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FUNCTIONALIZED MESOPOROUS SILICA NANOPARTICLES: ANALYSIS OF NEUROTOXICITY AND HEAVY METAL DETOXICATION CAPABILITY

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Aim. Mesoporous silica nanoparticles (MSN) are perspective materials for biomedical applications and environmental management.

Methods. MSN functionalized with SH groups (MSN-SH) and SO_3H (MSN-SO_3H) were synthesized. The content of elements in particles was determined according to EDX analysis. Assessment of neurotoxicity was performed using nerve terminals (synaptosomes).

Results. The powder diffraction patterns of MSN-SH and MSN-SO_3H showed reflections in the low-angle region, which were characteristic of the MCM-41 structure. According to TEM data, the MSN-SH particles had an oval shape. Both MSN-SH and MSN-SO_3H (0.25–0.5 mg/ml) did not change the synaptosomal ambient level of excitatory neurotransmitter L-[^{3}H] glutamate. Neither MSN-SH nor MSN-SO_3H was able to detox the harmful influence of Cd^{2+}, Pb^{2+}, and Hg^{2+} in nerve terminals by mitigating Cd^{2+}/Pb^{2+}/Hg^{2+}-induced neurotoxicity.

Conclusions. MSN-SH and MSN-SO_3H did not demonstrate acute neurotoxicity signs, and so they are biocompatible. MSN-SH and MSN-SO_3H did not mitigate acute Cd^{2+}/Pb^{2+}/Hg^{2+}-induced neurotoxicity in nerve terminals, and so did not adsorb these metals in biological systems. Therefore, MSN-SH and MSN-SO_3H have the potential to be used as adsorbents/carriers of other substances than heavy metals in medicine and biotechnology. From an environmental point of view, mesoporous silica nanoparticles were safe and did not bind heavy metals, thereby not serving as their carriers to the organism during dust storms.

Key words: mesoporous silica nanoparticles, SH and SO_3H groups, neurotoxicity risk, glutamate, detoxication of heavy metals, Cd^{2+}, Pb^{2+}, Hg^{2+}, presynaptic brain nerve terminals.

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Mesoporous silica nanoparticles (MSN) are nanostructured materials that have gained substantial attention due to their unique properties and versatile applications in various fields. These nanoparticles have the structure of silica-based frameworks with a highly ordered mesoporous structure. Among different types of inorganic nanoparticles, MSNs are of interest due to their high sorption capacity and discrete nature, ability to form colloid solutions or penetrate cells (under certain conditions), along with the flexibility of their structure and properties. MSN possesses the extraordinary features, including a large tunable surface area and pore volume, high thermal and chemical stability, the ability to functionalize their surfaces, and a variety of particle sizes and morphologies [1]. These properties make them an ideal candidate for numerous applications, including drug delivery, catalysis, biosensing, and environmental remediation. In drug delivery, for instance, MSNs offer controlled and targeted release of therapeutic agents, owing to their ability to encapsulate drugs within their porous structure and release them under specific physiological conditions. Their surface can be functionalized with different ligands, polymers, or biomolecules, enabling targeted delivery and enhancing their specificity in biological systems. Beyond healthcare, MSNs are widely utilized in catalysis due to their ability to support active sites and facilitate efficient catalytic reactions.

An advantage of MSN over other inorganic nanoparticles is their safety. MSN exhibit biocompatibility and low toxicity, making them promising materials for biomedical applications. MSN has a lot of advantages compared to other inorganic nanoparticles in the biomedical field [1]. Clinical trials and clinical studies demonstrated the safety of silica nanoparticles in oral drug delivery, bioimaging, and PTT. The physiological toxicity of MSN is related to particle size, morphology, and structural composition. In particular, MSN can effectively load therapeutic drugs, including small molecules, genes, peptides, and proteins, through electrostatic adsorption or chemical bonding, nanomaterials, e.g., carbon dots, gold, and iron oxide nanoparticles, resulting in inorganic nanocomposites [1]. Mesoporous silica with 100–200 nm nanoparticle dimensions was considered the optimal choice, preventing fast release and toxic effect without aggregating on physiological fluids, blood capillaries, and alveoli [2].

