Pediatric Surgery International (2019) 35:129–136
https://doi.org/10.1007/s00383-018-4384-4

ORIGINAL ARTICLE

Induction of angiogenic and inflammation-associated dermal biomarkers following acute UVB exposure on bio-engineered pigmented dermo-epidermal skin substitutes in vivo

Katarzyna Micka-Michalak[1,3] · Thomas Biedermann[1,3] · Ernst Reichmann[1,3] · Martin Meuli[2,3] · Agnes S. Klar[1,3]

Accepted: 18 October 2018 / Published online: 14 November 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Purpose Ultraviolet (UV) radiation adversely affects skin health at cellular and molecular levels. Hence, UV radiation can directly induce inflammatory responses in the dermis by inducing erythema, edema, inflammation, dermal fibroblasts alterations, and extracellular matrix modifications.

Methods Human keratinocytes, melanocytes, and fibroblasts were isolated from skin biopsies, cultured, and expanded in vitro. Fibroblasts were seeded into collagen type I hydrogels that were subsequently covered by keratinocytes and melanocytes. These pigmented dermo-epidermal skin substitutes (pigmDESS) were transplanted for 5 weeks onto full-thickness skin wounds on the back of immuno-incompetent rats, exposed to a single UVB dose of 250 mJ/cm^2 or unexposed and excised after 1 week. The effects onto the dermis were assessed regarding cell number, cell phenotype, and cell proliferation. Local inflammation by granulocytes (HIS48) or macrophages (CD11b, iNOS) was analyzed by immunohistochemistry staining.

Results We observed a significantly enhanced ingrowth rate of blood capillaries, but not of lymphatic capillaries at 1 week post-irradiation. Moreover, the enhanced vascularization of pigmDESS after UVB exposure was concomitant with a high infiltration of granulocytes and monocytes/macrophages to the dermal part of grafts. In addition, a heterogeneous expression of HIF-1α and TNFα was detected at this early phase after UVB exposure. In local cellular response examination, results only show a moderate cell proliferation in the dermis.

Conclusions We were able to define early markers of UVB-induced effects in the dermis of pigmDESS. Overall, a single UVB dose induces temporary acute angiogenic and immune responses during the early post-irradiation phase in vivo.

Keywords Acute UVB · Human skin substitute · Tissue engineering · Blood- and lymph-angiogenesis · Inflammatory response · Granulocyte infiltration · Monocyte/macrophage recruitment · HIF-1α expression

Introduction

The treatment of large full-thickness skin loss is hampered by insufficient donor skin for autografts. Bio-engineered dermo-epidermal skin replacement offers an alternative to meet this demand. For several years, our laboratory has developed such skin grafts and tested them in vitro as well as in pre- and clinical settings [1–7]. Recently, we reconstituted pigmented dermo-epidermal skin substitutes (pigmDESS), which perfectly restore the patients' native skin color [3, 5, 8–10]. Those skin equivalents offer not only a novel treatment method for severe and extensive burns, but also protect against ultraviolet irradiation (UV) and provide the original skin taint [8, 10, 11].

Solar UV irradiation is the most harmful external component threatening the skin and may cause severe sunburn, transient inflammation, premature skin aging, and even skin cancer [12, 13]. Based on their distinct physical properties, UVA and UVB penetrate into the skin and interact with cells, located at different depths, and induce distinct biological responses in the epidermal and dermal layers [12]. The induced photoaging is the most common form of

✉ Agnes S. Klar
Agnes.Klar@kispi.uzh.ch

1 Tissue Biology Research Unit, Department of Surgery, University Children's Hospital Zurich, Zurich, Switzerland

2 Department of Surgery, University Children's Hospital Zurich, Zurich, Switzerland

3 Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland

skin damage induced by chronic, repetitive UV exposure, in particular UVA and UVB [12, 14]. UVB (280–320 nm) is a highly energetic component of UV irradiation. As the amount of energy is inversely proportional to the wavelength, UVB delivers more energy than UVA (320–400 nm) [15]. Due to its short wavelengths, UVB penetrate mostly the epidermis but is also reaches the upper papillary dermis and therefore its harmful effects should not be neglected in the dermal skin layer [13, 16].

Previous studies have shown that long-term exposure to solar UV irradiation causes damage to cells and extracellular matrix (ECM) present in the dermis of human skin. As a result, degradation of collagen and elastin fibers increases and, in turn, de novo synthesis of collagen decreases in photodamaged skin [17]. Studies in mice [18] and rats [19, 20] suggested that repetitive UVB radiation causes degeneration of elastic fibers by elastases, which results in the loss of skin elasticity, and eventually in wrinkle formation.

Interestingly, Imokawa et al. identified a close correlation between UVB-exposed human keratinocytes and dermal fibroblasts [21]. The authors proposed a UVB-induced wrinkling mechanism by an increased secretion of IL-1α and GM-CSF by irradiated human keratinocytes that was responsible for the increased gene expression of skin fibroblast elastase, which then cleaves elastic fibers in the dermis [21].

