

Citrate-coated ultrasmall superparamagnetic iron oxide nanoparticles for efficient labeling and MRI tracking of olfactory ectomesenchymal stem cell-derived exosomes

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ABSTRACT

Objective(s): Exosomes are promising nanoscale carriers for cell-free regenerative medicine; however, limited *in vivo* traceability and inefficient targeting hinder their clinical translation. We investigated the feasibility of using citrate-coated ultrasmall superparamagnetic iron oxide nanoparticles (USPIONS) to efficiently label and MRI-track stem cell-derived exosomes.

Materials and Methods: USPIONS were synthesized and characterized using TEM (transmission electron microscopy) and DLS (dynamic light scattering), with complementary physicochemical characterization techniques. Olfactory ectomesenchymal stem cells (OE-MSCs) were labeled with USPIONS (50 µg/ml), and labeling efficiency was evaluated using cell viability assay, intracellular iron quantification, Prussian blue staining, and TEM. Exosomes were isolated from the USPIONS-labeled OE-MSCs (USPIONS-OE-MSCs) and characterized. All quantitative measurements were performed in triplicate, and data are presented as mean±SD.

Results: USPIONS exhibited a mean core size of 3.40±1.43 nm by TEM and a hydrodynamic peak at 6.53±1.71 nm by DLS, with excellent colloidal stability, crystallinity, and superparamagnetic-like properties. The USPIONS-OE-MSCs exhibited an intracellular iron content of 8.65±0.35 pg/cell. TEM revealed that exosomes derived from USPIONS-OE-MSCs (USPIONS-exosomes) had a mean diameter of 95.69±44.51 nm, whereas DLS showed a hydrodynamic peak at 121.5±33.89 nm. ICP-OES confirmed successful iron labeling (13.50±0.49 ng Fe/µg exosomal protein), and labeled exosomes produced a 32.6% signal reduction in *in vitro* T2-weighted MRI compared with unlabeled exosomes ($P\leq 0.0001$).

Conclusion: USPIONS provide an efficient, biocompatible platform for exosome labeling, enabling MRI-based tracking. This strategy may facilitate exosome engineering for regenerative nanomedicine and targeted delivery.

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Introduction

Mesenchymal stem cells (MSCs) are adult, multipotent, mesoderm-derived stromal cells that can self-renew and differentiate into multiple non-hematopoietic lineages within connective tissues. MSCs can be isolated from bone marrow, cord blood, muscle, fat, and other tissues (1).

Recently, OE-MSCs, a new member of the MSC superfamily, have received special attention as a therapeutic strategy (2, 3). OE-MSCs have a primitive origin from neural crest cells and are located in the olfactory lamina propria. These cells have a strong capacity to proliferate, self-renew, and differentiate into various cell lineages (4). OE-MSCs offer easy access and the potential for autologous grafts, which can address immune rejection and ethical concerns in clinical trials (2). Nerve regeneration and the production

of various types of neural cells, such as neurons, astrocytes, and oligodendrocytes, are other functions of OE-MSCs (5).

Literature findings revealed that MSC-based therapy has several defects, such as costly storage, transportation, and recovery. In addition, changes in transplanted MSCs due to complex *in vivo* environments can cause undesirable effects in patients (6).

Recently, a new cell-free therapy, MSC-released extracellular vesicles (EVs), has emerged as a promising therapeutic approach for numerous diseases (7-9). EVs are cellular products encapsulated in lipid bilayers. They comprise various subtypes, including microvesicles, exosomes, and apoptotic vesicles (10).

Almost all cell types can secrete exosomes. Exosomes are present in a variety of body fluids (11). Exosomes contain

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specific proteins, nucleic acids, lipids, and glycoconjugates to perform various activities, including intercellular signaling and molecular communication (12, 13).

Exosomes influence many facets of human physiology and pathology, including tissue homeostasis, development, immunity, cancer, and neurodegenerative diseases (13). New research shows that, given the physiological and pathological functions of exosomes, they can serve as diagnostic biomarkers, therapeutic agents, and molecular transporters (12).

Applying suitable labeling methods, noninvasive tracing, and imaging of stem cells and exosomes helps us identify the exact location where they exert their therapeutic function. Obtaining high-resolution *in vivo* images using nanotechnologies provides us with useful information about the stem cells' and exosomes' mechanisms of action (14, 15).

Among different labeling techniques, nanoparticles are particularly valuable in clinical studies (16). Recent reviews have highlighted the growing potential of metal nanoparticle-exosome hybrid systems, which combine the biological properties of exosomes with the unique physicochemical characteristics of engineered nanomaterials, including the magnetic properties of iron oxide nanoparticles (17).

Superparamagnetic iron oxide nanoparticles (SPIONs) are a nanomaterial that has become popular in biomedicine because of their unique characteristics, including easy synthesis, superparamagnetism, high saturation magnetization, biocompatibility, and low toxicity (18). The core of SPIONs usually comprises magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$). Magnetic nanoparticles are used as contrast media in MRI (19).

Before SPIONs were introduced as MRI contrast agents, gadolinium chelates were used. Gadolinium chelates are sensitive for micromolar-level imaging, whereas SPIONs are detectable in the nanomolar range and can be clearly observed using T1, T2, and T2* MRI parameters (20).

SPIONs cause negligible side effects (21). Iron-containing nanoparticles are biocompatible partly because the body is capable of metabolizing iron to its natural form of ferritin. Their superparamagnetic properties minimize the likelihood of nanoparticle aggregation and vascular occlusion. The organic and inorganic polymer coating around the nanoparticle core is another important factor in biocompatibility (19).

Labeling MSCs without transfection agents is challenging

due to their limited phagocytic capacity, resulting in low SPION uptake and reduced MRI sensitivity. Although transfection agents or high SPION concentrations can enhance uptake, their potential toxicity limits their application. Citrate is a type of polymer coating. This anionic monomer can increase cellular uptake and improve MR imaging (22). Citrate's strong negative surface charge attracts SPIONs to cells. As a result, SPIONs can be incorporated into cells more efficiently at lower concentrations and without transfection agents (19).

Exosomes can be labeled with SPIONs via multiple methods, such as electroporation, natural cellular uptake, or bioconjugation (23). As exosome research advances, attention has shifted toward labeling strategies and their impact on exosomal function. Choosing stable labeling agents, using proper labeling procedures, and optimizing labeling conditions are important for sustaining exosome detectability and trackability. Researchers can incorporate metal nanoparticles into exosomes through various approaches. Although membrane labeling is convenient, excessive modifications, such as using large dyes, hierarchical connections of antigen-antibody complexes, or particle chelation, can alter exosomal function. Therefore, small-molecule membrane labeling or intraluminal methods are preferred for functional studies (16).

Recent studies have further expanded the use of iron oxide nanoparticles to label and track EVs. Helsper *et al.* showed that USPIO-labeled human MSC-derived EVs could be visualized by MRI to assess their biodistribution and clearance in an experimental ischemic stroke model, and a subsequent study from the same research group further investigated MRI-based assessment of iron oxide-labeled EVs derived from hypoxia-conditioned hMSCs and their therapeutic potential (24, 25). More recently, researchers have explored iron oxide nanoparticle-engineered exosomes as multifunctional platforms that combine MRI-based imaging with therapeutic modulation of the disease microenvironment (26).

