Measurement of lactate dehydrogenase release on 3D reconstructed human epidermis: A rapid and cost-effective way to evaluate skin irritation

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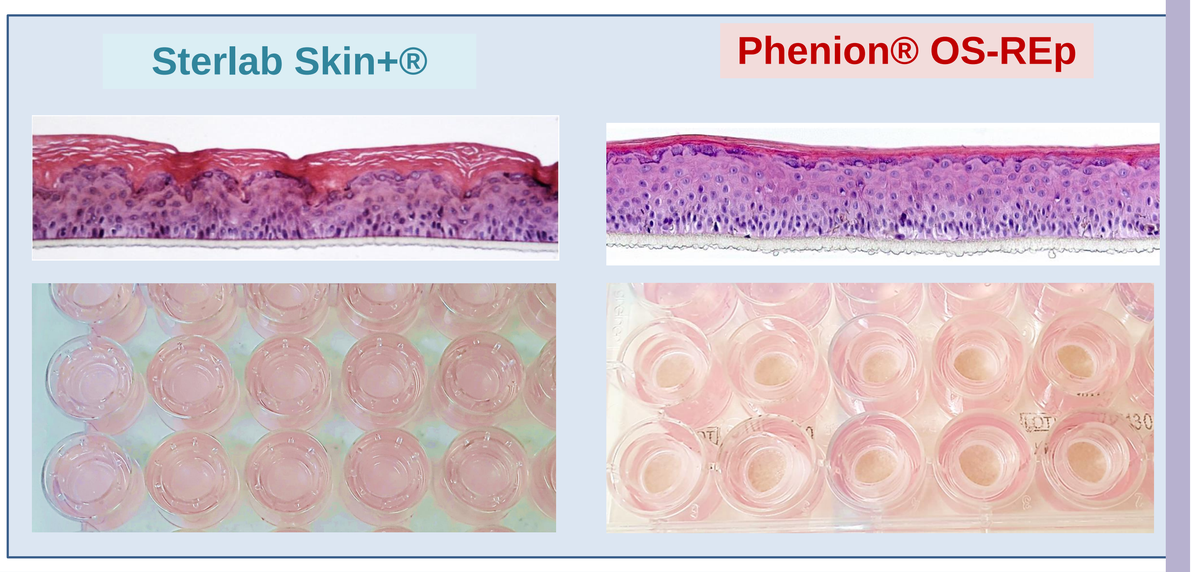
BACKGROUND

3D reconstructed human epidermis models are widely used in skin irritation safety testing of chemicals including personal care, cosmetic and pharmaceutical ingredients. *In vitro* skin irritation test based on reconstructed human epidermis (RHE) was adopted in the OECD test guideline 439. In this irritation test, RHE tissues are topically exposed to the test chemicals for a short period of time followed by a long post-incubation period. Viability of each tissue is then measured by the MTT assay. One of the drawbacks of the MTT assay lies in the fact that this assay is destructive and the RHE tissues cannot be further used to measure any other endpoints of interest, e.g. efficacy markers… Lactate dehydrogenase (LDH) leakage is a marker of plasma membrane integrity and cell viability. The aim of this work was therefore to evaluate if measurement of LDH release in culture medium could be a surrogate to MTT viability assay.

METHODS

Two different RHE models in 24-well plates were used: Skin+® Reconstructed Human Epidermis (0.5 cm²) from STERLAB, France, and Phenion® OS-REp 3D Reconstructed Human Epidermis (0.6 cm²) from Henkel, Germany. RHE tissues were exposed to 5% SDS or to DPBS (negative control) for 35 minutes. After an extensive washing, tissues were incubated for 42 hours at 37°C, 5% CO_2 and saturated humidity. Each treatment condition was performed in triplicates. Viability of each tissue was evaluated using Promega CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT viability assay). Release of LDH in culture medium was evaluated using Promega LDH-Glo™ Cytotoxicity Assay. Reduction of MTT was measured at 560 nm and LDH release was measured by luminescence using GloMax® Explorer plate reader (Promega France).



