Abstract

Safety concerns arising from cytotoxic behavior of nanoparticles (NPs) in complex biological environment remain the main problem limiting NPs application in biomedicine. In this study, we have investigated cytotoxicity of NPs with different composition, shape and size, namely SiO_2 NPs (SiNPs, 7-14 nm), superparamagnetic iron oxide NPs (SPIONs, 8 nm) and carboxylated multiwalled carbon nanotubes (CNTCOOHs, diameter: 60-100 nm, length: 1-2 μm). Cytotoxicity was evaluated with newly designed screening assay capable to simultaneously assess activity of cell dehydrogenases, activity of lactate dehydrogenase (LDH) released from cells into environment and number of intact cell nuclei and apoptotic bodies in human umbilical vein endothelial cell (HUVEC) culture growing in the very same well of the 96-well plate. Aforementioned attributes were subsequently utilized to obtain information about cell viability and necrotic and apoptotic aspects of cell death. Results from this „three-in-one" cell death screening (CDS) assay showed that SiNPs and CNTCOOHs evoked pronounced cytotoxic effect demonstrated as decrease of cell viability and development of apoptotic bodies formation. In contrast to this, SPIONs induced only mild cytotoxicity. Moreover, SiNPs impaired cell membrane leading to increased LDH release (necrotic type of activity) slightly visible also after application of SPIONs. In the case of CNTCOOHs, necrotic changes could not be evaluated due to remarkably strong interference of CNTCOOHs with components of LDH assay as an intern compound of CDS assay. In the subsequent investigation, we have focused on influence of protein coating (so called protein corona) composition on cell toxicity of CNTCOOHs. Results from CDS assay implied that preformed protein coating from immunoglobulin G (IgG) supported cytotoxic effect of CNTCOOHs which induced decrease in cell dehydrogenases activity, while coating with human serum albumin (HSA) had rather protective effects manifesting as increase in intact cell nuclei alongside with decrease in apoptotic bodies formation. Cytotoxic influence of CNTCOOHs without protein corona leading to accumulation of autophagic vesicles in HUVEC cells was also significantly attenuated specifically by stimulation of autophagic flux with ≤ 1 nM macrolide antibiotics bafilomycin A1. In summary, our results suggest that using of appropriate protein corona in combination with pharmacological stimulation of autophagic flux could be in the future one of the promissing approaches enabling to minimize the cytotoxic impact of at least some types of NPs.