

Developmental Neurotoxicity Screening for Nanoparticles Using Neuron-Like Cells of Human Umbilical Cord Mesenchymal Stem Cells: Example with Magnetite Nanoparticles

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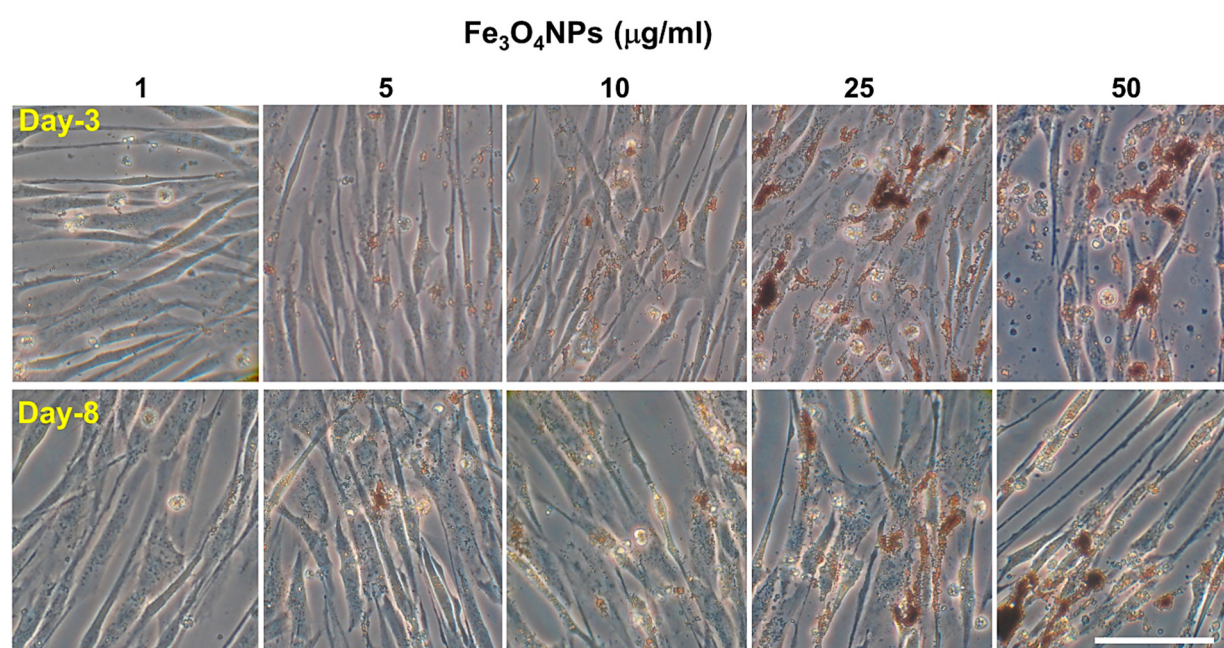


Figure 1. Morphological analysis of hNLCs cultured with increasing concentration of Fe₃O₄NPs. Representative micrographs, by phase-contrast microscopy, of hNLCs after 3 and 8 days of culture in neurogenic medium with Fe₃O₄NPs (from 1 to 50 µg/ml). No morphological changes were induced by Fe₃O₄NPs exposure in hNLCs at day 3 and 8 of transdifferentiation. However, a cell density decrease was observed from 25 µg/ml Fe₃O₄NPs at both time points considered. Brownish aggregates/agglomerates of Fe₃O₄NPs were also observed in culture medium. Scale bar: 100 µm.

