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Utility of 3D HepaRG spheroid model for testing genotoxicity using high-throughput CometChip platform

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Three-dimensional (3D) culture systems are becoming more popular than 2D cultures for genotoxicity evaluation as several studies have demonstrated improved cell-to-cell interactions and tissue-like structures in 3D that are limited or lacking in 2D cultures. In the current study, HepaRG spheroids were formed using metabolically competent human HepaRG cells. 3D spheroids formed in 96- or 384-well ultra-low attachment plates were then exposed to various concentrations of 34 test articles that encompassed 8 direct-acting and 11 indirect-acting genotoxicants/carcinogens as well as 15 compounds that show different genotoxic responses in vitro and in vivo. High-throughput CometChip assay was employed to evaluate DNA damage along with concurrent cytotoxicity assessment by the ATP assay in both 2D and 3D cultures. 3D HepaRG spheroids compared to 2D cultures appeared to maintain a stable phenotype for up to 30 days with higher levels of albumin secretion, cytochrome P450 gene expression, and enzyme activities. 3D spheroids also demonstrated a higher sensitivity than 2D cultures for detecting both direct- and indirect-acting genotoxicants/carcinogens, indicating a better reporter of in vivo genotoxicity. DNA damage dose-response data when quantified using PROAST software, 3D spheroids showed generally lower or similar benchmark dose values compared to 2D HepaRG cells, but they were more comparable to primary human hepatocytes. These results demonstrate that 3D models can be adapted to the CometChip technology for high-throughput genotoxicity testing and that 3D HepaRG spheroids may be a reliable and pragmatic in vitro approach for the hazard identification and risk assessment of potential human genotoxic carcinogens.