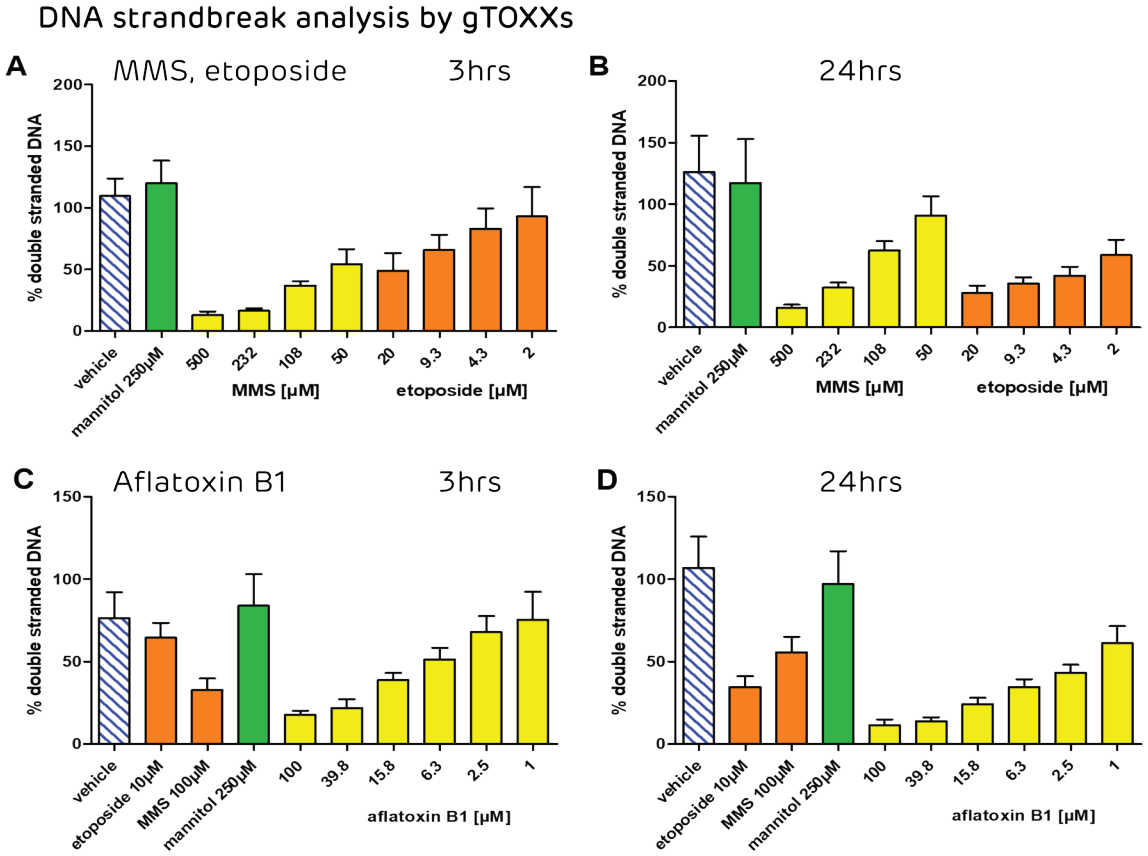
AUREA gTOXXs - Automated Genotox Solution

Selected Application

AUREA gTOXXs, automated DNA strand break analysis, is a complete solution for assessing the genotoxic potential of chemicals, botanicals and nanoparticles. In AUREA gTOXXs, high-end liquid handling is combined with automation robotics and powerful temperature control systems. The endpoint measurement platform of DNA damage enables reliable prediction of gene toxicity likewise in human cell lines, organotypic 3D tissue or human organ cells.