The aim of the present study was to elucidate further the effects of UVB in the dermis of pigmDESS in vivo. In particular, we examined the effect of an acute high dose of UVB irradiation on dermal angio- and lymph-angiogenesis as well as local tissue inflammation. Our data show that acute UVB induces a progressive increase of blood vessel ingrowth and enhanced density of neutrophils and monocytes/macrophages in the dermis. In addition, UVB-treated pigmDESS revealed an increased HIF-1α expression in the dermis.

Materials and methods

Human skin samples

The investigation has been conducted according to the Declaration of Helsinki principles and after acceptance of the Ethic Commission of the Canton Zurich. Childrens' parents gave informed consent to use skin samples. Human epidermal keratinocytes, melanocytes, and dermal fibroblasts were isolated from the skin samples. Tissue samples for histological examinations were embedded in paraffin, or in OCT compound (Sakura Finetek, Switzerland) and kept at − 20 °C.

Isolation and culturing of primary cells

Human keratinocytes and fibroblasts were isolated and cultured as described by Pontiggia et al. [22] and Biedermann et al. [23], melanocytes as specified in Böttcher-Haberzeth et al. [3].

Preparation of tissue-engineered skin analogs

Transwell cell culture plates in six-well format containing inserts with 3.0 µm pore-size membranes (BD Falcon, Switzerland) were used to prepare skin analogs [24]. To reconstruct the dermal compartment, rat collagen type I was mixed with 0.2 ml neutralization buffer containing 0.15 M NaOH and with 1×10^5 human dermal fibroblasts (passage 1–3). After 10 min polymerization at room temperature and 45 min in an incubator at 37 °C, the dermal equivalents were grown for 7 days in Dulbecco's modified Eagle's medium (DMEM) enriched with 10% FCS and Hepes (DMEM, Invitrogen, Switzerland). Subsequently, 5×10^5 melanocytes and keratinocytes (both passage 1–3) of the corresponding donor skin were seeded in 1:5 ratio onto dermal equivalents. Subsequently, skin analogs were cultured for 1 week in a 1:5 mix of melanocyte growth medium (Promocell, Germany) and keratinocyte medium (SFM, Invitrogen, Switzerland), and subsequently transplanted.

Transplantation of cultured pigmDESS

All animal studies have been approved by the local Committee for Experimental Animal Research (permission number: 76/2011). Immuno-deficient female nu/nu rats, 8–10-week-old (Harlan Laboratories, Netherlands) were anesthetized as previously described [25, 26]. Full-thickness skin wounds were created on the backs of the rats. Subsequently, custom-made steel rings (diameter 2.6 cm) were sutured into the skin wounds using non-absorbable polyester sutures (Ethibond®, Ethicon, USA) and the skin analogs were sutured into those rings. Steel rings protected the skin analogs and prevented the closure of the wound by the surrounding rat skin. The transplants were then covered with a silicone foil (Silon-SES, BMS, USA), a polyurethane sponge (Ligasano, Ligamed, Austria), a cohesive conforming bandage (Sincohaft, Theo Frey AG, Switzerland), and tape as wound dressing. Dressing changes and photographic documentations were performed once per week. After 6 weeks control (non-irradiated) ($N = 8$) or UVB-irradiated ($N = 8$) skin transplants were excised in toto and processed for paraffin- and cryo-sections.

Exposure of grafted pigmDESS to UVB

Five weeks after transplantation, a single dose of UVB irradiation with an intensity of 250 mJ/cm^2 was applied on grafted

pigmDESS. The employed device (Excimer System, Alma Lasers) is emitting monochromatic narrowband UVB light with a wavelength of 308 nm. Grafted pigmDESS were then kept for another 1 week on the rats and excised.

Immunohistochemical staining

Immunofluorescence staining was performed as described in Böttcher-Haberzeth et al. [2, 26]. Double immunofluorescence stainings were performed to visualize rat blood and/or lymphatic vessels (CD31 [clone TDL-3A12, 1:50, BD Pharmingen, Switzerland] and LYVE1 [polyclonal, 1:200, Novus Biologicals, UK]). To visualize immune cells, pigmDESS were stained with HIS48 (neutrophils/granulocytes) [clone HIS48, 1:100, Santa Cruz, USA], CD11b (monocytes/macrophages) [clone 44, 1:100, Santa Cruz, USA], and iNOS (macrophages) [polyclonal, 1:200, abcam, UK]. HIF-1α [clone 28b, 1:50, Santa Cruz, USA] was applied to detect hypoxia and CD90 [clone 5E10, 1:50, Dianova, Germany] to stain specifically human fibroblasts.

Pictures of immunofluorescence stainings were taken with a DXM1200F digital camera connected to a Nikon Eclipse TE2000-U inverted microscope. The device is equipped with Hoechst 33342-, FITC-, and TRITC-filter sets (Nikon AG, Switzerland; Software: Nikon ACT-1 vers. 2.70). Images were processed with Photoshop 7.0 (Adobe Systems Inc, Germany).

