Identification of dermal components influencing UV-induced DNA damage repair in epidermal cells

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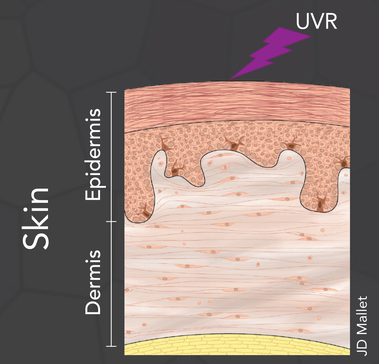
Introduction



Human skin contains two main cellular layers, i.e. the epidermis and the dermis. Epidermis mainly comprises keratinocytes whereas dermis consists of extracellular matrix with dispersed fibroblasts.

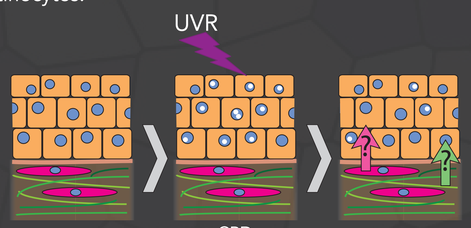
The exposure of skin to solar ultraviolet radiations (UVR) leads to the generation of cyclobutane pyrimidine dimers (CPD), a highly mutagenic DNA damage responsible for skin cancer driver mutations in the epidermis. An efficient CPD repair is one major way to avoid the conversion of CPD into mutations.

It has been previously shown that the three-dimensional environment of the skin plays a role in promoting epidermal CPD repair (1). Surprisingly, very little is known about the mechanisms and the components involved in the potential dermal-epidermal crosstalk that modulates UV-induced DNA damage repair in keratinocytes.

Objectives

Evaluate the impact of dermal components on epidermal CPD repair in tissue-engineered skin models.

Investigate the factors responsible for the dermal-epidermal crosstalk modulating UV-induced DNA damage repair in keratinocytes.



Methods and Results

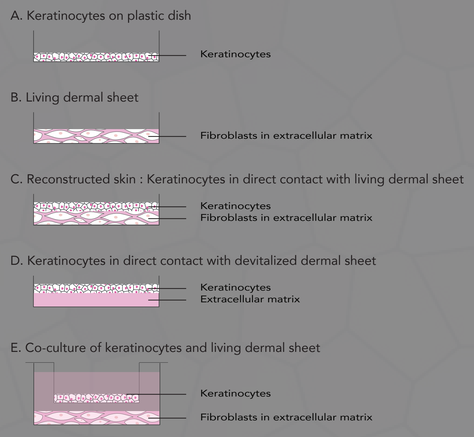


Figure 1 – Schematic representation of the tissue-engineered skin models

Tissue-engineered skin models are produced exclusively from human diploid fibroblasts and keratinocytes. Keratinocytes were seeded and grew during 10 days directly on culture dish plastic, on a living dermal sheet, on a devitalized matrix or in co-culture in a Transwell cell culture plate above a dermal sheet. Living dermal sheets are formed by cultivating fibroblasts for 35 days in the presence of ascorbic acid to bring them to secrete and assemble their own extracellular matrix (2). Devitalized dermal matrix is obtained by incubating and rinsing dermal sheet with deionized sterile water (3).

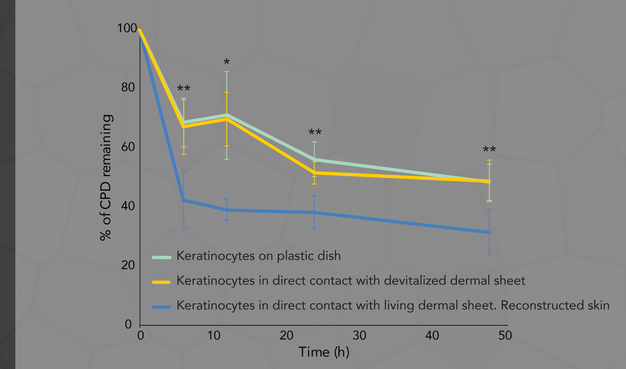


Figure 2 – Effect of different keratinocytes culture supports on UV-induced CPD repair

Keratinocytes seeded on living dermal sheet, on devitalized dermal sheet or on plastic culture dish were exposed to 400 J/m² of UVB. Keratinocytes were then harvested at different time points post-irradiation. CPD were quantified by ELISA using an anti-CPD antibody to derive the repair kinetic. Experiments were performed using 4 cellular populations (N=4) at least in duplicate (n=2). Student's t-test (* p<0.05; ** p<0.01).