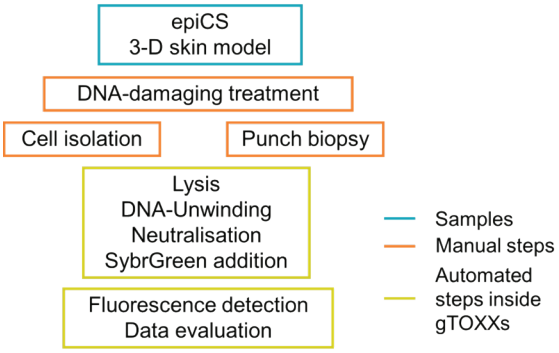
Applicability of HepaRG™ cell line for automated assessment of DNA strand breaks



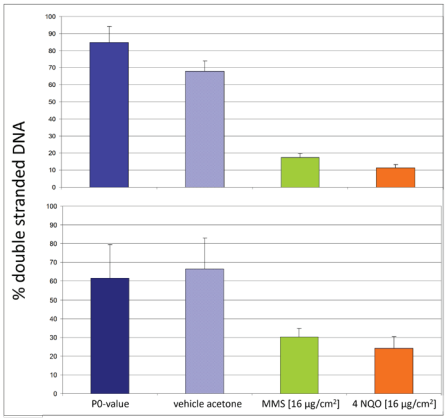
The HepaRG™ cell line from Biopredic International is an excellent model for mapping the metabolic response of human hepatocytes to chemicals like aflatoxin B1 which unfolds the toxic effects only upon metabolic activation. The AUREA gTOXXs solution based on the automated FADU (Fluorimetric Detection of Alkaline DNA Unwinding) assay provides a sensitive and reliable in vitro technique to detect DNA strand breaks. DNA damage is shown to occur dose-dependent as well as time-dependent upon exposure of HepaRG™ to genotoxic agents, etoposide, MMS and aflatoxin B1. The high time resolution of the gTOXXs analysis permits unique insight into the DNA repair capacity of HepaRG™ cells. % DNA strand breaks recovers after 24 hrs exposure to MMS as compared to three hrs exposure. The applicability of the HepaRG™ cell line for gTOXXs based genotoxicity studies of drugs with the need for bio-activation by liver cells is demonstrated.

Organotypic human epidermal 3D skin models and genotoxicity – safety assessment for epidermal applications

Topical treatment of skin model



DNA strand break analysis



❑ **epiCS skin model are eligible to gTOXXs testing**

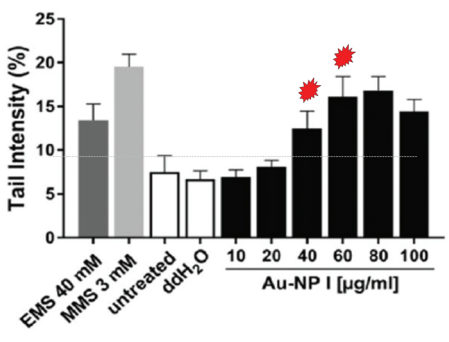
❑ Punch biopsies: an alternative for cell separation procedures

The route of administration of how substances are introduced or adsorbed into the body is critical for their cellular impact. Drugs, for example, which are delivered by the trans-dermal route, entail the barrier function of the skin. Reconstructed skin models have recently emerged as tools for drug absorption studies. Here, the epiCS skin model is shown to be applicable for risk assessment by AUREA gTOXXs.

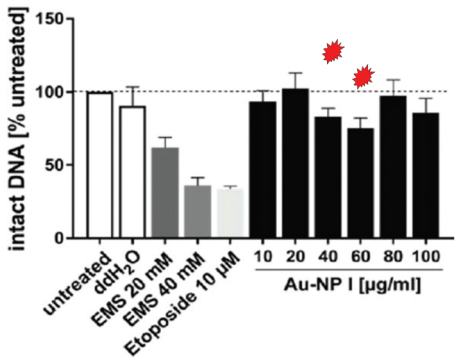
Transient DNA damage following exposure to gold nanoparticles