Quantification of human blood and lymphatic capillaries and expression of HIS48, CD11b, and HIF-1α in vivo

6–8 µm-thick cryo-sections of transplanted pigmDESS were double-stained for human CD90 and other markers and imaged by the confocal microscope. Distinct parameters such as number of CD31- (blood) and LYVE1-positive (lymphatic) vessels, as well as positive expression of HIS48 (neutrophils/granulocytes), CD11b (monocytes/macrophages), and positive area (mm^2) of HIF-1α expression were quantified per 3×10^{-1} mm^2 of skin transplant. 3D confocal projection images were subjected to a series of image analyses using NIH ImageJ allowing quantification of distinct parameters. Graphs show values as the mean (± SD). Comparison between two groups was performed using the unpaired Student's *t* test. Results were considered significant with a $p < 0.05$.

Results

Blood and lymphatic vessel distribution in transplanted human pigmDESS without and after acute UVB exposure

Six weeks post-transplantation, non-treated and UVB-exposed pigmDESS were excised and analyzed. The anti-human CD90-staining delineates the human dermal compartment of skin analogs (green, Fig. 1). The ingrowth of host blood vessels into the human transplants was analyzed using a specific anti-rat-CD31 antibody (red, Fig. 1a, b) and thereafter quantified (Fig. 3a).

UVB-irradiated pigmDESS demonstrated a significantly higher number of $CD31^+$ blood vessels (31 ± 9) throughout the human CD90-positive dermal compartment as compared to non-irradiated skin analog (7 ± 2) (p value = 0.0109, statistically significant) (Fig. 3).

Moreover, we also analyzed the density of $LYVE1^+$ lymphatic vessels in the human dermis of pigmDESS (Fig. 1c, d). However, the overall lymphatic vessel density within the non-irradiated and UVB-irradiated group did not show any obvious differences: 4 ± 2 versus 8 ± 2 (p value = 0.1052, not significant) (Fig. 3a).

Expression of granulocyte and monocyte/macrophage markers in transplanted human pigmDESS

The human skin analogs with or without UVB irradiation were examined for the presence of granulocyte (HIS48) and monocyte/macrophage (CD11b, iNOS) markers in the human CD90-stained neodermis and quantified (Figs. 2, 3a). The expression of all those markers in normal human or rat skin was low in the dermis (data not shown).

We detected a significantly enhanced density of neutrophils/granulocytes (HIS48) (97 ± 21) throughout the dermis of UVB-treated pigmDESS, whereas $HIS48^+$ cells were present at quite low numbers in the control group without UVB treatment (8 ± 2) ($p = 0.0019$, statistically very significant) (Figs. 2a, b, 3a).

Similar, $iNOS^+$ (data not shown) and $CD11b^+$ monocytes/macrophages were detected at significantly higher frequencies in UVB-exposed skin analogs (56 ± 4) than in untreated transplants (12 ± 3) (p value = 0.0001, extremely statistically significant) (Figs. 2c, d, 3a).