Despite the potential of OE-MSC-derived exosomes as a cell-free therapeutic platform, noninvasive tracking remains challenging. Our USPIO-labeling strategy (Figure 1) enables sensitive MRI tracking of these exosomes and provides a basis for magnetic guidance and targeted delivery.

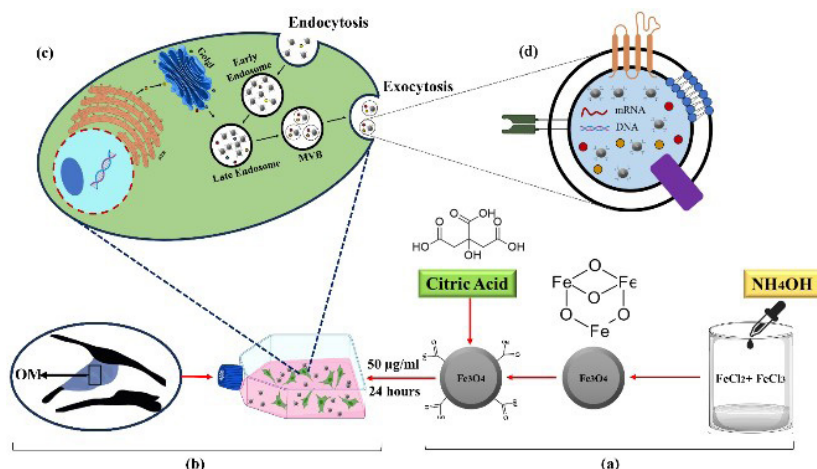


Figure 1. USPIOs provide an efficient nanoparticle-based approach for labeling OE-MSC-derived exosomes

(a) The synthesis of USPIOs was conducted. (b) Next, OE-MSCs were isolated and cultured. Then, OE-MSCs were incubated with characterized USPIOs (50 µg/ml) diluted in culture medium for 24 hr. (c) Cells internalized the nanoparticles via endocytosis. These endocytosed USPIOs mostly accumulated within multivesicular bodies (d), which released their cargo as exosomes after fusing with the plasma membrane.

OM: olfactory mucosa; MVB: multivesicular body; RER: rough endoplasmic reticulum

Materials and Methods

Synthesis of USPIONS

The first solution was prepared by dissolving 6 mM citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$, 99.5%, LR grade, CDH Fine Chemicals, India) in 50 ml of deionized water using a magnetic stirrer at room temperature. The second solution was produced by dissolving 4 mmol of anhydrous iron (III) chloride ($FeCl_3$, $\geq 97\%$, Sigma-Aldrich, St. Louis, MO, USA) and 2 mmol of iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 98%, Sigma-Aldrich, St. Louis, MO, USA) in 50 ml of deionized water and adding it to the first solution. Then, 3 ml of 25% ammonia solution (EMPARTA®, Merck, Darmstadt, Germany) was mixed dropwise. The combination was stirred gently for an hour at 80 °C. Finally, dialysis was used to purify the product and remove unreacted ions. The citrate-coated Fe_3O_4 was collected and stored at 4 °C until use (27).

Characterization of USPIONS

The Fe concentration in the sample was measured using inductively coupled plasma mass spectrometry (ICP-MS, Varian - 573 Radial). The sample was then diluted in deionized water and sonicated for 10 min before assessment.

Transmission electron microscopy (TEM; Carl Zeiss LEO 906; Jena, Germany) was conducted to evaluate morphological features and measure the average core size of nanoparticles. For sample preparation, ultrasmall superparamagnetic iron oxide nanoparticles (USPIONS) were diluted in distilled water, then sonicated for 10 min to prevent particle aggregation. Then, 5 μ l of the particle suspension was placed on a carbon-coated copper grid, air-dried, and TEM imaging was performed at 100 kV. The mean size of nanoparticle cores (Fe_3O_4) was measured from at least 100 particles.

Dynamic light scattering (DLS) and zeta potential evaluations were acquired with a Malvern Zetasizer apparatus (Malvern Instruments Co., Malvern, UK) at a backscatter detection angle of 173° (NIBS).

Fourier transform infrared (FTIR) spectra were obtained on a Frontier infrared spectrophotometer (PerkinElmer). Low-angle X-ray diffraction (XRD) analysis was carried out using a PW1730-Philips system with $K\alpha$ (1.54 Å) radiation to determine the crystalline nature of the nanoparticles. The magnetic characteristics of the synthesized nanoparticles were assessed using a Vibrating Sample Magnetometer (VSM).

OE-MSCs isolation and culture

OE-MSCs were isolated from the olfactory mucosa of 8-week-old male Wistar rats (230–280 g) purchased from the IUMS animal lab (Tehran, Iran) and maintained according to IUMS ethical guidelines (IR.IUMS.FMD.REC.1401.433). Animals were deeply anesthetized by intraperitoneal injection of ketamine (100 mg/kg; Rotexmedica, Trittenau, Germany) and xylazine (20 mg/kg; Alfasan, the Netherlands). Following confirmation of deep anesthesia, rats were euthanized by CO_2 inhalation in accordance with the approved institutional animal ethics protocol. Death was confirmed by the absence of respiration and heartbeat before tissue collection. Olfactory mucosal tissues were collected from nine rats and pooled for primary cell isolation. The pooled tissue was subsequently processed and seeded into three T25 culture flasks. Next, the cribriform plate was removed, and the turbinates were delicately discarded. Then, the olfactory mucosa was extracted from the nasal cavity. The tissue specimens were washed three times with culture medium containing penicillin (100 IU/ml)/streptomycin (100 μ g/ml) (28). Then, enzymatic dissociation was performed by submerging the

biopsies in 0.5 ml serum-free culture medium containing 0.5% collagenase type I enzyme (Gibco, Billings, USA) at 37 °C for 10 min. Mechanical dissociation was performed at the beginning, middle, and end of the incubation period using a pipette. The cell pellet was obtained. The cells were resuspended in the complete culture medium (Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 10% fetal bovine serum (FBS, Gibco, Billings, USA) and 1% penicillin-streptomycin antibiotic (final concentration: 100 IU/ml penicillin and 100 μ g/ml streptomycin; Invitrogen, Waltham, USA) and plated in flasks. After 72 hr, the culture medium was renewed to discard non-adherent cells. The medium was then replaced with fresh medium every 2–3 days. When confluence was reached, the adherent cells were trypsinized using 0.05% trypsin-ethylenediaminetetraacetic acid at 37 °C for 2 min and expanded for 3 passages.

Characterization and confirmation of OE-MSCs

OE-MSCs differentiation induction

Osteogenic and adipogenic differentiation of OE-MSCs at passage 3 was induced by seeding nearly 5×10^4 cells/well in a 24-well culture plate. The OE-MSCs were incubated in StemPro® osteogenic induction medium (Gibco, USA) and adipocyte differentiation medium (Cell Applications, Inc., USA) at 37 °C with 5% CO_2 . We replaced the media every 3 days. After 21 days, Osteogenic and adipogenic differentiation were evaluated using alizarin red S and oil red O dye, respectively, following the manufacturer's instructions (29).