DNA damage induced by gold nanoparticles

Comet assay



gTOXXs analysis



❑Dose dependent DNA damage

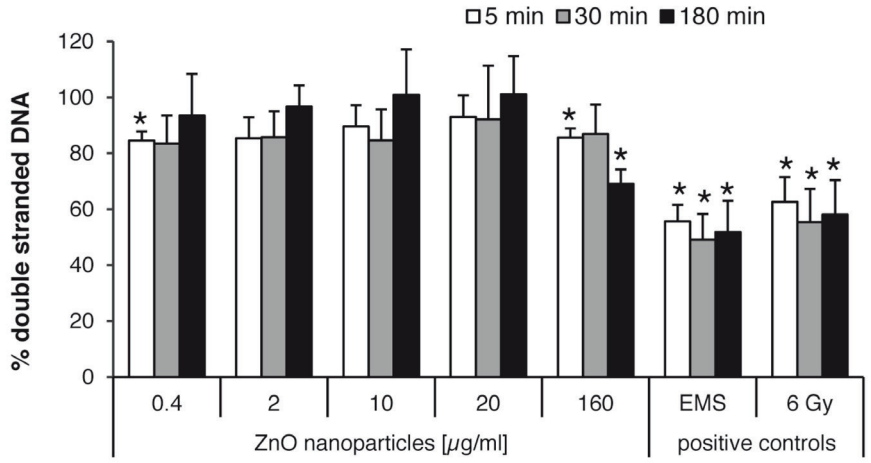
❑**Degree of DNA strand breaks assessed by AUREA gTOXXs and Comet assay conform (as indicated by the red stars)**

Gold nanoparticles (Au-NPs) are in the focus of the field of biomedicine and are under consideration for use in drug delivery and bioimaging, or as radio-sensitizers and nano-based vaccines.

Thorough evaluation of the genotoxic potential of Au-NPs, hitherto limited or controversial, is required, since damage to the genome can remain undetected in standard hazard assessments. Here, the influence of three surface modified 3–4 nm Au-NPs on human A549 cells is examined according to the reactive oxygen species paradigm. After 24 hrs of Au-NP treatment, nanoparticles were taken up by cells as agglomerates. No influence on cell viability or inflammation was detected, however, all three types of Au-NPs induced DNA damage, as suggested by the alkaline comet assay. The strongest effect is observed for positively charged Au-NP I. Further analysis by neutral comet assay, AUREA gTOXXs, and γH2AX staining, revealed that i) AU-NP I induced DNA lesions are predominantly alkali-labile sites, ii) AU-NP I induced DNA damages amount to 15-20% single or double strand breaks and iii) Au-NP-induced DNA damage is largely repaired over time, indicating that the observed damage is of transient nature. (May, Hirsch et al. 2018, Nanoscale; DOI: 10.1039/c8nr03612h).

Clastogenicity of ZnO nanoparticles

Zinc oxide (ZnO) nanoparticles are widely used in a broad range of applications, including manufacturing of rubber, cosmetics, pigments, food additives, medicine, chemical fibers, electronics, paints, and solar cells. Most in vitro studies show a rather high toxicity of ZnO nanoparticles towards cells of different tissues and organisms. The concentration-response relationship reveals a very steep curve where toxic effects abruptly start at concentrations between 10 and 20 µg/ml. Although the exact mechanism is not fully understood and remains controversial, zinc ions and reactive oxygen species may be involved in the toxic effects.
In this study statistically significant differences in DNA strand break formation have been detected by AUREA gTOXXs in A549 cells incubated at higher concentrations (≥160 µg/ml) of ZnO.



