat epidermis transplanted on engineered acellular collagen hydrogel does not survive, only single epidermal cells are occasionally detectable. The collagen gel is easily discernable and there are almost no cells. (*acg* engineered acellular collagen hydrogel). *Scale bars* 100 μm (**a–i**)

detectable and was only partially colonized by ingrown rat cells (Fig. 4i).

Basal lamina deposition and epidermis stratification

Grafts excised 21 days post-transplantation were sectioned and immunofluorescence stainings were performed. Staining with an antibody to the basal lamina component laminin 5 revealed the deposition of a continuous basal lamina in human bioengineered grafts in all three groups (Fig. 5a–c). In bioengineered grafts, all suprabasal keratinocyte layers expressed keratin 1 (Fig. 5a–c). Similar expression patterns for laminin 5 and keratin 1 were observed in transplanted human split-thickness skin in all three groups (Fig. 5d–f). In the specimens where neonatal rat epidermis was transplanted, neither basal lamina deposition nor keratin 1 expression was detected in either group (Fig. 5g–i).

In human bioengineered grafts, the ingrowth of rat blood vessels, as detected by staining with CD31, was similar in all three groups (Fig. 6a–c). The human fibroblasts of the bioengineered graft could be detected by staining with CD90 in all groups (Fig. 6a–c). Similarly, in specimens covered with human split-thickness skin, rat blood vessels were found in proximity to the epidermis (Fig. 6d–f). The human origin was confirmed by CD90 staining, which was expressed by human fibroblasts in the split-thickness skin (Fig. 6d–f). In specimens covered with neonatal rat epidermis, the ingrowth of rat blood vessels was similar in all three groups (Fig. 6g–i). No human fibroblasts expressing CD90 were detected (Fig. 6d–i).