Flow cytometry analysis

The expression of CD90 and CD105, as MSC positive markers, and CD34 and CD45 as negative markers, from the isolated OE-MSCs in passage three was determined using Flow cytometry analysis. The cells were incubated with antibodies against CD90 (APC-conjugated anti-rat CD90, Cat. No. 202526, Biolegend, USA), CD105 (PE-conjugated, Cat. No. GTX79979, GeneTex, USA), and the hematopoietic markers CD34 (PE-conjugated, Cat. No. 551387, BD Biosciences, USA) and CD45 (FITC-conjugated anti-rat CD45, Cat. No. 202205, Biolegend, USA) at 4 °C for 30 min according to the manufacturer's instructions. Isotype-matched control antibodies were used to determine non-specific staining. The antibody binding levels were detected using a BD FACS Calibur (BD Biosciences, San Jose, CA, USA).

Labeling of OE-MSCs with USPIONS

When OE-MSCs reached 80% confluency, they were incubated with USPIONS diluted in DMEM/F12 medium for 24 hr. Based on previous optimization studies and our preliminary evaluation of nanoparticle labeling efficiency (14, 30), along with MTT-based cell viability assessment, we selected 50 μ g/ml USPIONS and a 24 hr incubation period for OE-MSC labeling. During the first 4 hr, cells were deprived of FBS (the starvation period). The addition of 10% FBS was performed during the remaining 20 hr. After incubation, cells were washed three times with phosphate-buffered saline (PBS) to remove extracellular nanoparticles (30).

Evaluation of OE-MSCs labeling efficiency

Prussian blue (Perl's) staining

Prussian blue (PB; Perl's acid ferrocyanide) staining was carried out to detect the internalized USPIONS by OE-MSCs. Briefly, cells were fixed with 4% paraformaldehyde (PFA; Merck, Kenilworth, NJ, USA) for 20 min at room temperature and washed three times with PBS. The cells

were then incubated with a 1:1 mixture of 2% potassium ferrocyanide and 1% hydrochloric acid (HCl) (Merck, Germany) for 20 min. After washing three times with PBS, the cells were counterstained with nuclear fast red for 15 sec. USPION internalization was observed using an Olympus IX71 inverted microscope (Olympus, Japan).

TEM

We performed primary fixation on NP-labeled cells in 2.5% glutaraldehyde buffer (diluted from 25% stock, Cat. No. 104239, Merck, Germany) in a fridge at 4 °C overnight. They were postfixed with 1% osmium tetroxide (OsO₄; diluted from 4% stock, Cat. No. 75632, Sigma-Aldrich, Buchs, Switzerland) for 1 hr, then washed with PBS. The samples were then dehydrated and placed in graded concentrations of ethanol. TEM images were obtained using a PHILIPS EM208S microscope (Netherlands) at 100 kV.

Iron quantification in USPIONs-OE-MSCs

USPIONs-labeled and unlabeled cells were lysed in 0.5 ml concentrated HCl, and the inductively coupled plasma optical emission spectrometry (ICP-OES) analysis (ICP-OES 730-ES, Varian) was performed to ascertain the amount of intracellular iron.

Cytotoxicity assay

The cytotoxicity of the USPIONs was analyzed using the 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay. At the beginning, 100 µl of OE-MSC suspension per well with a concentration of 5×10^4 cells/ml was seeded in a 96-well plate and allowed to attach to the bottom of the wells. After 24 hr, the medium was changed and cells were treated with a fresh medium containing the optimum concentration of nanoparticles (50 µg/ml) for 24, 48, and 72 hr as described and confirmed to be a nontoxic concentration for MSCs in some previous studies (14, 30, 31). Afterward, the medium containing the USPIONs was removed, and fresh serum-free medium containing MTT (5 mg/ml in PBS; Sigma, Missouri, USA) was added to each well and incubated for 4 hr. Then, the medium was replaced with 100 µl of dimethyl sulfoxide (DMSO; Sigma, Missouri, USA) and shaken for 15 min to dissolve the formazan crystals. Finally, the absorbance was measured at 570 nm using an ELISA reader. Cell viability was calculated relative to the corresponding untreated control group at each time point, which was set as 100%.

USPIONs-exosomes isolation

OE-MSCs (10^6 cells) with 80% confluency were incubated with 50 µg/ml of USPIONs for 24 hr. After three PBS washes, the cells were incubated again in fresh culture medium containing 10% EVs-depleted FBS to prevent impurities from serum vesicles. The differential centrifugation method was used to isolate exosomes from OE-MSCs conditioned medium (OE-MSCs-CM) with minor modifications to a previously described protocol (32). The isolation and characterization of EVs were performed in accordance with the MISEV2018 guidelines (33). Ultracentrifugation was carried out using a Beckman L5-50 ultracentrifuge (Beckman Instruments, Fullerton, CA, USA) equipped with a Type 60 Ti fixed-angle rotor, and all centrifugation conditions are reported as relative centrifugal force (×g). Briefly, the collected OE-MSCs-CM was sequentially centrifuged at 300 ×g for 10 min to remove dead cells, at 2000 g for 10 min to eliminate debris, and at 10,000 ×g for 30 min to remove microvesicles. Then, the samples were filtered through a 0.22-µm syringe filter. Finally, the

supernatants were centrifuged again at 100,000 ×g at 4 °C for 1 hr. The exosome pellet was resuspended in PBS and either used immediately or stored at -80 °C.

Characterization of USPIONs-exosomes

USPIONs-Exosomes concentration measurement

The Bradford assay (DNABioTech, Cat. No. DB0017, Iran) was used to measure the exosome fraction protein content according to the manufacturer's instructions.

Western blotting

Western blotting was used to characterize the proteins of exosomes following previously established methods, with some modifications (34, 35). First, exosomes were lysed with RIPA buffer. Then, centrifuged at 14,000 rpm for 20 min at 4 °C to remove the lysates. Protein concentration was measured using the Bradford Protein Quantification kit according to the manufacturer's instructions. (DB0017, DNABioTech, Iran). The lysates were then blended with an equal volume of 2X Laemmli sample buffer. After that, Equal amounts of protein (20 µg per lane) were applied to SDS-PAGE following a 5-min minute boil and were subsequently transferred to a 0.2 µm Immune-Blot™ polyvinylidene difluoride (PVDF) membrane (Cat No: 162-017777; Bio-Rad Laboratories, CA, USA). 5% BSA (Cat No: A-7888; Sigma-Aldrich, MO, USA) in 0.1% Tween 20 was used for 1 hr to block the membranes. Then, the membranes were incubated with Anti-Calnexin (Cat No: ab22595, Abcam), Anti-CLC3 (Cat No: A01080, Boster Biological Technology, USA), and Anti-TSG101 (Cat No: ab125011, Abcam) for 1 hr at room temperature. After washing with TBST, the membranes were incubated with goat anti-rabbit IgG H&L (HRP) (Cat. No.: ab6721; Abcam) diluted 1:3000 for 1 hr at room temperature to detect the primary antibody. The membranes were then incubated with enhanced chemiluminescence (ECL) for 1–2 min. Protein expression was normalized to β-actin. Densitometry of protein bands was performed using the Gel Analyzer Version 2010a software (NIH, USA).

