The Influence of Different Types of Upconversion Nanoparticles Surface Coatings on Neurotoxicity

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The aim of the investigation was to study the effect of upconversion nanoparticles (UCNPs) with different surface coatings on viability and functional neural network activity of primary hippocampal cultures.

Materials and Methods. The UCNPs with three surface coating modifications (tetramethylammonium hydroxide (TMAH), polymaleic acid octadecene and polyethyleneimine (PEI)) were synthesized by a solvothermal technique with further hydrophilization. Primary hippocampal cultures, obtained from C57BL/6 mice embryos (E18), were incubated with tested UCNPs in the concentration of 0.8 and 8 mg/ml during 72 h. The cell viability detection, evaluation of morphological changes by immunocytochemical staining as well as the UCNPs influence on the main parameters of the functional neural network calcium activity and on the endocytosis processes were carried out.

Results. Our studies revealed that UCNPs caused a dose-dependent cytotoxic effect on primary hippocampal cultures, wherein the severity of this effect directly related to the type of UCNPs surface coating. The greatest cytotoxicity was identified for UCNP–PEI, and the least — for UCNP–TMAH. UCNPs toxicity is manifested in significant morphological changes of neural networks and in the increase the number of dead cells ($p<0.05$) in primary hippocampal cultures. Moreover, a significant decrease ($p<0.05$) in the main parameters of spontaneous functional calcium activity was shown.

Conclusion. A comprehensive investigation of the nanoparticles effects on primary hippocampal cultures showed that all tested UCNPs have the strong toxic effect to the nervous system cells.

Key words: upconversion nanoparticles; particle toxicology; primary hippocampal cultures; neural network; functional network activity.

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Introduction. Application of nanomaterials in biological research regarded as a modern technology, allowing to investigate not only intravital processes occurring in tissues at cellular and molecular levels but also actively adjust the metabolism of certain cell populations. Upconversion nanoparticles (UCNPs) is unique molecular probes which benefits are associated with excitation/emission falling into the biological tissue and therefore with the possibility to conduct imaging due to the minimized absorption and scattering of biotissue in near-infra-red spectral range, unlimited photostability, chemical inertness and physical stability of the solid core. Among advantages of UCNPs a low toxicity compared to the most currently used organic dyes and the possibility of autofluorescence reduce in bioimaging research should be noticed. Nanoparticles application in the therapeutic correction of various diseases as a substrate for drugs delivery is a promising approach. For instance, photoluminescent nanoparticles loading with drug molecules can be target-delivered to pathological tissue sites rendering them conspicuous for imaging and diagnostic purposes. The concomitant drug release, which can be either externally controlled [1] or triggered by the altered environment of the pathology site, induces therapeutic effect that can be monitored by means of the photoluminescence imaging. Despite the early development stage, upconversion nanoparticles have demonstrated considerable promise for theranostics applications, as widely reported in the literature [2].

UCNPs application in neurobiological research is of special interest. The blood-brain barrier is impermeable for most substances, including UCNPs [3]. However, in certain pathological conditions nanoparticles able to penetrate into the brain tissue [4, 5]. In this case the use of UCNPs opens wide prospects in the therapy and fundamental research. It has been demonstrated that labeling of brain cancer lesions by means of protoporphyrins (ALA-PP9)13 provided sufficient contrast to aid the cancer visualization and guided surgery, nowadays known as fluorescence-guided surgery [6].

In this regard, the question concerning cytotoxicity of UCNPs and their coatings, possible providing the specificity of nanoparticles interaction with certain surface proteins, is of particular importance.

However, according to a wealth of information published on this topic, the majority of studies were conducted on cell lines that are more resistant to the toxic agents' action [7, 8]. In terms of the cell viability, the average impact of *in vitro* cell exposition to UCNPs for 1–2 days was estimated as 10–20% of the population loss [9]. Recently, we have reported the systematic cytotoxicity studies of normal immortalized human skin cells, fibroblasts and keratinocytes, incubated with bare UCNPs ($NaYF4:Yb^{3+}:Er^{3+}/NaYF4$), UCNPs surface-coated with several types of polymers commonly used in biological procedures, and UCNPs embedded in sub-micron polyacrylic spheres. While the fibroblast cell culture appeared to be immune to the tested nanomaterials, keratinocytes displayed variable sensitivity ranging from the most cytotoxic polyethyleneimine (PEI) — through to mildly cytotoxic polymaleic acid octadecene (PMAO)-coated UCNPs to almost non-cytotoxic bare UCNP surface-modified by small-molecular-weight tetramethylammonium hydroxide (TMAH) [10].

In addition, during the investigation of nervous cells particular attention should be paid to cellular viability preservation as well as to the maintaining the functional state of neural networks — the minimum functional unit of the nervous system.

