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Biocompatibility of Bare Nanoparticles Based on Silicon and Gold for Nervous Cells

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Abstract

This work aimed to investigate the biocompatibility of bare (ligand-free) laser-synthesized nanoparticles (NPs) based on silicon (Si) and gold (Au) with primary hippocampal cultures. 1%, 5% and 7% of culture medium were replaced by 0.1 mg/mL NP solution on day 14 of culture development *in vitro*. Our studies revealed that the NPs caused a dose-dependent cytotoxic effect, which was manifested by an increase the number of dead cells and a decrease of the spontaneous functional calcium activity of neural networks. Au NPs revealed less pronounced cytotoxic effect than Si ones and it can be explained by larger size and better solubility of Si NPs.

Keywords: bare nanoparticles, primary hippocampal cultures, neurotoxicity

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1. Introduction

Nanoparticles (NPs) are widely investigated for applications in different areas of human activity. Particular attention of specialists in biology and medicine is given to solid state NPs. Due to small size and unique properties, such type of NPs can penetrate deeply into tissues, cells and nuclei, which is promising for theranostics (simultaneous therapy and diagnostics) of cancer [1]. NPs are considered as agents for a number of optical imaging techniques, carriers of drugs and their controlled release, as well as targets for directed tumor cells destruction by physical actions [2-4].

One of the main requirements for the development of NPs for biomedical application is nontoxicity for healthy cells, lack of reactions from the immune system and risks of other side effects, safe NPs biodegradation with subsequent naturally elimination from the organism. However, despite a wide range of existing nanomaterials, few of them meet the above criteria. Recent studies reveled that some types of NPs provide

cytotoxic effects to the human organism, especially to the nervous system [5]. Therefore, it is so important to evaluate biosafety of the emerging NPs for brain cells and tissues. Development of new non-toxic NPs will open new possibilities in molecular neuro diagnostics and therapy.

This paper is devoted to studies of the biocompatibility of bare laser-synthesized NPs based on silicon (Si) and gold (Au) with primary hippocampal cells.

2. Materials and Methods

2.1. Fabrication of NPs

Aqueous suspensions of silicon and gold nanoparticles were obtained by femtosecond laser ablation (FLA) of monocrystalline Si and polycrystalline Au targets placed at the bottom of a glass vessel filled with 20 mL of deionized water (18.2 MΩcm). Radiation of an Yb:KGW femtosecond laser with wavelength 1025 nm, pulse duration 480 fs, pulse energy 500 mJ, and repetition rate 1–5 kHz was focused with the help of a 750 mm lens onto the target surface to provide the ablation of material. The target was moved at a scanning velocity of 0.35 mm/s in the focusing plane to obtain identical surface conditions during the laser ablation, while the thickness of the water layer above the target was about 1 cm. Such ablation geometry normally leads to grey (Si NPs) or red (Au NPs) coloration of the aqueous solution after 2–5 minutes of the experiment. The concentration of NPs was determined by comparing the weight of the targets before and after FLA and it was accounted 0.1 mg/mL [6-8]. Before *in vitro* experiments, the hydrodynamic sizes (diameter) of nanoparticles were measured by using an Zetasizer Nano-SZ analyzer (Malvern Instruments LTD, UK). The mean sizes of Au and Si NPs were about 40±14 nm and 110±20 nm, respectively.

2.2. Cell culture

Dissociated hippocampal cells were taken from C57BL/6 mice embryos (E18) and cultured on coverslips during 14 days *in vitro* (DIV) according to the previously developed protocol [9]. The cell viability was maintained under constant conditions of 35.5°C, 5% CO_2 and a humidified atmosphere in a cell culture incubator. 1%, 5% and 7% of culture medium were replaced by NPs solution on 14 DIV.

2.3. Cell viability detection

Viability determination of primary hippocampal cultures was conducted on the 7^{th} day after NPs application by estimation the ratio of the number of dead cells stained by propidium iodide (Sigma, P4170, Germany) and the total number of cells stained by bisbenzimide (Invitrogen, H3570, USA). Cells were observed using a Leica DMIL HC inverted fluorescence microscope (Leica, Germany) with a 10×/0.2Ph1 objective.

The metabolic activity of primary hippocampal cells was studied by using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) test according to protocol described in [10, 11].

The viability (E) was calculated by the following equations:

$$E = A570 - A620, \tag{1}$$

$$\%\ \text{of viable cells} = (\text{E experiment/E control}) \times 100\%, \tag{2}$$

where A is the optical density measured at 570 and 620 nm wavelengths.

2.4. Calcium imaging

Analysis of the functional neuronal network activity in primary hippocampal cultures in response to NPs application on day 7 after treatment was conducted according to a protocol described in Ref.[7]. To detect Ca^{2+} oscillations we used Ca^{2+}-sensitive dye Oregon Green 488 BAPTA-1 AM (0.4 μM, Invitrogen, O-6807, USA) and a confocal laser-scanning microscope (Zeiss LSM 510, Germany) with a W Plan-Apochromat 20×/1.0 objective.

