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Characterization of an autologous pigmented human dermo-epidermal skin substitute upon UVB irradiation *in vivo*

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The skin is a vital organ for the human body. Full thickness skin defects in patients suffering from burns, soft tissue trauma, congenital giant nevi or chronic necrotic skin diseases are usually covered by split-thickness or full-thickness skin grafting. There is donor site shortage, when the defect exceeds 50-60% of the total body surface area and hypertrophic scarring and keloid formation is frequently seen. To solve both main problems a human autologous skin graft with a dermal and an epidermal compartment has been developed and is presently applied in clinical phase I studies on first patients. Moreover, a pigmented version (MelSkin) has been engineered and demonstrated successfully restoration of skin homeostasis and original donor skin color in an *in vivo* model.
It was the aim of this study, as a next step, to analyze this pigmented autologous human dermo-epidermal skin substitute (MelSkin) upon UVB irradiation *in vivo*. Therefore, keratinocytes and melanocytes were seeded on fibroblast containing collagen hydrogels, (with all cells from the same donor). The substitutes were transplanted onto full-thickness wounds of immuno-incompetent rats and UVB irradiation was applied *in vivo* when skin transplants showed stable skin homeostasis and color after 4 weeks. Transplants were followed to measure skin color and punch biopsies were taken for immunohistochemically staining, regarding skin morphology, differentiation and proliferation.
Exposed to UVB irradiation, MelSkin showed temporary tanning as well as Keratin 16 induction, indicative for a wound healing response. Moreover, temporary induction of Keratin19/Keratin15 positive basal keratinocytes was observed. In MelSkin tissue homeostasis is characterized, as in foreskin, by few proliferating keratinocytes which are mostly located in the first suprabasal layer, directly adjacent to the basal layer. After UVB exposure proliferation of keratinocytes, but not melanocytes or fibroblasts, massively increased, notably in the basal layer, and normalized 4 weeks after irradiation. In accordance, the expression of the Wnt signaling pathway inhibitors Dickkopf 3 and Wnt inhibitory factor 1 present in the basal keratinocytes upon tissue homeostasis, was lost after UVB irradiation, in particular in proliferating keratinocytes. Importantly and contrasting the keratinocytes, melanocytes continued to express Wnt inhibitors after UVB treatment, correlating well with their preserved non-proliferative state.
Taken together characterization of MelSkin upon UVB exposure *in vivo* considered various aspects that are relevant for a clinical application.