Loss of neural network activity, even the cells remain viable, is detrimental to the whole microorganism, and should be considered as a limiting factor when choosing a therapeutic or diagnostic agent.

Therefore, the aim of our investigation was to study the effect of UCNPs with different surface coatings on viability and functional neural network activity of primary hippocampal cultures.

Materials and Methods

Synthesis of UCNPs. The following combination of substances: Y_2O_3 (0.78 mM), Yb_2O_3 (0.2 mM), Er_2O_3 (14 mM) and Tm_2O_3 (6 mM) was dissolved in 20 ml 70% trifluoroacetic acid up to clear solution through gently refluxing and further cooling to room temperature. The obtained solution undergoes evaporation to a fine homogenous powder. A rare-earth trifluoroacetate mixture and sodium trifluoroacetate (2 mM) was added to 10 ml oleic acid and 10 ml 1-octadecene with subsequent heating at 120°C and stirring in a vacuum for 30 min. Next, the mixture was gradually heated up to 318°C on a Wood's alloy bath with further cooling to a room temperature. Isopropanol (150 ml) was applied to the mixture and then centrifuged at 6,000 rpm for 20 min.

The obtained nanocrystals were washed in 100% ethanol, dried, dissolved in 10 ml chloroform, precipitated with 50 ml isopropanol, and centrifuged at 4,000 rpm for 10 min.

Design of UCNPs. To estimate the toxicity of the as-synthesized UCNPs, the ligand exchange reaction was carried out with TMAH. TMAH is adsorbed on the surface of UCNPs [11], partially displacing oleic acid (OA) moieties from the OA–UCNP surface. UCNPs were transferred into an aqueous fraction by preparation of a UCNP microemulsion, followed by solvent evaporation.

The following amphiphilic polymers were adsorbed onto the UCNP surface by a solvent evaporation method: poly(maleic anhydride-alt-1-octadecene) (UCNP–PMAO), polyethyleneimine (UCNP–PEI). Evaporation of the solvent resulted in an aqueous dispersion of UCNPs surface-modified with the polymer.

Hydrophilization of UCNPs with pentahydrate TMAH. 20 μl UCNP dispersion (10 mg/mL) in chloroform was applied dropwise to 1 ml 1% aqueous solution of TMAH and thoroughly shaken. After UCNPs transferring to the water phase, chloroform was evaporated. The UCNP suspension was washed three times with water

with subsequent centrifugation at 13,400 rpm for 10 min. The pellet was dispersed in 1 ml water.

Surface modification of UCNPs with polymers. The solvent evaporation technique, described previously in [12], was applied for coating the as-synthesized UCNPs with the amphiphilic polymer PMAO.

The mixture was stirred, sonicated and incubated during 1 h at room temperature. Next, under vigorous stirring and sonication the mixture was applied dropwise to 1 ml water, PBS (phosphate-buffered saline) buffer (pH 7.2) or Na-borate buffer (pH 8.2) and then undergo heat and centrifugation (10 min at 13,400 rpm) with water addition. After a threefold reiteration of this procedure, the mixture was dispersed in 1 ml water, PBS buffer (pH 7.2) or Na-borate buffer (pH 8.2).

Ethics statement. All experimental protocols were reviewed and approved by the Bioethics Committee of Nizhny Novgorod State Medical Academy; experiments were conducted in strict accordance with Act 708n (23.08.2010) the National Ministry of Public Health of Russian Federation approving the rules of laboratory practice for the care and use of laboratory animals and European Convention for the Protection of Vertebrata used for Experimental and other Scientific Purposes (the Convention was passed in Strasburg, March 18, 1986, adopted in Strasburg, June 15, 2006).

Cell cultures. Primary hippocampal cultures were performed according to the previously developed protocol [13]. Briefly, hippocampi were isolated from embryonic C57BL/6J mice (E18) and undergo mechanical and enzymatic (0.25% trypsin, 25200-056, Invitrogen, USA) dissociation. Then the cells were carefully resuspended in Neurobasal medium (21103-049, Invitrogen, USA) containing 2% B27 (17504-044, Invitrogen, USA), 0.5 mM L-glutamine (25030-024, Invitrogen, USA), 5% fetal calf serum (K055, PanEco, Russia) and plated at initial density of approximately 9,000 cells/mm^2 on coverslips, pre-treated with positively charged hydrophilic substance — polyethyleneimine (P3143, Sigma, Germany).

The next day, 30% of the cultural medium was replaced by a medium with a lower content of fetal calf serum (0.4%) without any antibiotics or antimycotics. Then one-half of the medium was changed every 2 days. The viability of the cells was maintained under constant conditions at 37°C in a humidified atmosphere containing 95% air and 5% CO_2.

