

Use of three dimensional skin models for estimation of genotoxic effects in an automated assay

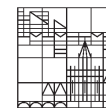


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In vitro risk assessment of cosmetic ingredients already include tests using reconstructed skin models (skin corrosion and irritation). For the assessment of genotoxic effects, another aspect of risk assessment, BioTeSys together with the University of Konstanz is developing an assay for the detection of DNA strand breaks and repair by means of the FADU-assay and EST-1000 skin models.

The automated FADU-assay is validated for use of cell suspensions. The first trials were conducted to adapt the protocol to three-dimensional skin models. Models were treated with known genotoxic substances as well as non toxic reference compounds. After defined treatment cells of the tissue were separated and subsequently DNA-strand breaks were examined by an automated FADU technique.

Method: The tests were performed according to the technique applied by Pfuher S. et al. 2009 with minor variations. EST-1000 skin models (CellSystems, Germany) were treated with three dilutions of methylmethanesulfonate (MMS) diluted in acetone or pur acetone (10µL per tissue, 3h). Subsequently the skin models were treated with phosphate buffered saline for 15 min followed by 15 min Trypsin/EDTA (0.25 % / 1mM). The reaction was stopped by addition of 10% FBS and the cells were pipetted through a cell strainer. The suspensions was centrifuged (5 min; 280 x g) and the resulting pellets were resuspended in cell culture media. The cells were analysed on DNA strand breaks by FADU according to the schematic flow chart below



Figure 1: EST1000 skin model transversal section, HE staining

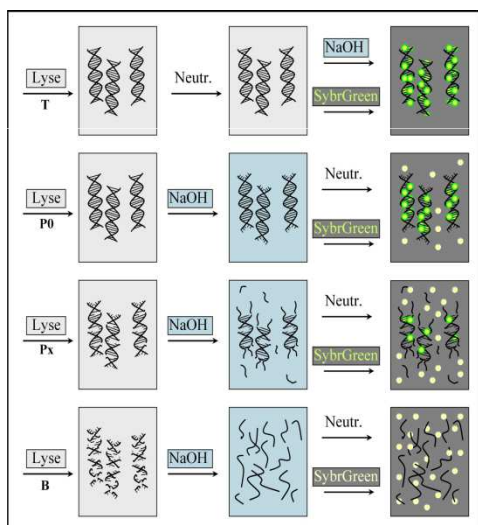


Figure 2: Scheme of FADU protocol

Measure of DNA strand breaks by the FADU assay bases on double strand unwinding under alkaline conditions. After damaging, cells lysed. Strand breaks in the DNA are sites where unwinding starts, this occurs under tightly controlled conditions of pH and temperature. Intact DNA can not be unwound. To stop the unwinding, neutralisation buffer is added. To visualize the double stranded part of DNA, SybrGreen is added to all samples as a dye that only fluoresces upon binding to double stranded DNA (green circles). T, P0 and B are controls. To the T samples neutralisation buffer is added before the unwinding buffer (NaOH), so that the unwinding is blocked. Here, fluorescence signal is highest. Alkaline unwinding in P0 samples starts only at the ends of the chromosomes., basic strand breaks and replication forks conform to physiological conditions. In B samples DNA is completely unwound, so no double stranded DNA is present and no fluorescent signal is observed. Px (P1, P2, P3... Px) are the damaged samples for measuring DNA strand breaks. The whole process, starting from lysis of the cells is performed by a robotised system in order to strictly keep to the time, temperature and pipetting accuracy defined by the protocol. Total runtime on the robotised system = 2 h.

Result: Treatment of skin models with MMS dose dependently increased the number of DNA strand breaks compared to acetone.

Conclusion: The automated FADU technique was able to measure an elevated number of DNA-strand breaks induced by a known genotoxic reference compound compared to the acetone control in this first attempt. Applicability of the three-dimensional skin models to the automated FADU technique in future will offer a method to reproducible and objectively measure the frequency of DNA-strand breaks induced by chemicals in the skin. FADU also is applicable to cells in suspension. However, tests with skin models with or without cell types other than keratinocytes represent a more physiologic answer to potentially genotoxic compounds including repair processes. Possibly offering a more realistic picture of genotoxic potentials.

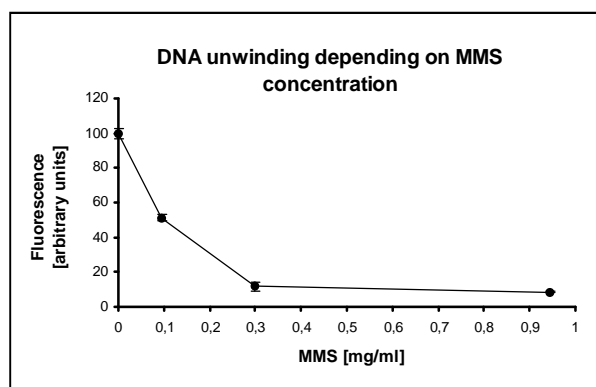


Figure 2: Fluorescence of SybrGreen is reduced by increasing numbers of DNA strand breaks, dose dependently induced by MMS