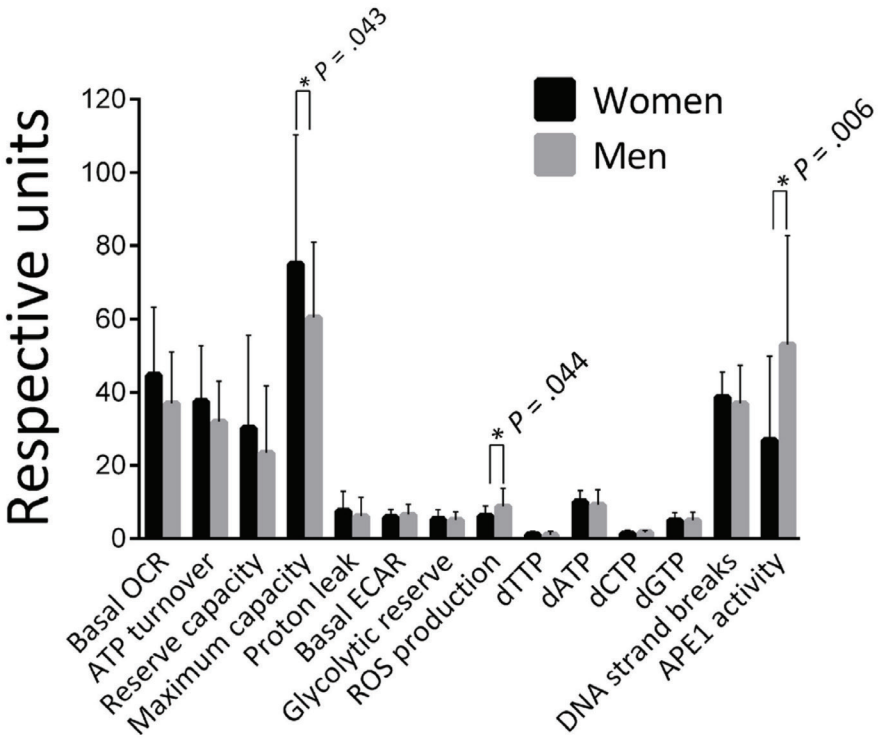
A549 cells were incubated for 5 (white bars), 30 (grey bars), or 180 min (black bars) at 37°C with 0.4, 2, 10, 20, and 160 µg/ml ZnO nanoparticles. As a positive control, two samples were exposed to 15 mM ethyl methane sulfonate (EMS) or 6 Gy of X-irradiation. Error bars represent standard deviations of three independent experiments. *The values are significantly different from control (Student's t-test, $p<0.01$) (Moreno-Villanueva et al. 2011, Altex).

Reduced DNA repair activity in peripheral blood mononuclear cells of Alzheimer's disease patients

Accurate biomarkers for early diagnosis of Alzheimer's disease (AD) are badly needed. Recent reports suggest that dysfunctional mitochondria and DNA damage are associated with AD development. In this report, various cellular parameters, related to mitochondrial bioenergetics and DNA damage, have been measured in peripheral blood mononuclear cells (PBMCs) of AD and control participants, for biomarker discovery.