To record the culture status, phase-contrast images of primary hippocampal cultures were taken daily using inverted microscope Leica DMIL HC (Leica, Germany) with a 10×/0.2Ph1 objective.

UCNPs in the concentration of 0.8 and 8.0 mg/ml were added into the cultural medium on day 14 of the culture development *in vitro* (DIV).

Cell viability detection. The viability of primary hippocampal cells was calculated as the percentage ratio between the number of dead cells stained by propidium iodide (P4170, Sigma, Germany) and the total number of cells stained by bisBenzimide (H3570, Invitrogen, USA) 72 h after UCNPs treatment [14].

Immunocytochemical staining. Primary hippocampal cultures were fixed by 4% formaldehyde dissolved in PBS and were subsequently treated with 0.1% Triton X-100 (93443-100ML, Sigma, Germany) containing 2% bovine serum albumin. Next, the cultures were incubated with following primary antibodies solution: mouse monoclonal MAP2a+MAP2b (ab36447, Abcam, USA) to stain neurons and chicken polyclonal GFAP (ab4674, Abcam, USA) to stain astrocytes. After 2 h incubation at room temperature, the samples were carefully washed in PBS and undergo further 30-minute treatment by the mixture of secondary antibodies: rabbit anti-mouse conjugated Alexa Fluor 555 (A21427, Invitrogen, USA) and goat anti-chicken conjugated Alexa Fluor 647 (ab150171, Abcam, USA).

The immunostained cultures were embedded into Fluomount™ Aqueous Mounting Medium (F4680, Sigma, Germany) and further examined under a confocal laser-scanning microscope (LSM 710, Carl Zeiss, Germany) equipped by femtosecond pulsed Ti:Sapphire laser (Chameleon vision II, Coherent Inc., USA) and C-Apochromat 63×/1.20 W Corr UV-VIS-IR M27 objective lens. A confocal pinhole of 1 airy unit was used to obtain axial optical slices of the axial resolution of 1.6 µm. Quantitative analysis was performed using ImageJ (Research Service Branch, NIH).

Detection of endocytosis in primary hippocampal cultures. To investigate endocytosis processes a specific fluorescence dye for intravital staining of acidic organelles (lysosomes) LysoTracker Red DND-99 (L7528, Molecular Probes, USA) was used. LysoTrackers are capable of specifically binding with organelles, having low pH values, and are designed to study the biosynthesis and lysosomes' moving in the cell cytoplasm.

75 nM of the fluorescence dye was added into the cultural medium with subsequent 1 h incubation with primary hippocampal cells. In experimental group UCNPs in the concentration of 0.8 mg/ml was applied simultaneously with LysoTrackers. Next the complete replacement of the cultural medium with a free fluorescent dye medium with subsequent organelles visualization was carried out.

To detect the stained organelles a laser scanning microscope (LSM 710, Carl Zeiss, Germany) with a W Plan-Apochromat 63×/1.2 objective as well as 543 nm line of He-Neon laser radiation and emission detection with a 575–663 nm filter were used.

Ca^{2+} imaging. The functional calcium imaging was conducted by following published protocol [13]. In brief, Oregon Green 488 BAPTA-1 AM (OGB-1) (0.4 µM; O-6807, Invitrogen, USA), dissolved in dimethyl sulfoxide (D8418, Sigma, Germany) with 4% Pluronic F-127 (P-3000MP, Invitrogen, USA), was added into the cultural medium for 40 min. Then the cultures were incubated in a dye-free medium for 15 min. A confocal laser scanning microscope (LSM 510, Carl Zeiss, Germany) with a W Plan-Apochromat 20×/1.0 objective as well as 488 nm line of Argon laser radiation and emission detection with a 500–530 nm filter were used for detection of cytosolic

Ca^{2+} distribution in neurons and astrocytes through OGB-1 excitation. Time series of 256×256-pixel images with a 420×420 µm field of view were recorded at 4 Hz. A confocal pinhole of 1 airy unit was applied to obtain an axial optical slice resolution of 1.6 µm. Quantitative evaluation of Ca^{2+} transients was performed using custom-made software C++ Builder to manually select the regions of interest in obtained fluorescent images. The Ca^{2+} fluorescence of selected cells was evaluated as an average fluorescence intensity (relative units from 0 to 255) of the pixels within the defined region. Single Ca^{2+} signals were detected by averaging two neighboring points in the sample set with subsequent detection the difference in each pair of consequent points for a simple derivative calculation. A threshold detection algorithm was applied to determine the Ca^{2+} events from the derivative of the trace. The threshold was evaluated as the detection accuracy coefficient multiplied by the standard deviation of the derivative of the trace. Suprathreshold points on the derivative of the trace considered as the beginnings and ends of the pulses.