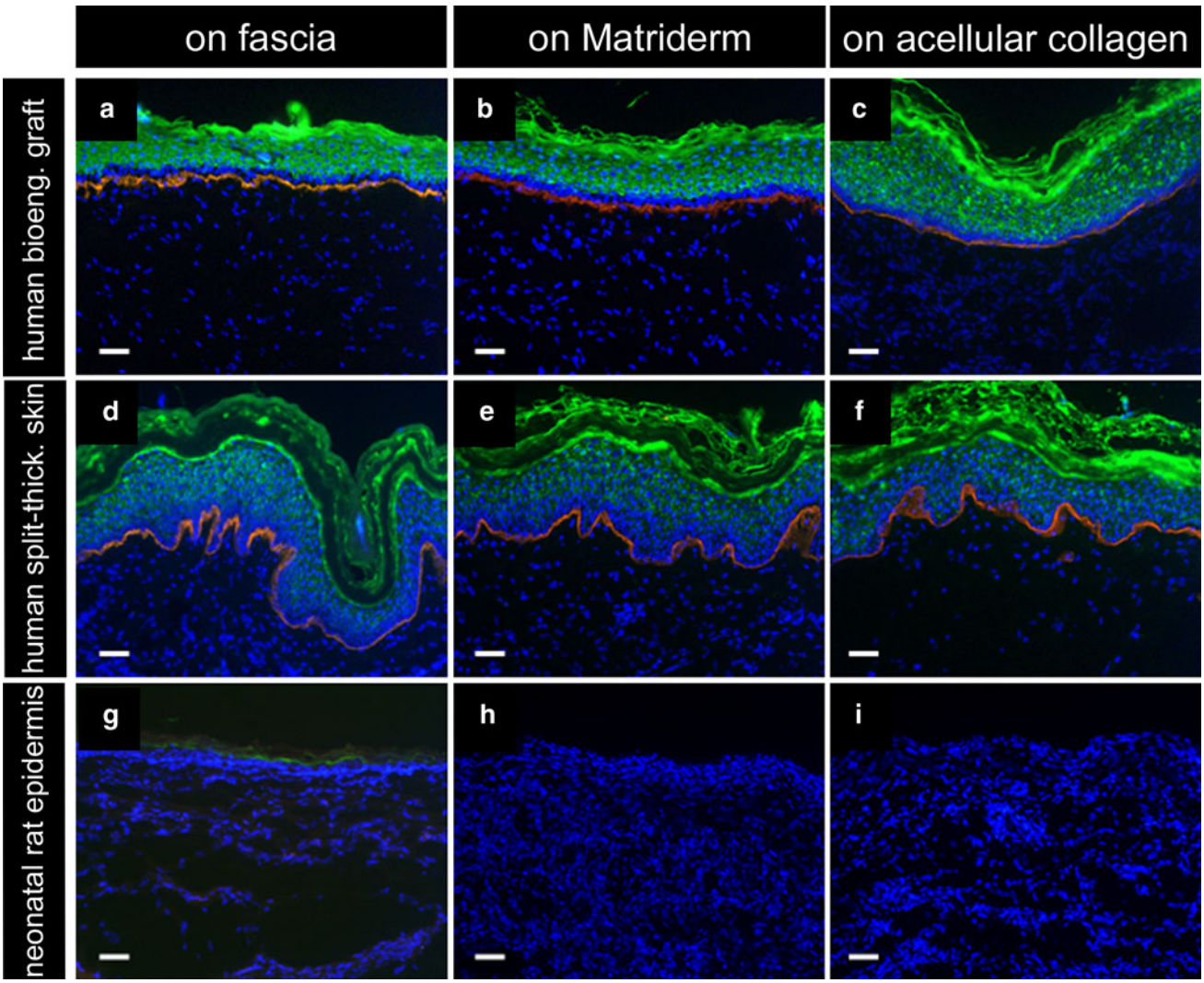

Fig. 5 Immunofluorescence stainings (laminin 5 and keratin 1) of grafts excised 21 days post-transplantation. **a–c** In human bioengineered grafts in all three groups, staining of the basement membrane component laminin 5 (*red*) reveals the deposition of a continuous basement membrane. In all three groups, all suprabasal cells express keratin 1 (*green*). **d–f** Human split-thickness skin in all three groups shows similar expression of laminin 5 (*red*) and keratin 1 (*green*) as described for **a–c**. **g–i** In all three groups with neonatal rat epidermis transplantation, there is no laminin 5 (*red*) nor keratin 1 (*green*) expression. *Scale bars* 50 μm

