

Toxicity and bioresorption of magnetite and manganese-zinc ferrite nanoparticles with oleic acid-based and amino-silica coatings

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Abstract

Introduction: The prospects for using magnetic hyperthermia in cancer therapy are largely determined by the properties of the magnetic nanoparticles (MNPs), including heating efficiency, biosafety, and bioresorption capacity. In this study, we performed *in vivo* assessment of the toxicity and bioresorption of Fe₃O₄- and Zn_{0.2}Mn_{0.8}Fe₂O₄-based MNPs stabilized with oleic acid/sodium oleate (OA) or amino-functionalized silicon dioxide (SiO₂-NH₂).

Materials and Methods: Four types of MNPs were synthesized: Fe₃O₄@OA, Zn_{0.2}Mn_{0.8}Fe₂O₄@OA, Fe₃O₄@SiO₂-NH₂ and Zn_{0.2}Mn_{0.8}Fe₂O₄@SiO₂-NH₂. The particles were characterized using DLS, TEM and vibrating sample magnetometry. Acute toxicity was evaluated in BALB/c mice after a single intraperitoneal or intramuscular administration. Biosafety and bioresorption after intramuscular administration were assessed on days 2, 14, and 90, using hematological and biochemical blood tests, histological examination, sample magnetization, and total iron content in tissues.

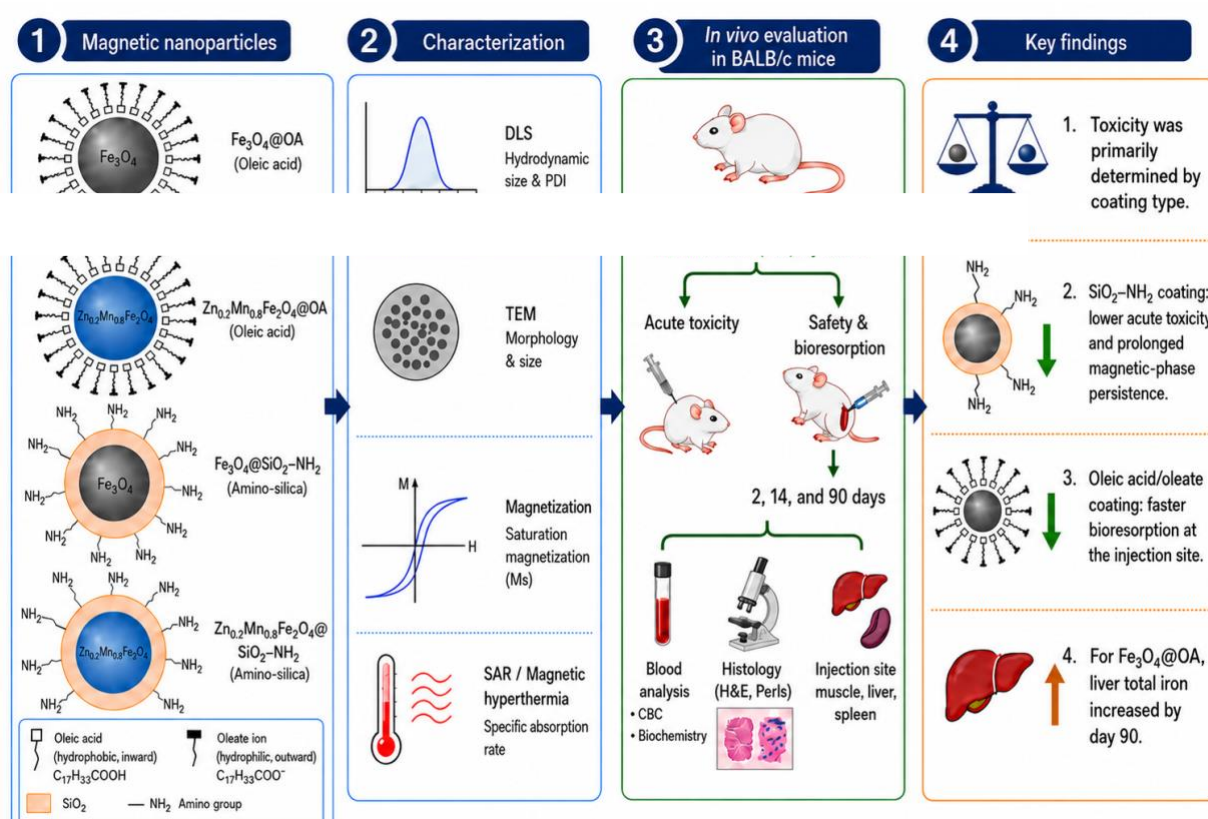
Results and Discussion: After intraperitoneal administration, the LD₅₀ values were 904 mg/kg for Fe₃O₄@OA, 1145 mg/kg for Zn_{0.2}Mn_{0.8}Fe₂O₄@OA, and 2955 mg/kg for Zn_{0.2}Mn_{0.8}Fe₂O₄@SiO₂-NH₂, whereas the LD₅₀ for Fe₃O₄@SiO₂-NH₂ was not reached. After intramuscular administration at a dose of 1020 mg/kg, neither deaths nor pronounced systemic toxicity was observed. By day 90, MNPs with an oleic acid/sodium oleate-based coating showed a decrease in magnetic signal and total iron content at the injection site, whereas SiO₂-NH₂-coated nanoparticles remained in muscle tissue.

Conclusion: The study showed that the chemical nature of the coating is one of the main factors determining the toxicity and bioresorption of Fe₃O₄ and Zn_{0.2}Mn_{0.8}Fe₂O₄ MNPs. Particles with a SiO₂-NH₂ coating were characterized by lower acute toxicity after intraperitoneal administration compared with MNPs coated with oleic acid/sodium oleate. After intramuscular administration at a dose of 1020 mg/kg, neither mortality nor pronounced signs of systemic toxicity were detected for any type of MNPs. It was also shown that the coating type affects the possibility and rate of bioresorption: after intramuscular administration at a dose of 256 mg/kg, MNPs with a SiO₂-NH₂ coating remained at the injection site for 90 days, whereas the oleic acid/sodium oleate-based coating promoted bioresorption.



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Graphical Abstract



Keywords

acute toxicity; bioresorption; magnetic hyperthermia; magnetic nanoparticles; oleic acid

Introduction

The development of nanotechnology has expanded the possibilities for creating biomedical materials for tumor diagnosis and therapy. Nanostructured systems are considered a platform for targeted delivery of therapeutic agents, increasing the local efficacy of treatment, and reducing systemic toxicity (Brodovskaya et al. 2023; Nikolaeva et al. 2025). One direction of this approach is the use of magnetic nanoparticles (MNPs) for local heating of tumor tissue, namely magnetic hyperthermia (MHT) (Baldea et al. 2025). It is based on the ability of MNPs to convert the energy of an alternating magnetic field (AMF) into heat (Fatima et al. 2021). Depending on the heating intensity, MHT can achieve either direct ablation at temperatures above 46 °C, causing necrosis of tumor tissues, or moderate hyperthermia in the range of 42–45 °C, increasing cell sensitivity to chemotherapy and radiotherapy (Spirou et al. 2018; Ribeiro et al. 2022).

