Usefulness of Reconstructed Skin Micronucleus (RSMN) Assay Beyond Testing of Cosmetics and Dermally Applied Chemicals: A Proof of Principle Study

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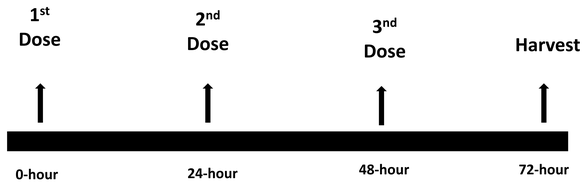
Abstract

The *in vitro* human reconstructed skin micronucleus (RSMN) assay in EpiDerm™ is a promising, novel, animal alternative for evaluating genotoxicity that is highly relevant to the development and implementation of New Approach Methodologies (NAMs). The assay was primarily developed for the safety assessment of dermally applied chemicals and cosmetic ingredients and is now routinely used as a follow up approach for positive results observed in the standard in vitro micronucleus assay. Skin is the largest organ, and basal cells are constantly exposed to chemicals present in the systemic circulation. We evaluated the usefulness of the EpiDerm™ model by adding the test substance to culture medium to mimic the exposure of basal cells in vivo. Three reference test substances with different modes of action were evaluated by topical application as well as direct addition to the culture medium. Mitomycin C (direct acting Clastogen), Cyclophosphamide (metabolically dependent Clastogen), and Carbendazim (Aneugen) were dissolved in acetone and tested by both routes of administration. Three tissues per dose level were used. Cytotoxicity was evaluated using cytokinesis blocked proliferation index (CBPI) and relative viable cell count (RVCC) methods. Significant induction of micronucleus was observed in both routes of application. Exposure of the test substance via culture media was overall more sensitive compared to the topical application. The results indicated the RSMN assay is suitable to use with test substances applied via culture medium, the standard approach used in chemical and pharmaceutical testing.

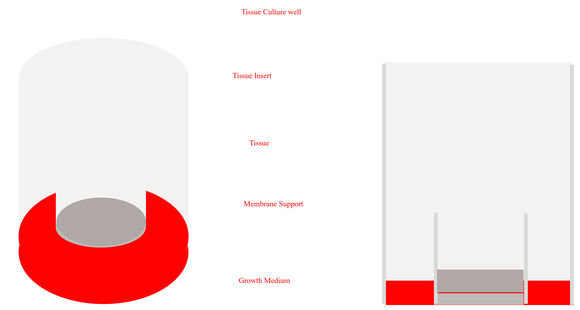
Introduction

Assessment of the genotoxic potential of chemicals presents a challenge specifically, when the standard battery of assays, built upon the conventional two-dimensional (2D) tissue culture system, yields positive results that may be biologically irrelevant. The limitations of traditional *in vitro* testing are driving an increasing need for three-dimensional (3D) tissue culture testing systems as a scientifically relevant alternative to animal testing. The 2D model fundamentally lacks the complexity required to accurately mimic the *in vivo* environment. In response to the global ban on animal testing for cosmetic products, notably by the European Union (EU), the reconstructed human skin model-based micronucleus assay (RSMN-assay) has been rigorously validated. This assay successfully addresses the regulatory demands for hazard assessment of dermally applied chemicals.

Methodology

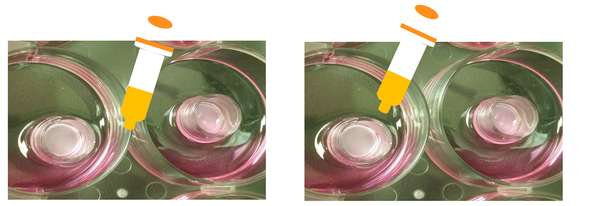


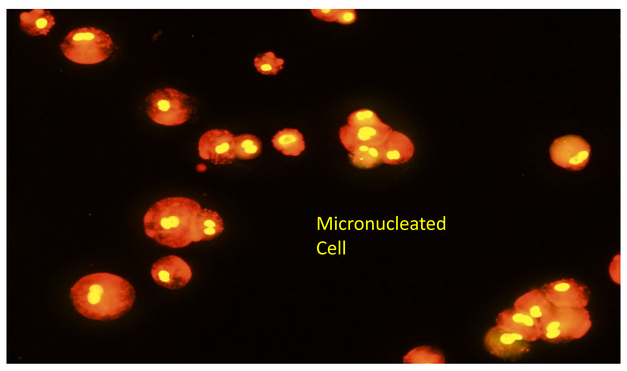
Three tissues per dose in presence of Cytochalasin B (3 µg/mL)
Dahl et al ., Mutation Res. 2011, 720: 42-52.



Dosing into Culture medium

Topical dosing





Results

	% Cytotoxicity (Mean ± SD)[1]	
	CBPI	RVCC
Acetone	0 ± 0.0	0 ± 0.0
MMC-3 µg/mL	52 ± 6	58 ± 3
MMC-5 µg/mL	100 ± 0	100 ± 0
CP - 50 mg/mL	56 ± 14	48 ± 8
CP -60 mg/mL	88 ± 3	76 ± 2
CBZ- 0.25 mg/mL	52 ± 6	58 ± 3
CBZ- 0.50 mg/mL	100 ± 0	98 ± 2

MMC = Mitomycin C; CP = Cyclophosphamide; CBZ = Carbendazim
CBPI = Cytokinesis Blocked Proliferation Index
RVCC = Relative Viable Cell Count
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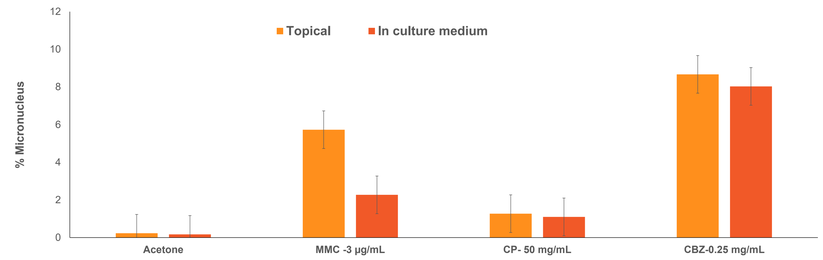

Figure 1: Values represented in the graph is based on an average of two independent experiments with topical dosing and dosing into the culture medium methods. The three reference test compounds showed statistically significant induction in micronucleus frequency compared to vehicle control.

Summary and Conclusion

- Among the two cytotoxicity markers evaluated concurrently, the CBPI was slightly more sensitive than the RVCC for clastogenic compounds (MMC and CP) while for aneugenic test compound (CBZ), the RVCC was more sensitive, irrespective of the methos of dosing used.
- In the topical method of dosing that is routinely used for the genotoxicity evaluation in RSMN assay, the three independent experiments with reference test compounds (MMC, CP, CBZ) showed a statistically significant induction in the micronucleus frequency. This was in the line of historical control data of the laboratory and to the published literature for this assay.
- Dosing of test compounds directly into the culture medium showed increased cytotoxicity (measured by CBPI and RVCC) for these reference test compounds, although the MN induction was slightly milder compared to the topical dosing method. It is possible that cytotoxicity has some inhibitory influence on the cell cycle since an active cell division/cell cycle has crucial role in the MN formation and therefore sensitivity of the micronucleus assay assay.
- The results also indicated that RSMN assay is suitable to use with test compounds applied into the culture medium, the standard approach used in the genotoxicity testing of chemicals and pharmaceuticals.