OMICS Group Conferences
Accelerating Scientific Discovery

2nd International Conference and Exhibition on
Neurology & Therapeutics
June 17-19, 2013 Hilton Chicago/Northbrook, Chicago, USA

Three-dimensional multicellular neurospheroids for neurological assessment of neurotoxic compounds

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To date, *in vitro* models for biological assays have depended mostly on 2-dimensional (2D) monolayer cultures. However, virtually all cells in brain tissues reside in a 3-dimensional (3D) environment, which is important for the regulation of intercellular communication. Therefore, although *in vitro* assay systems using 2D cultures are appropriate for toxicological evaluation of many toxicants in a high-throughput mode, the usefulness of 2D systems is limited because of the absence of the 3-dimensionality that is present in normal tissues. In particular, the multicellular neurospheroids (MCS) obtained using human or mouse neuroblastoma cell lines are very useful tools to assess the efficacy of chemo-, immuno-, and radio-therapy or for the neurotoxicity testing of chemicals. In this study, we report a facile formation of MCS by liquid overlay culturing of SH-SY5Y or N2a cells to use as the models for the evaluation of the cytotoxic effects perfluorinated compounds (PFCs). Initially, we examined the growth characteristics of spheroids with respect to their macroscopic morphology, viability, and proliferation. We also systematically compared the differences among monolayer cells and spheroids grown under undifferentiated and RA-differentiated conditions in terms of the expression levels of cell adhesion molecules and extracellular matrix proteins, neurite extension, and the contents of neuronal markers. Under undifferentiating and RA-differentiating conditions, the MCS were exposed to PFCs for 3 h and 24 h, and the resulting toxic effects were studied with respect to morphological features, cell-cell interaction, neuronal marker expression, reactive oxygen species (ROS) production, caspase-3 mRNA expression, and cell injury mechanisms (i.e. apoptosis and necrosis). Finally, we compared these cytotoxic effects with those of a conventional 2D culture-based cytotoxicity assay. Our results establish the significance of 3D spheroid cultures in *in vitro* testing of the inhibitory effects of PFCs on contact-mediated intercellular communication.

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