Critical parameters determining the applicability of MNPs in magnetic hyperthermia are their biocompatibility and specific absorption rate (SAR), an index reflecting the efficiency with which nanoparticles convert AMF energy into heat (Vilas-Boas et al. 2020). To date, the most extensively studied objects are iron oxide nanoparticles, primarily magnetite (Fe_3O_4), which has proven biocompatibility (Ganapathe et al. 2020). A fundamental contribution to the development of clinical MHT using magnetic nanoparticles was made by the studies by A. Jordan and co-authors, who applied MNPs in tumor therapy (Jordan et al. 1999; Johannsen et al. 2005). A clinical example of this approach is NanoTherm AS1 (MagForce Nanotechnologies), a highly concentrated aqueous suspension of superparamagnetic Fe_3O_4 magnetite nanoparticles with an aminosilane coating, intended for intratumoral administration (Maier-Hauff et al. 2011). However, recent studies show that

magnetite is not always the optimal material in terms of SAR (Gavilán et al. 2021). Ferrite nanoparticles MeFe_2O_4 ($\text{Me} = \text{Zn}^{2+}, \text{Mn}^{2+}, \text{Ni}^{2+}, \text{Cu}^{2+}, \text{Co}^{2+}$) may serve as an alternative, since their magnetic properties can be purposefully optimized by incorporating other metals into their composition. In particular, manganese-zinc ferrites, such as $\text{Zn}_{0.2}\text{Mn}_{0.8}\text{Fe}_2\text{O}_4$, demonstrate enhanced heating efficiency under AMF exposure due to an optimal combination of composition, particle size, and nonlinear magnetic response (Nandwana et al. 2016; Liu et al. 2022a). In addition, Mn-containing ferrites are considered promising theranostic agents because they can provide pronounced T1 contrast in MRI, which may allow magnetic hyperthermia to be combined with tumor tissue imaging (Islam et al. 2020). Despite the physical advantages of Zn-Mn ferrites, their biosafety remains less studied than their magnetic and magnetothermal characteristics. A substantial part of the available studies is limited to cytotoxicity assessment in cell cultures (Iacovita et al. 2019; Slavů et al. 2021). However, *in vitro* tests do not allow full prediction of systemic toxicity, including the effects of nanoparticles on blood parameters and the functional state of organs (Ajday et al. 2018; Awashra and Mlynarz 2023). For administered MNPs, the processes occurring after local administration are also important: particle distribution in tissue, their bioresorption, and the possible involvement of nanomaterial components in systemic metabolism.

Therefore, another important aspect is the chemical nature of the nanoparticle coating, since it may affect not only the colloidal stability of MNPs but also their interaction with the biological environment, local distribution, retention in tissue, and subsequent bioresorption. In the commercial NanoTherm formulation, Fe_3O_4 magnetite particles are coated with amino-functionalized silicon dioxide ($\text{SiO}_2\text{-NH}_2$). This coating provides long-term stability of nanoparticles in tumor tissue; however, their prolonged presence may be associated with the risk of local inflammatory reactions and, in some cases, the need for subsequent surgical removal of the nanoparticles (Grauer et al. 2019). In this regard, the use of bioorganic substances, such as oleic acid, is promising, as they may provide biocompatibility and subsequent nanoparticle bioresorption. In addition, oleic acid effectively stabilizes MNPs, prevents their aggregation, and enables the preparation of highly concentrated suspensions suitable for magnetic hyperthermia (Soares et al. 2016; Ong et al. 2020, Kulikov et al. 2022; Liu et al. 2023). Thus, despite the promising magnetothermal properties of Zn-Mn ferrites, their toxicity and bioresorption after local administration remain insufficiently studied.

The aim of this study was to comparatively assess the safety and bioresorption of four types of MNPs differing in magnetic core composition and coating type: $\text{Fe}_3\text{O}_4@\text{OA}$, $\text{Zn}_{0.2}\text{Mn}_{0.8}\text{Fe}_2\text{O}_4@\text{OA}$, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$, and $\text{Zn}_{0.2}\text{Mn}_{0.8}\text{Fe}_2\text{O}_4@\text{SiO}_2\text{-NH}_2$. To the best of our knowledge, this study is the first to compare, within a single *in vivo* design, the effects of magnetic core composition and coating chemistry on the safety and bioresorption of Fe_3O_4 and $\text{Zn}_{0.2}\text{Mn}_{0.8}\text{Fe}_2\text{O}_4$ -based MNPs.

Materials and Methods

Reagents

Deionized water was prepared using a Nova U water purification system (Innova Bio-Meditech, China). Iron (II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), zinc chloride (ZnCl_2), manganese (II) chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), oleic acid ($\text{C}_{18}\text{H}_{34}\text{O}_2$), ammonium hydroxide (25% NH_4OH), sodium hydroxide (NaOH), and hydrochloric acid (37% HCl) were purchased from Vekton, Russia. N-(3-(Trimethoxysilyl)propyl) ethylenediamine was purchased from Sigma-Aldrich, USA. Zoletil was purchased from Virbac, France, and Rometar – from Bioveta, Czech Republic. All reagents used in this study were of at least chemically pure grade.

Synthesis and characterization of magnetic nanoparticles

Fe_3O_4 and $\text{Zn}_{0.2}\text{Mn}_{0.8}\text{Fe}_2\text{O}_4$ magnetic nanoparticles were synthesized by co-precipitation in an alkaline medium (Laurent et al. 2008). To obtain Fe_3O_4 , 1.35 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and 3.5 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were dissolved in 50 mL of deionized water preheated to 100 °C, after which 9 mL of NH_4OH was added in one step, and the mixture was incubated for 1 h at 90 °C. $\text{Zn}_{0.2}\text{Mn}_{0.8}\text{Fe}_2\text{O}_4$ was synthesized by dissolving 0.1768 g of ZnCl_2 , 1.03 g of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ and 3.5 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 35 mL of water preheated to 120 °C followed by rapid addition to 50 mL of 1.25 M NaOH solution and further incubation for 2 h at 180 °C in a closed system. The obtained MNPs were purified by magnetic decantation for 10 cycles and washed with a water-ethanol solution to neutral pH, with ultrasonic treatment at 500 W for 10 min after every two washing cycles.

To obtain nanoparticles stabilized with oleic acid and sodium oleate ($\text{Fe}_3\text{O}_4@\text{OA}$ and $\text{Zn}_{0.2}\text{Mn}_{0.8}\text{Fe}_2\text{O}_4@\text{OA}$), a mixture of 0.16 g NaOH , 2 mL water, 3 mL ethanol, and 1.75 mL

oleic acid was added to the purified MNPs. The mixture was then incubated for 2 h at 150 °C and subjected to ultrasonic treatment at 500 W for 10 min every 30 min. Large aggregates and unstabilized particles were then removed by centrifugation at 12,000 rpm for 15 min, and the resulting nanoparticle supernatant was concentrated by evaporation to 154 mg/mL, calculated as magnetic material. The SiO₂-NH₂ shell (Fe₃O₄@SiO₂-NH₂, Zn_{0.2}Mn_{0.8}Fe₂O₄@SiO₂-NH₂) was formed using a modified aminosilanization method, similar to approaches used for the development of NanoTherm AS1 (MagForce AG), by hydrolysis of the precursor N-(3-(Trimethoxysilyl) propyl) ethylenediamine (Waldoefer and Jordan 2016). Under stirring at 900 rpm, 520 µL of the precursor and 550 µL of 37% HCl were added dropwise to the purified MNPs to maintain pH 2.5–3.0. After ultrasonic treatment at 150 W for 24 h and dialysis using a cellulose membrane with a molecular weight cut-off of 12–14 kDa for 72 h, the nanoparticle suspension was centrifuged at 2000 rpm for 10 min and evaporated to a concentration of 154 mg/mL, calculated as magnetic material.

As a result, four highly concentrated suspensions of magnetic nanoparticles were obtained: Fe₃O₄@OA (Fe@OA); Zn_{0.2}Mn_{0.8}Fe₂O₄@OA (ZnMn@OA); Fe₃O₄@SiO₂-NH₂ (Fe@SiO₂); Zn_{0.2}Mn_{0.8}Fe₂O₄@SiO₂-NH₂ (ZnMn@SiO₂).

