# Gd Nanoparticles' Impact on Living Cells in Presence of Low Frequency EMF and Strong MF

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**Introduction.** Nanoparticles are defined as particles with size less than 100 nm and include natural, incidental and engineered nanoparticles [1]. It is recognized that nanoparticles produce reactive oxygen species (ROS) inside and outside the cell and are the key factor in toxicological effects [2]. Several studies have been conducted on effect of oxidative stress on animal cells, including on the cell membrane, after exposure in ELF EMF (50 Hz) [3, 4, 5]. Extremely low frequency electromagnetic fields (ELF EMF) occur primarily from 50 and 60 Hz electric power lines and from electric devices and installations in buildings. In medicine MRI scanners use strong magnetic fields, radio waves, and field gradients to form images of the body [6]. Majority of research shows no genotoxic, or otherwise harmful, effects caused by any part of MRI [7]. Gadodiamide is a gadolinium-based MRI contrast agent, used in MR imaging procedures to assist in the visualization of blood vessels. It is commonly marketed under the trade name Omniscan [8]. Plant immature gametic cells were used as model cells in the research as very vulnerable to environmental influence [9]. ROS production as a result of oxidative stress in plant cells has great influence on cell self fluorescence that could be detected by flow cytometry. Flow cytometry (FCM) is widely used method for investigation of different cell parameters, including cell oxidative stress determination on base of educed peroxidase intensity [10, 11, 12]. FCM has several advantages: large number of cells can be evaluated in a very short time, the results are statistically significant and represent the whole population, on the base of changes of cell relative fluorescence it is possible to collect and analyze more than 20 parameters of each cell. All mentioned make the method an excellent investigation tool in many areas [13, 14, 15].

The aim of this study was to determine by FCM the combined effects of Gd nanoparticles (GdNPs) on intracellular concentration of ROS in plant immature gametic cells in presence of both 50 Hz ELF EMF and strong MF.

**Materials and methods.**

*Plant material and immature microspore cell culture preparation*. The cyclamens (*Cyclamen persicum*) immature gametic cells in one nuclear stage were used as a model cells. 1 ml of prepared cell sediment contained about 600 000 cells [16]. The liquid phase was poured off and 1 ml of cell sediment was suspended in 4 ml liquid MS medium (Murashige and Skoog [17]) and mixed

in cultivation tubes. The cell culture quality was determined by light microscope (magnification $\times 10^3$). Gd nanoparticles were added to the cell culture in concentration 0.5 µl of „Omniscan" (0.5 mmol/ml ) per 1 ml of cell culture. Then cells were incubated in speed shaking regime for 1 hour at 20 °C. The control cells were incubated in the same conditions without adding of GdNPs. The experiment was made in three repetitions.

*Exposure to electromagnetic radiation.* A 50 Hz electromagnetic field (sinusoidal) was provided by a specific- shape signal generator and through an extremely low-frequency amplifier fed into a vertically positioned induction coil. Applied B-field monitoring was ensured throughout the whole experiment by magnetic flux density measurements in the place of the coil using a three-axis magnetometer THM1176 (Metrolab Technology, Switzerland). Samples of experimental cultures were placed into the induction coil and exposed to time-varying electromagnetic fields for 30 minutes at 20 °C. The control samples were not exposed. Density of the electromagnetic field exposure was 50, 100, 200 and 400 µT. Density of background magnetic fields in the laboratory used for preparation of cyclamens gametic cells culture was 0.09 µT.

*Exposure to strong magnetic field* was provided using „Brivo MR 355" 1.5 T MRI Scanner from „Ge Healthcare". The temperature during cell cultivation was 22-23 °C, exposure time 0.5 hour. The experiment was made in three replications.

