Automated Genotox-Assay (FADU)
to Quantify Formation and Repair of DNA Strand Breaks

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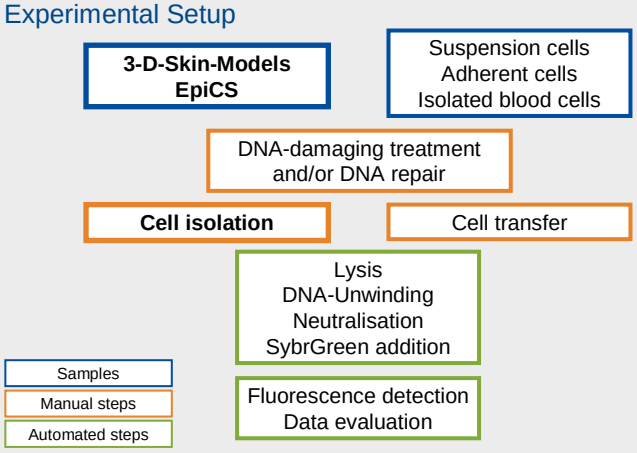
Introduction

The increasing demand of chemical safety assessment calls for alternative methods to reduce animal experimentation. Furthermore registration of pharmaceuticals also requires a comprehensive assessment of their genotoxic potential. Mammalian cell-based alternatives open up new opportunities for fast and reliable tests to screen and identify genotoxic potential of substances and possible modifications of their toxicity profile in substance mixtures. Indicator tests, such as the FADU assay measuring DNA damage and repair, provide additional useful information for initial classification.
Even if no single test can cover all the different mechanisms of genotoxicity, FADU is a valuable part of a test battery.
This assay is able to identify DNA strand breaks and by reasonable experimental design also physiologically based alteration. The formation of DNA strand breaks was tested with different cell samples (e.g. 3-D skin models) and agents.

Experimental Setup



Principle of the Assay

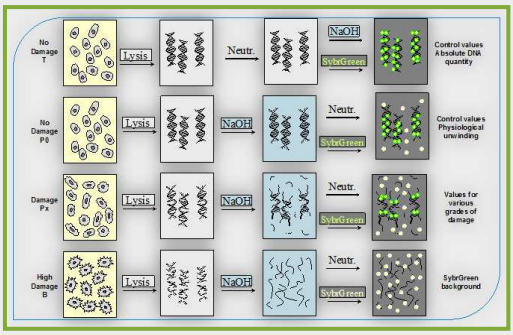
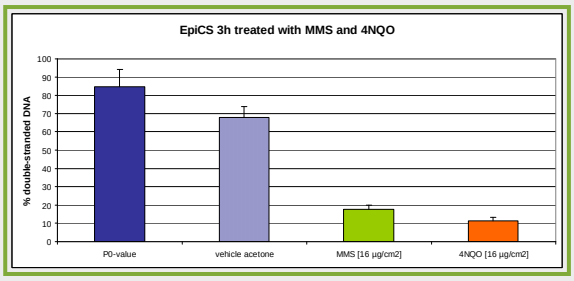


Figure 1: Scheme of the principle of the FADU assay [3]

FADU assay detects DNA strand breaks and repair. The detection is based on progressive DNA unwinding under highly controlled conditions of alkaline pH, time and temperature. DNA 'open sites' are the starting points for the unwinding process. A fluorescence dye is used as marker for the remaining double- stranded DNA. A decrease in the fluorescence intensity indicates a greater number of DNA strand breaks.
Controls are to be run in parallel with experimental treated cells. **T-value**: absolute DNA quantity; **P0-value**: physiological unwinding; B-value: completely unwound = background.

Exemplary Results

Data obtained using the 96-well automated FADU assay is comparable with literature data concerning the comet[4].



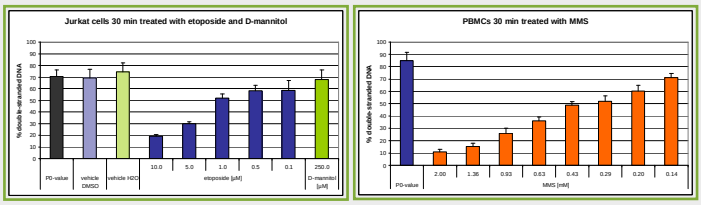


Figure 2: Exemplary results obtained performing the FADU assay

After treatment with methyl methane-sulfonat (MMS), 4-nitroquinoline N-oxide (4NQO) and etoposide the expected DNA damaging was detected, whereas D-mannitol shows no effect on the DNA (negative control)[1, 2].

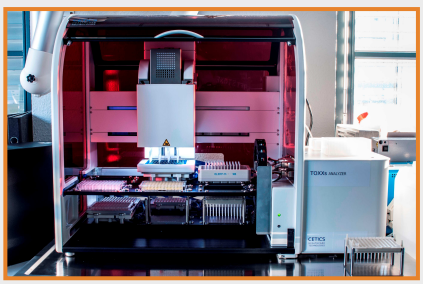


Figure 3: Picture of the TOXX workstation

Conclusion

We presented an automated in vitro method to asses DNA strand breaks and repair. The main advantages of this assay are:

- Automation – high reproducibility, accuracy and robustness
- 96-well format – high throughput
- Easy handling
- Cost saving due to the speed (2 hours versus minimum 12 hours for Comet assay)
- Successfully implemented in a EU-project (NANOSOLUTIONS, FP7)

Using EpiCS for genotoxic tests opens the safety assessment of compounds with the dermal route of exposure and completes the already existing 3-D skin test battery (corrosion, irritation and sensitivity).

References
1. Moreno-Villanueva, M. et al., BMC Biotechnology, 2009, 9:39.
2. Moreno-Villanueva, M., et al., ALTEX, 2011, 28:4.
3. Moreno-Villanueva, M., Bürkle, A., High-Throughput Screening Methods in Toxicity Testing, (Hrsg.: P. Steinberg), John Wiley & Sons, New Jersey, 2013.
4. Reus, A. A., et al., Mutagenesis, 2013, 28:6.