Characterization of the obtained nanoparticles included determination of hydrodynamic size by dynamic light scattering (DLS), measurement of ζ-potential, analysis of morphology and size by transmission electron microscopy (TEM), assessment of magnetic properties by vibrating sample magnetometry (VSM), and investigation of magnetocaloric properties in an alternating magnetic field. DLS and ζ-potential measurements were performed using NANO-flex and STABINO instruments (Microtrac, Germany). The polydispersity index (PDI) from DLS data was calculated using the formula $PDI = \sigma^2 / d^2$, where d^2 is the weighted mean of the size distribution and σ^2 is the weighted mean squared deviation (Bhattacharjee 2016). TEM was performed using a Tecnai Osiris microscope (FEI, USA), and magnetization was measured using an EZ11 vibrating sample magnetometer (Microsense, USA). The nanoparticle concentration was determined photocolometrically by the absorbance of the Fe³⁺ complex with sulfosalicylic acid at pH 9–12 and $\lambda = 430$ nm using a Varioscan Lux instrument (Thermo Scientific, USA) (Pozdnyakov et al. 2006). Magnetocaloric properties were assessed under exposure to an alternating magnetic field with a frequency of 100 kHz and an amplitude of 8 kA/m. Temperature was recorded using a SeekThermal Compact PRO thermal imager, and SAR was calculated from the initial linear region of the temperature curve according to a previously described method (Pimentel et al. 2018).

According to TEM data, all synthesized MNPs had spherical or near-spherical morphology. The mean TEM diameters were 9 ± 1 nm for Fe@OA, 9 ± 2 nm for ZnMn@OA, 9 ± 2 nm for Fe@SiO₂, and 8 ± 2 nm for ZnMn@SiO₂. The mean hydrodynamic diameters were 29 ± 10 , 31 ± 9 , 37 ± 17 , and 35 ± 16 nm, respectively. The corresponding PDI values were 0.107, 0.089, 0.205, and 0.151. The ζ-potentials were -65 ± 3 , -67 ± 5 , $+46 \pm 4$, and $+41 \pm 4$ mV, respectively. The saturation magnetization values were 60, 76, 58, and 72 emu/g, and the SAR values were 12.6, 15.4, 11.2, and 13.2 W/g for Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂, respectively.

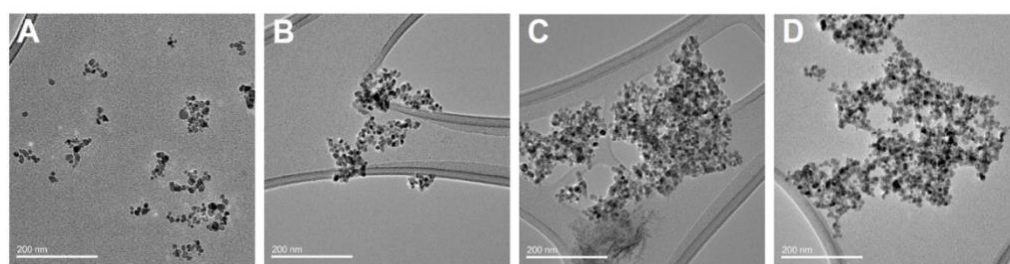


Figure 1. TEM images of magnetic nanoparticles. Note: **A** – Fe@OA; **B** – ZnMn@OA; **C** – Fe@SiO₂; **D** – ZnMn@SiO₂.

In vivo toxicity study

Animals

The study included male BALB/c laboratory mice aged 40–45 days with weight of 18–24 g. The animals were obtained from the Stolbovaya Breeding Facility of the Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency of Russia. The animals were housed under standard laboratory conditions: temperature of 20–22 °C, relative humidity of 40–60 %, and a 12 h light/dark cycle, with free access to food and filtered water. All

procedures were performed in strict accordance with European Directive 2010/63/EU on the protection of animals used for scientific purposes. The study protocol was reviewed and approved by the Biomedical Research Ethics Committee of National Research Ogarev Mordovia State University, approval protocol No. 1 dated 30 October 2025.

Acute toxicity testing in mice

Acute toxicity of magnetic nanoparticles (Fe@OA, ZnMn@OA, Fe@SiO₂, ZnMn@SiO₂) was assessed after a single intramuscular (i.m.) or intraperitoneal (i.p.) administration to mice (Gajdosíková et al. 2006). To assess acute toxicity after a single intraperitoneal administration of MNP suspensions, seven groups of animals were formed (n = 6). Nanoparticle suspensions (154 mg/mL) were administered at increasing doses: 274, 678, 1024, 1823, 2984, 4057, and 4896 mg nanoparticles/kg. For intramuscular administration, four groups were formed (n = 6 per group). The nanoparticle suspension (0.2 mL; dose 1020 mg/kg) was administered once into the anterior and posterior muscle groups of one thigh. The dose corresponded to the maximum possible volume for intramuscular administration. Since no deaths were observed at this dose, lower doses were not studied. All mice were observed for 14 days after administration, with monitoring of their general physical condition, including respiration, feeding, and activity. In addition, body weight was recorded every 48 h and expressed as a percentage of the baseline value. Toxicity parameters were calculated by probit analysis, and survival analysis was performed using the Kaplan–Meier method in Prism 8.0.1 software (GraphPad Software Inc., USA).

Histopathological study and blood analysis

Blood analysis and histological examination were performed after a single intramuscular administration of nanoparticle suspensions at doses of 1020 and 256 mg/kg, limited by the minimum and maximum injection volumes of 0.05 and 0.2 mL, respectively. The animals were anesthetized with Zoletil and Rometar and then euthanized by cervical dislocation on days 2, 14, and 90 after administration (n = 6 per group and time point). Blood was collected into heparin-containing tubes. Hematological analysis was performed using a URIT-5160 analyzer (Medical Electronic Group, China), and biochemical analysis of blood plasma was performed using a FUJI DRI-CHEM 4000ie analyzer (FUJIFILM, Japan).

For histological examination, organs (lung, spleen, liver, and kidneys) and muscle tissue were fixed in 10% neutral formalin. The samples were embedded in paraffin, and 7–8 µm-thick sections were prepared using a PFM Rotary 3003 (PMF Medical, Germany) rotary microtome. Deparaffinized sections were subjected to the Perls' Prussian blue reaction to detect iron in tissues, and background staining was performed with hematoxylin and eosin (Meguro et al. 2007). Histological specimens were examined using a Nikon Eclipse NI-SS (Nikon Corporation, Japan) light microscope. Microphotographs were obtained using a Nikon DS-Fi2 (Nikon Corporation, Japan) camera attachment at ×40, ×100, and ×400 magnification.

Bioresorption study of magnetic nanoparticles

Bioresorption of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles was assessed by magnetization and total iron content in muscle tissue samples from the MNP injection site, as well as in the liver and spleen, organs involved in iron deposition and nanomaterial metabolism (Yaremenko et al. 2022). Magnetization was determined by vibrating sample magnetometry using an EZ11 magnetometer (Microsense Inc., USA) at 25 °C, and Fe³⁺ content was determined spectrophotometrically using a Varioskan LUX instrument (Thermo Scientific, USA) (Pozdnyakov et al. 2006).

The animals were randomized, and then four groups of three animals each were formed (n = 5 for each group and time point). On days 2, 14, and 90 after intramuscular administration of nanoparticle suspensions at a dose of 256 mg/kg, the animals were anesthetized and euthanized, after which hindlimb muscle tissue and organs were collected for magnetization analysis. The obtained biomaterial was frozen at –80 °C and then homogenized to a uniform consistency. Standardized 0.2 g homogenate samples were placed in plastic containers and dried to constant weight.

For quantitative determination of total iron, biological samples were weighed and dried at 60 °C to constant weight. The dehydrated samples were mineralized with a mixture of concentrated hydrochloric and nitric acids at a 3:1 ratio (v/v) and evaporated to dryness. The dry mineralized residue was dissolved in 5 mL of deionized water and centrifuged, after which 1 mL of the supernatant was collected for analysis. The total iron concentration was determined photocolometrically by the absorbance of the complex with sulfosalicylic acid at pH 9–12 and λ = 430 nm (Pozdnyakov et al. 2006). Calculations were performed using a calibration curve (Supplementary materials, Fig. S1). The magnetic moment of the samples and total iron were normalized to the mass of the biological sample.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 24 software (IBM, USA). Physicochemical characterization data for MNPs, as well as quantitative bioresorption data based on magnetization and total iron content in biological samples, are presented as the mean $\pm$ standard deviation ($M \pm SD$). Body weight dynamics and hematological and biochemical blood parameters are presented as the mean $\pm$ standard error of the mean ($M \pm SEM$). The statistical significance of differences between two independent groups was assessed using the nonparametric Mann–Whitney U test. Acute toxicity parameters LD_{16} , LD_{50} , and LD_{84} were calculated in GraphPad Prism 8.0.1 using probit analysis based on 14-day animal mortality in each dose group. Differences were considered statistically significant at $p < 0.05$.