2.5. Statistical analysis

All quantified data are presented as the mean ± standard error of the mean (SEM). Statistical analyses were performed using two-way ANOVA implemented in Sigma Plot 11.0 software (Systat Software, Inc.). Student-Newman-Keuls (SNK) test was used as a post hoc test following analysis of variance (ANOVA). Differences between groups were considered significant if the corresponding p-value was less than 0.05.

3. Results and discussion

Carried out experiments reveled that substitution of the culture medium by 7% suspensions of Si and Au NPs exerted pronounced toxic effect to nervous cells. The number of dead cells in primary hippocampal cultures day 7 after addition of NPs based on Au and Si amounted to 31±5% and 32±5%, respectively (Figure 1). Data obtained from MTT test also indicate low metabolic activity of cells in both experimental groups (Table 1).

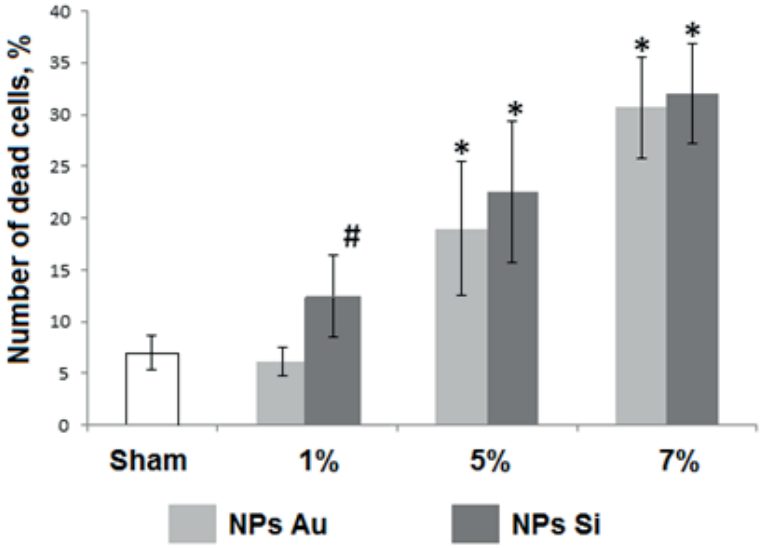

Figure 1: Analysis of cell viability in primary hippocampal cultures day 7 after NPs treatment. * - versus "Sham"; # - versus "NPs Au", $p < 0.05$, ANOVA, N = 6.

5% replacement of the culture medium to the corresponding volume of the tested nanoparticles did not induce significant metabolic changes in the primary hippocampal cultures (Table 1).

On the other hand, this concentration of NPs was not optimal, since its application led to marked morphological changes of the primary culture, manifested as an increase in the number of dead cells (19±7% for Au NPs and 23±7% for Si ones).

Replacing of the culture medium with 1% solutions of both types of NPs did not result in morphological and metabolic changes in primary hippocampal cells. Nevertheless, increased number of dead cells in Si NPs experimental group compared with Au NPs can be noted.

1% solution was used in further investigation the influence of tested NPs on functional neural network activity in primary hippocampal cultures.

Table 1: MTT reductase activity of primary hippocampal cultures day 7 after NPs application.

Experimental sample	% of living cells relative to "Sham" group
Au NPs	
1%	129
5%	113
7%	34
Si NPs	
1%	88
5%	53
7%	26

As previously shown, synchronous calcium oscillations on DIV 14 is a characteristic feature of neuron-glial networks of primary hippocampal cultures [12]. The number of cells exhibited calcium activity is approximately 77±10%, the duration of Ca^{2+} oscillations amounted to 7.8±0.5 sec with the frequency of 1.4±0.2 per min. These parameters point to full-developed neural network, accompanied by the development of complex chemical synapses and stable neural network bioelectrical activity [13].

Our studies revealed that NPs application caused changes in the main parameters of neural network activity (Figure 2 D-F). On day 7 after Si NPs treatment there was a significant increase of the duration of Ca^{2+} oscillations (11±1 sec) whereas the frequency was decreased up to 0.9±0.2 oscillations per min. Moreover, the proportion of cells exhibited calcium activity was decreased and amounted to 53±10%. Construction of raster diagrams also indicates changes in the activation pattern, manifested in a decrease of calcium synchrony events day 7 after Si NPs addition (Figure 2 G-I). Therefore, the effect exerted by 1% Si NPs replacement on primary hippocampal cultures considered as moderately toxic.

