Assessment of nano particle induced DNA strand breaks in vitro by the gTOXXs analyzer

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Background
High energy radiation, like UV light or radiation, as well as chemicals may cause defects to the DNA which possibly will be manifested in mutations. Types of genome damages are numerous. Therefore to foresee genotoxic effects a battery of tests is needed which adequately cover possible endpoints. Over the past years great efforts have been made to establish reliable, reproducible assays detecting DNA strand breaks in vitro. Here a new technology, AUREA gTOXXs is presented by exemplarily showing recent results on the genotoxicity of nanoparticles.

Objectives
• To demonstrate the principle applicability of the AUREA gTOXX solution for detection of genotoxic effects caused by engineered nanomaterials (ENM).
• To uncover possible interference of ENM with the gTOXXs assay.
• To investigate the effect of surface modifications of the ENM on the genotoxicity.

Method
Jurkat cells were supplemented with a variety of ENM (31) obtained from partners of FP7 NANO SOLUTIONS project. Subsequently to range finding experiments (MTT-assay) the cells were treated with applicable concentrations of the ENM for 30 min. The detection of DNA strand breaks by gTOXXs is based on the FADU assay (Fluorimetric Detection of Alkaline DNA Unwinding) and the determination of progressive DNA unwinding under highly controlled conditions of alkaline pH, time and temperature. A fluorescence dye is used to label remaining double-stranded DNA.

Controls were treated in parallel (untreated and etoposide-treated). T-value: absolute DNA quantity (100%); P0-value: physiological unwinding.

Experiments were performed on the AUREA gTOXXs analyzer (3T Analytik).

Experimental set up:

Suspension cells
Jurkat

DNA-damaging treatment:
supplementation with nano particle

Cell transfer
Lysis
DNA-Unwinding
Neutralisation
SybrGreen addition

Fluorescence detection
Data evaluation

Results
• Ag-NH ENM display dose-dependent genotoxic effects below the cytotoxicity concentration level (Fig. 1, 2). This shows the DNA damaging effects of Ag-NH containing ENM.
• Ag-COOH ENM do not display DNA strand breaking activity even at higher, nearly cytotoxic concentrations (Fig. 3, 4). Concentrations higher than 500 μg/mL were not available due to technical reasons.
• The positive control (etoposide) exerts the expected dose dependent genotoxic effects (Fig. 2, 4).

Conclusion
• Surface modifications significantly contribute to the genotoxicity of Ag-ENM.
• Non existent (Ag-COOH) and high level DNA damage (Ag-NH) is reproducibly and reliably detected by AUREA gTOXXs in less than 2 hrs.
• The potential genotoxicity of ENM is shown by short exposure time. The gTOXXs solution, however, also permits long treatments and thus the simultaneous analysis of DNA repair activity if wanted.

References

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