Statistical analysis. All data are presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using a two-way ANOVA test (SigmaPlot 11.0, Systat Software Inc., USA). Student–Newman–Keuls was used as a post hoc ANOVA test. The difference between groups was considered significant if $p<0.05$.

Results. During the observation period the formation of neural networks, characterized by morphological stability starting with 10–14 days of culture development *in vitro*, were shown. The number of dead cells in control cultures did not exceed 7% during the cultivation period. The UCNPs in the concentration of 0.8 and 8.0 mg/ml were added into the cultural medium on DIV 14. 0.8 mg/ml regards as a typical concentration for investigations of UCNPs cytotoxic effects. In addition, in our studies we used the tenfold UCNPs concentration (8.0 mg/ml). A viability and functional activity determination of primary hippocampal cultures was conducted 72 h after incubation with UCNPs.

The cytotoxic effect of UCNPs with three surface coating modifications was investigated. Our selection of the tested surface modifications was focused on the following polymers: TMAH, PMAO and PEI. Such surface modifications are characterized not only by chemical properties but also by their surface charge, which is an essential point for carrying out experiments on nervous system cells. This cell type is preferred for negatively charged coatings, thus positively charged nanoparticles are supposed not to be engaged in close interaction with the neurons and astrocytes. Among the selected materials TMAH is the most simple coating, whereby the UCNPs remain almost bare with the subsequent appearance of the hydroxide radical (OH^-) on their surface, which in turn providing a negative charge. PMAO is a polymer, also providing a negative charge on the UCNPs surface, wherein ζ-potential of this type of nanoparticles in 2 times higher compared to UCNPs–TMAH and amounts –41.65±3.3 [10]. On the other hand, PEI has a positive charge and probably should not engage in close interaction with differentiated neurons.

The cytotoxicity evaluation showed that upconversion nanoparticles with all three types of surface coatings have strong toxicity to the nervous system cells (Figure 1).

A significant increase ($p<0.01$) in the proportion of dead cells in primary cultures was observed 72 h after UCNPs treatment. The most pronounced and dose-dependent toxic effect was shown in UCNP–PEI group, where the percentage of dead cells was 74.3±5.5% for UCNP–PEI (0.8 mg/ml) and 93.6±1.8% for UCNP–PEI (8.0 mg/ml).

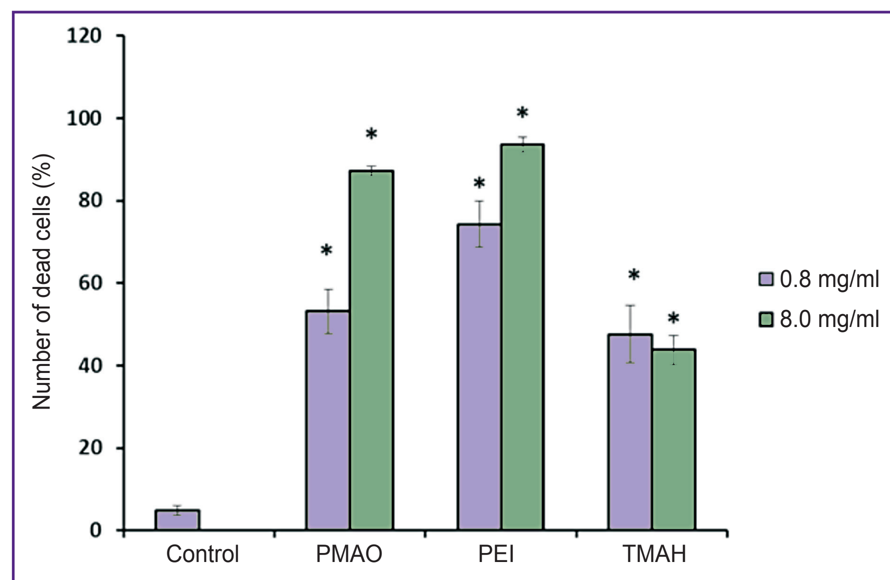

Figure 1. A histogram of the primary hippocampal culture (DIV 17) cell viability 72 h after the incubation with upconversion nanoparticles, presented in terms of the percentage of dead cells (UCNPs); * statistically significant, $p<0.05$

The UCNP–PMAO material also caused an increase in the number of dead cells to 53.1±5.44 and 87.1±1.17% during incubation with nanoparticles in the concentration of 0.8 and 8.0 mg/ml, respectively.