Replacement of 1% culture medium to Au NPs led to less destructive action. For this experimental group showed the decrease the percentage of cells exhibited functional metabolic activity (67±9%). An insignificant decrease in the frequency of Ca^{2+} oscillations (0.8±0.2 oscillations per min) in the maintaining the duration of Ca^{2+} oscillations (7.7±0.8 sec) were also observed on day 7 after Au NPs application. Analysis of raster diagrams revealed the decrease in synchronism of calcium events. However, these changes were less essential than in Si NP group.

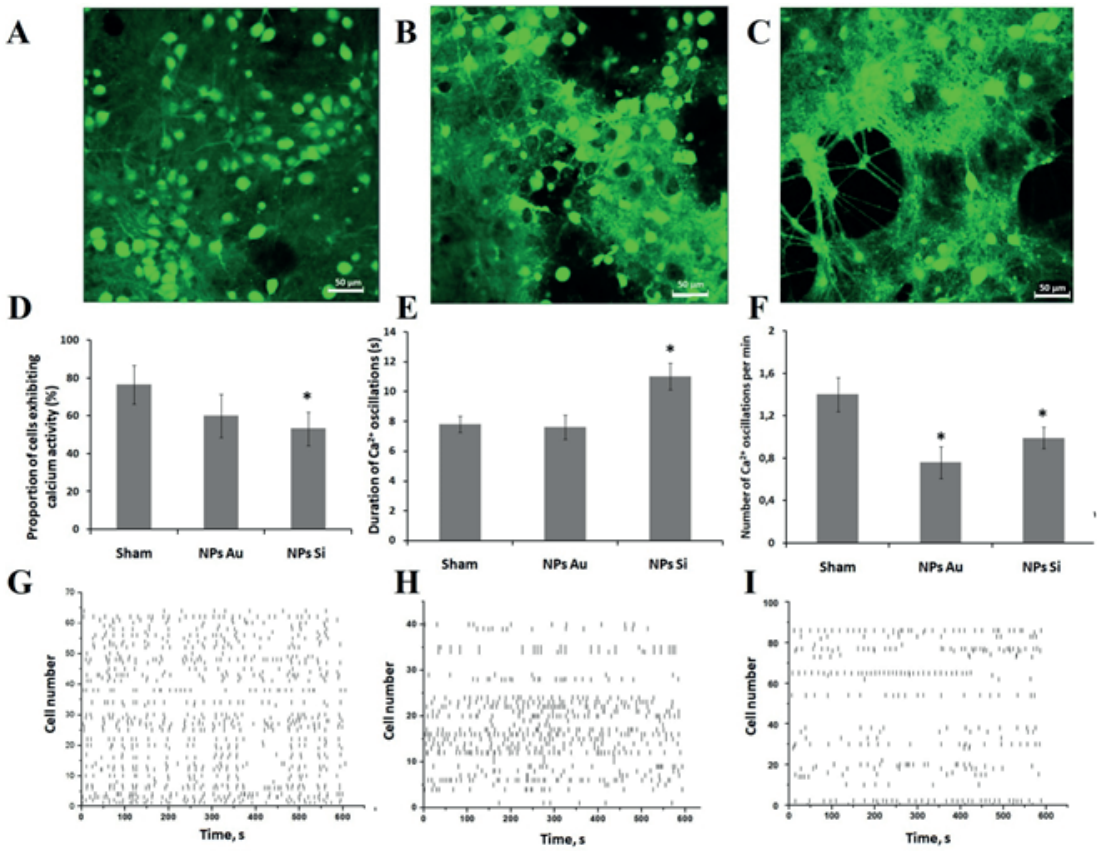

Figure 2: Analysis of spontaneous calcium activity in primary hippocampal cultures day 7 after NPs application. **(A-C)** representative confocal microscopy images of primary cultures stained by Oregon Green 488 BAPTA-1 AM: A – Sham, B – Au NPs, C – Si NPs. Scale bar – 50 μm; **(D-F)** the main parameters of spontaneous calcium activity in primary neuronal cultures. * - versus "Sham", $p < 0.05$, ANOVA, N = 6; **(G-I)** representative example of Ca^{2+} activity patterns. Ca^{2+} oscillations are plotted as dashes: G – Sham, H – Au NPs, I – Si NPs.

4. Conclusions

In this study the neurotoxicity properties of solid state nanoparticles prepared by femtosecond laser ablation of silicon and gold were investigated. It was shown that both types of NPs caused a dose-dependent cytotoxic effect on primary hippocampal cultures. Application of high concentrations of nanoparticles led to significant increase the number of dead cells in neuronal cultures. 1% replacement of the culture medium to the NPs resulted in the moderately toxic effect. Application of Au NPs exerted less toxic effect compared with Si NPs, manifesting in moderate influence on neuronal network calcium activity. It seems that larger size and higher solubility of Si NPs in water could be responsible for their larger neurotoxicity. Nevertheless, both types of NPs at desired concentration can be considered as possible theranostic agents for nervous system pathologies. Further investigations of the effect of NPs on different aspects

of neural network activity are required to determine all areas of their applications in neuroscience.

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