

The Comet Assay using Full-Thickness Skin Models

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Introduction

- The skin is the first site of contact facing maximum exposure for environmental stimuli and a wide range of industrial products.
- However, *in vitro* genotoxicity test methods show low predictivity especially if combined to test batteries (Kirkland *et al.*, 2005; 2006).
- Organ- and species-specific characteristics like xenobiotic metabolism are not adequately represented so far.
- Recently, a micronucleus test (MNT) was adapted to an epidermal skin model (Epiderm™, MatTek, Ashland, MA) (Aardema *et al.*, 2010).
- To address complementary DNA-damages as compared to the MNT the Comet assay was transferred to two full-thickness skin models in this project.
- The aim of the project is to supplement existing *in vitro* test batteries with biological more relevant test systems with regard to dermal application.

Protocol

Epiderm™ Full Thickness Model
(MatTek, Ashland, MA)



Separation of compartments using forceps

PBS, EDTA, Trypsin



Separation of compartments after enzyme treatment

Mincing



Single Cell Suspension

0.5% LMA
At least 2 slides/tissue

Lysis overnight

Unwinding 20min pH 13

Electrophoresis
Keratinocytes 30min
Fibroblasts 45min

Neutralization
Ethanol
Slides air-dried

Phenion® Full-Thickness Skin Model
(Henkel, Germany)

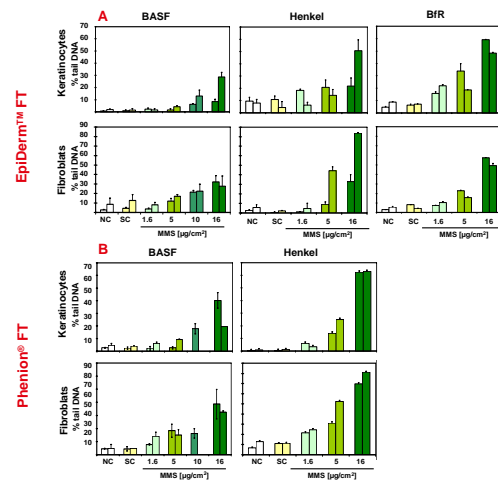
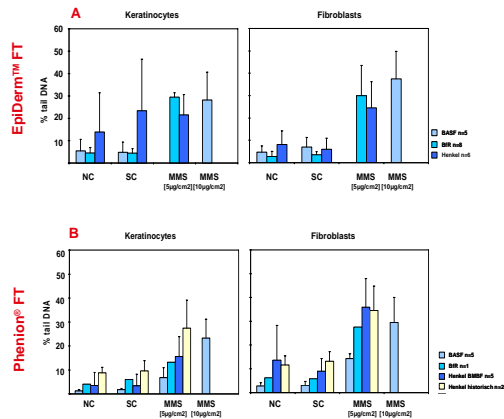
Experimental Design

- Three tissues per dose group were used.
- The experiments contained a negative and solvent (acetone) control as well as 3 concentrations of the test chemical.
- MMS (16µl/cm²) was applied on top of each model for 3h.
- Intracellular ATP concentration was used as cytotoxicity measurement.

Analysis

- Slides were stained with SYBR Gold and analyzed regarding % tail intensity.
- Two slides per tissues and 50 comets/slide were investigated.
- Perceptive Instruments was used at BASF and Henkel; MetaSystems at the BfR.
- Comets values were summarized as medians, and finally given as mean within a group of tissues.

Results



Conclusion and Outlook

- Epiderm™FT and Phenion®FT are well suited as test systems for the Comet Assay:
 - With both full thickness skin models low values in the negative and solvent controls could be obtained in all three participating laboratories.
 - A clear dose-dependent increase in %tail DNA was observed after the topical application of the direct acting mutagen MMS.
- In a second funding period a protocol for testing pro-mutagens will be established.
- Finally, approx. 30 substances are planned to be tested under double-blind condition to prevalidate the test system.