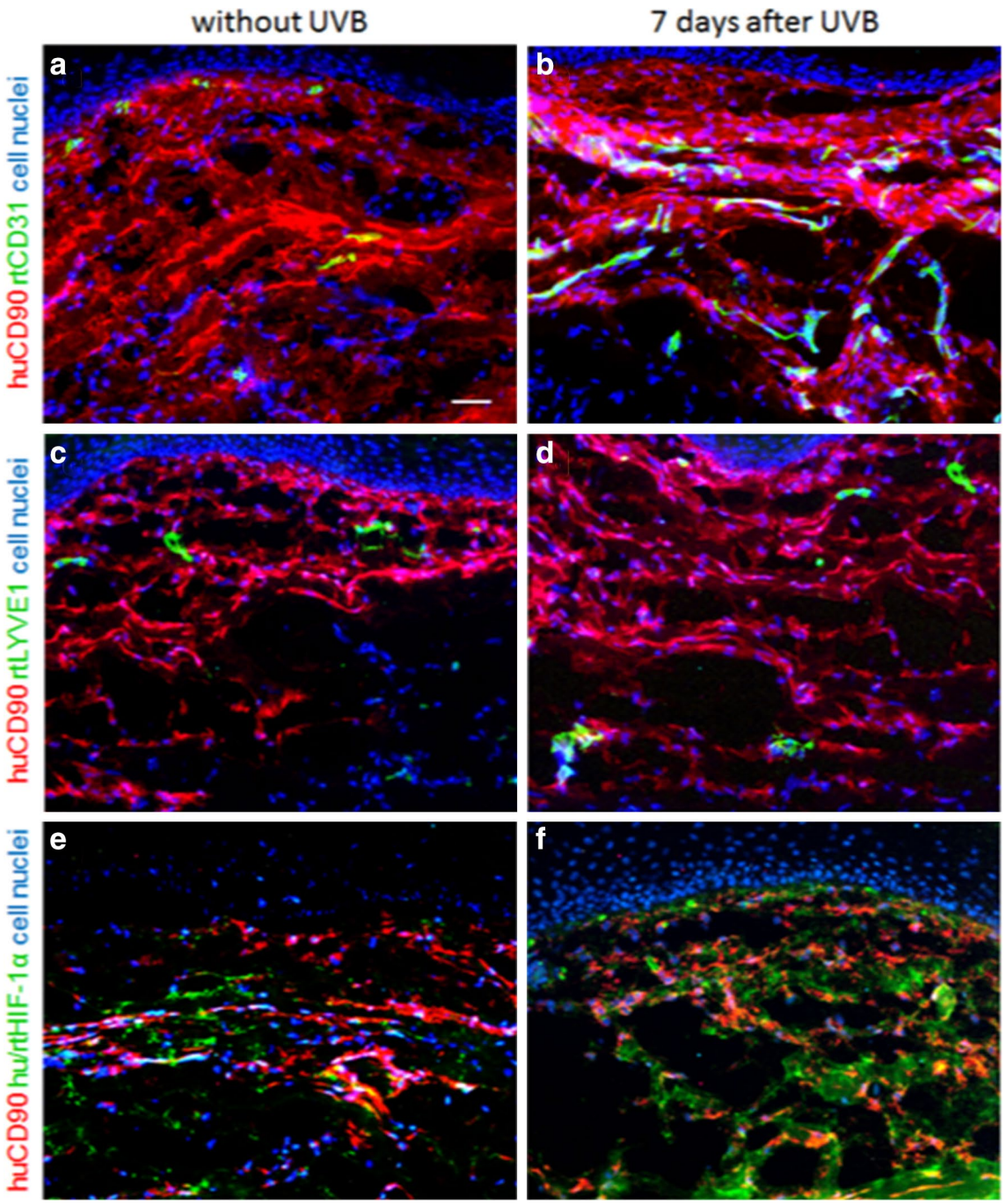

Fig. 1 Expression of blood- and lymphatic-specific markers in unexposed pigmented dermo-epidermal skin substitutes (pigmDESS) and 7 days after UVB radiation in vivo. **a, b** Immunofluorescence double staining of pigmDESS representing the ingrowth of rat blood capillaries marked with an antibody against rat CD31 (green) into human dermal compartment highlighted by human CD90 (red). **c, d** Immunofluorescence counterstaining with anti-rat LYVE-1 showing lymphatic endothelium (green) and anti-human CD90 (red) markers. Note that UVB-irradiated pigmDESS show a markedly higher number of CD31-positive blood vessels in the human dermal compartments. **e, f** Cells expressing HIF-1-α (green) are counterstained with human CD90 (red). The number of HIF-1-α positive cells is significantly higher in the human neodermis of UVB-treated pigmDESS. Cell nuclei are stained with Hoechst (blue). Scale bars 50 µm