Results

Acute toxicity

The results of the acute toxicity assessment of magnetic nanoparticles are presented in Tables 1 and 2. After intraperitoneal administration, animal mortality showed a dose-dependent pattern in the Fe@OA, ZnMn@OA, and ZnMn@SiO₂ experimental groups (Table 1).

Table 1. Mortality and median survival of BALB/c mice during 14 days after a single intraperitoneal administration of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles at doses of 274 – 4896 mg/kg

Dose, mg/kg	Dose, mL/kg	Mortality within 14 days							
		Fe@OA		ZnMn@OA		Fe@SiO ₂		ZnMn@SiO ₂	
		Dead/total	MS, days	Dead/total	MS, days	Dead/total	MS, days	Dead/total	MS, days
274	1.76	0/6	-	0/6	-	0/6	-	0/6	-
678	4.5	2/6	-	1/6	-	0/6	-	0/6	-
1024	6.6	4/6	5	3/6	7.5	0/6	-	1/6	-
1823	12.17	4/6	6.5	4/6	2.5	0/6	-	0/6	-
2984	18.8	6/6	3.5	6/6	1	0/6	-	4/6	10
4057	25	6/6	1	6/6	1	1/6	-	5/6	1
4896	31.25	6/6	1	6/6	1	0/6	-	6/6	1

Note: MS – median survival (days).

In the Fe@OA and ZnMn@OA groups, after nanoparticle administration at doses of 1823–4896 mg/kg, lethargy, reduced motor activity, and a delayed response to external stimuli were observed immediately after injection. Most deaths in these groups were recorded within the first day. At a dose of 1024 mg/kg, these reactions were also observed but were less pronounced. The calculated LD_{50} values were 904 mg/kg for Fe@OA and 1145 mg/kg for ZnMn@OA (Table 2). For Fe@SiO₂, a short-term decrease in activity was observed only at the maximum dose of 4896 mg/kg. A single death was recorded in this group. Therefore, the LD_{50} value was not reached within the tested dose range. In the ZnMn@SiO₂ group, at doses of 4057–4896 mg/kg, reduced motor activity, slower respiratory movements, and crouching against the bedding were visually observed in mice. Mortality in this group increased sharply at doses ≥ 2984 mg/kg, and the LD_{50} value was 2955 mg/kg (Table 2). After intramuscular administration of all types of MNPs at a dose of 1020 mg/kg, no deaths were recorded during the 14-day observation period. No visible disturbances in behavioral activity were observed in the animals.

Table 2. Acute toxicity parameters (LD_{16} , LD_{50} , and LD_{84}) of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles after a single intraperitoneal administration in BALB/c mice

Nanoparticles	LD_{16} , mg/kg [95% CI]	LD_{50} , mg/kg [95% CI]	LD_{84} , mg/kg [95% CI]
Fe@OA	484 [225 – 743]	904 [635 – 1172]	1687 [906 – 2468]
ZnMn@OA	676 [463 – 889]	1145 [917 – 1372]	1939 [1329 – 2250]
ZnMn@SiO ₂	-	2955*	-
Fe@SiO ₂	-	***	-

Note: * – The 95% confidence interval could not be reliably estimated because of the steep dose–response transition and the limited number of intermediate mortality points. ** – For Fe@SiO₂, the LD_{50} value was not reached within the tested dose range.

Figure 2 shows the dynamics of animal body weight during 14 days after intraperitoneal and intramuscular administration of MNPs. Nanoparticles with a $\text{SiO}_2\text{-NH}_2$ shell did not cause statistically significant changes in body weight from the control over the entire dose range. Administration of Fe@OA and ZnMn@OA at a dose of 1024 mg/kg led to a significant decrease in body weight of up to 15%. At a dose of 1823 mg/kg, a significant decrease in body weight of up to 20% from baseline was recorded, and mortality in the group exceeded 50% by day 7 of observation. No statistically significant change in body weight was detected after intramuscular administration of MNPs at a dose of 1020 mg/kg.

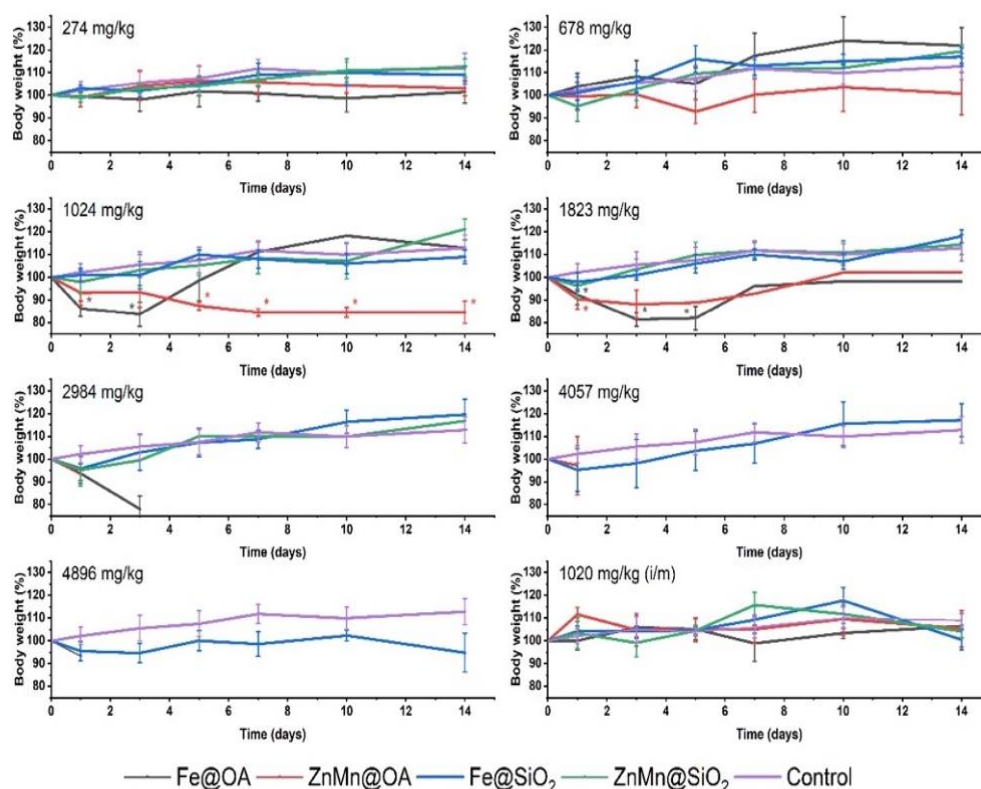


Figure 2. Body weight dynamics in BALB/c mice during 14 days after a single intraperitoneal or intramuscular administration of magnetic nanoparticles. Data are shown as $M \pm \text{SEM}$ (% relative to day 0) during 14 days after single intraperitoneal administration of Fe@OA , ZnMn@OA , Fe@SiO_2 and ZnMn@SiO_2 nanoparticles at doses of 274–4896 mg/kg and single intramuscular administration at a dose of 1020 mg/kg. **Note:** * – $p < 0.05$ compared with the time-matched control.

Blood analysis

Hematological and biochemical blood parameters on days 2, 14, and 90 after intramuscular administration of MNPs at doses of 256 and 1020 mg/kg are presented in Figures 3, 4. No pronounced dose-dependent response was detected for the studied parameters.