TEM

The morphology of USPIONs-exosomes was assessed using TEM. Briefly, isolated exosomes were resuspended in 100 µl of sterile-filtered PBS. 10 µL of exosomes were placed on a Formvar/Carbon coated copper grid, 300 mesh (Agar Scientific, UK; Cat. No. AGS162-3). Afterward, the grid was washed three times with sterile-filtered PBS, and the exosome specimen was fixed with 2% PFA (EMS, EM grade) for 10 min. The grid was washed again and incubated with 2.5% glutaraldehyde for 10 min at room temperature. Then, negative staining was conducted using 2% uranyl acetate (pH 7.4) (EMS, EM grade). TEM images were acquired by Carl Zeiss LEO 906 (Jena, Germany) at 100 kV.

Size distribution analysis

Exosome sample at 100 µg of protein/ml was diluted in PBS. The particle size of the exosomes was evaluated by dynamic light scattering using a Malvern Zetasizer (Malvern, UK).

Quantification of iron associated with isolated exosomes

The iron content associated with the isolated exosome fraction was measured by ICP-OES (ICP-OES 730-ES, Varian) analysis, as described by Hood *et al.* (36). Briefly, NP labeled and unlabeled exosome samples were combined with 0.5 ml Optima-grade nitric acid (HNO₃),

0.2 ml hydrochloric acid (HCl), and 0.1 ml of hydrogen peroxide (H_2O_2) and mixed by pipetting. The samples were microwaved for 2 hr, with the power reaching 1000W within 20 min. Then, the samples were cooled for 10 min. The digested specimens were diluted to 10 ml in DI water. This was the counterpart volume to that of the iron calibration standards. The calibration standards contained iron concentrations of 0.5, 1, and 2 ppm.

In vitro MRI of USPIONs-exosomes

300 micrograms of characterized USPIONs-exosomes were fixed homogeneously in a 2% agarose gel matrix in a 1.5 ml microtube. A similar amount of unlabeled exosomes was dispersed and fixed in the gel matrix as a control. MR images were then acquired on a 3-Tesla clinical MRI scanner (Siemens, Magnetom Prisma, Henkestr, Germany) to assess the *in vitro* detectability of USPIONs-exosomes. The T2 relaxation mapping was conducted using a multi-echo spin-echo (MESE) sequence (32 echoes). The imaging parameters were as follows: Repetition time (TR): 3000 ms; echo time (TE): 28-58 ms; Field of View (FOV): $130 \times 200 \text{ mm}^2$; and slice thickness: 3.5 mm. Then, quantitative image analysis was performed using Fiji (ImageJ 2.16.0, Java 21.0.7, NIH, USA) software. For each sample, regions of interest (ROIs) of identical size were placed on the central portion of each microtube from three independent slices. The mean gray value for each ROI was used as the signal intensity. The noise standard deviation (σ noise) was obtained by averaging the standard deviation values derived from three identically sized background ROIs selected in regions without sample-related signals across the corresponding DICOM images. The Contrast to Noise Ratio (CNR) was calculated to investigate MRI detectability using the following formula.

$$\text{CNR} = \frac{S_{\text{Control}} - S_{\text{USPIONs-exosomes}}}{\sigma_{\text{noise}}}$$

Signal drop (%) induced by USPIONs-exosomes was also calculated relative to the control exosomes using the following equation:

$$\text{Signal drop}(\%) = \frac{S_{\text{Control}} - S_{\text{USPIONs-exosomes}}}{S_{\text{Control}}} \times 100$$

Where S_{Control} and $S_{\text{USPIONs-exosomes}}$ represent the mean signal intensities of unlabeled and USPIONs-exosomes, respectively. σ noise defined as the standard deviation of the background noise measured from a signal-free region of the background.

Statistical analysis

All quantitative experiments were performed in at least triplicate, and the results are presented as mean $\pm$ standard deviation (SD). Statistical analyses were conducted using GraphPad Prism software. Comparisons between groups were performed using Student's t-test or two-way ANOVA, where appropriate. A *P*-value of < 0.05 was considered statistically significant. Qualitative analyses, such as TEM, FTIR, and XRD, were evaluated descriptively.

Results

Characterization of USPIONs

The ICP-MS analysis showed an iron concentration of $3000.37 \pm 741.0 \mu\text{g/ml}$ in the stock nanoparticle suspension. The morphology of USPIONs was investigated using TEM imaging (Figure 2A). It shows that these nanoparticles are spherical, relatively uniform in shape and size, with good dispersion. The mean size of USPIONs was obtained to be $3.40 \pm 1.43 \text{ nm}$ by TEM. DLS measurement indicated that the hydrodynamic diameter (d_h) of the particles is $6.530 \pm 1.71 \text{ nm}$ (Figure 2B). The zeta potential, or surface charge, was found to be $-25.57 \pm 3.18 \text{ mV}$ (Figure 2C), confirming the stability of nanoparticles in aqueous solutions. FTIR

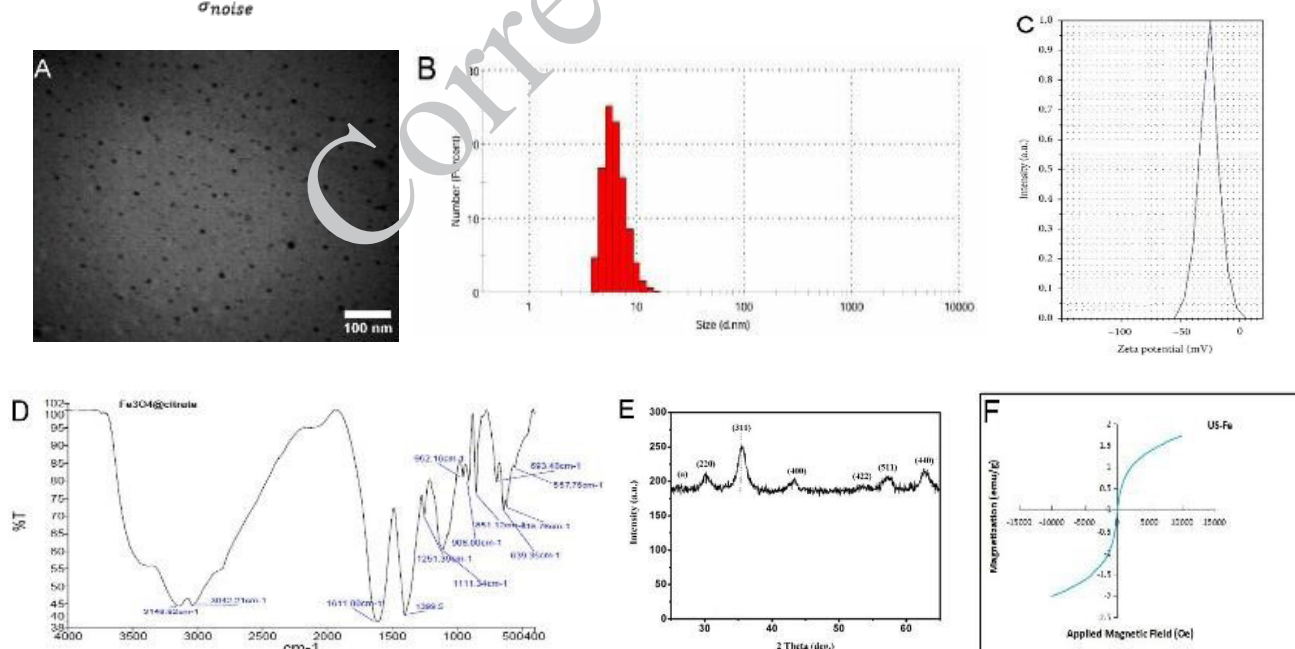


Figure 2. Characterization of USPIONs

(A) TEM images showed that USPIONs are uniformly dispersed and spherical. The average core diameter of nanoparticles was $3.40 \pm 1.43 \text{ nm}$ ($n=350$). (B) The hydrodynamic diameter and (C) Zeta potential of the USPIONs measured by DLS were $6.53 \pm 1.71 \text{ nm}$ and -25.57 mV , respectively. (D) FTIR spectrum, (E) XRD pattern, and (F) VSM hysteresis curve of the USPIONs

