Abstract

Nanomaterials possess unique physicochemical properties such as high reactivity, large surface to volume ratio, exceptional electronic characteristics and quantum size effects. A probable downside of these remarkable properties would be their enhanced interactions with biological systems, which may lead to potential toxic effects. Zinc oxide (ZnO) and graphene are two important nanomaterials that are extensively studied by the nanoscience community owing to their unique physico-chemical properties rendering wide range of potential applications. In the current Ph.D. work, we have extensively studied the specific role of size scale, and surface chemistry of zinc oxide nanocrystals (ZnO NCs) on its toxicity towards prokaryotic and eukaryotic cells. The results show that the toxicity towards bacteria increased with reduction in particle size (40nm −1.2μM) and with increasing concentrations (1mM − 7.5mM). Flow cytometry and confocal microscopy studies revealed that ZnO NCs undergo rapid dissolution in acidic (pH ~ 5-6) cancer microenvironment causing elevated ROS stress, mitochondrial superoxide formation, depolarization of mitochondrial membrane, and cell cycle arrest at S/G2 phase leading to apoptosis whereas normal cells having neutral pH remained unaffected at the same concentration. These results point to the interesting role of dissolution chemistry of nanomaterials under various bio-chemical environments in determining their toxicity. In case of graphene, where dissolution is a remote possibility, we have studied the role of surface chemistry in its nano-bio interaction. The interaction of graphene with kidney epithelial cells and macrophage cells showed that pristine graphene (*p*-G) accumulated preferentially on the plasma membrane leading to high oxidative stress. In contrast, carboxyl functionalized hydrophilic graphene (*f*-G) was internalized by the cells without causing much toxicity up to 48 h. However, cellular and mechanistic toxicity studies demonstrated that irrespective of surface functionalization, both *p*-G and *f*-G induced cellular toxicity in human umbilical vein endothelial cells (HUVECs) through oxidative stress mechanism. Toxicogenomic studies in HUVEC cells also revealed that both graphene systems causes altered expression of various critical genes governing key cellular functions. More importantly, single cell gel electrophoresis analysis showed that both *p*-G and *f*-G has the potential to cause DNA damage in HUVEC cells. This pattern of toxicity effects has changed in the case of blood cells, hemocompatibility studies (Hemolysis, platelet activation/aggregation, plasma coagulation, immune cell response) using human primary blood components indicated no differential toxicity effects between *p*-G and *f*-G and both samples remained compatible up to 75 μg/ml. The *in vitro* findings were further reinforced through *in vivo* studies using Swiss Albino mice model. *In vivo* bio-imaging analysis using

radio labeled (^{99m}Tc) graphene displayed longer retention ability of graphene in lung tissue, which was further corroborated by histology studies. Histological examinations revealed that both graphene systems have accumulated in major organs such as lung, liver, spleen, and kidney and induced severe pathological changes, including inflammation, pulmonary edema and granuloma formation at a dosage of 20 mg kg^{-1} body weight. In lung tissue, *p*-G caused more damages in bronchial epithelium while *f*-G entered in to the alveolae and caused damages in the alveolar epithelium. In liver tissue, *p*-G caused more irritations in the sinusoidal line while *f*-G was shown to enter the peri-sinusoidal space and cause damage to the hepatocytes. In the case of spleen, *p*-G samples remained in the red pulp while *f*-G showed more damages in marginal zone, indicating loss of demarcation between white pulp and red pulp. In order to confirm the identity of graphene in mouse organs, we have performed confocal Raman spectral mapping. Raman signature of graphene from tissue samples showed no sign of degradation under in *in vivo* conditions even after three months. RT-PCR analysis of mice serum samples demonstrates that both *p*-G and *f*-G induced acute immune and inflammatory responses. Blood biochemical analysis revealed elevated levels of biochemical markers, indicating altered liver functions. Overall, our in vitro studies demonstrate the surface functionalization as well as cell type dependent toxicity nature of graphene. *In vivo* studies demonstrated that *p*-G induced more structural damages to mice tissue while *f*-G shows the capability to enter in to the cells and cause extensive cellular damages. The above results should be perceived by considering the lateral dimension of 200 nm and dose of 20mg/kg used in this study. These effects may vary with reduction in lateral size and dose. Further, additional surface functionalization like PEGylation may also modify the in vivo response. However, our investigations clearly suggest that, graphene has definite toxicity effects, at both organ and cellular levels and hence extensive investigations are needed before its potential applications in biomedicine.