Transient fluctuations were observed for WBC, LYM, and NEU (Fig. 3A–F). The values of these parameters remained within the reference interval. At a dose of 1020 mg/kg, a statistically significant increase in platelet count was observed on day 14 in the Fe@OA , Fe@SiO_2 , and ZnMn@SiO_2 groups, but this increase was reversible by day 90 (Fig. 3H). Erythroid lineage parameters (RBC, HCT, and HGB) in the ZnMn@OA , Fe@SiO_2 , and ZnMn@SiO_2 groups remained within the reference interval (Fig. 3I–N). However, in animals receiving Fe@OA at a dose of 256 mg/kg, statistically significant increases in RBC, HCT, and HGB relative to the time-matched control were observed on day 90 (Fig. 3I, K, M). Nevertheless, the values of these parameters did not exceed the reference interval.

In the Fe@OA and ZnMn@OA groups, increased ALT activity relative to the control was recorded by day 90 (Fig. 4A–F). Nitrogen metabolism parameters in these groups remained stable throughout the experiment. For MNPs with a $\text{SiO}_2\text{-NH}_2$ coating, transient increases in glucose and urea levels were observed at early time points, on days 2–14. By day 90, a significant increase in creatinine concentration was observed in these groups at doses of 256 and 1020 mg/kg (Fig. 4G–L). The values of most studied parameters in all experimental groups remained within the reference interval.

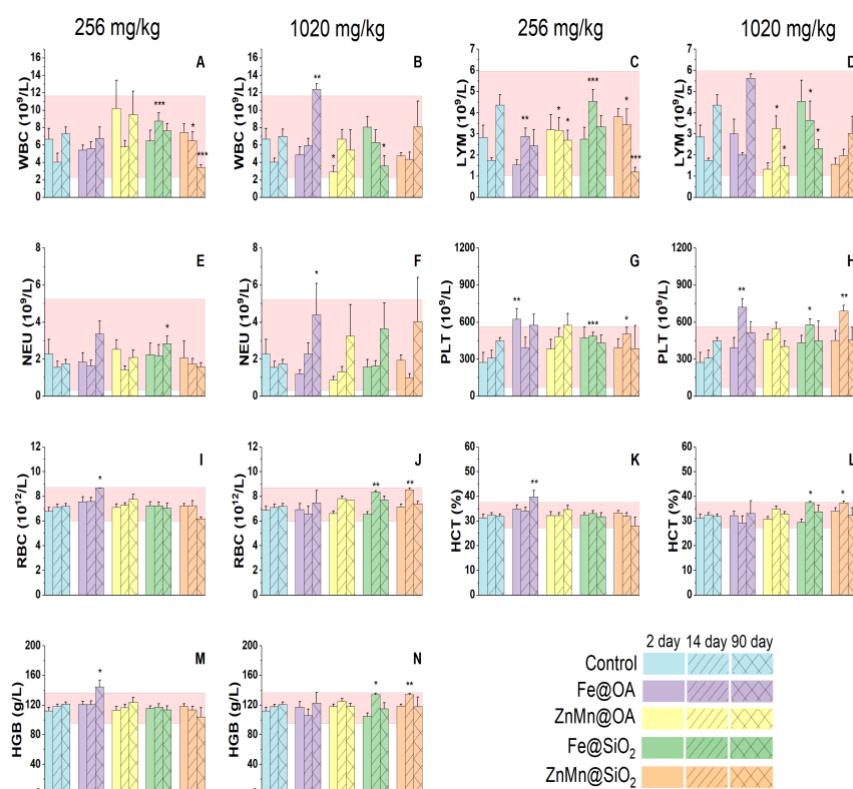


Figure 3. Hematological parameters in BALB/c mice on days 2, 14, and 90 after a single intramuscular administration of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles. **Note:** A, B – White blood cells (WBC); C, D – Lymphocytes (LYM); E, F – Neutrophils (NEU); G, H – Platelets (PLT); I, J – Red blood cells (RBC); K, L – Hematocrit (HCT); M, N – Hemoglobin (HGB), for doses of 256 and 1020 mg/kg, respectively. Data are shown as M ± SEM (n = 6). Statistical significance compared with the time-matched control: * – p < 0.05; ** – p < 0.01; *** < 0.001. The red band indicates the in-house reference interval (2.5th and 97.5th percentiles) for intact animals (n = 25).

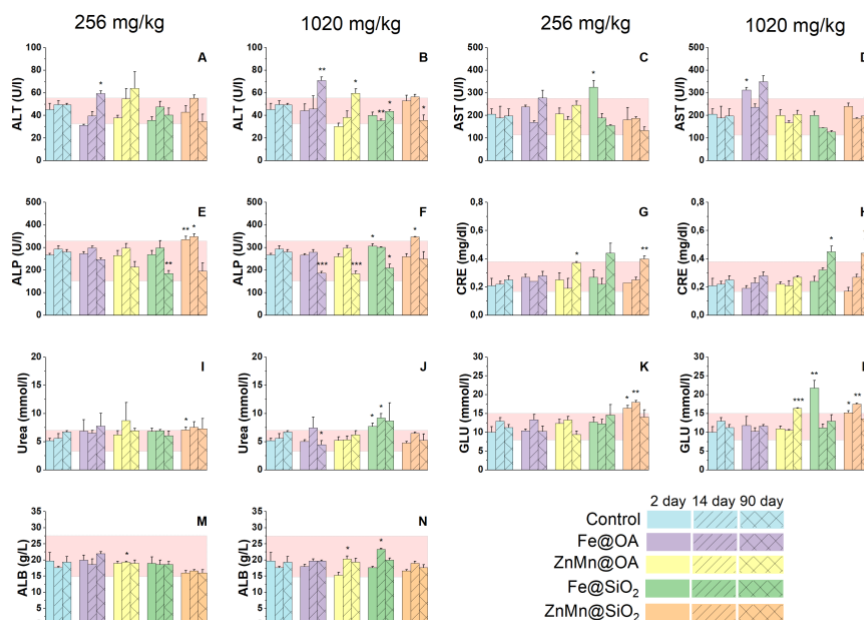


Figure 4. Biochemical parameters in BALB/c mice on days 2, 14, and 90 after a single intramuscular administration of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles. **Note:** A, B – Alanine aminotransferase (ALT); C, D – Aspartate aminotransferase (AST); E, F – Alkaline phosphatase (ALP); G, H – Creatinine (CRE); I, J – Urea; K, L – Glucose (GLU); M, N – Albumin (ALB), for doses of 256 and 1020 mg/kg, respectively. Data are shown as M ± SEM (n = 6). Statistical significance compared with the time-matched control: * – p < 0.05; ** – p < 0.01; *** – p < 0.001. The red band indicates the in-house reference interval (2.5th and 97.5th percentiles) for intact animals (n = 25).

Bioresorption

To assess the bioresorption of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles after intramuscular administration at a dose of 256 mg/kg, magnetization and total iron content were determined in the muscle tissue at the injection site, liver, and spleen on days 2, 14, and 90 (Fig. 5).

In muscle tissue after administration of Fe@OA and ZnMn@OA, the magnetic signal ($A \cdot m^2/g$) decreased by approximately 2.5-fold by day 90 compared with day 2 (Fig. 5A). No statistically significant decrease in magnetic signal was detected for Fe@SiO₂ and ZnMn@SiO₂. The total iron content in muscle tissue after administration of Fe@OA and ZnMn@OA decreased by approximately 1.5-fold by day 90 (Fig. 5D). No significant changes either in magnetization or total iron content were detected in the spleen (Fig. 5C, F). In the liver, the magnetic signal for all MNPs remained comparable to that of the liver of intact animals (Fig. 5B). For Fe@OA, a significant increase in total iron content in the liver was observed by day 90 relative to days 2 and 14 (Fig. 5E). By day 90, the total iron content in the liver after Fe@OA administration was 1.46-fold higher than after Fe@SiO₂ administration at the same time point (Fig. 5E).

In the present study, a decrease in the magnetic signal is considered not as direct evidence of nanoparticle removal from the tissue, but as one of the signs of their bioresorption, since the decrease in magnetic signal may be associated both with the degradation and clearance of MNPs from the injection site and with transformation of the magnetic phase, changes in the crystal structure, or the formation of weakly magnetic/non-magnetic compounds (Levy et al. 2011; Gutiérrez et al. 2015).

