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Cytotoxic action of graphene nanoplatelets functionalized by serum albumin on leucocytes

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The prospects for carbon nanomaterials in biomedical applications depend on their ability to decompose in organism into products which can be easily eliminated from the body [1-5]. The nanomaterials after enter the body interact with different blood cells including leucocytes. Phagocytes such as neutrophils and macrophages can implement enzymatic digestion of carbon nanoparticles for several days [4-6]. Manifestations of carbon nanotubes interaction with blood leucocytes can be detected already in the early hours of their contact. Previously, we found that multi-walled carbon nanotubes induce an initial amplification of reactive oxygen species (ROS) generation in neutrophils, after which cell activity is suppressed [7]. In this study, we have investigated the influence of graphene nanoplatelets (GNPs) on human blood polymorphonuclear and mononuclear leucocytes in the first hour of their interaction *in vitro*.

GNPs were functionalized by serum albumin (SA). GNPs were added in water solution of SA (0.5 µmol/L) and sonicated at frequency of 44 kHz for 30 min. Polymorphonuclear and mononuclear cells were isolated from heparinized donor blood by histopaque-1077 density gradient centrifugation. Cells were resuspended in Earle's medium at pH 7.3. Suspensions of cells were exposed to GNPs-SA (10-100 µg/mL) or SA (controls) for 1 hour. The cellular structure and the number of cells were studied by light microscopy and nephelometry methods. Cell viability was investigated using fluorescent stains propidium iodide (PI) and fluorescein diacetate (FDA). PI fluorescence is revealed with emission at λ_{em} 600-700 nm and excitation maximum at λ_{ex} 543 nm after interaction of PI with DNA in cells with damaged membranes, but cell membrane integrity excludes PI from staining viable and apoptotic cells. FDA is a non-fluorescent molecule, which is hydrolyzed intracellularly by esterases in live cells and then fluorescence is observed at emission maximum 518 nm and excitation maximum 490 nm. The cell activity was assessed using 2,7-dichlorofluorescein diacetate (DChFDA, cell-permeable form). DChFDA is de-esterified and converted to membrane-impenetrable dichlorofluorescein (DChF) which remains in-

side the cells. Fluorescence of DChF is appeared under the action of ROS. In this work, the kinetic dependencies of DChF fluorescence intensity I_{fl} during its oxidation in leucocytes activated by n-formyl-methionyl-leucyl-phenylalanine (fMLP, 1 μM) was recorded, and then values of I_{fl} were integrated for 10 min. Suspensions of cells were incubated in the presence of 2.5 μmol/L DChFDA or 60 μmol/L PI and 10 μmol/L FDA for 30 min at 37 °C and then the cells were washed from the unbound probe by centrifugation for 8 min at 1500 rpm.

It was shown that ability to ROS generation of polymorphonuclear and mononuclear leucocytes is decreased under GNPs-SA impact and suppressed significantly in the presence of higher concentration of GNPs-SA. The decline of ability of cells to ROS generation under the influence of GNPs-SA does not correlate with changes of cell viability (in experiments with fluorescence stains PI and FDA), but is accompanied by cellular deformation. A decrease in the number of cells was revealed as a result of GNPs-SA effect in 1 hour after their addition in higher concentration to cell suspension. We found that along with a reduction in the total number of cells exposed to GNPs-SA, there is a decrease in the number of polarized and spread out cells is observed. Thus, the obtained data indicate the cytotoxic effect of GNPs-SA on leucocytes.

References

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