Despite the fact that MSNs are generally considered biocompatible with minimal non-specific or adverse effects, several factors affect their biocompatibility, e.g., size, shape, and surface chemistry. At present, the MSN biocompatibility remains inconclusive [3, 4]. The interaction that appears between MSN and the cells, allowing their assimilation by the tumor cells, could result in unpredicted side effects in the healthy cells [5]. The surface properties may influence MSN biocompatibility. Cationic charges on the surface may produce considerable immune reaction and cytotoxicity in contrast with the neutral and anion counterparts [6–8]. A negative zeta potential was considered related to the serum opsonin. MSN biocompatibility was affected by the silanol groups at the outer layer, negatively interacting with the biological molecules and destroying their structure [2, 9]. So, despite numerous advantages, specific challenges remain in the development of MSN for biological applications. These include the need for precise control over particle size, surface functionalization, and biodegradability. Moreover, understanding the long-term safety and pharmacokinetics of MSN *in vivo* is critical for their clinical translation. Future research is focused on addressing these challenges and exploring new approaches, such as personalized medicine and organ-specific drug delivery.

In addition, from an environmental point of view, and especially during sand dust storms, silica accounts for the most substantial part of the mass of air pollution, particulate matter components. The WHO has revealed that sand dust particles can be mixed with industrial pollutants and induce inflammatory lung injury [10, 11]. In this context, it is not excluded that silica particles can be combined with toxic pollutants, e.g., heavy metals, in the environment.

In our previous study, amino-grafted mesoporous silica nanoparticles (MSN-NH_2) were synthesized by means of co-condensation of tetraethoxysilane and 3-aminopropyltriethoxysilane [12]. It was shown using rat cortex nerve terminals that MSN-NH_2 did not have excitotoxic signs and so were biocompatible, possessed weak antioxidant properties, and did not mitigate the excitotoxic effects of heavy metals Cd^{2+}, Pb^{2+}, and Hg^{2+}.

Taking into account above mentioned, the aims of this study were:

(1) to assess neurotoxicity of mesoporous silica nanoparticles, functionalized with SH groups (MSN-SH) and SO_3H (MSN-SO_3H);

(2) to analyze a capability of MSN-SH and MSN-SO_3H to adsorb heavy metals Cd^{2+}, Pb^{2+} and Hg^{2+} in biological model using isolated rat cortex nerve terminals (synaptosomes).

Materials and Methods

Synthesis of MSN-SH and MSN-SO_3H

MSN-SH and MSN-SO_3H were synthesized according to the published procedures [13, 14].

Characterization of MSN-SH and MSN-SO_3H

Energy dispersive X-ray analysis (EDX) was performed on the FEI Inspect S50 equipped with Apollo XL SDD EDAX. X-ray diffraction (XRD) analysis was performed on a Bruker D8 Advance diffractometer, CuKα, λ = 0.154 nm. Transmission electron microscopy (TEM) images of MSN-SH and MSN-SO_3H were obtained using a Selmi TEM125K microscope with 100 kV accelerating voltage.

Experiments using brain nerve terminals

Ethics

Experiments involving animals were carried out in accordance with the Guidelines of the European Community (2010/63/EU), Scientific Requirements and Research Protocols; Research Ethics Committees of the Declaration of Helsinki; and ARRIVE Guidelines for reporting experiments involving animals [15, 16], and Ukrainian laws and policies. The protocols of the experiments were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol # 1 from 10/01/2024). Wistar rats were kept at 22–23 °C in a quiet temperature-controlled room in the vivarium of the Palladin Institute of Biochemistry, NASU. Rats were supplied with dry food pellets and water *ad libitum*. The overall number of animals was 12.