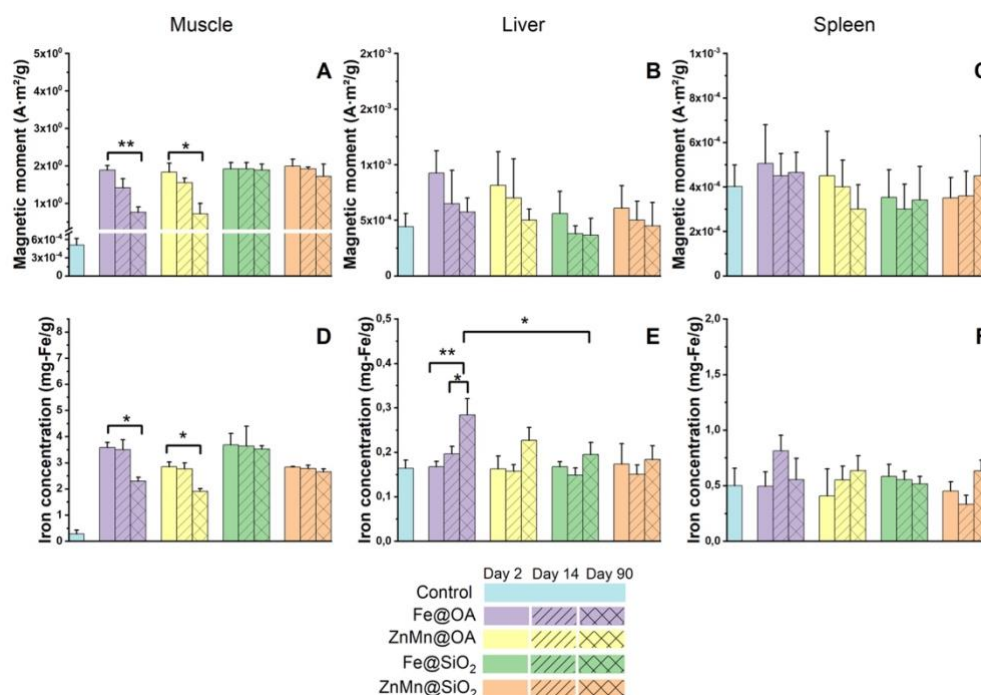


Figure 5. Magnetization and total iron content in injection-site muscle tissue, liver, and spleen of BALB/c mice on days 2, 14, and 90 after a single intramuscular administration of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles at a dose of 256 mg/kg. Magnetization and total iron content were normalized to biological sample mass. Data are shown as $M \pm SD$ ($n = 5$ for each time point). **Note:** * – $p < 0.05$; ** – $p < 0.01$.

Histopathological study results

Histological examination was performed after intramuscular administration of MNPs at a dose of 256 mg/kg to assess the muscle tissue at the injection site, liver, spleen, kidneys, and lungs on days 2, 14, and 90 of the experiment.

In the muscle tissue at the injection site, on day 2 after intramuscular administration of MNPs, dark nanoparticle accumulations in the form of dark-blue aggregates were detected in all experimental groups (Fig. 6). Most of the material was located in the perimysium spaces in the Fe@SiO₂ and ZnMn@SiO₂ groups, with penetration into the endomysium of individual muscle fibers in the Fe@OA and ZnMn@OA groups (Fig. 6). In the case of subcutaneous localization of the magnetic material, Prussian blue staining was observed over a larger area than with

intramuscular localization, due to the looser structure of subcutaneous adipose tissue (Fig. 6). No substantial differences in the amount of detected magnetic material were observed on days 2 and 14 in any of the experimental groups. However, by day 90, different dynamics in the distribution of magnetic material in the tissue became apparent. After administration of Fe@OA and ZnMn@OA, a reduction in the area of histology with Perls' Prussian blue staining regions was observed by day 90 of the experiment. At the same time, Fe@SiO₂ and ZnMn@SiO₂ occupied larger areas of the sections by day 90 of observation (Fig. 6). The localization pattern of Fe@OA and ZnMn@OA was also more fragmented. MNP foci were small and scattered, mainly in the endomysium, whereas Fe@SiO₂ and ZnMn@SiO₂ were located by day 90 as large aggregates in the perimysium. Both types of magnetic nanoparticles were resorbed from the subcutaneous layer by day 90 but remained in the muscle tissue. The muscle tissue architecture was preserved and showed no signs of either damage or inflammation. The observed differences in Perls staining suggest more active resorption of MNPs with an oleic acid/sodium oleate shell than of those with a silicon dioxide coating, which may be associated with the lipophilicity of the OA shell and its physicochemical affinity for tissue membrane structures. Considering the above, the histologically observed resorption of magnetic material should have affected the total iron concentration in reticuloendothelial system organs and their magnetization.

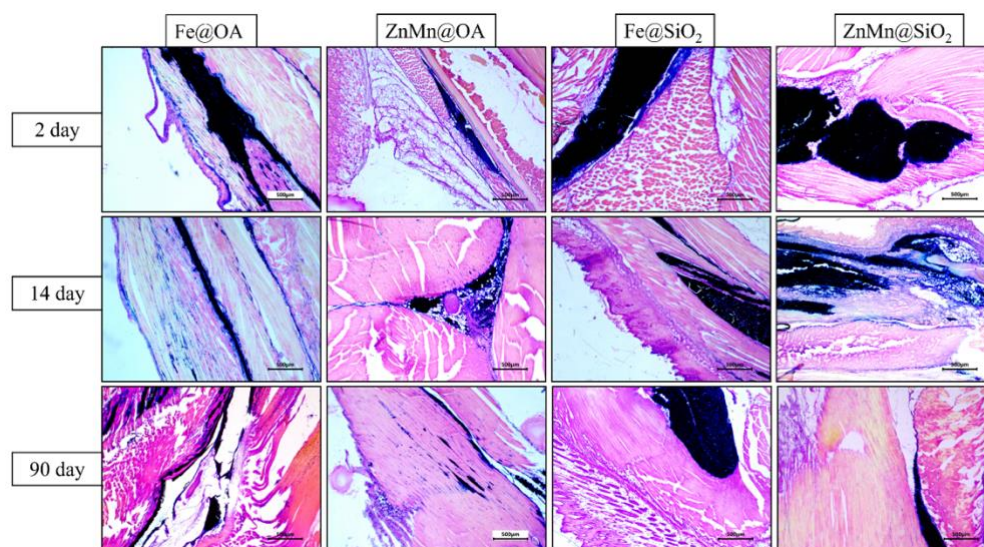


Figure 6. Histological analysis of injection-site muscle tissue in BALB/c mice on days 2, 14, and 90 after a single intramuscular administration of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles at a dose of 256 mg/kg. Representative Perls-stained microphotographs are shown. Dark deposits correspond to local accumulations of iron-containing material in intermuscular and connective tissue spaces. Scale bars: 500 μ m.

In spleen tissue, the general architecture of the organ was preserved in all experimental groups and at all observation time points (Fig. 7). The red and white pulp, lymphoid follicles, and trabecular structures were visualized. Perls' Prussian blue staining revealed Prussian blue-positive areas in individual fields of view, predominantly in the red pulp. No pronounced destructive changes were detected on days 2 and 14. By day 90, the spleen structure also remained preserved. In individual samples, signs of red pulp congestion and stained areas were observed. No signs of widespread necrosis, pronounced hemorrhages, or gross disruption of spleen tissue structure were observed.

In liver tissue, the general architecture of the organ was preserved in all groups and at all observation time points (Fig. 8). Hepatic cords, sinusoids, and vascular structures were visualized without gross disruption of the parenchymal structure. No signs of widespread necrosis, pronounced inflammatory infiltration, or gross liver tissue damage were detected. Perls' Prussian blue staining of liver tissue did not reveal morphologically pronounced areas of blue staining characteristic of iron deposits in any of the groups.