Therefore, PEI and PMAO materials exhibited the greatest toxic dose-dependent effect. Interestingly, the number of dead cells in UCNP–PEI (0.8 mg/ml) group was significantly higher than in cultures incubated with UCNP–PMAO (0.8 mg/ml) ($p<0.05$).

Less pronounced toxic properties were shown for the nanoparticles coated with TMAH. In this group of cultures the dose-dependent cytotoxic effect was not observed. The number of dead cells, caused by 0.8 and 8.0 mg/ml UCNP–TMAH treatment, was equal.

Furthermore, a significant decrease of non-viable cells in primary hippocampal cultures with UCNP–TMAH application in the concentration of 8.0 mg/ml compared to cultures, incubated with UCNP–PEI and UCNP–PMAO in similar concentration, was detected.

Therefore, the obtained data revealed that all tested nanoparticles have strong neurotoxicity, wherein the severity of this effect directly related to the type of UCNPs surface coating. The greatest toxicity was shown for UCNP–PEI, the lowest cytotoxic effect for UCNP–TMAH. In this regard, the toxicity of UCNPs should be considered in choosing the nanoparticles' type to carry out specific investigations.

For more detailed analysis the morphological changes in primary hippocampal cultures an immunocytochemical staining was performed.

Microtubule-associated protein (MAP) and glial fibrillary acidic protein (GFAP) were chosen to stain the mature neurons and astrocytes in primary cultures, respectively.

Compared to control cultures, significant morphological changes in the cultures, incubated with UCNP–TMAH and UCNP–PEI, were detected (Figure 2). Moreover, the body shapes' modification of neurons as well as the partial reduction of neuronal outgrowth up to their almost complete destruction was observed in primary hippocampal cultures with UCNP–PEI treatment.

UCNP–TMAH application did not cause a clearly marked effect on the morphological structure of the neural network (Figure 2 (d)).

In cultures, incubated with UCNP–PMAO and UCNP–PEI during 72 h, the changes in the morphological structure of astrocytes, manifested in stretching their shape and more close localization of the cells, were also detected. Such morphological changes are typical for neuronal cultures affected by different stress-factors and toxic substances. In contrast, in control cultures the astrocytes have a normal star shape and arrange freely, connecting to each other by the end of outgrowth only.

The endocytosis processes occurring in primary hippocampal cultures were investigated. In order to