RESULTS

Figure 1: MTT reduction measured at 560 nm and release of LDH in culture medium in 3D reconstructed human epidermis. Data represent Mean + SD (N=3)

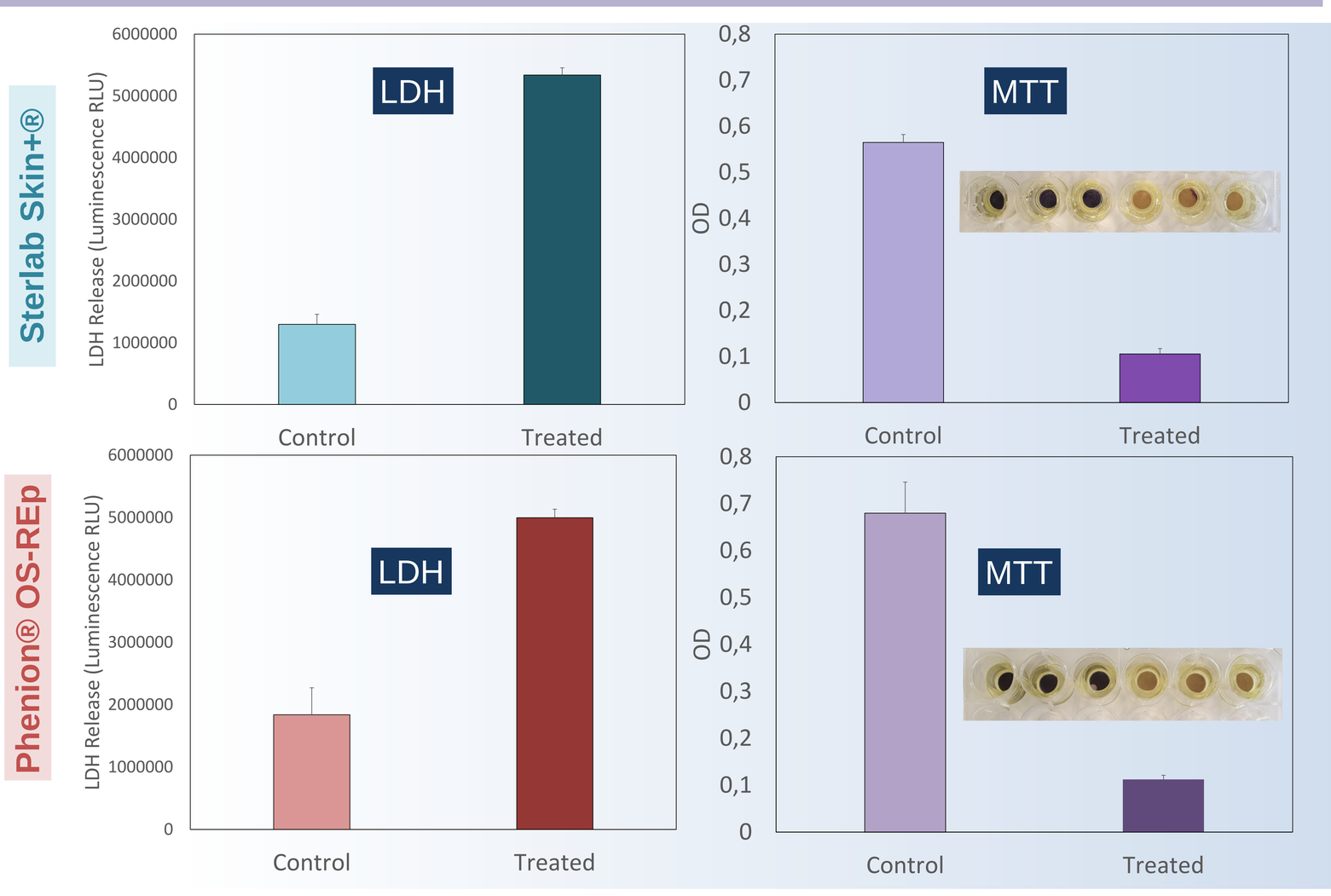
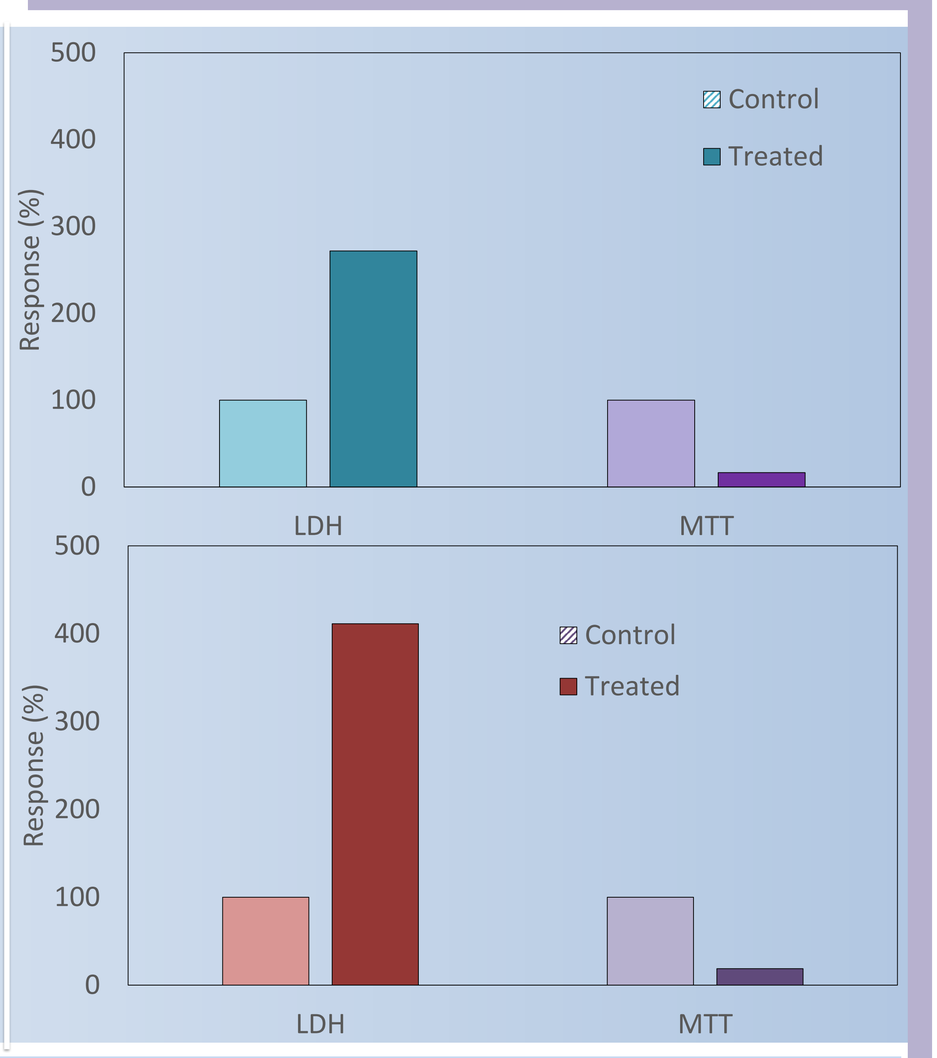


Figure 2: Correlation between LDH release and MTT reduction



In both Sterlab Skin+® and Phenion® OS-Rep models, exposure of RHE tissues to 5% SDS induced a decrease in cell viability by more than 80% as measured by the MTT reduction assay, and an increase of LDH release by more than 2.5 times indicating a decrease in cell viability. These results clearly show a good correlation between MTT viability assay and LDH release assay on RHE. However, for a more accurate correlation between both assays, it is important to measure total LDH content (100% lysis) in RHE tissues. On the other hand, this study shows that both RHE models are suitable alternative models for the evaluation of skin irritation and prediction of the safety of cosmetic, personal care and pharmaceutical products.

CONCLUSION

Measurement of LDH release in culture medium can be a good surrogate to MTT assay for *in vitro* skin irritation evaluation. The LDH-Glo™ Cytotoxicity Assay can monitors cytotoxicity from the same sample over time and can provides more data per well through multiplexing with other cell-based assays, this makes this assay very useful and cost-effective.

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