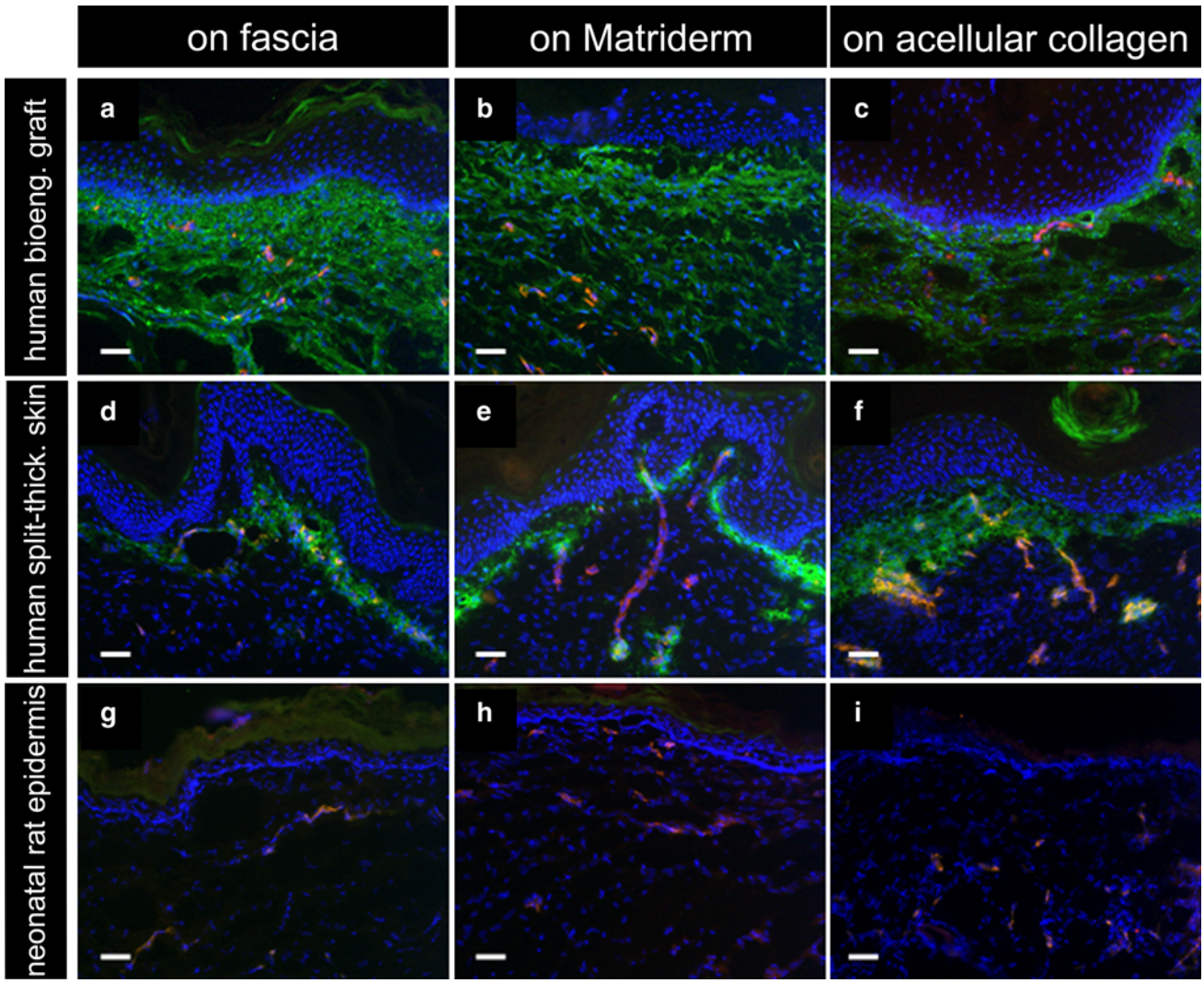

Fig. 6 Immunofluorescence stainings (CD31 and CD90) of grafts excised 21 days post-transplantation. **a–c** In human bioengineered grafts in all three groups, staining with CD31 (*red*) reveals the ingrowth of rat blood vessels into the dermal part of the coverage graft. Also, human fibroblasts expressing CD90 (*green*) are detected in all groups. **d–f** In human split-thickness skin, rat blood vessels grew into the dermal part as detected by CD31 staining (*red*). Human fibroblasts are detected by staining with CD90 (*green*). **g–i** In neonatal rat epidermis in all three groups, staining with CD31 (*red*) reveals the ingrowth of rat blood vessels into the transplants. In all three groups, no human fibroblasts expressing CD90 (*green*) are detected. *Scale bars* 50 μm

Discussion

The goal of this experimental study was to test whether a human bioengineered skin graft can be used to replace human split-thickness skin for the pre-clinical testing of acellular dermal substitutes used for skin reconstruction.

These bioengineered grafts were compared to the current standard coverage of dermal substitutes, i.e. split-thickness skin, and to neonatal rat epidermis. In the global picture, human bioengineered grafts demonstrated a very similar biological behavior as split-thickness skin, while neonatal rat epidermis performed significantly poorer.

Several points deserve a detailed comment: Split-thickness skin was used as positive control for the new model, as split-thickness skin transplantation is the gold standard for complex dermo-epidermal skin reconstruction in clinical practice since more than 10 years [1, 25, 26]. Interestingly, we found that the human bioengineered graft behaved as well as split-thickness skin. When transplanted on Matriderm as well as on engineered acellular collagen hydrogel, the bioengineered skin grafts show similar biological performance as seen with transplantation of split-thickness skin. A continuous basal lamina was deposited as evidenced by staining for the basal lamina component laminin 5, and all suprabasal cells expressed keratin 1 as is seen in normal homeostatic skin. Rat blood vessels grew into the dermal part of the bioengineered grafts and so ensured sufficient oxygen and nutrient supply for the

epidermis. The presence of human fibroblasts, which are indispensable key players regarding skin architecture and functionality, in the bioengineered skin grafts could be confirmed by the detection of human CD90-specific cells.

In sharp contrast, the performance of neonatal rat epidermis alone was significantly poorer. The most tenable explanation for the good performance of the bioengineered graft and split-thickness skin as opposed to rat epidermis is the presence of dermal components in the former and a complete lack of those elements in the latter. It is well documented in the literature that viable and functional dermal elements are crucial for epidermal viability and functionality [27–31].