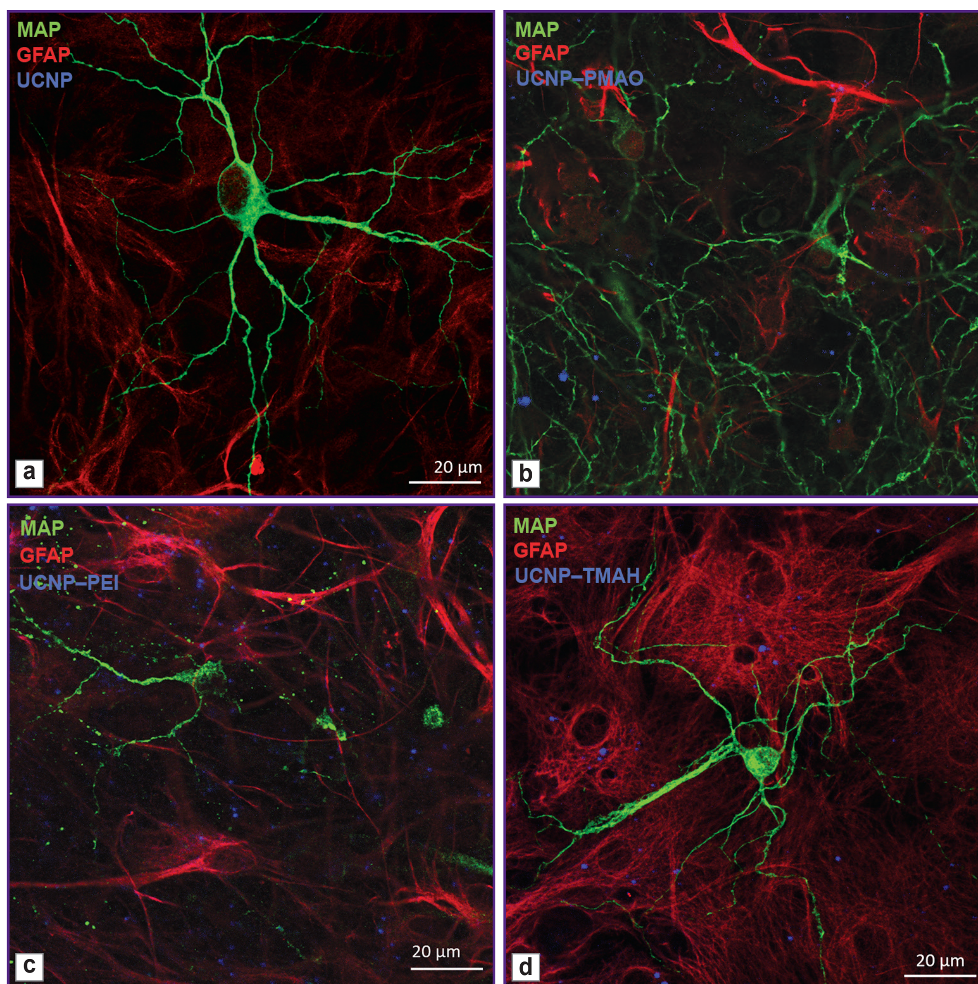

Figure 2. Immunocytochemical images of primary hippocampal cultures stained by MAP (neuronal marker, green) and GFAP (astrocytes marker, red). Dark blue — UCNPs distribution in primary cultures. (a) Control; (b)–(d) cultures with UCNP–PMAO, UCNP–PEI and UCNP–TMAH incubation, respectively

assess the presence of vacuoles containing internalized nanoparticles, the primary hippocampal cultures we incubated UCNP and LysoTracker Red DND-99 in accordance with the protocol (See in Materials and Methods). We have shown the presence of a significant number of stained lysosomes in glial cells as well as in neurons, indicating the active processes of endocytosis and lysosomes formation in cells (Figure 3 (a)). Interestingly, the primary hippocampal cultures are characterized by a high rate of endocytosis (the formation of 10–25 lysosomes per 1 h) and it is logical to assume that the toxicity of nanoparticles for nervous cells is related to their internalization processes by cells. However, the lysosomes, containing UCNPs, were not observed. Our results supposed to be associated with the fact that lysosomal organelle are small (1 mm), and may contain nanoparticles with size up to 100 nm, while during visualization we were able to detect a significantly larger in linear dimensions of nanoparticles' aggregates only.

The next part of our study was associated with the evaluation of the functional neural networks activity with using Ca^{2+} imaging technique. A synchronized spontaneous calcium activity was registered in primary hippocampal cultures on DIV 14. We have previously shown, such activity is typical for this period of culture development *in vitro* [15]. In control cultures there was 85–95% of cells, exhibiting Ca^{2+} activity, the duration of Ca^{2+} oscillations was approximately 8 s at a frequency of 2 oscillations per minute on DIV 17 (See the Table).

A significant Ca^{2+} activity modification was observed in primary hippocampal cultures 72 h after UCNPs application (Figure 4). Upconversion nanoparticles with PEI surface coating have the most pronounced effect on neural networks functionality. In cultures, incubated with UCNP–PEI, the spontaneous Ca^{2+} activity was completely negated.

Incubation with UCNP–TMAH and UCNP–PMAO caused a significant decrease in the amount of cells, exhibiting Ca^{2+} oscillations in 4 and 12 times, respectively ($p<0.05$). In UCNP–PMAO experimental group the