Isolation of nerve terminals from the rat cortex (rat cortex synaptosomes)

The synaptosomes were isolated from the rat cortex. The rat cortex was homogenized in the following solution: sucrose 0.32 M; HEPES-NaOH 5 mM, pH 7.4; EDTA 0.2 mM. The isolation was carried out according to the method of Cotman with minor modifications [17]. The standard saline solution in the synaptosome experiments was as follows: NaCl 126 mM; KCl 5 mM; MgCl2 2.0 mM; NaH_2PO_4 1.0 mM; HEPES 20 mM, pH 7.4; and D-glucose 10 mM. Protein concentrations were recorded according to the method of Larson [18] using a spectrophotometer Shimadzu UV-1900i.

The ambient level of L-[^{3}H] glutamate in synaptosomes

The synaptosome suspension (2 mg of protein/ml) was preincubated at 37 °C for 10 min; after that, the suspension was loaded with L-[^{3}H] glutamate (1 μCi/ml) at 37 °C for 10 min. The synaptosome suspension was diluted with 10 volumes of the standard saline solution, and centrifuged at10,000×g for 20 s. The pellets were suspended in the standard saline solution (1 mg protein/ml). The ambient L-[^{3}H] glutamate level was monitored in 125 μl aliquots with a concentration of 0.5 mg of protein/ml. The aliquots were preincubated for 8 min and after that Cd^{2+} (300 μM $CdCl_2$), Pb^{2+} (2.5 mM Pb acetate (PbAc)), Hg^{2+} (10 μM $HgCl_2$), MSN-SH (0.1–1.0 mg/ml), MSN-SO_3H (0.1–1.0 mg/ml) *per se* or their mixtures (after preliminary incubation in water for 30 min) were added. The suspension was incubated at 37 °C for 0 and 6 min. Then the suspension was centrifuged at 10,000 × *g* for 20 s. The value of the ambient L-[^{3}H] glutamate level was recorded in the supernatant and pellets (preliminary treated with SDS, 100 ml of 10% SDS stock solution) using the liquid scintillation counting with Sigma-Fluor® High Performance LSC Cocktail and the liquid scintillation counter Hidex 600SL (Finland). The experimental data were from "n" independent experiments with different synaptosome preparations.

Statistical analysis

The experimental data were expressed as the mean ± S.E.M. of *n* independent experiments. One-way and two-way ANOVA were applied; the accepted significance level was $P < 0.05$. Two-way ANOVA followed by Tukey's post hoc test was used to assess the interactions between MSN-SH/ MSN-SO_3H and Cd^{2+}, Pb^{2+}, and Hg^{2+} (MSN-SH/ MSN-SO_3H treatment and Cd^{2+}/Pb^{2+}/Hg^{2+} treatment were the independent factors).

Materials

HEPES, EGTA, EDTA, salts of the analytical grade, High Performance LSC Cocktail were obtained from Sigma, USA; L-[^{3}H] glutamate and [^{3}H] GABA were from Revvity, Waltham, MA, USA. Tetraethyl orthosilicate (TEOS), 3-mercaptopropyl triethoxysilane (MPTES), and cetyltrimethylammonium bromide (CTAB) of the analytical grade were purchased from Ukrorgsyntez Ltd, Ukraine.

Results and Discussion

Characterization of MSN-SH and MSN-SO_3H

The synthesis of MSN-SH was carried out according to a published method by co-condensation of TEOS and MPTES in the presence of CTAB [13]. In this case, 3-mercaptopropyl triethoxysilane was the source of SH-groups, which were linked to MSNs via propylene bridges. Incorporation of 3-mercaptopropyl moieties into MSNs was achieved simultaneously with the formation of the MSN itself, in contrast to post-synthetic modification. The procedure usually results in the formation of porous material of MCM-41 type; in this case, CTAB serves as a template for the formation of cylindrical pores with *a ca*. 3 nm diameter. MSN-SO_3H was synthesized by oxidation of MSN-SH with H_2O_2 according to the reported procedure [14]. The elements content in the obtained samples was determined by EDX analysis. The content of sulfur was 2.7% and 2.2% for MSN-SH and MSN-SO_3H, respectively (Table 1). Lower content of S in MSN-SO_3H is consistent with "addition" of oxygen upon oxidation of SH to SO_3H; however, partial loss of sulfur due to complete oxidation can't be excluded.