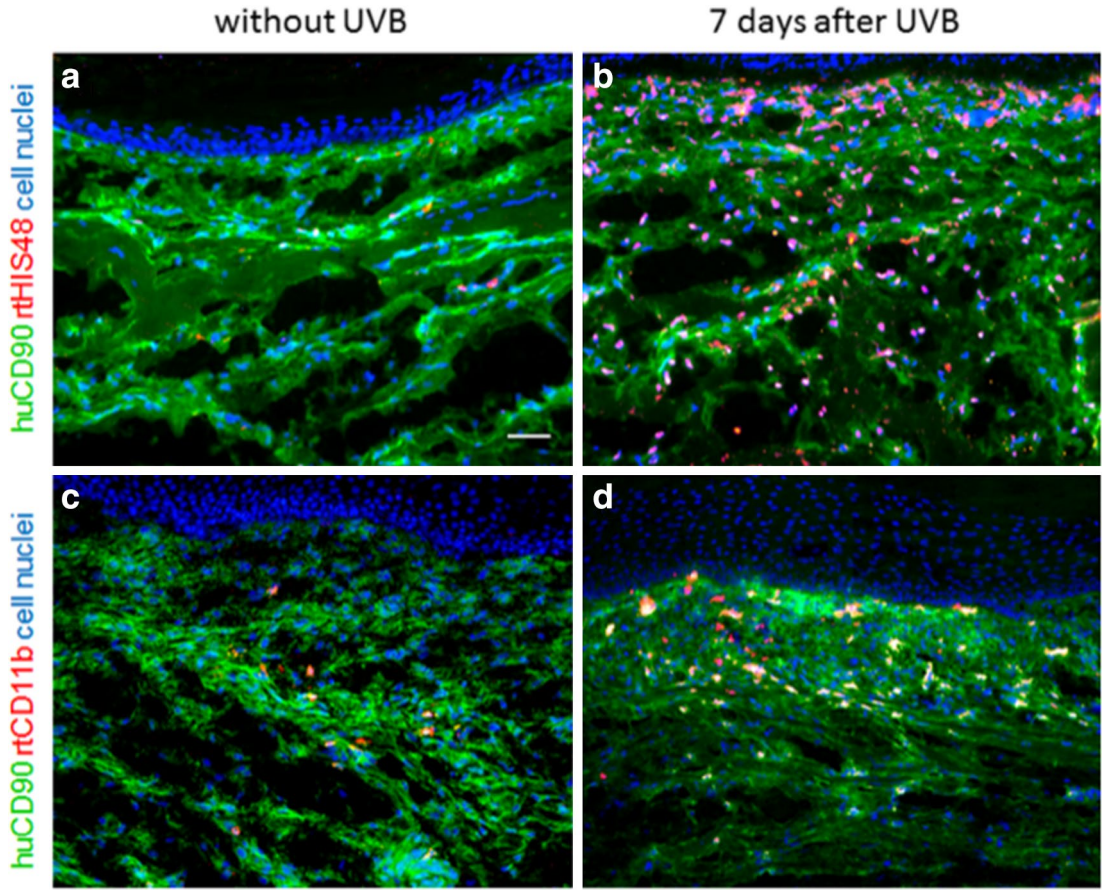

Fig. 2 Infiltration of rat granulocytes and monocytes/macrophages into unexposed pigmDESS and 7 days after UVB exposure in vivo. **a, b** Immunofluorescence co-staining of pigmDESS for rat granulocyte marker HIS48 (green) and for human neodermis visualized by human CD90 (red). **c, d** Distribution of rat monocytes/macrophages stained with CD11b (green) in the human dermal compartments (CD90). Note a significantly enhanced density of HIS48- and CD11b-positive cells in the UVB-exposed dermal compartments. Cell nuclei are stained with Hoechst (blue). Scale bars 50 µm

Expression of HIF-1α and TNFα in transplanted human pigmDESS

As hypoxia may occur in UVB-exposed tissue, we analyzed the expression of HIF-1α in pigmented skin analogs after transplantation (Figs. 1e, f, 3b). Quantitative evaluation revealed the highest level ($2 \times 10^2 \pm 0.006$ mm^2) of HIF-1-alpha expression 7 days after UVB treatment, whereas it was low ($5 \times 10^2 \pm 0.012$ mm^2) in the untreated controls (Fig. 3b) ($p = 0.0016$, statistically very significant). HIF-1-alpha was expressed in the dermal compartment delineated by human CD90-staining. Furthermore, we did not detect any significant difference in the expression of TNFα in transplanted human pigmDESS before and after UVB exposure (data not shown).

Discussion

Sun exposure is clearly one of the most important factors causing skin damage and photoaging. Here we have assessed the in vivo effects of acute solar UVB radiation on pigmDESS, a well-established pigmented skin model [3, 5, 10]. Our data indicate that UVB exposure of skin rapidly induces cutaneous angiogenesis and inflammatory responses by increased dermal neutrophil, granulocyte, and monocytes/macrophage infiltration. Furthermore, we detected an enhanced expression of HIF-1α in treated pigmDESS. Several aspects will be highlighted and discussed in more detail.

Angiogenesis, the growth of blood vessels from the existing vasculature, is regulated by positive and negative stimulators [27]. Under normal homeostatic conditions, the