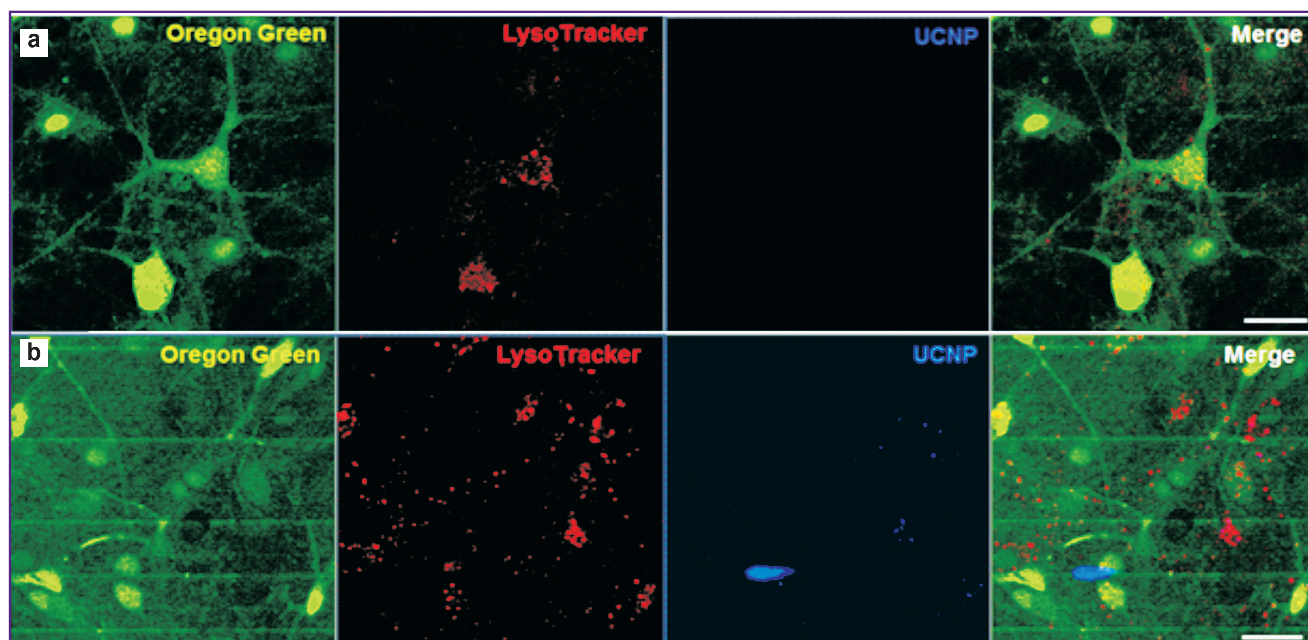

Figure 3. Example of primary hippocampal images stained by LysoTracker Red DND 99: (a) control; (b) culture, incubated with nanoparticles. Scale bar 20 µm

The main parameters of spontaneous calcium activity of primary hippocampal cultures 72 h after UCNPs treatment

Groups	Proportion of cells exhibiting calcium activity	The duration of Ca^{2+} oscillations per second	Number of Ca^{2+} oscillations per minute
Control	90.5±6.3	7.87±0.17	1.82±0.12
UCNP–PMAO	5.8±2.6*	5.01±0.5*	1.17±0.09*
UCNP–PEI	0.5±0.3	0	0
UCNP–TMAH	17.0±2.8*	8.91±0.65	1.28±0.3

* Statistically significant, p<0.05, ANOVA.