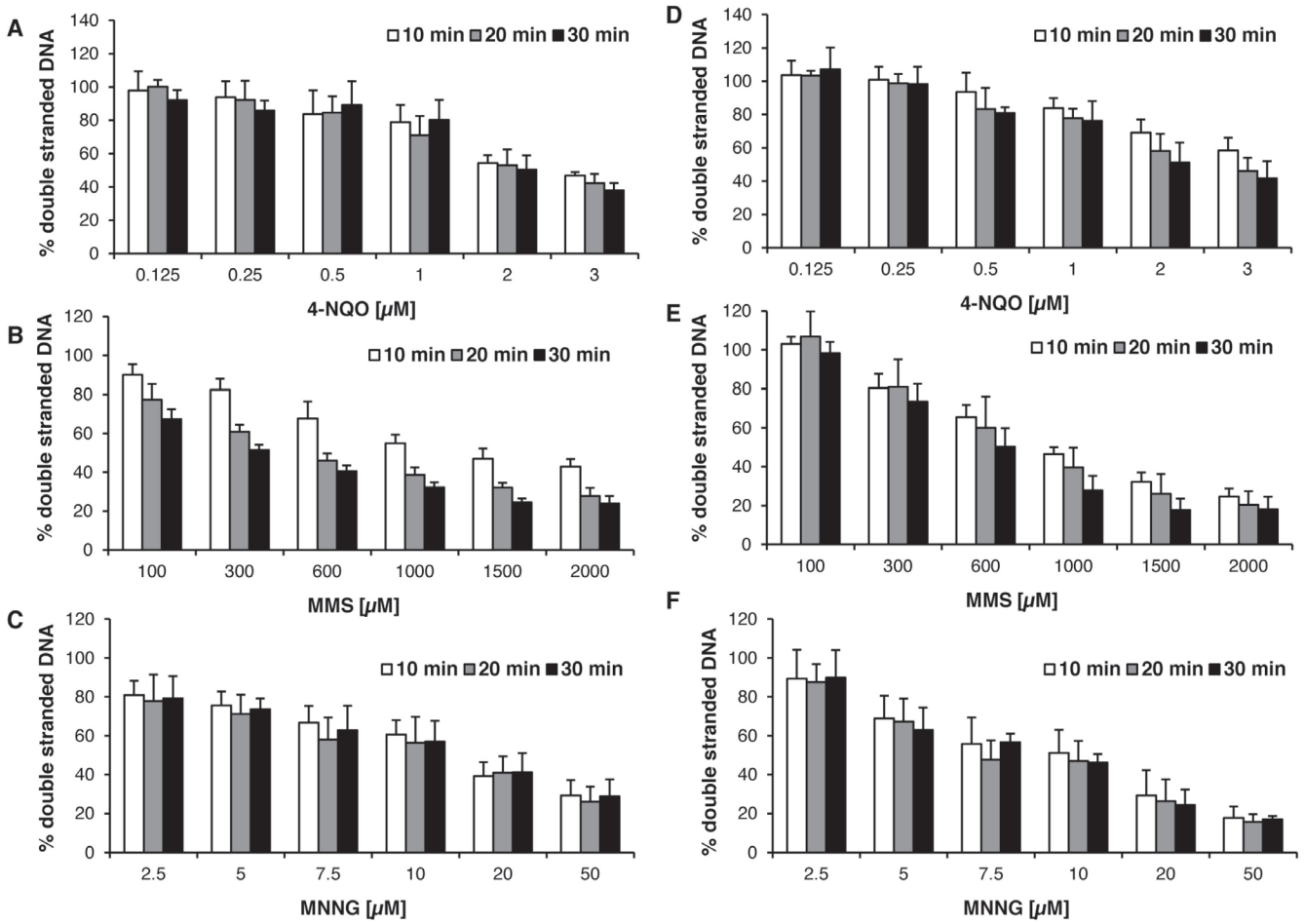
PBMCs were isolated from 53 patients with AD of mild to moderate degree and 30 age-matched healthy controls. Tests were performed on the PBMCs from as many of these participants as possible. Glycolysis and mitochondrial respiration fluxes are assessed by using the Seahorse Bioscience flux analyzer, mitochondrial reactive oxygen species production by using flow cytometry, dNTP levels by way of a DNA polymerization assay, DNA strand breaks by using AUREA gTOXXs, DNA repair efficiency by APE1 incision activity (in cell lysates) on a DNA substrate containing an AP site.
In the PBMCs of AD patients, reduced basal mitochondrial oxygen consumption is observed as well as reduced proton leak, higher dATP level, elevated DNA strand break and lower AP endonuclease 1 activity, depending on adjustments for gender and/or age.