The powder diffraction patterns of MSN-SH and MSN-SO_3H contain reflections in the low-angle region, which are characteristic of the MCM-41 structure (Fig. 1). The most intense reflection was observed at $2\theta = 2.2°$ and corresponded to diffraction from (100) planes. However, the intensity of the reflections at $2\theta = 2.2$ and 3.9° (diffraction from (110) and (200) planes) was lower compared to bulk samples of pure MCM-41; such a decrease may be due to the partial disorder of the MCM-41 structure caused by the incorporation of functional groups in the silica matrix [19].

Table 1

Content of elements in functionalized MSNs according to EDX analysis

Element	MSN-SH	MSN-SO_3H
Wt(Si), %	23.2	21
Wt(O), %	48.3	49.5
Wt(C), %	25.8	27.3
Wt(S), %	2.7	2.2

According to TEM data, the MSN-SH particles have an oval shape with a typical length of 500 nm and a typical width of 250 nm (Fig. 2, *A*). The MSN-SO_3H sample contains predominantly oval-shaped particles (Fig. 2, *B*). The width and length of the MSN-SO_3H particles are 250 and 650 nm, respectively. Single bean-shaped particles are present in both samples (Fig. 2, *A, B*).

Analysis of acute neurotoxicity risk of MSN-SH and MSN-SO_3H in rat brain nerve terminals

Acute neurotoxicity risk assessment of MSN-SH and MSN-SO_3H was carried out in the nerve terminal preparations, measuring their influence on the ambient level of L-[^{3}H] glutamate, the key excitatory neurotransmitter in the central nervous system. The change in the ambient level of L-[^{3}H] glutamate reflects possible disintegration of the plasma membrane of the nerve terminals and impaired transportation of neurotransmitters. It was found that MSN-SH within the effective concentration range 0.25–0.5 mg/ml (mass of MSNs suspended in liquid media) did not change the ambient level of L-[^{3}H] glutamate in the nerve terminal preparations. An increase in the MSN-SH concentration up to 1.0 mg/ml