USPIONs: ultrasmall superparamagnetic iron oxide nanoparticles; TEM: transmission electron microscopy; DLS: dynamic light scattering; FTIR: fourier- transform infrared spectroscopy; XRD: X-ray diffraction; VSM: vibrating sample magnetometer

analysis was carried out to verify the binding of citric acid to the surface of iron oxide nanoparticles. FTIR spectrum of USPIONs (Figure 2D) indicated an absorption peak at 557 cm^{-1} corresponding to the Fe–O stretching vibration. The peaks at 1611 and 1399 cm^{-1} are attributed to the asymmetric and symmetric stretching of the carboxyl group, corroborating the binding of citrate to the USPION surface. The FTIR results show that two COOH groups from citric acid are covalently attached to the nanoparticle surface, leaving one COOH group free. This free acid group can be utilized for further functionalization of the nanoparticles (37). The crystalline structure of the sample was investigated by XRD analysis. The powder XRD pattern (Figure 2E) demonstrated that all Bragg's planes can be indexed to cubic inverse spinel magnetite with the indices (311), (440), (220), (511), and (400) (37, 38), which are consistent with the reference (JCPDS card no. 19-0629). VSM analysis indicated that the synthesized Fe_3O_4 nanoparticles reached 1.78 emu g^{-1} at an applied magnetic field of $10,000\text{ Oe}$. This magnetic response is consistent with the ultrasmall magnetic core size and the contribution of the non-magnetic citrate coating to the total sample mass. Overall, the nanoparticles exhibited a superparamagnetic-like magnetic response at room temperature. (Figure 2F).

Generation and characterization of OE-MSCs

Extraction and culture of OE-MSCs from the olfactory mucosa of normal rats were performed successfully (Figure 3A). After three passages, the isolated cells appeared as a relatively homogeneous population, and most of them presented a fibroblast-like morphology *in vitro* (Figure 3B). According to the multi-lineage differentiation capability of MSCs, OE-MSCs can also differentiate into adipocytes and osteocytes after culturing under induction conditions. Figures 3C and 3D revealed lipid droplets and calcium deposits after Oil Red O and Alizarin Red S staining, respectively.

Quantitative flow cytometry analysis showed that 98.9% and 96.3% of OE-MSCs stained strongly for the MSC surface markers CD105 and CD90, respectively (Figures 3F and 3E). However, OE-MSCs were negative for the hematopoietic markers CD34 and CD45, at 0.62% and 0.9%, respectively (Figure 3G and 3H).

Evaluation of OE-MSCs labeling efficiency

PB staining results indicated that USPIONs ($50\text{ }\mu\text{g/ml}$) were efficiently taken up by the OE-MSCs. After staining of the labeled cells, the nanoparticles were present as blue spots inside the cells, and the nuclei appeared red (Figure 4B). These blue sediments are formed from the reduction

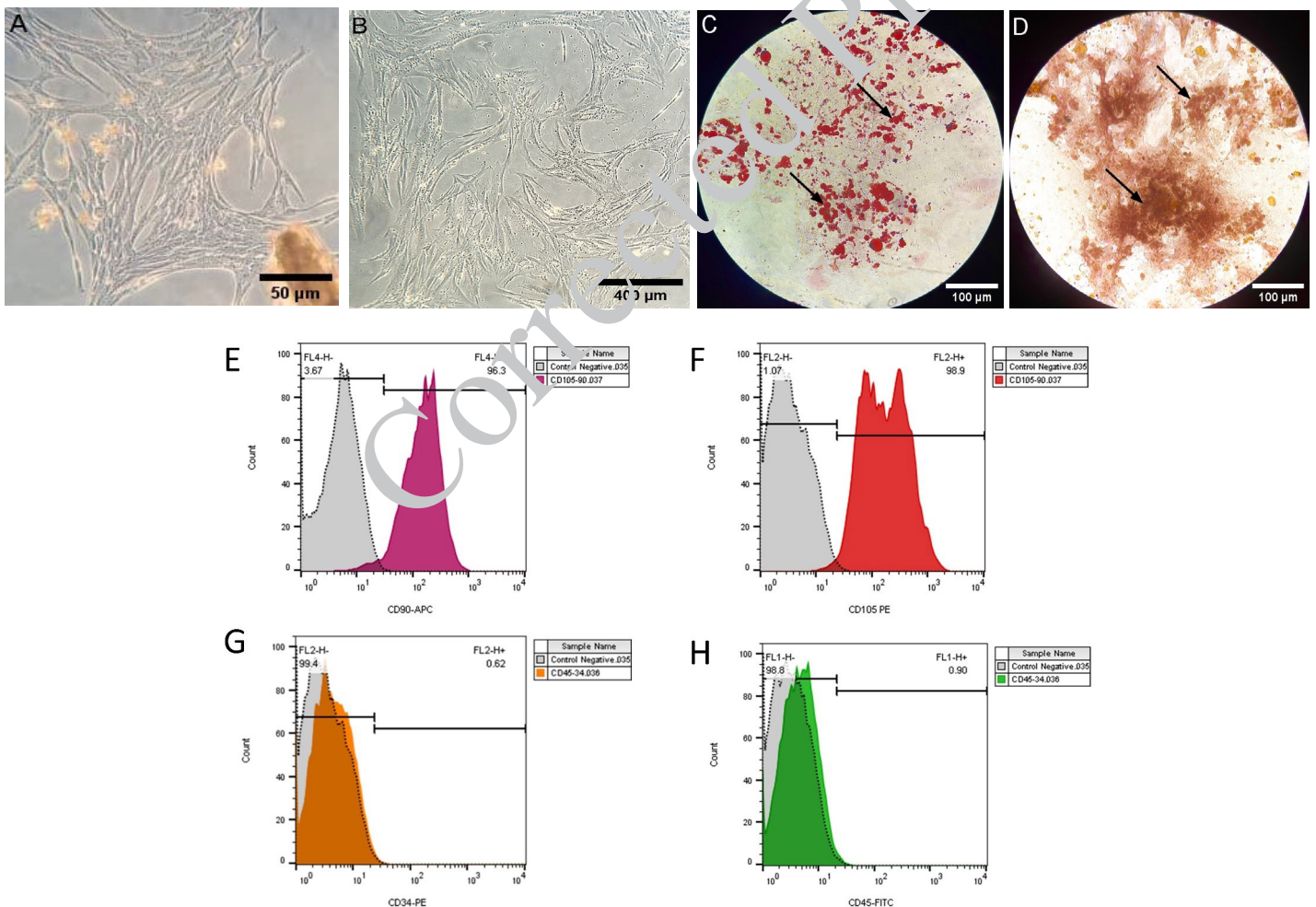


Figure 3. OE-MSC characterization

(A) Morphology of OE-MSCs shortly after isolation, before the first passage. (B) Isolated OE-MSCs in passage three, which show a spindle-like morphology. (C) Adipogenic and (D) osteogenic differentiation of OE-MSCs. Lipid droplets (arrows in C) and calcium deposits (arrows in D) are seen under adipogenic and osteogenic induction conditions, respectively. Characterization of OE-MSC surface markers using Flow cytometry analysis indicated that OE-MSCs were positive for mesenchymal markers (E) CD90 and (F) CD105 and negative for hematopoietic markers (G) CD34 and (H) CD45
OE-MSCs: olfactory ectomesenchymal stem cells