In conclusion, this study reveals impaired mitochondrial respiration, altered dNTP pools and reduced DNA repair activity in PBMCs of AD patients, thus suggesting that these biochemical activities may be useful as biomarkers for AD (Maynard, Hejl et al, 2015, Aging).



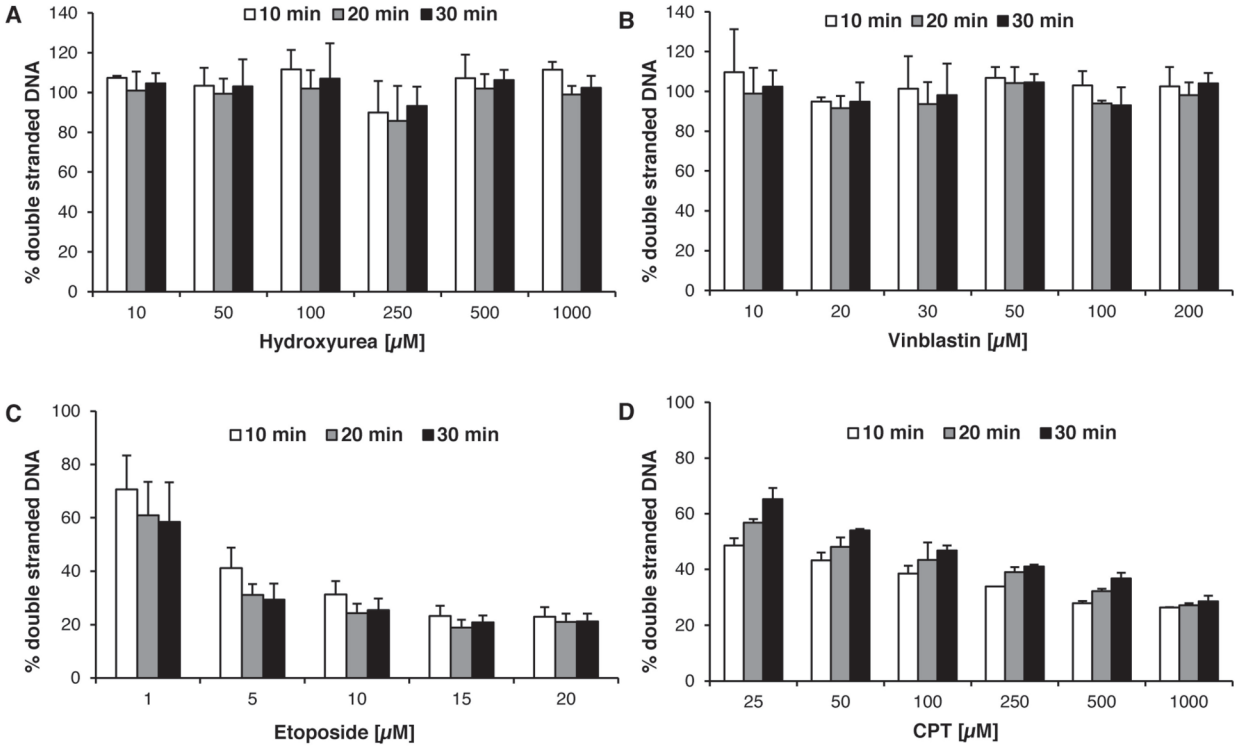
Effects of gender on the average values of the biochemical parameters. Stratified for gender. Population numbers are as follows: OCRs (Basal OCR, ATP turnover, Reserve capacity, Maximum capacity, Proton leak) and ECARs (Basal ECAR, Glycolytic reserve), men = 33, women = 35; ROS production, men = 18, women = 21; all dNTPs, men = 22, women = 33; DNA strand breaks, men = 31, women = 39; APE1 activity, men = 19, women = 17. Error bars represent ± standard deviation. * Significant difference ($P < .05$) in the average level of the parameter between men and women.

DNA strand breaks induced by carcinogens



Jurkat cells (A,B,C) and peripheral blood mononuclear cells (D,E,F) were treated with several concentrations of 4-nitroquinoline-1-oxide (4-NQO), methyl methanesulfonate (MMS) and methyl-nitronitrosoguanidine (MNNG) for 10, 20 and 30 min at 37°C as indicated. DNA strand break formation was measured by AUREA gTOXXs. In all cases, an increase in DNA strand breaks is detected in a concentration-dependent manner. In addition, a statistically significant difference in time dependent DNA strand break formation is observed upon MMS treatment. Error bars represent standard deviations of three independent experiments. Statistical significance ($p<0.05$ by two-way ANOVA with Bonferroni post-test) between exposure times was found only in Jurkat cells for MMS comparing 10 with 20 and 10 with 30 min. Results comply with the ones reported in literature (Moreno-Villanueva et al, 2011, Altex).

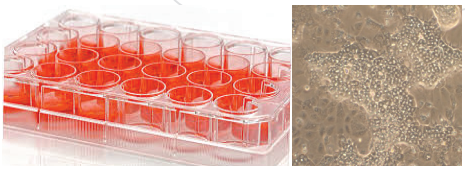
DNA strand breaks induced by anti-tumor drugs



Jurkat cells were treated with several concentrations of hydroxyurea, vinblastine, etoposide, or camptothecin (CPT) for 10, 20 and 30 min at 37°C. DNA strand break formation was measured using the AUREA gTOXXs. In the case of etoposide and camptothecin, an increase of DNA strand breaks could be detected in a concentration-dependent manner. By contrast, for hydroxyurea and vinblastine, no DNA strand breaks were detectable after 30 min drug exposure. Error bars represent standard deviations of three independent experiments (Moreno-Villanueva et al, 2011, Altex).

AUREA gTOXXs / Samples

Cell culture experiments
- Human derived cell: suspension and adherent cell culture, e.g. HepaRG™, TERT-1, Jurkat



3D Skin models
- epiCS, Cellsystems

Samples from test persons
- Peripheral Blood Mononuclear Cells out of the blood of subjects from different studies

AUREA gTOXXs

Scan QR-Code for more information
https://qr-b.de/aureagtoxxs

3T analytik

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