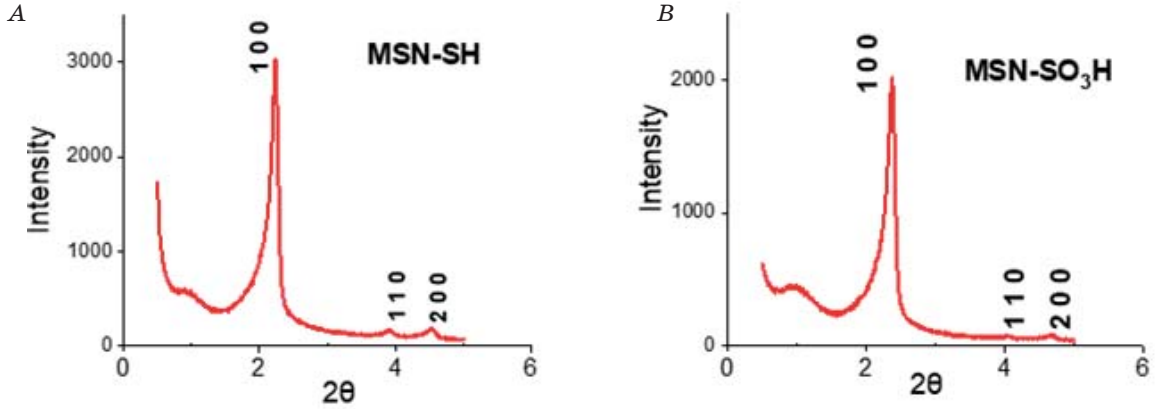

Fig. 1. **Powder diffraction patterns of MSN-SH and MSN-SO_3H**

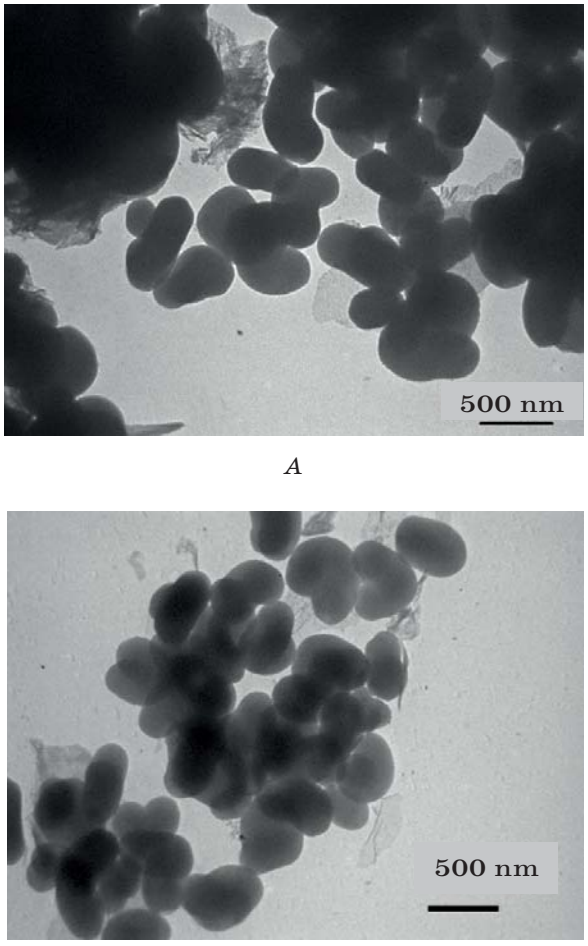

Fig. 2. **TEM images of MSN-SH (*A*) and MSN-SO_3H (*B*)**

resulted in an increased ambient level of L-[^{3}H] glutamate in the nerve terminal preparations (Table 2). MSN-SO_3H remained inert regarding the modulation of the ambient level of L-[^{3}H] glutamate in the nerve terminal preparations within the concentration range 0.25–1.0 mg/ml (Table 3). Therefore, it can be concluded that MSN-SH and MSN-SO_3H were biocompatible and did not demonstrate acute neurotoxic signs at concentrations less than 0.5 mg/ml (Tables 2, 3).

Analysis of the capability of MSN-SH and MSN-SO_3H to detox heavy metals in rat brain nerve terminals

In the following sets of experiments, the capability of MSN-SH and MSN-SO_3H to mitigate acute heavy metal-induced neurotoxicity was analysed using our recently developed model [20]. It was demonstrated that neither MSN-SH nor MSN-SO_3H was capable of reducing the harmful effects of heavy metals Cd^{2+} (300 μM $CdCl_2$), Pb^{2+} (2.5 mM Pb acetate (PbAc)), Hg^{2+} (10 μM $HgCl_2$) in rat brain nerve terminals (Fig. 3).

The experimental data were analyzed by two-way ANOVA followed by Tukey's post hoc test. It was revealed no interaction between Cd^{2+} and MSN-SH [$F_{(1,44)} = 3.11$; $P = 0.08$; $n = 12$], between Pb^{2+} and MSN-SH [$F_{(1,44)} = 2.67$; $P = 0.11$; $n = 12$] and between Hg^{2+} and MSN-SH [$F_{(1,44)} = 2.47$; $P = 0.12$; $n = 12$].

Two-way ANOVA revealed no interaction between Cd^{2+} and MSN-SO_3H [$F_{(1,44)} = 2.99$; $P = 0.09$; $n = 12$], between Pb^{2+} and MSN-SO_3H [$F_{(1,44)} = 0.52$; $P = 0.47$; $n = 12$] and between Hg^{2+} and MSN-SO_3H [$F_{(1,44)} = 2.63$; $P = 0.11$; $n = 12$].