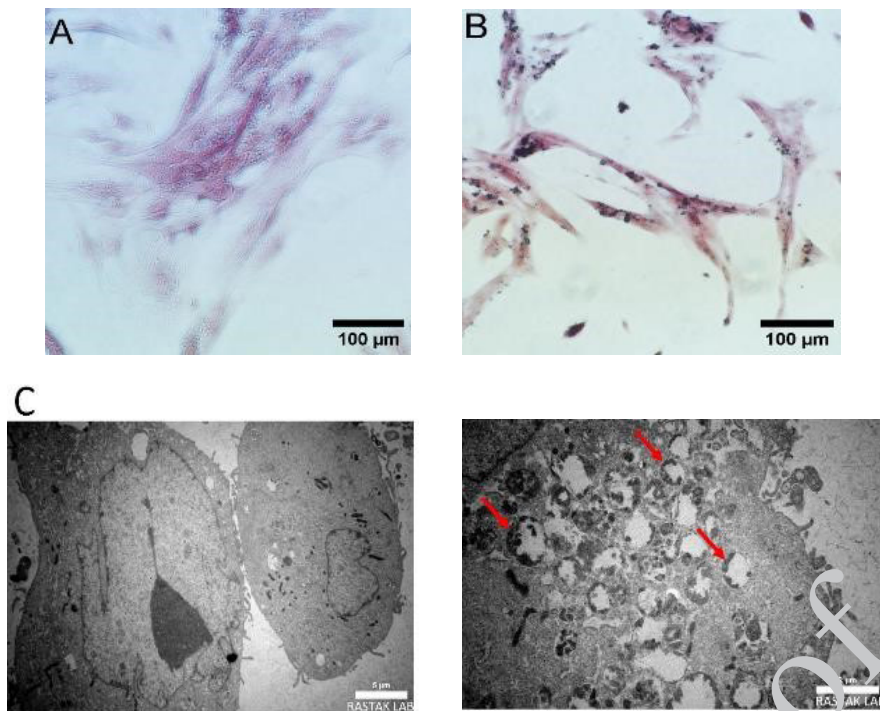


Figure 4. USPIONs internalization

PB staining of: (A) unlabeled and (B) USPIONs-OE-MSCs to evaluate intracellular USPIONs uptake. In (B), red nuclei and blue perinuclear cytoplasmic inclusions confirm the existence of iron nanoparticles within the cells. (C) TEM images of USPIONs-OE-MSCs. The internalized USPIONs (arrows) are observed within membrane-bound cytoplasmic vesicles. USPIONs: ultrasmall superparamagnetic iron oxide nanoparticles; PB: prussian blue; TEM: transmission electron microscopy; OE-MSCs: olfactory ectomesenchymal stem cells

of ferric to ferrous iron. Unlabeled control cells were not positive for PB (Figure 4A)

TEM imaging of USPIONs-OE-MSCs also confirmed that nanoparticles accumulated in the cytoplasm. In several images, membrane fragments enclosed aggregates, suggesting they may have been in endocytic vesicles (Figure 4C).

ICP-OES analysis quantified the amount of iron internalized by OE-MSCs. The iron content of USPIONs labeled cells, at approximately 8.65 ± 0.35 pg/cell, was significantly higher than that of unlabeled cells.

Cytotoxicity assay

The cytotoxicity of citrate-USPIONs at a concentration of 50 $\mu\text{g}/\text{ml}$ was investigated in OE-MSCs after 24, 48, and 72 hr of incubation using the MTT assay. A time-dependent reduction in the viability of OE-MSCs was observed following treatment with citrate-USPIONs at 50 $\mu\text{g}/\text{ml}$. While cell viability remained statistically comparable to the control group after 24 hr of incubation ($P=0.3153$), significant reductions were observed after 48 hr ($P=0.0098$) and 72 hr ($P=0.0013$) (Figure 5), indicating a time-dependent cytotoxic effect with prolonged exposure. Based on these findings, incubation of OE-MSCs with 50 $\mu\text{g}/\text{ml}$ citrate-USPIONs for 24 hr was considered the optimal labeling condition, providing efficient nanoparticle uptake while preserving cell viability.

USPIONs-exosome isolation and characterization

Exosomes were isolated from labeled OE-MSC-CM. The exosome yield per 10^6 OE-MSCs was 150–200 $\mu\text{g}/\text{ml}$ of exosome proteins for each isolation, as measured by the Bradford assay.

TEM imaging was performed to assess exosome ultrastructure and evaluate USPION association with isolated exosomes. Electron micrographs illustrated that exosomes are round, cup-shaped, and double-membraned

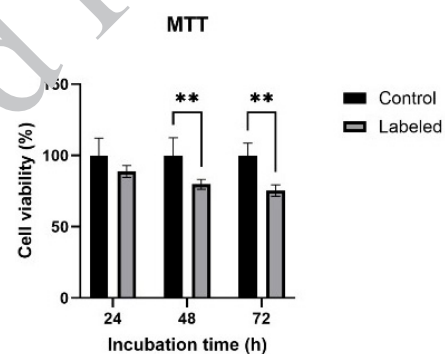


Figure 5. Viability of OE-MSCs treated with 50 $\mu\text{g}/\text{ml}$ citrate-USPIONs over different incubation times (24, 48, and 72 hr)

MTT assay results indicate that incubating cells with USPIONs at the specified concentration for 24 hr did not result in a statistically significant change in cell viability between the unlabeled and labeled groups (Mean $\pm$ SD, $n=5$, $**P \leq 0.01$).

OE-MSCs: olfactory ectomesenchymal stem cells; USPIONs: ultrasmall superparamagnetic iron oxide nanoparticles; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

structures. They showed a homogeneous size distribution with an average diameter of 95.69 ± 44.91 nm. TEM images also revealed electron-dense nanoparticles associated with the exosomes, consistent with successful USPION labeling (Figure 6A).

Western blot analysis revealed that the exosomal markers, CD63, a member of the tetraspanin family, and TSG101, an endosomal pathway protein, were expressed in the exosome fraction. The endoplasmic reticulum protein calnexin, as a negative control marker of exosomes, was not detected in OE-MSC-derived exosomes (Figure 6C).

DLS-measured hydrodynamic size indicated that the exosome size-distribution peak was 121.5 ± 33.89 nm (Figure 6D), matching the theoretical size of exosomes.