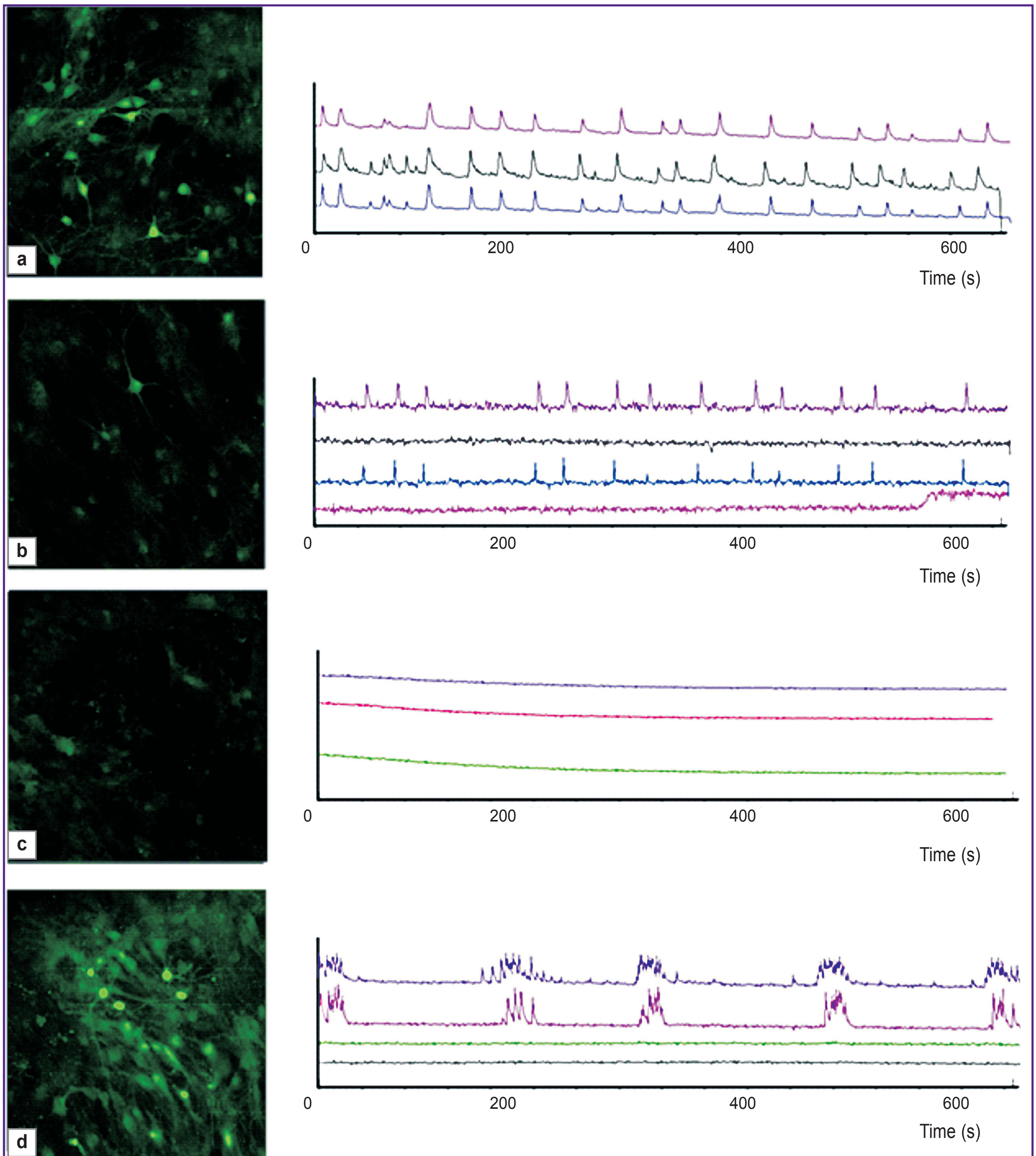

Figure 4. An example of spontaneous Ca^{2+} oscillation recordings in primary hippocampal cultures, stained with calcium-dependent fluorescent dye (Oregon Green), 72 h after UCNPs incubation: (a) control; (b) UCNP–TMAH; (c) UCNP–PMAO; (d) UCNP–PEI

frequency and the duration of Ca^{2+} oscillations were also reliable decreased ($p<0.05$).

The least negative effect on the spontaneous calcium activity was noted for cultures with UCNP–TMAH application, since the duration and frequency of calcium events were comparable to control values with maintaining of the Ca^{2+} oscillations patterns.

Therefore, a comprehensive investigation of the nanoparticles effects on primary hippocampal cultures showed that all tested UCNPs have the strong toxic effect

on the nervous system cells. The greatest cytotoxicity was identified for UCNP–PEI, and the least — for UCNP–TMAH.

Discussion. Optical properties, small in size and an opportunity for the creation of nanoparticles, having a high affinity to the certain targets, represent UCNPs as an extremely promising technology for investigation of various types of cellular receptors and their tracking as well as a potential method of directed delivery of pharmacological agents to the target cells. We have shown that the nanoparticles, interacting with the nervous cells of primary hippocampal cultures, exhibit cytotoxic properties. Our data revealed that the UCNPs concentration and the type of surface modification could be optimized to achieve the efficient of UCNPs interaction with cells and to decrease their toxicity.

Different types of surface coating modifications with specific chemical and physical properties were investigated. Among tested nanoparticles the less toxic effects were typical for UCNP–TMAH, the most pronounced — for UCNP–PEI. Our findings consistent with published data, according to which bare nanoparticles have the least pronounced cytotoxic properties. In the reported studies by Zhao et al. [16], a dose-dependent toxicity of UCNPs, conjugated with PEI, on rat mesenchymal stem cells were shown.

In this research, it was also found the toxicity increase according to the increasing the concentration of nanoparticles with polymer surface modification (for UCNP–PEI and UCNP–PMAO, respectively). Such dynamic for UCNP–TMAH was not identified. UCNP–TMAH toxicity is presumably determined by the property of nanoparticles core, capable of activating the free radical processes, or with the ability of all types of UCNPs nonspecifically bind with cellular surface molecules. In the second case, the absence of increased number of dead cells during the increasing the UCNP–TMAH concentrations could be associated with disabilities of OH^- radicals on the UCNP–TMAH surface bind with cell surface structures.

It was shown that the cellular death in primary hippocampal cultures is followed by morphological changes, manifested in the dendritic destruction and their morphological modifications as well as in changes of astrocytes mutual. Besides the influence on morphological parameters of neuron-glial networks, the UCNPs effects on the functional calcium homeostasis were also shown.

It could be assumed that the decrease of spontaneous calcium activity in primary hippocampal cultures after incubation with the nanoparticles associated with the destruction and loss of key elements of neural networks. Nanoparticles' ability to be absorbed by cells via endocytosis is one of the possible mechanisms of UCNPs influence on the cells. The literature data suggest the possibility of nanoparticles absorption by cell lines [16–18]. Active UCNPs internalization by primary hippocampal cells was not found, therefore, it could be assumed that cytotoxic effects of nanoparticles are caused by the UCNPs interaction with cell surface proteins.

Conclusion. A comprehensive investigation of the nanoparticles effects on primary hippocampal cultures showed that all tested UCNPs have the strong toxic effect on the nervous system cells. The greatest cytotoxicity was identified for UCNP–PEI, and the least — for UCNP–TMAH.

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Conflicts of Interest. The authors have no conflicts of interest.

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