Therefore, both MSN-SH and MSN-SO_3H did not change the ambient L-[^{3}H] glutamate level in the rat brain nerve terminals at concentrations less than 0.5 mg/ml. Taking into account the above fact, it can be suggested that functionalized MSN, MSN-SH, and MSN-SO_3H, due to their biocompatibility, represent a versatile platform with immense potential in diverse scientific domains. Their unique structural and functional attributes continue to drive innovation and research in materials science, nanotechnology, and related fields. In particular, the tunable properties of MSN make them ideal candidates for numerous biological applications, including drug delivery, bioimaging, biosensing, and tissue engineering. MSN has the following key advantages in biological applications. The mesoporous structure, high surface area, and pore volume allow for the encapsulation and delivery of a wide variety of biomolecules, including drugs, proteins, genes, and peptides. MSNs are highly versatile in surface chemistry, enabling functionalization with targeting ligands, polymers, and bioactive molecules to enhance specificity and therapeutic efficacy.

The porous structure of MSN enables sustained release of therapeutic agents, while surface modifications allow for targeted delivery to specific cells or tissues. MSNs are widely used for the controlled release of anticancer drugs, antibiotics, and other therapeutic agents. For example, doxorubicin-loaded MSN have been shown to enhance drug efficacy while minimizing side effects through targeted delivery [21]. MSN can be engineered to carry imaging agents (e.g., fluorescent dyes, quantum dots, magnetic nanoparticles) alongside therapeutic molecules, enabling simultaneous diagnosis and treatment (theranostics). Also, the tunable properties of

Table 2

The ambient level of L-[^{3}H] glutamate in nerve terminal preparations in control and in the presence of MSN-SH

Samples description	The ambient level of L-[^{3}H] glutamate in nerve terminal preparations (% of total accumulated label)	F; *P*-value
Control	15.92 ± 0.36	
MSN-SH; 0.1 mg/ml	16.16 ± 0.40; n.s.	$F_{(1.22)} = 0.23$; $P = 0.64$
MSN-SH; 0.5 mg/ml	16.86 ± 0.51; n.s.	$F_{(1.22)} = 2.53$; $P = 0.13$
MSN-SH; 1.0 mg/ml	18.63 ± 0.27***	$F_{(1.22)} = 40.21$; $P < 0.001$

Note. Data are the mean ± SEM. ***, $P < 0.001$; n.s., no significant differences as compared to the control, $n = 12$

