Supplement to the paper: V. I. Popenko, O. G. Leonova, V. I. Salyanov, N. N. Orlova, P. V. Spirin, V. S. Prasolov, and Yu. M. Evdokimov "Dynamics of Penetration of "Rigid" Nanostructures of Double-Stranded DNA

Complexed with Gadolinium into CHO Cells"



Fig.1. CHO cell of sample incubated with NS--Gd nanostructures for 1 h with small accumulations of NS--Gd particles in cytoplasm. The number of such cells is less than 5% of the population. Here and below, N stands for nucleus and C, for cytoplasm, arrows indicate NS--Gd outside cells and short arrows, NS--Gd inside cells. Scale bar is 2 μ m.



Fig. 2. CHO cell of sample incubated with NS--Gd nanostructures for 3h. NS--Gd nanostructures are observed in about 50% of the population. Cavities with NS-Gd structures are $\sim 0.5 - 2 \ \mu m$ in size. Scale bar is 2 μm .



Fig. 3. CHO cell of sample incubated with NS--Gd nanostructures for 12 h. Cell morphology does not change significantly, although the number of NS--Gd inside of the cells increases. Cytoplasmic membrane remains undamaged. Scale bar is 2 µm.



Fig. 4. Confocal microscopy of CHO cells of sample incubated with NS--Gd nanostructures for 24 h. NS-Gd nanostructures were identified in Leica TCS SP5 confocal microscope using SYBR Green I fluorescent dye. Optical sections at the levels $\alpha - \alpha$ (at the top), $\beta - \beta$ (in the middle) $\mu \gamma - \gamma$ (at the bootom) along Z axis are shown. Above each optical section a corresponding cross section along I-I line is shown. The boundaries of the nucleus and the cell are marked by blue and violet colors, respectively. A schematic drawing of the cell and optical section levels is shown on the right side. It is clearly seen that NS--Gd nanostructures are located inside the cells, in the cytoplasm.



Fig. 5. An increase in incubation time leads to an increase in the number of NS--Gd inside of the cells. NS-Gd nanostructures were identified in Leica TCS SP5 confocal microscope using SYBR Green I fluorescent dye (upper row). In the middle row one can see bright field images of the same cells. Bottom row shows overlapped images. Separated large particles 0.5 and more μ m in size are situated on the Petri dish bottom and on the cell surfaces. Many small fluorescent NS--Gd particles are located inside the cells.



Fig. 6. CHO cells of sample incubated with NS--Gd nanostructures for 72 h. A significant portion of the cells dies. Many NS--Gd particles are observed inside the survived cells. The cell with broken cytoplasmic membrane is seen at the left. Scale bar is 5 μ m.



Fig. 7. CHO cell of the sample not incubated with NS--Gd nanostructures after irradiation. The cells were removed from substrate without a scraper by trypsin. The procedure allowed for the complete exclusion of mechanical damage to cells in the process of sample preparation; however, it resulted in a loss of the characteristic spindle-like shape and some swelling of the cytoplasm and nuclear structures. It is well seen that cytoplasmic membrane is not damaged. Scale bar is $2 \mu m$.