CPD repair rate is similar between keratinocytes grown on devitalized dermal sheet and on culture dish.

CPD repair is significantly more efficient in keratinocytes seeded on living reconstructed dermis than on culture dish.

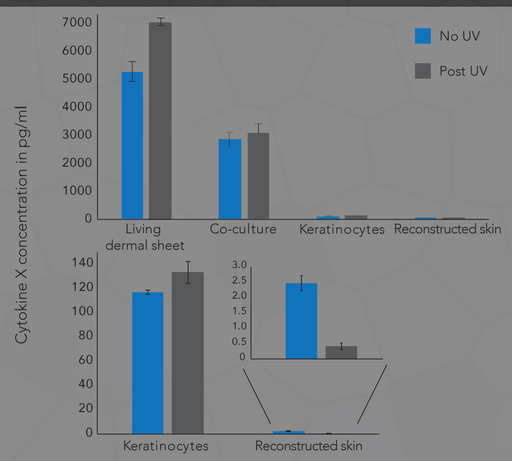


Figure 3 – Quantification of cytokine X secretion

Cytokine X secretion by a living dermal sheet, by co-cultured keratinocytes and living dermal sheet, by keratinocytes and by reconstructed skin were quantified by ELISA. Culture media were obtained from the different conditions before and 3h after 400 J/m² UVB. N=4, n=3

Cytokine X is highly secreted by fibroblasts and co-cultured cells (Before UVB irradiation 5265 and 2828 pg/ml respectively), but poorly by keratinocytes (117 pg/ml) and even less by the complete reconstructed skin (2.5 pg/ml).

3h following UVB radiation, in the reconstructed skin where the CPD repair is the faster, the cytokine X secretion in the medium is almost absent (0.4 pg/ml).

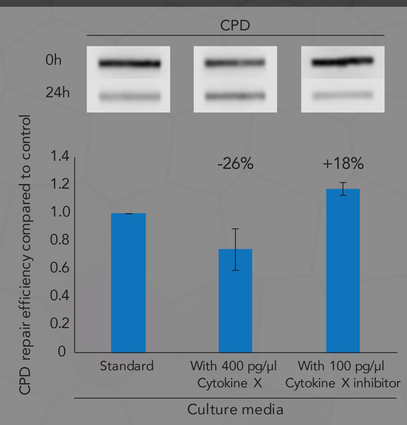


Figure 4 – Effect of cytokine X on UV-induced CPD repair efficiency in human diploid keratinocytes

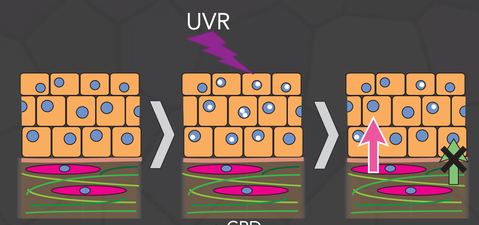
Keratinocytes were cultured with standard culture media, or with culture media supplemented with 400 pg/µl of cytokine X, or with 100 pg/ul of neutralizing antibody against cytokine X for 12h prior to irradiation with 400 J/m² UVB. 0h and 24h post irradiation, DNA was extracted and CPD were quantified by dot-blot to determine the repair efficiency. N=3, n= 2

Adding cytokine X in the culture media of keratinocytes leads to a decrease of CPD repair by 26% at 24h. On the contrary, with media supplemented with cytokine X neutralization antibody, CPD removal is 18% faster at 24h.

This indicates that the cytokine X inhibits UV-induced CPD repair in human keratinocytes.

Conclusions

- UV-induced CPD repair in keratinocytes is enhanced by the direct contact with a living dermal sheet.
- The effect of the dermal sheet on epidermal UV-induced CPD repair does not come from the extracellular matrix.
- The positive impact of the dermis on epidermal DNA repair seems to be driven by secreted molecules.



- A secreted molecule, here called cytokine X for confidentiality purpose, is greatly reduced in reconstructed skin and is further lowered following UV radiation.
- Cytokine X is an inhibitor of CPD repair in keratinocytes. Indeed, the presence of cytokine X leads to a reduction in epidermal CPD repair. This is further confirmed by the fact that an inhibition of cytokine X by neutralizing antibody leads to an increase of CPD repair efficiency in keratinocytes.

References

1. Fernandez et al., Photochem Photobiol, 2014
2. Michel et al., In VitroCell.Dev.Biol, 1999
3. Bourget et al., Biomaterials, 2012

Acknowledgements

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