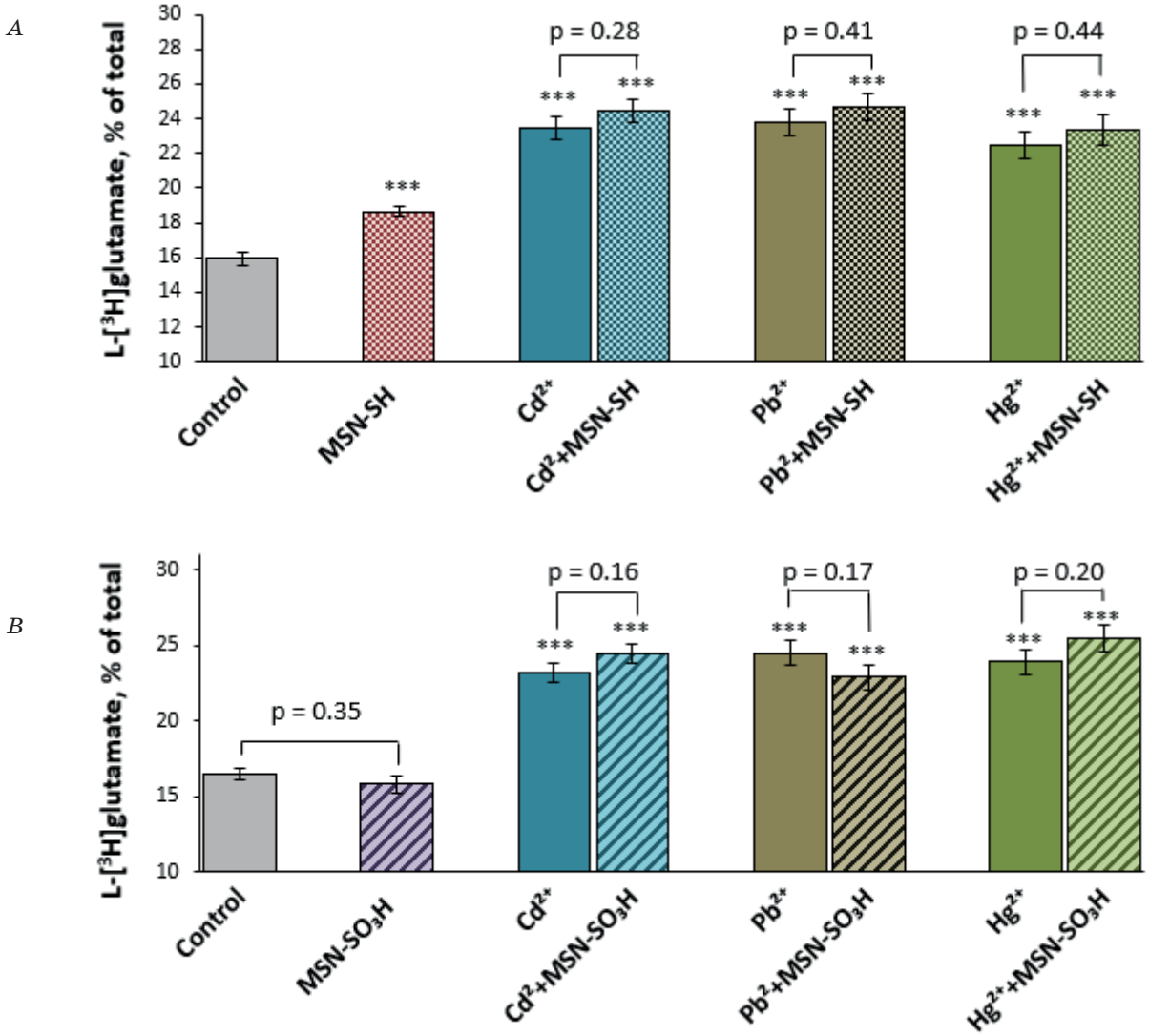

Fig. 3. **Effects of MSN-SH (*A*) and MSN-SO_3H (*B*) at a concentration of 1.0 mg/ml on Cd^{2+}/Pb^{2+}/Hg^{2+}-induced increase in the ambient level of L-[^{3}H] glutamate**

Note. Cd^{2+} (300 μM $CdCl_2$), Pb^{2+} (2.5 mM Pb acetate (PbAc)), Hg^{2+} (10 μM $HgCl_2$). Data are the mean ± SEM. ***, $P < 0.001$; as compared to the control; $n = 12$.

Table 3

The ambient level of L-[^{3}H] glutamate in nerve terminal preparations in control and in the presence of MSN-SO_3H

Samples description	The ambient level of L-[^{3}H] glutamate in nerve terminal preparations (% of total accumulated label)	F; P-value
Control	16.43 ± 0.38	
MSN-SO_3H; 0.1 mg/ml	15.79 ± 0.61; n.s.	F(1.22) = 0.90; $P = 0.35$
MSN-SO_3H; 0.5 mg/ml	16.62 ± 0.42; n.s.	F(1.22) = 0.12; $P = 0.74$
MSN-SO_3H; 1.0 mg/ml	16.10 ± 0.48; n.s.	F(1.22) = 0.33; $P = 0.57$

Note. Data are the mean ± SEM. n.s., no significant differences as compared to the control, $n = 12$

MSN make them suitable scaffolds for tissue regeneration and engineering applications.