In kidney tissue, the general structure was preserved in all groups. Glomeruli and tubules were visualized at all observation time points. On days 2 and 14, no pronounced morphological changes were detected. On day 90, focal interstitial cellular infiltrates were observed in individual fields of view, predominantly in the Fe@OA and ZnMn@SiO₂ groups. No signs of widespread necrosis, pronounced hemorrhages, or gross disruption of kidney tissue architecture were detected (Fig. S2).

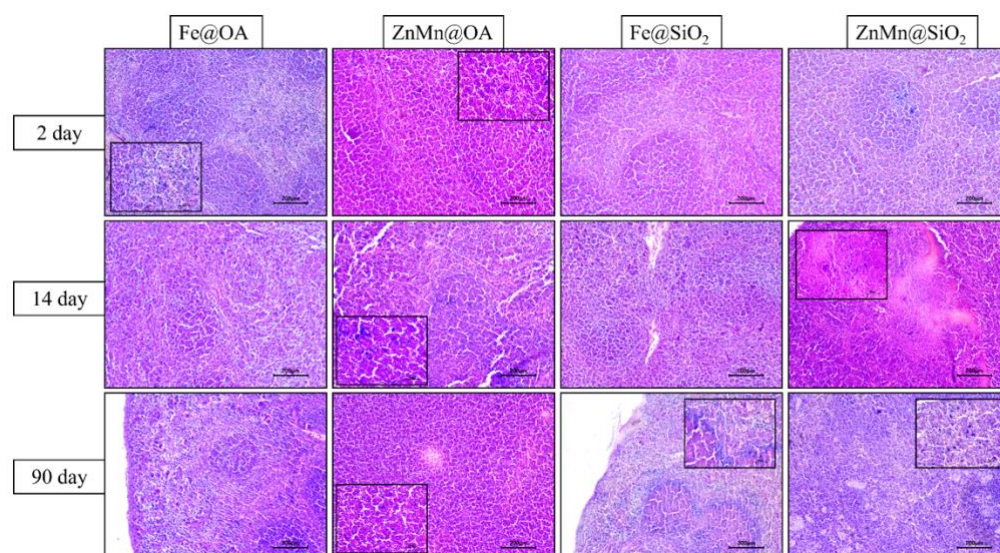


Figure 7. Histological analysis of spleen tissue in BALB/c mice on days 2, 14, and 90 after a single intramuscular administration of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles at a dose of 256 mg/kg. Representative Perls-stained microphotographs are shown. Insets show representative areas of splenic parenchyma. Scale bars: 200 µm.

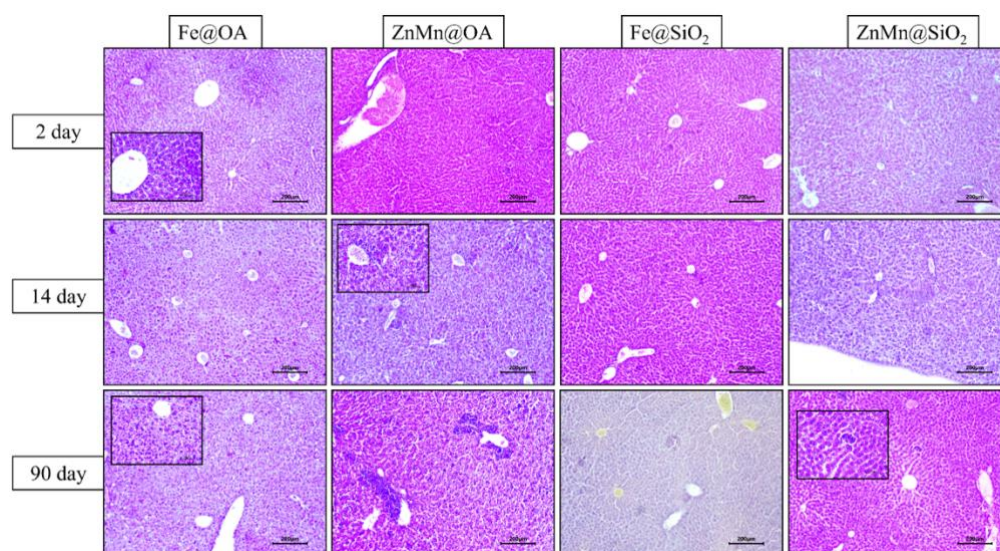


Figure 8. Histological analysis of liver tissue in BALB/c mice on days 2, 14, and 90 after a single intramuscular administration of Fe@OA, ZnMn@OA, Fe@SiO₂, and ZnMn@SiO₂ nanoparticles at a dose of 256 mg/kg. Representative Perls-stained microphotographs are shown. Insets show representative areas of hepatic parenchyma. Scale bars: 200 µm.

In lung tissue, the general alveolar structure was preserved in all groups. On days 2 and 14, no pronounced widespread morphological changes were observed. Focal peribronchial or perivascular cellular infiltrates were detected in individual fields of view and were more noticeable in some samples on days 14 and 90. No signs of massive hemorrhage, widespread alveolar damage, or gross disruption of lung tissue structure were detected (Supplementary materials, Fig. S3).

Discussion

The synthesized MNPs showed a combination of properties relevant for magnetic hyperthermia, including nanoscale core size, colloidal stability, magnetic response suitable for field-induced heating, and efficient heating in an alternating magnetic field (Laurent et al. 2011). Zn_{0.2}Mn_{0.8}Fe₂O₄ containing particles had higher saturation magnetization and SAR values than the corresponding Fe₃O₄ particles, indicating the contribution of magnetic core composition to

heating efficiency. This agrees with the concept that the magnetothermal properties of ferrites can be optimized by changing the cation composition (Slavu et al. 2021; Liu et al. 2022b).

Acute toxicity after intraperitoneal administration depended to a greater extent on the chemical nature of the coating than on the magnetic core composition. The LD₅₀ values for MNPs with an oleic acid/sodium oleate-based coating were 904 mg/kg for Fe@OA and 1145 mg/kg for ZnMn@OA. For nanoparticles stabilized with SiO₂-NH₂, the LD₅₀ value was higher: 2955 mg/kg for ZnMn@SiO₂, whereas for Fe@SiO₂ it was not reached within the tested dose range (Tables 1 and 2). Thus, after intraperitoneal administration, Fe@OA MNPs can be classified as slightly toxic substances, ZnMn@OA and ZnMn@SiO₂ as practically non-toxic substances, and Fe@SiO₂ MNPs as relatively harmless substances according to the Berezovskaya classification (Berezovskaya 2003). After intramuscular administration of MNPs at a dose of 1020 mg/kg, all tested nanoparticle types can be classified as practically non-toxic or relatively harmless substances according to the same classification.

The lower toxicity of MNPs with a SiO₂-NH₂ coating may be associated with the chemical inertness of SiO₂-based material. It has previously been shown that SiO₂ surface modification of MNPs can improve biocompatibility and reduce toxicity (Malvindi et al. 2014). The lower toxicity of SiO₂-NH₂-stabilized magnetic nanoparticles observed in the acute experiment may be related to the greater pharmacological inertness of the shell compared with the OA coating, which may specifically affect some biological targets. Lipophilic shells based on fatty acids, including oleic, lauric, palmitic, and stearic acids, can interact with phospholipid cell membranes and influence nanoparticle cellular uptake and the severity of the biological response (Matshaya et al. 2014; Zaloga et al. 2014). After intramuscular administration at a dose of 1020 mg/kg, neither deaths nor significant decrease in body weight was observed for any type of MNPs (Fig. 2). Hematological and biochemical blood parameters remained predominantly within the reference interval (Figs 3, 4). These data indicate good tolerability of MNPs after intramuscular administration. This is especially important for local magnetic hyperthermia, since this approach involves direct injection of nanoparticles into tumor tissue and the adjacent area.