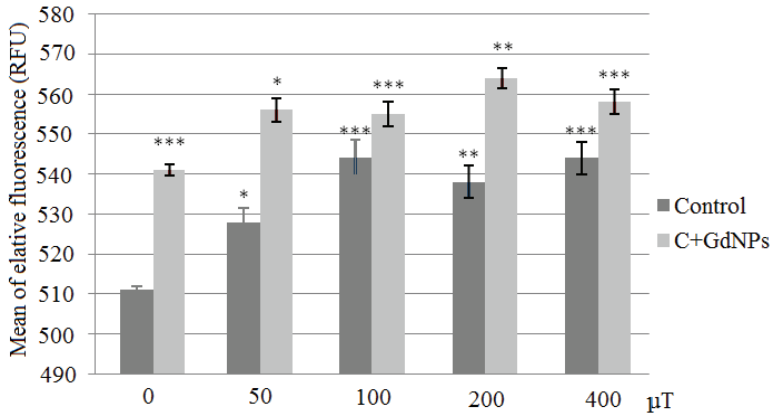


**Fig. 1.** Relative fluorescence (RF) of *Cyclamen persicum* gametic cells incubated in presence of Gd nanoparticles after effect of 50 Hz EMF. Statistical analysis was carried out using the Student's paired t test. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus control cultures. Data represent mean ± SE, n = 20 independent measurements in three replications.

*Measurement of cell relative fluorescence.* A BD FACSJazz® cell sorter (BD Biosciences, USA) with flow cytometer function was used to test relative fluorescence of plant cells. The excitation of the cell fluorescence was made by

488 nm Coherent Sapphire Solid State (blue) laser. The fluorescence emission was measured at 585 nm. Cell counts were gated by the intensity in both fluorescence-detecting channels to include approximately 90% of target cells. More than $1x10^4$ cells were evaluated. Error of method did not exceed 3%.

**Results**. Cell fluorescence increased in presence of Gd nanoparticles in cell cultivation medium (Fig.1). A significant difference of fluorescence was observed between control cultures of gametic cells and gametic cells after 1 hour incubation with suspension of GdNPs in all used densities of ELF EMF. The most effective influence on cells were observed using ELF EMF with density 200 μT.

The experiment with MR showed that GdNPs did not have any significant influence on immature gametic cells (Fig. 2). The MR dramatically decreased the cell fluorescence. The cells incubated in presence of GdNPs had considerable extent of relative fluorescence (RF) after influence of MR in comparison to cells cultivated without GdNPs.

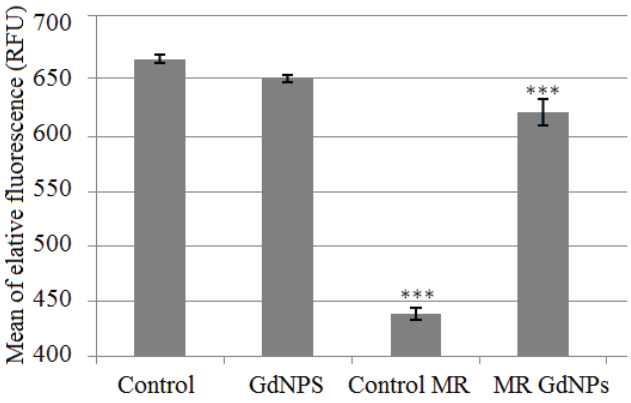


**Fig. 2.** Relative fluorescence (RF) of *Cyclamen persicum* gametic cells incubated in presence of Gd nanoparticles after effect of strong (1,5T) magnetic field for 30 minutes. Statistical analysis was carried out using the Student's paired t test. ***$p < 0.001$ versus control cultures. Data represent mean ± SE, n = 20 independent measurements in three replications.

**Conclusions/Discussion.** The significant influence of both studied factors - ELF EMF and MR on immature plant gametic cells was found. The difference of RF in control cell groups of the experiments was probably caused by differences in room temperature during the cell cultivation, because in higher temperature the cell metabolism processes are more intensive and cell RF increases. There is no doubt in GdNPs' influence on cells' RF. The RF of cells cultivated in presence of GdNPs changed after exposure to ELF EMF and MR. Apparently, the cells' fluorescence's changes were caused by the ability of all investigated factors, during their interaction with cells, to change the metabolic processes of cladding oxidative stress in cells.

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