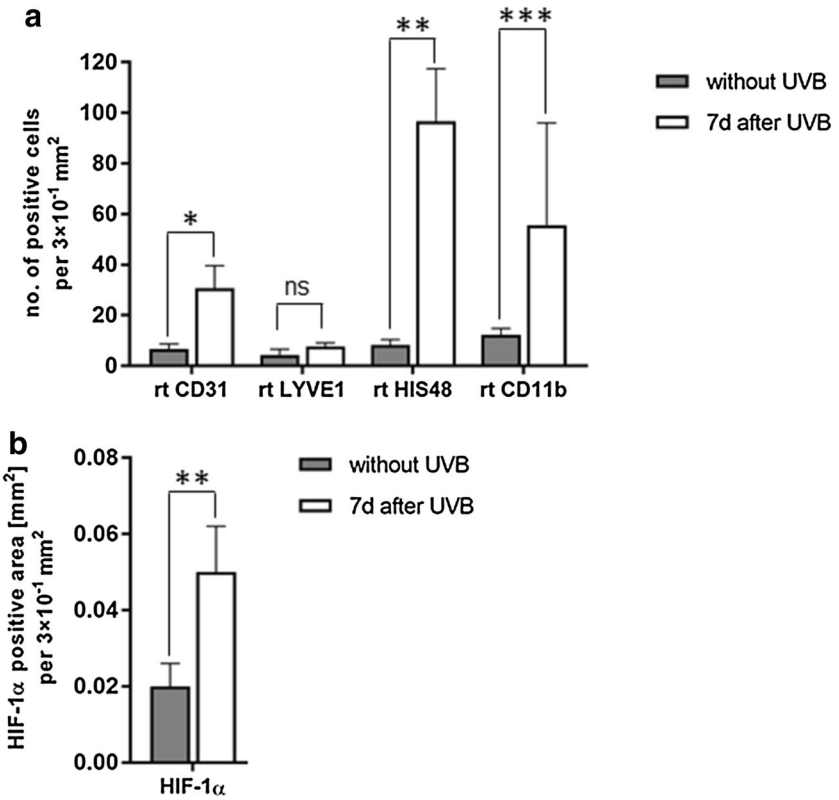

Fig. 3 The quantification of the number of blood and lymphatic vessels as well as immune cell infiltration in transplanted pigmDESS without and 7 days after UVB radiation in vivo. **a** The number of rat CD31-positive blood and LYVE-1-positive lymphatic vessels in human neodermis of pigmDESS without (white bars) and 7 days after UVB exposure (grey bars). The density of granulocytes (HIS48) and monocytes/macrophages (CD11b) in dermal compartments of unexposed pigmDESS (white bars) and 7 days after UVB irradiation (grey bars). **b** Area of HIF-1-α-positive staining in human dermal compartments of pigmDESS without (white bars) and after UVB exposure (grey bars). Note that UVB-irradiated pigmDESS demonstrate a significantly higher number of blood vessels as well as enhanced density of immune cells throughout the CD90-positive neodermis as compared to non-irradiated controls. Graphs show values as the mean (±SD) quantified per 3×10^{-1} mm^2 of skin transplant. $N=5$ for each group

negative regulators are usually dominant [28, 29]. Interestingly, our results demonstrate increased capillary density in the dermis of UVB-treated pigmDESS. Hence, we concluded that UVB induces a rapid pro-angiogenic switch in those transplants. This is in line with previous studies reporting enhanced endothelial cell proliferation in mice after acute UVB irradiation of the skin [30]. Furthermore, Bielenberg et al. found prolonged angiogenesis over at least 4 weeks after a single dose of UVB irradiation of ear skin [30]. Dejana et al. also reported a significant increase of both vessel density and vessel size as well as increased proliferation of endothelial cells after UVB treatment in vivo [31]. Similar findings were detected in chronically photodamaged skin in mice [32]. Moreover, Kawada et al. suggested that the hyperplasia of cutaneous blood vessels is directly associated with skin wrinkle formation in acute UVB-irradiated mouse skin [33].

Our data imply that pronounced vascular upregulation may be mediated by a high level of HIF-1α detected in the dermis of UVB-treated transplants. It is known that angiogenesis is activated under conditions such as hypoxia (oxygen saturation level lower than 90%), UVB irradiation, or upon activation of signaling pathways by growth factors such as fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs). Under hypoxia, HIF-1α is stabilized, interacts with hypoxia-responsive elements (HREs) in the nucleus, and finally promotes the activation of pro-angiogenic genes such as nitric oxide synthases, VEGF, and genes regulating extracellular matrix metabolism (urokinase-type plasminogen activator receptor; uPAR) [34, 35]. Of note, the study of Kawada et al. showed that exposure to hyperoxia (90% oxygen level) immediately after each UVB irradiation attenuated increased HIF-1α expression and angiogenesis, that in turn, reduced wrinkle formation in mouse skin [33]. These findings confirm that hypoxia might be indeed a direct inducer of UVB-induced skin damage.

Nevertheless, the specific regulation of skin angiogenesis still remains not completely understood. Some authors suggested that UVB radiation induced cutaneous angiogenesis in mice may be associated with an imbalance between

positive and negative angiogenic growth factors [30]. Bielenberg et al. showed that a single exposure to UVB radiation (15 kJ/m^2) first increased the expression of bFGF and then decreased the expression of IFN-β [30]. These events were associated with epidermal hyperplasia and enhanced angiogenesis in the underlying skin. This, in turn, implies that not only dermal cells but also epidermal cells might stimulate cutaneous neovascularization [36]. As epidermis is avascular, keratinocytes are dependent on the dermal blood supply to deliver sufficient nutrients and oxygen, in particular during wound healing or hyperproliferative disorders such as psoriasis [37].

In contrast, acute UVB irradiation resulted in almost no changes of lymphatic microvasculature in transplanted pig-mDESS. These findings indicate a specific regulation of the vascular response to acute UVB irradiation, with an initial phase of enhanced blood-angiogenesis, but not in lymph-angiogenesis. This implies that the formation of new lymph vessels is not essential in acute photodamaged skin. It is reasonable to argue that the establishment of a rapid vascular supply within the dermis has the highest priority to ensure an adequate nourishment of UVB-damaged tissue and cells, e.g., keratinocytes. Similarly, we previously observed that during wound healing in human skin substitutes in vivo, the ingrowth of host lymphatic vessels also appeared later than the one of blood vessel networks [5]. This may be also caused by severely impaired lymphatic regeneration across scar (fibrotic) tissues due to reduced lymphatic endothelial cell proliferation and abnormal lymphatic microarchitecture [38, 39]. Unfortunately previous studies on photodamaged skin focused all only on angio- and not on lymph-angiogenesis [30, 31].