The almost identical biological behavior of the bioengineered grafts compared to split-thickness skin indicates that human bioengineered grafts might be used in the future instead of split-thickness skin as standard dermo-epidermal coverage to test any newly designed dermal substitutes. Of note, human bioengineered grafts can be prepared in a very standardized way in terms of cell numbers, cell types, and thickness of the graft. The use of a humanized model represents an additional advantage, given the fact that the mentioned preclinical testing procedures may lead to applications in human patients.

In summary and conclusion, this series of experiments provides evidence that human bioengineered skin grafts can successfully be employed to test different acellular dermal substitutes designed for skin reconstruction. Such testing is indispensable before new products can be applied clinically.

Acknowledgments This work was financially supported by the EU-FP6 project EuroSTEC (soft tissue engineering for congenital birth defects in children: contract: LSHB-CT-2006-037409), by the EU-FP7 project EuroSkinGraft (FP7/2007-2013: grant agreement no. 279024), and by the University of Zurich. We are particularly grateful to the Fondation Gaydoul and the sponsors of "Dona Tissue" (Thérèse Meier and Robert Zingg) for their financial support and interest in our work.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Schiestl C, Stiefel D, Meuli M (2010) Giant naevus, giant excision, eleg(i)ant closure? Reconstructive surgery with Integra artificial skin to treat giant congenital melanocytic naevi in children. J Plast Reconstr Aesthet Surg 63(4):610–615
2. Schiestl C, Neuhaus K, Biedermann T, Böttcher-Haberzeth S, Reichmann E, Meuli M (2011) Novel treatment for massive lower extremity avulsion injuries in children: slow, but effective with good cosmesis. Eur J Pediatr Surg 21(2):106–110
3. Stiefel D, Schiestl CM, Meuli M (2009) The positive effect of negative pressure: vacuum-assisted fixation of Integra artificial skin for reconstructive surgery. J Pediatr Surg 44(3):575–580
4. Stiefel D, Schiestl C, Meuli M (2010) Integra artificial skin for burn scar revision in adolescents and children. Burns 36(1): 114–120
5. Böttcher-Haberzeth S, Biedermann T, Reichmann E (2010) Tissue engineering of skin. Burns 36(4):450–460
6. Heitland A, Piatkowski A, Noah EM, Pallua N (2004) Update on the use of collagen/glycosaminoglycate skin substitute-six years of experiences with artificial skin in 15 German burn centers. Burns 30(5):471–475
7. Branski LK, Herndon DN, Pereira C, Mlcak RP, Celis MM, Lee JO, Sanford AP, Norbury WB, Zhang XJ, Jeschke MG (2007) Longitudinal assessment of Integra in primary burn management: a randomized pediatric clinical trial. Crit Care Med 35(11): 2615–2623
8. Schneider J, Biedermann T, Widmer D, Montano I, Meuli M, Reichmann E, Schiestl C (2009) Matriderm versus Integra: a comparative experimental study. Burns 35(1):51–57
9. Cervelli V, Lucarini L, Cerretani C, Spallone D, Palla L, Brinci L, De Angelis B (2010) The use of Matriderm and autologous skin grafting in the treatment of diabetic ulcers: a case report. Int Wound J 7(4):291–296
10. Böttcher-Haberzeth S, Biedermann T, Schiestl C, Hartmann-Fritsch F, Schneider J, Reichmann E, Meuli M (2012) Matriderm® 1 mm versus Integra® single layer 1.3 mm for one-step closure of full thickness skin defects: a comparative experimental study in rats. Pediatr Surg Int 28(2):171–177
11. Keck M, Haluza D, Lumenta DB, Burjak S, Eisenbock B, Kamolz LP, Frey M (2011) Construction of a multi-layer skin substitute: simultaneous cultivation of keratinocytes and preadipocytes on a dermal template. Burns 37(4):626–630
12. Haslik W, Kamolz LP, Nathschläger G, Andel H, Meissl G, Frey M (2007) First experiences with the collagen-elastin matrix Matriderm as a dermal substitute in severe burn injuries of the hand. Burns 33(3):364–368
13. Shakespeare PG (2005) The role of skin substitutes in the treatment of burn injuries. Clin Dermatol 23(4):413–418
14. Kearney JN (2001) Clinical evaluation of skin substitutes. Burns 27(5):545–551
15. Cervelli V, Brinci L, Spallone D, Tati E, Palla L, Lucarini L, De Angelis B (2011) The use of MatriDerm® and skin grafting in post-traumatic wounds. Int Wound J 8(4):400–405
16. Lamme EN, van Leeuwen RT, Mekkes JR, Middelkoop E (2002) Allogeneic fibroblasts in dermal substitutes induce inflammation and scar formation. Wound Repair Regen 10(3):152–160
17. Middelkoop E, van den Bogaerdt AJ, Lamme EN, Hoekstra MJ, Brandsma K, Ulrich MM (2004) Porcine wound models for skin substitution and burn treatment. Biomaterials 25(9):1559–1567
18. van den Bogaerdt AJ, Ulrich MM, van Galen MJ, Reijnen L, Verkerk M, Pieper J, Lamme EN, Middelkoop E (2004) Upside-down transfer of porcine keratinocytes from a porous, synthetic dressing to experimental full-thickness wounds. Wound Repair Regen 12(2):225–234
19. Philandrianos C, Andrac-Meyer L, Mordon S, Feuerstein JM, Sabatier F, Veran J, Magalon G, Casanova D (2012) Comparison of five dermal substitutes in full-thickness skin wound healing in a porcine model. Burns 38(6):820–829
20. Pontiggia L, Biedermann T, Meuli M, Widmer D, Böttcher-Haberzeth S, Schiestl C, Schneider J, Braziulis E, Montaño I, Meuli-Simmen C, Reichmann E (2009) Markers to evaluate the quality and self-renewing potential of engineered human skin substitutes in vitro and after transplantation. J Invest Dermatol 129(2):480–490
21. Costea DE, Loro LL, Dimba EA, Vintermyr OK, Johannessen AC (2003) Crucial effects of fibroblasts and keratinocyte growth factor on morphogenesis of reconstituted human oral epithelium. J Invest Dermatol 121(6):1479–1486