Conclusions

Both nanoparticles MSN-SH and MSN-SO_3H did not demonstrate acute neurotoxicity signs and high biocompatibility. MSN-SH and MSN-SO_3H did not mitigate acute Cd^{2+}/Pb^{2+}/Hg^{2+}-induced neurotoxicity in nerve terminals, and so did not adsorb these metals in biological systems. Therefore, MSN-SH and MSN-SO_3H have the potential to be used as safe adsorbents/carriers of substances other than heavy metals in biotechnology and medicine.

From an environmental point of view, it was concluded that mesoporous silica nanoparticles (even functionalized) were safe and did not bind heavy metals, thereby not serving as their carriers to the organism during dust storms.

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Competing interests

The authors declare no financial and non-financial competing interests exist.

Author Contributions

S. O. Sotnik, I. M. Pavliei, O. O. Pariiska — Chemical methodology, Investigation, Data curation, Formal analysis; N. G. Pozdnyakova, N. V. Krisanova — Biochemical methodology, Investigation, Data curation, Formal analysis, Writing — review & editing, Writing — original draft; A. O. Pastukhov, M. V. Dudarenko, R. V. Sivko, L. M. Kalynovska, M. M. Driuk, I. D. Panas — Methodology, Investigation; S. V. Kolotilov — Conceptualization, Data curation, Formal analysis, Writing — review & editing, Writing — original draft; T. A. Borisova — Conceptualization, Project administration, Funding acquisition, Data curation, Formal analysis, Writing — review & editing, Writing — original draft.

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ФУНКЦІОНАЛІЗОВАНІ НАНОЧАСТИНКИ МЕЗОПОРИСТОГО КРЕМНЕЗЕМУ: АНАЛІЗ НЕЙРОТОКСИЧНОСТІ ТА ЗДАТНОСТІ ДО ДЕТОКСИКАЦІЇ ВАЖКИХ МЕТАЛІВ

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Мета. Мезопористі наночастинки кремнезему (MSN) є перспективними матеріалами для біомедичного застосування та екологічного менеджменту навколишнього середовища.

Методи. Були синтезовані MSN, функціоналізовані групами –SH (MSN-SH) та -SO_3H (MSN-SO_3H). Вміст елементів частинок було визначено за допомогою EDX-аналізу. Оцінку нейротоксичності проводили з використанням нервових закінчень (синаптосом).

Результати. На дифрактограмах порошків MSN-SH та MSN-SO_3H наявні відбиття в малокутовій області, що характерно для структури MCM-41. Згідно з даними TEM, частинки MSN-SH мають овальну форму. Як MSN-SH, так і MSN-SO_3H (0,25–0,5 мг/мл) не змінювали позаклітинний рівень збуджувального нейромедіатору L-[^{3}H] глутамату в синаптосомах. Було виявлено, що MSN-SH та MSN-SO_3H не здатні пом'якшувати нейротоксичний вплив, викликаний Cd^{2+}, Pb^{2+} та Hg^{2+} у нервових закінченнях.

Висновки. MSN-SH та MSN-SO_3H не продемонстрували ознаки гострої нейротоксичності та мали високу біосумісність. MSN-SH та MSN-SO_3H не пом'якшували гостру нейротоксичність, індуковану Cd^{2+}/Pb^{2+}/Hg^{2+}, в нервових закінченнях, і тому не адсорбували ці метали в біологічних системах. Отже, MSN-SH та MSN-SO_3H мають перспективи для використання як адсорбенти/носії інших речовин, окрім важких металів, у медицині та біотехнології. З екологічної точки зору було зроблено висновок, що мезопористі наночастинки кремнезему є безпечними та не зв'язують важкі метали, тим самим не слугуючи їх переносниками до організму під час пилових бур.

Ключові слова: мезопористі наночастинки кремнезему, SH та SO_3H групи, ризик нейротоксичності, глутамат, детоксикація важких металів, Cd^{2+}, Pb^{2+}; Hg^{2+}, пресинаптичні нервові закінчення мозку.