The 3D Reconstructed Human Skin Comet Assay: Transferability and Reproducibility Within and Between Laboratories

T Downs1, M Bartel2, V Blatz3, J Brinkmann4, U Engels5, A Fischer6, F Henkel7, S Hoffmann8, S Jeschke9, C Kruh10, M Liebsch11, A Luch12, R Piron13, A Reus14, M Schulz12, S Pfuhler1, K Reisinger15

1Procter & Gamble Co, USA; 2BASF SE, Germany; 3Federal Institute for Risk Assessment, Safety of Consumer Products, Germany; 4Henkel AG & Co KGaA, Germany; 5seh consulting + services, Germany; 6TNO, The Netherlands; 7TNO Triskelion, The Netherlands

Introduction

- Currently used in vitro cytogenetic assays show a high rate of false positive results. In order to improve the in vitro prediction, we adapted the Comet assay procedure to 3D skin models that mimic exposure route and potential metabolism of cosmetic ingredients.
- The assay reflects now the first-site-of-contact for dermally applied ingredients and considers the species (human) and organ-specific metabolism of the skin.

The work was funded both by Cosmetics Europe (CE) and the German Ministry of Education and Research (BMBF).

Methods

3D Skin project: 3 Phases of validation

- Phase 1: Optimisation with 3D-cultured keratinocytes
- Phase 2: Intra- and inter-lab reproducibility with 8 coded compounds
- Phase 3: Validation with 3D-cultured fibroblasts

Selection of compounds

- The validation included testing of 3D chemicals, selected by external experts (Raffaella Cini, ECAMM, and David Kirkland, consultant), in an incomplete block design.
- The coded test chemicals are equally balanced i.e. 15 with an expected negative outcome and 15 with an expected positive outcome. The negatives include true negatives and chemicals that generally yield false positive results in the in vitro genotoxicity battery.
- The coding and shipment was performed by the BfR.

Results

Aphidicolin (APC) protocol

- Results of phase 1 showed that the standard Comet protocol was generally not robust enough for detecting pro-mutagens. A proof-of-concept study showed that APC, a DNA-polymerase inhibiter, could increase the sensitivity of the assay without compromising its high specificity. Negative and equivocal findings are now confirmed by additional APC experiments before finalizing its classification. An example with 7,12-dimethylbenz[a]anthracene (DMBA) is shown. The % tail DNA in solvent controls (acetone) was not increased by APC, confirming that it generally does not increase the background DNA damage.

- Comparison of the % tail DNA in keratinocytes from Phenion® FT skin models treated with DMBA in the absence or presence of APC (5 µg/ml). SC: solvent control; B0P = 12.5 µg/cm² benzopyrene (positive control for APC).

Conclusions

- Data support use of the Phenion® FT 3D skin model in the Comet assay since the predictivity for 8 coded chemicals tested across 5 labs was 90%. When APC was included in the protocol, predictivity of the assay was improved since it enabled more efficient detection of pro-mutagens like BaP and DMBA.
- MMC, a DNA cross-linker, caused borderline, but significant increases in the standard and APC protocols. A cross-linker specific protocol clearly confirmed MMC cross-linking activity.
- Phase 3 testing (data base development) is continuing with the Phenion® FT model to obtain a complete data set for 3D chemicals.
- Once validated, the 3D Skin Comet assay is envisioned to be used as a follow-up test for positive results from the current in vivo genotoxicity test battery 2.
- These results support the use of 3D reconstructed skin models as a direct replacement for animal testing of dermally exposed chemicals.