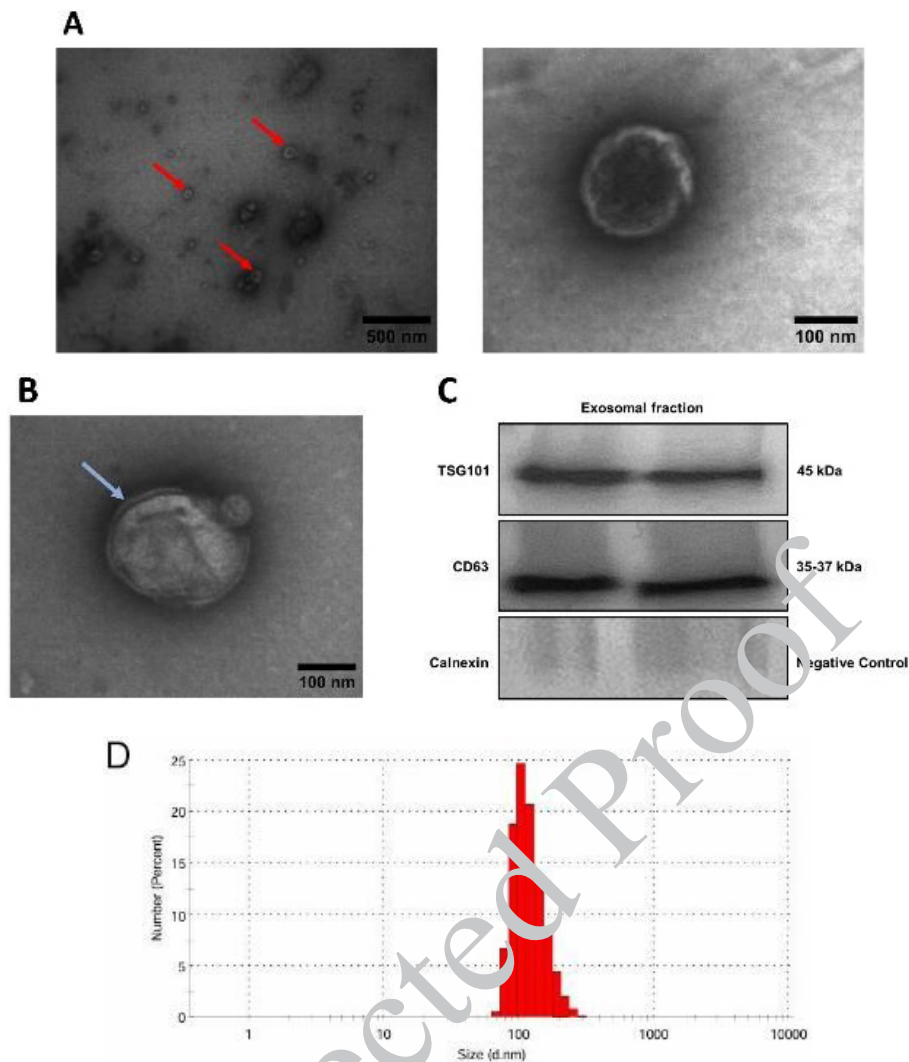


Figure 6. USPIONs-exosomes characterization

(A) TEM images of USPIONs-labeled and (B) unlabeled exosomes. Electron-dense nanoparticles associated with exosomes and the exosomal membrane are displayed with red and blue arrows, respectively. (C) Western blot analysis of surface protein markers of USPIONs-exosomes: CD63 and TSG101. Calnexin, as a negative control marker, was not detected in the exosomes. Two independent replications are shown. (D) DLS was used to evaluate the size profile of USPIONs-exosomes. The exosome size distribution peak was 121.5 ± 33.89 nm.

USPIONs: ultrasmall superparamagnetic iron oxide nanoparticles; TEM: transmission electron microscopy; DLS: dynamic light scattering

ICP-OES analysis showed that the iron content associated with the isolated exosome fraction derived from USPIONs-OE-MSCs was 13.50 ± 0.49 ng Fe/ μ g exosomal protein, which was markedly higher than that of the unlabeled exosome fraction, confirming successful iron labeling.

In vitro MRI of USPIONs-exosomes

Three hundred micrograms of USPIONs-exosomes were immobilized in an agarose gel matrix. A similar amount of unlabeled exosomes was also loaded into the gel matrix as a control. T2 mapping (multi-echo spin-echo, 32 echoes) images showed hypointense signals in the USPIONs-exosomes (Figure 7A). The MR images demonstrated a signal reduction in USPIONs-exosomes with a signal drop of 32.6% compared to unlabeled control exosomes. The noise standard deviation (σ noise) used for CNR calculation was 63.38. A high Contrast-to-Noise Ratio (CNR=13.4) confirmed robust MRI detectability of USPIONs-exosomes

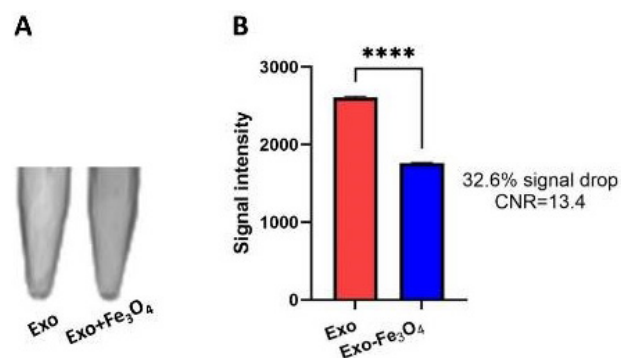


Figure 7. *In vitro* MRI assessment

(A) Coronal images of USPIONs-exosomes (Exo+Fe₃O₄) and unlabeled exosomes (Exo) embedded in gel were acquired using a MESE sequence (32 echoes). (B) USPIONs-exosomes showed a 32.6% signal drop relative to controls with a CNR of 13.4. Bars represent mean signal intensities $\pm$ SD (n=3) (**** $P \leq 0.0001$).

USPIONs: ultrasmall superparamagnetic iron oxide nanoparticles; Exo: exosomes; MESE: multi-echo spin-echo

(*** $P \leq 0.0001$, Figure 7B).

Discussion

OE-MSCs are easily accessible, maintain lifelong potency, and serve as a potent source of neural stem cells (39). Autologous transplantation and non-invasive isolation make them more desirable for cell therapy in neurodegenerative disorders (40). OE-MSCs have the potential to differentiate into different neurons *in vitro* (28). Several recent studies show that, despite their similarity to BM-MSCs and WJ-MSCs, OE-MSCs divide more frequently and express higher levels of neurogenesis-related genes than BM-MSCs. Given these characteristics, OE-MSCs are a better choice for transplantation strategies in neurodegenerative disorders (3, 41).

Exosomes secreted by MSCs play a key role in mediating their paracrine effects (42). Compared to MSC transplantation, MSC-derived exosomes offer advantages such as better tissue targeting due to their small size, higher stability, and lower side effects owing to their acellular status, simpler production and storage, non-oncogenicity, low immunogenicity, and tissue-specific homing, making them a promising therapeutic strategy (42, 43).

Researchers need suitable labeling methods and imaging techniques to investigate exosome characteristics and biological properties (16). Detection and tracking of exosomes are essential for understanding their biological mechanisms, including homing, functional capabilities, and site of action (44). Among labeling techniques, nanoparticles are particularly valuable in clinical studies (16).