To the best of our knowledge, this appears to be the first study demonstrating the effects of acute UVB doses on both angio- and lymphangiogenesis in pigmented human tissue-engineered dermo-epidermal skin analogs in a preclinical rat model. Of note, our data show a near physiological pattern of blood and lymphatic vessel ingrowth, which is regulated by a fine-tuning of the expression of specific pro-angiogenic factors such as HIF-1α. It appears likely that a similar vascularization dynamics take place when autologous tissue-engineered skin analogs are transplanted onto human patients.

Acknowledgements This work was financially supported by the Clinical Research Priority Programs (CRPP) of the Faculty of Medicine of the University of Zurich. We are particularly grateful to the Gaydoul Foundation and the sponsors of "DonaTissue" (Thérèse Meier and Robert Zingg) for their generous financial support and interest in our work.

Compliance with ethical standards

Conflict of interest ER and MM are co-founding members and shareholders of "Cutiss AG", a company to fund the further development of the tissue-engineered skin substitutes. All other authors declare that they have no conflict of interest.

References

1. Braziulis E, Biedermann T, Hartmann-Fritsch F, Schiestl C, Pontiggia L, Bottcher-Haberzeth S et al (2011) Skingineering I: engineering porcine dermo-epidermal skin analogues for autologous transplantation in a large animal model. Pediatr Surg Int 27:241–247
2. Bottcher-Haberzeth S, Biedermann T, Pontiggia L, Braziulis E, Schiestl C, Hendriks B et al (2013) Human eccrine sweat gland cells turn into melanin-uptaking keratinocytes in dermo-epidermal skin substitutes. J Investig Dermatol 133:316–324
3. Bottcher-Haberzeth S, Klar AS, Biedermann T, Schiestl C, Meuli-Simmen C, Reichmann E et al (2013) "Trooping the color": restoring the original donor skin color by addition of melanocytes to bioengineered skin analogs. Pediatr Surg Int 29:239–247
4. Schiestl C, Biedermann T, Braziulis E, Hartmann-Fritsch F, Bottcher-Haberzeth S, Arras M et al (2011) Skingineering II: transplantation of large-scale laboratory-grown skin analogues in a new pig model. Pediatr Surg Int 27:249–254
5. Klar AS, Bottcher-Haberzeth S, Biedermann T, Schiestl C, Reichmann E, Meuli M (2014) Analysis of blood and lymph vascularization patterns in tissue-engineered human dermo-epidermal skin analogs of different pigmentation. Pediatr Surg Int 30:223–231
6. Klar AS, Guven S, Biedermann T, Luginbuhl J, Bottcher-Haberzeth S, Meuli-Simmen C et al (2014) Tissue-engineered dermo-epidermal skin grafts prevascularized with adipose-derived cells. Biomaterials 35:5065–5078
7. Marino D, Luginbuhl J, Scola S, Meuli M, Reichmann E (2014) Bioengineering dermo-epidermal skin grafts with blood and lymphatic capillaries. Sci Transl Med 6:221ra14
8. Bottcher-Haberzeth S, Biedermann T, Klar AS, Widmer DS, Neuhaus K, Schiestl C et al (2015) Characterization of pigmented dermo-epidermal skin substitutes in a long-term in vivo assay. Exp Dermatol 24:16–21
9. Biedermann T, Klar AS, Bottcher-Haberzeth S, Michalczyk T, Schiestl C, Reichmann E et al (2015) Long-term expression pattern of melanocyte markers in light- and dark-pigmented dermo-epidermal cultured human skin substitutes. Pediatr Surg Int 31:69–76
10. Klar AS, Biedermann T, Michalak K, Michalczyk T, Meuli-Simmen C, Scherberich A et al (2017) Human adipose mesenchymal cells inhibit melanocyte differentiation and the pigmentation of human skin via increased expression of TGF-beta1. J Investig Dermatol 137:2560–2569
11. Michalczyk T, Biedermann T, Bottcher-Haberzeth S, Klar AS, Meuli M, Reichmann E (2018) UVB exposure of a humanized skin model reveals unexpected dynamic of keratinocyte proliferation and Wnt inhibitor balancing. J Tissue Eng Regen Med 12:505–515
12. Gilchrest BA (2013) Photoaging. J Investig Dermatol 133:E2–E6
13. Sanches Silveira JE, Myaki Pedroso DM (2014) UV light and skin aging. Rev Environ Health 29:243–254
14. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ (1997) Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med 337:1419–1428
15. Debacq-Chainiaux F, Leduc C, Verbeke A, Toussaint O (2012) UV, stress and aging. Dermato-endocrinology 4:236–240
16. Bruls WAG, Vanweelden H, Vanderleun JC (1984) Transmission of UV-radiation through human epidermal layers as a factor influencing the minimal erythema dose. Photochem Photobiol 39:63–67

