3D Spheroid Cultures from Human Astrocyte- and Neuronal- like Cells: New In Vitro Models to assess Magnetite Nanoparticle-Induced Adverse Effects on CNS.

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ABSTRACT (caratteri: 1922 spazi eslclusi)

Several lines of evidence demonstrate that nanoparticles (NPs) can translocate to brain and impact on the highly vulnerable central nervous system (CNS). The distribution of NPs in the bloodstream raises a particular concern of NP transfer from placenta to the fetal CNS, and since BBB develops gradually in the fetal brain, a direct exposure to NP *in utero*,that may have the most detrimental consequences. A recognized need is to combine nanotoxicology and neurology knowledge and calls for novel specific tools and investigation methods for this discipline including the integration of new *in vitro* models into safety assessment strategies.
In this study two types of CNS cell spheroids have been developed and optimised in combination with standard assays (viability readout and cell morphology) to test neurotoxic effects caused by magnetite NPs (Fe_3O_4NP), as NP-model, after short- (24-48h; 1-100 µg/ml) and long-term repeated exposure (up to 30 days; 0.1-25 µg/ml). Three-dimensional (3D) spheroids were generated from human D384 astrocyte- and SH-SY5Y neuronal-like cells: they formed reproducible well-rounded spheroids, with homogeneous size distribution, viability and functionality over long period.
Short-term exposure to Fe_3O_4NPs induced cytotoxicity in both 3D astrocytes and neurons spheroids, starting at 10 and 25 µg/ml, respectively.
After long-term repeated dose regimen, cell spheroids showed concentration- and time-dependent accumulation of Fe_3O_4NPs. Cell mortality appeared at 10 and 0.5 µg/ml, for D384 and SH-SY5Y, respectively, indicating a higher susceptibility, to Fe_3O_4NPs, of neurons compared to astrocytes. Both spheroid types failed to maintain their canonical shape and displayed cell disaggregation occurring within the first week of treatment beginning at ≥ 0.1 µg/ml (cumulative total exposures of ≥ 1.2 µg/ml) and becoming considerably evident at higher concentrations and over the 30 day-period.
Recreating the 3D spatial environment of CNS allows cells to behave *in vitro* more closely to the *in vivo* situation, therefore providing a model that can be used as stand-alone test or as a part of integrated testing strategies. These models could drive an improvement in the species-relevant predictivity of toxicity testing.

Key words: Fe_3O_4NPs, *in vitro* screening, D384 cells, SH-SY5Y cells, neurotoxicity, nanotoxicology