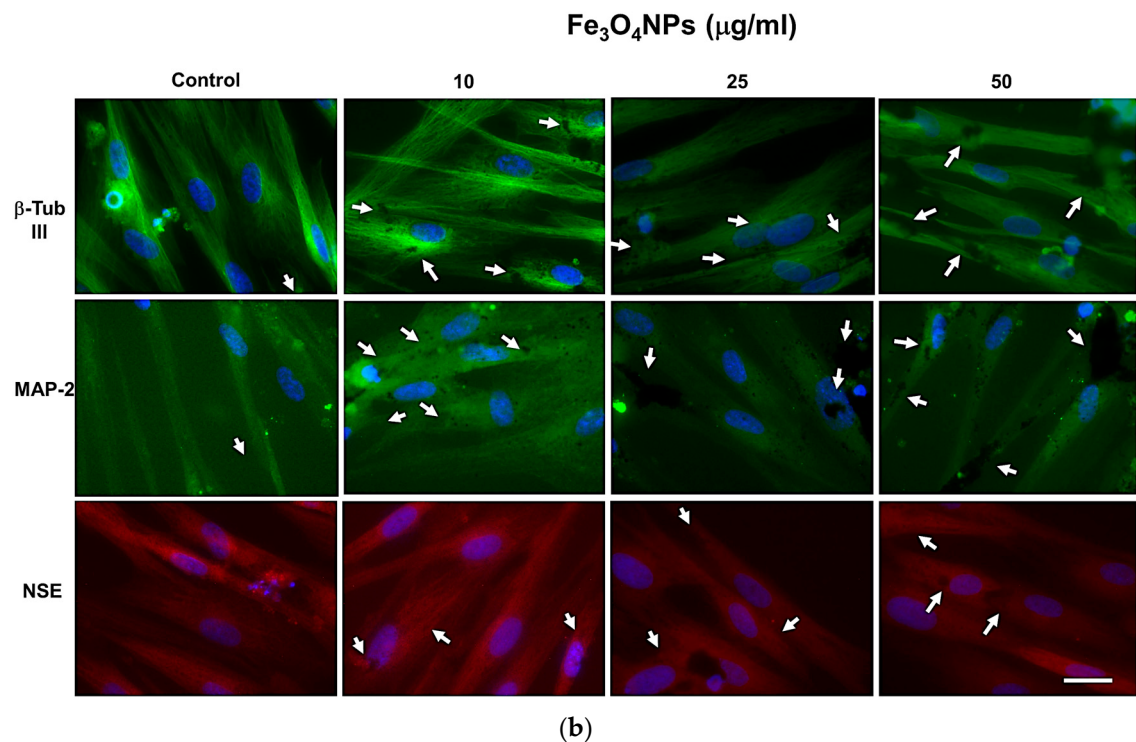
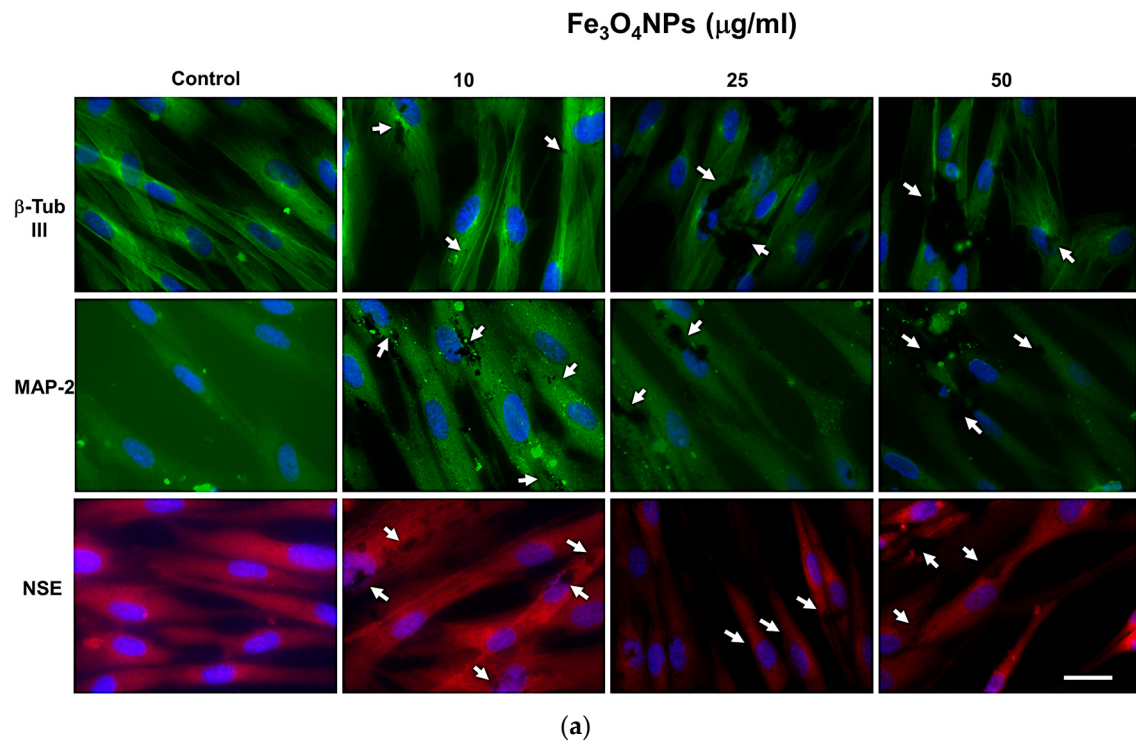


Figure S2. Neuronal marker protein expressions in hNLCs cultured with Fe₃O₄NPs (10-50 μg/ml). Representative fluorescence merged images of hNLCs at day 3 (a) and day 8 (b) of transdifferentiation. Fe₃O₄NPs affected the expression of neuronal proteins such as β-Tub III (green fluorescence), MAP-2 (green fluorescence) and NSE (red fluorescence): a light fluorescence intensity decrease of the neuron markers was observed starting from ≥ 25 μg/ml. Black spots of Fe₃O₄NPs (intracellularly and on the cell membrane) were also visible. Nuclei were stained with Hoechst 33258. White arrows indicat Fe₃O₄NPs (dark spots). Scale bar: 100 μm.