Statistically significant changes in individual biochemical blood parameters were not accompanied by pronounced morphological signs of organ damage. Therefore, they should be considered limited functional deviations. In the Fe@OA and ZnMn@OA groups, increased ALT activity was observed by day 90 (Fig. 4A). These changes may reflect a delayed functional liver response associated with MNP bioresorption and the involvement of released iron in systemic metabolism (Gu et al. 2012; Vogt et al. 2021). This interpretation is consistent with data showing that degradation products of iron oxide nanoparticles could enter physiological pathways of iron metabolism, including ferritin, transferrin, and hemoglobin (Gu et al. 2012; Vogt et al. 2021). However, these changes were not accompanied by pronounced morphological signs of liver damage. According to histological examination, the general liver architecture was preserved, and no signs of widespread necrosis, pronounced inflammatory infiltration, or gross liver tissue damage were recorded (Fig. 8). Therefore, the detected biochemical deviations cannot be unambiguously interpreted as a manifestation of hepatotoxicity. Perls' Prussian blue staining of liver tissue in the Fe@OA and ZnMn@OA groups on days 14 and 90 did not reveal foci of pronounced blue staining (Fig. 8). By day 90, the total iron content in the liver in the Fe@OA group was significantly higher than on days 2 and 14 and was 1.46-fold higher than in the Fe@SiO₂ group at the same time point (Fig. 5E). However, this was insufficient for the formation of morphologically pronounced iron deposits characteristic of hemosiderosis on Perls' Prussian blue staining (Salomao 2021). In contrast, iron released during MNPs resorption at the injection site could have contributed to the significant increase in erythrocyte count, hematocrit, and hemoglobin concentration observed in animals of the Fe@OA group at a dose of 256 mg/kg on day 90 relative to the time-matched control (Fig. 3I, K, M), while these parameters remained within the reference interval. Taken together, this may indicate the involvement of released iron in physiological metabolism, including deposition in the liver as ferritin/hemosiderin or further use in erythropoiesis (Gu et al. 2012).

For MNPs with a SiO₂-NH₂ coating, transient increases in glucose and urea levels were observed at early time points (Fig. 4I-L). These changes may reflect a short-term stress or metabolic response of the organism to nanomaterial administration. By day 90, an increase in creatinine concentration was observed in these groups (Fig. 4G, H). However, histological examination of the kidneys in these groups did not reveal widespread necrosis, pronounced hemorrhages, or gross disruption of kidney tissue architecture (Supplementary materials, Fig. S2). Only focal interstitial cellular infiltrates were observed in individual fields of view (Supplementary materials, Fig. S2). Therefore, the increase in creatinine requires further verification and cannot be considered an unambiguous sign of nephrotoxicity.

Histological examination of the injection site is consistent with the results of magnetometry and total iron determination. In muscle tissue, local nanoparticle accumulations were detected

in all groups at early time points (Fig. 6). However, by day 90, the distribution of the material in the tissue differed depending on the coating. In the Fe@OA and ZnMn@OA groups, a visual decrease in the amount and compactness of MNPs at the injection site was observed (Fig. 6). The particles became more fragmented and less localized. This is consistent with the quantitative data: by day 90, in the muscle tissue of the Fe@OA and ZnMn@OA groups, the magnetic signal decreased by approximately 2.5-fold, while the total iron content decreased by approximately 1.5-fold compared with that on day 2 (Fig. 5A, D). In contrast, Fe@SiO₂ and ZnMn@SiO₂ persisted in muscle tissue as more pronounced local accumulations (Fig. 6). Magnetization in these groups was also maintained throughout the entire observation period (Fig. 5A). Thus, the results of magnetometry, quantitative determination of total iron, and histological examination indicate the absence of resorption of particles with a SiO₂-NH₂ shell at the injection site over 3 months.

The discrepancy between the dynamics of magnetization and total iron at the injection site in the Fe@OA and ZnMn@OA groups may be associated with transformation of the magnetic phase. The magnetic signal decreased more strongly than the total iron content (Fig. 5A, D). This may be related to partial transformation of the initial magnetic material into weakly magnetic Fe-containing phases, such as hematite, goethite, or ferrihydrite (Levy et al. 2011; Gutiérrez et al. 2015). For manganese-zinc ferrite nanoparticles, an additional factor may be acid-induced release of Mn²⁺ and Zn²⁺ from the crystal lattice, followed by a decrease in magnetic properties (Ma et al. 2022).

In the spleen, Perls' Prussian blue staining revealed areas of blue staining in some tissue regions, predominantly in the red pulp (Fig. 7). However, quantitative determination of total iron did not show a significant increase in its content in the spleen in any experimental group compared with the control (Fig. 5F). A positive Perls reaction in the spleen alone is not evidence of accumulation of the administered MNPs. This is related to the physiological role of the spleen in iron metabolism: red pulp macrophages remove senescent erythrocytes and ensure reuse of the released iron, while endogenous forms of deposited iron, including hemosiderin and ferritin-bound iron, can be detected in spleen tissue by Perls' Prussian blue staining (Kovtunovych et al. 2010; Slusarczyk et al. 2023; Sonoda et al. 2025). Therefore, the observed staining may reflect the physiological presence of endogenous iron in cells of the mononuclear phagocyte system rather than specific accumulation of MNPs. This interpretation is consistent with the absence of a significant increase in total iron content and spleen magnetization compared with the control (Fig. 5C, F).

Taken together, the results of magnetometry, total iron determination, and histological examination show that the coating type affects MNPs persistence in muscle tissue and their subsequent bioresorption after intramuscular administration. MNPs with an oleic acid/sodium oleate-based coating were characterized by an approximately 2.5-fold decrease in magnetic signal at the injection site and an approximately 1.5-fold decrease in total iron content by day 90, whereas MNPs with a SiO₂-NH₂ coating remained in muscle tissue by day 90 of the experiment. Therefore, when developing MNPs for local magnetic hyperthermia, the coating should be considered not only as a toxicity factor but also as a parameter determining MNP retention and particle bioresorption after administration.

Conclusion

In this study, a comparative assessment of the safety and bioresorption of four types of MNPs differing in magnetic core composition and coating type was performed for the first time within a single *in vivo* design: Fe₃O₄@OA, Zn_{0.2}Mn_{0.8}Fe₂O₄@OA, Fe₃O₄@SiO₂-NH₂, and Zn_{0.2}Mn_{0.8}Fe₂O₄@SiO₂-NH₂. The obtained data showed that the toxicity of the studied MNPs was determined to a greater extent by the chemical nature of the coating than by the core composition. Particles with a SiO₂-NH₂ coating demonstrated lower acute toxicity after intraperitoneal administration compared with MNPs coated with oleic acid/sodium oleate. After intramuscular administration at a dose of 1020 mg/kg, neither mortality nor marked signs of systemic toxicity were detected for any type of MNPs. The obtained results also showed that the coating type determines the rate of bioresorption. MNPs with a SiO₂-NH₂ shell remained in the injection area for 90 days, whereas the oleic acid/sodium oleate-based coating was accompanied by more pronounced nanoparticle resorption. Iron from resorbed Fe₃O₄@OA and Zn_{0.2}Mn_{0.8}Fe₂O₄@OA nanoparticles is incorporated into systemic iron metabolism without disrupting it. These results may be used to optimize MNPs for local magnetic hyperthermia of tumors, including the selection of coating materials that determine the stability, toxicity, distribution uniformity, and bioresorption of magnetic nanoparticles in tissue.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

Funding

The authors have no funding to report.

Ethics statement

The study was approved by the Biomedical Research Ethics Committee of National Research Ogarev Mordovia State University, approval protocol No. 1 dated 30 October 2025.

Data availability

All relevant data generated and analyzed during this study are included in this article and its Supplementary material.

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Supplementary material 1

Calibration curve for total iron determination

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Data type: pdf

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Link: <https://rrpharmacology.ru/index.php/journal/article/view/1165/747>

Supplementary Material 2

Histological analysis of kidney tissue after intramuscular nanoparticle administration

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Supplementary Material 3

Histological analysis of lung tissue after intramuscular nanoparticle administration

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