17. Dai G, Freudenberger T, Zipper P, Melchior A, Grether-Beck S, Rabausch B et al (2007) Chronic ultraviolet B irradiation causes loss of hyaluronic acid from mouse dermis because of down-regulation of hyaluronic acid synthases. Am J Pathol 171:1451–1461
18. Tessem MB, Bathen TF, Cejkova J, Midelfart A (2005) Effect of UV-A and UV-B irradiation on the metabolic profile of aqueous humor in rabbits analyzed by H-1 NMR spectroscopy. Investig Ophthalmol Vis Sci 46:776–781
19. Imayama S, Nakamura K, Takeuchi M, Hori Y, Takema Y, Sakaino Y et al (1994) Ultraviolet-B irradiation deforms the configuration of elastic fibers during the induction of actinic elastosis in rats. J Dermatol Sci 7:32–38
20. Imokawa G, Takema Y, Yorimoto Y, Tsukahara K, Kawai M, Imayama S (1995) Degree of ultraviolet-induced tortuosity of elastic fibers in rat skin is age-dependent. J Investig Dermatol 105:254–258
21. Imokawa G (2009) Mechanism of UVB-induced wrinkling of the skin: paracrine cytokine linkage between keratinocytes and fibroblasts leading to the stimulation of elastase. J Investig Dermatol Symp Proc 14:36–43
22. Pontiggia L, Biedermann T, Meuli M, Widmer D, Bottcher-Haberzeth S, Schiestl C et al (2009) Markers to evaluate the quality and self-renewing potential of engineered human skin substitutes in vitro and after transplantation. J Investig Dermatol 129:480–490
23. Biedermann T, Pontiggia L, Bottcher-Haberzeth S, Tharakan S, Braziulis E, Schiestl C et al (2010) Human eccrine sweat gland cells can reconstitute a stratified epidermis. J Investig Dermatol 130:1996–2009
24. Pontiggia L, Klar A, Bottcher-Haberzeth S, Biedermann T, Meuli M, Reichmann E (2013) Optimizing in vitro culture conditions leads to a significantly shorter production time of human dermoepidermal skin substitutes. Pediatr Surg Int 29:249–256
25. Schneider J, Biedermann T, Widmer D, Montano I, Meuli M, Reichmann E et al (2009) Matriderm versus integra: a comparative experimental study. Burns 35:51–57
26. Bottcher-Haberzeth S, Biedermann T, Schiestl C, Hartmann-Fritsch F, Schneider J, Reichmann E et al (2012) Matriderm (R) 1 mm versus Integra (R) single layer 1.3 mm for one-step closure of full thickness skin defects: a comparative experimental study in rats. Pediatr Surg Int 28:171–177
27. Hanahan D, Christofori G, Naik P, Arbeit J (1996) Transgenic mouse models of tumour angiogenesis: the angiogenic switch, its molecular controls, and prospects for preclinical therapeutic models. Eur J Cancer 32:2386–2393
28. Fidler IJ, Ellis LM (1994) The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell 79:185–188
29. Iruela-Arispe ML, Dvorak HF (1997) Angiogenesis: a dynamic balance of stimulators and inhibitors. Thromb Haemost 78:672–677
30. Bielenberg DR, Bucana CD, Sanchez R, Donawho CK, Kripke ML, Fidler IJ (1998) Molecular regulation of UVB-induced cutaneous angiogenesis. J Investig Dermatol 111:864–872
31. Dejana E, Corada M, Lampugnani MG (1995) Endothelial cell-to-cell junctions. FASEB J 9:910–918
32. Yano K, Oura H, Detmar M (2002) Targeted overexpression of the angiogenesis inhibitor thrombospondin-1 in the epidermis of transgenic mice prevents ultraviolet-B-induced angiogenesis and cutaneous photo-damage. J Investig Dermatol 118:800–805
33. Kawada S, Ohtani M, Ishii N (2010) Increased oxygen tension attenuates acute ultraviolet-B-induced skin angiogenesis and wrinkle formation. Am J Physiol Regul Integr Comp Physiol 299:R694–R701
34. Streit M, Velasco P, Riccardi L, Spencer L, Brown LF, Janes L et al (2000) Thrombospondin-1 suppresses wound healing and granulation tissue formation in the skin of transgenic mice. EMBO J 19:3272–3282
35. Weidemann A, Johnson RS (2008) Biology of HIF-1 alpha. Cell Death Differ 15:621–627
36. Malhotra R, Stenn KS, Fernandez LA, Braverman IM (1989) Angiogenic properties of normal and psoriatic skin associate with epidermis, not dermis. Lab Investig J Tech Methods Pathol 61:162–165
37. Detmar M (1996) Molecular regulation of angiogenesis in the skin. J Investig Dermatol 106:207–208
38. Warren AG, Slavin SA (2007) Scar lymphedema: fact or fiction? Ann Plast Surg 59:41–45
39. Avraham T, Clavin NW, Daluvoy SV, Fernandez J, Soares MA, Cordeiro AP et al (2009) Fibrosis is a key inhibitor of lymphatic regeneration. Plast Reconstr Surg 124:438–450