22. Braziulis E, Diezi M, Biedermann T, Pontiggia L, Schmucki M, Hartmann-Fritsch F, Luginbühl J, Schiestl C, Meuli M, Reichmann E (2012) Modified plastic compression of collagen hydrogels provides an ideal matrix for clinically applicable skin substitutes. Tissue Eng Part C Methods 18(6):464–474
23. Biedermann T, Pontiggia L, Böttcher-Haberzeth S, Tharakan S, Braziulis E, Schiestl C, Meuli M, Reichmann E (2010) Human eccrine sweat gland cells can reconstitute a stratified epidermis. J Invest Dermatol 130(8):1996–2009
24. Böttcher-Haberzeth S, Biedermann T, Pontiggia L, Braziulis E, Schiestl C, Hendriks B, Eichhoff OM, Widmer DS, Meuli-Simmen C, Meuli M, Reichmann E (2013) Human eccrine sweat gland cells turn into melanin-uptaking keratinocytes in dermo-epidermal skin substitutes. J Invest Dermatol 133(2):316–324
25. Meuli M, Raghunath M (1997) Burns (Part 2) tops and flops using cultured epithelial autografts in children. Pediatr Surg Int 12(7): 471–477
26. Gobet R, Raghunath M, Altermatt S, Meuli-Simmen C, Benathan M, Dietl A, Meuli M (1997) Efficacy of cultured epithelial autografts in pediatric burns and reconstructive surgery. Surgery 121(6):654–661
27. Rheinwald JG, Green H (1975) Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 6(3):331–343
28. Leary T, Jones PL, Appleby M, Blight A, Parkinson K, Stanley M (1992) Epidermal keratinocyte self-renewal is dependent upon dermal integrity. J Invest Dermatol 99(4):422–430
29. Botchkarev VA, Kishimoto J (2003) Molecular control of epithelial-mesenchymal interactions during hair follicle cycling. J Investig Dermatol Symp Proc 8(1):46–55
30. Schultz GS, Wysocki A (2009) Interactions between extracellular matrix and growth factors in wound healing. Wound Repair Regen 17(2):153–162
31. Helary C, Zarka M, Giraud-Guille MM (2012) Fibroblasts within concentrated collagen hydrogels favour chronic skin wound healing. J Tissue Eng Regen Med 6(3):225–237