SPIONs are metallic nanoparticles with superparamagnetic properties, enabling targeted delivery under a magnetic field and facilitating MRI-based imaging (45). USPIONs are stable, biocompatible, and small enough in size to be internalized in exosomes (14). This targeted delivery, combined with low toxicity and high sensitivity, results in optimal imaging of the lesion site even at low doses (46). Because of this sensitivity and resolution, nanoparticles are suitable for studying exosome biodistribution *in tissues* and for *in vivo* imaging (16). SPIONs are more appropriate than gadolinium chelates for cell labeling due to their high signal contrast on T2/T2*-weighted MR images, non-toxicity, and biodegradability (22).

In addition to choosing the appropriate labeling agent, using a suitable labeling procedure also helps achieve diagnostic and therapeutic goals. Electroporation is a routine method for loading cargo into cells and exosomes (15, 47). During electroporation, membrane integrity may be lost due to exposure to a strong electric field, which can induce spontaneous pores. Exosomes exert their biological functions on target cells through interactions between the exosome and cell membranes, followed by membrane fusion. Then, exosomes are incorporated and release their contents into the target cells (48, 49). Therefore, maintaining exosomal membrane integrity is essential for their proper function. Incubating cells with culture medium containing SPIONs enables direct cellular labeling. Subsequently, nanoparticle-associated EVs can be released from labeled cells into the surrounding medium. Busato *et al.* (14) and Li *et al.* (31) isolated Fe₃O₄-labeled exosomes from labeled MSCs using a similar approach. Likewise, in our study, USPIONs-exosomes were also isolated from USPIONs-OE-MSCs. This strategy is advantageous because it avoids direct manipulation of the exosomal membrane.

The SPION cell internalization rate is directly related to the incubation time and the iron concentration, and the amount of iron associated with EVs correlates with the level of cellular iron uptake (50). Therefore, selecting an appropriate nanoparticle and optimizing labeling conditions to achieve maximal iron loading in donor cells is essential for optimal outcomes. In the present study, the selection of 50 µg/ml USPIONs was based on our previous optimization study together with published evidence. Although previous investigations employed different MSC sources and nanoparticle formulations, including dextran-coated SPIONs in human umbilical cord-derived MSCs (30) and USPIONs in adipose-derived MSCs (14), they provide a valuable reference for selecting appropriate labeling concentrations in MSCs. Busato *et al.* (14) evaluated USPION concentrations ranging from 25 to 200 µg/ml and demonstrated that 50 µg/ml achieved efficient cell labeling while preserving cell viability. Although higher concentrations (100-200 µg/ml) produced a slight increase in labeling efficiency, the improvement was less than 10% despite a two- to fourfold increase in nanoparticle concentration. In the present study, MTT analysis at 24, 48, and 72 hr further supported the suitability of this concentration. While cell viability remained acceptable after 24 hr, longer incubation periods reduced viability further. Therefore, a concentration of 50 µg/ml with a 24 hr incubation period was considered an appropriate working condition for OE-MSCs labeling and was used in all subsequent experiments.

Studies have shown that citrate-coated SPIONs in the MSC culture medium, like the other investigated commercial nanoparticles, were internalized into endosomes through endocytic uptake (51, 52). The non-specific endocytic uptake pathway leads to efficient uptake of anionic SPIONs into endosomes. The interaction between highly negatively charged SPIONs and negative domains of the plasma membrane leads to local neutralization and non-specific absorption of SPIONs at the cell membrane. Then, the cell membrane bends, and the nanoparticles are efficiently internalized into the endosomes (22). These endocytosed nanoparticles mostly accumulate within multivesicular bodies, which may release their cargo as exosomes after fusion with the plasma membrane (53). Electron micrographs of USPIONs-OE-MSCs in the present study showed intracellular localization of nanoparticles within membrane-bound cytoplasmic vesicles, consistent with previous reports of endocytic uptake (14, 31, 54).

After confirming nanoparticle presence in cells by Prussian blue staining and TEM, we measured cellular iron content using ICP-OES. The results showed that the iron content of USPIONs-OE-MSCs was approximately 8.65±0.35 pg/cell. Busato *et al.* (14) showed that the intracellular concentration of iron in the commercial magnetite nanoparticle-labeled ASCs was about 6.20 pg/cell (50 µg/ml, 24 hr). This difference likely reflects the higher negative charge of citrate-coated USPIONs and, consequently, their higher absorption rate into cells.

In line with our findings, previous studies also confirm the superior efficiency of citrate-coated SPIONs labeling. Dextran-coated Endorem and carboxydextran-coated Resovist are clinically approved SPIONs primarily used for liver and spleen imaging due to efficient uptake by Kupffer cells. However, MSCs internalize them poorly because of their low phagocytic activity. In contrast, citrate-coated

SPIONs exhibit greater cellular uptake at similar iron concentrations, likely owing to their higher negative charge (22). Safi *et al.* suggested that the enhanced uptake of SPIONs coated with anionic monomers, such as citrate, may be attributed to their instability in physiological culture media (55). In cell culture medium, previously well-dispersed SPIONs may become destabilized, leading to aggregation and increased accumulation on the cell membrane, thereby enhancing cellular uptake. To achieve an equal loading rate in MSCs, commercial SPIONs require approximately 20-fold higher concentrations than citrate-coated SPIONs (22). Fleige *et al.* confirmed that citrate-coated USPIONs are taken up by macrophages more efficiently than carboxydextran-coated ones (56). Anionic SPION labeling preserves cellular functions, including surface markers, proliferation, and differentiation (22).

Busato *et al.* isolated USPIONs-exosomes from labeled adipose-derived stem cells and showed that these exosomes could be detected by both *in vitro* and *in vivo* MRI (14). Similarly, in the present study, MRI successfully detected the USPIONs-exosomes, as evidenced by a significant reduction in mean signal intensity compared with unlabeled exosomes. Previous studies have also reported that citrate-coated SPIONs provide strong MRI contrast and enhanced Prussian Blue staining (56).

Although the saturation magnetization of the synthesized citrate-coated USPIONs was relatively low, this finding can be explained by the ultrasmall core size (approximately 3.5 nm) of the Fe_3O_4 nanoparticles. In this size range, the high surface-to-volume ratio enhances surface-related magnetic effects, including spin disorder and spin canting, reducing the overall magnetic moment. Furthermore, the reported magnetization was normalized to the total mass of the citrate-coated nanoparticles rather than the Fe_3O_4 core alone, which also contributes to the relatively low apparent M_s value (57). Importantly, MRI contrast generation depends not only on saturation magnetization but also on several physicochemical characteristics, including particle size, surface coating, aggregation state, and relaxivity (58). Accordingly, despite the relatively low apparent saturation magnetization, the synthesized nanoparticles showed sufficient magnetic responsiveness for exosome labeling and MRI detection, as shown by reduced MRI signal intensity after nanoparticle labeling.

Labeling exosomes with iron oxide nanoparticles enables targeted delivery across biological barriers, supporting their potential clinical applications. Moreover, such labeling allows exosomes to serve as drug carriers for targeted therapeutic delivery. Several studies have explored this approach; for instance, Kim *et al.* indicated that iron-oxide-labeled exosome-mimetic nanovesicles (NV-IONP) can target injured spinal cord tissue using an external magnetic field (54). Li *et al.* isolated exosomes from Fe_3O_4 -labeled MSCs and showed that nanoparticles enabled magnet-guided accumulation of labeled exosomes at the wound area (31).

Although the proposed labeling strategy demonstrated efficient association of USPIONs with OE-MSC-derived exosomes and enabled their MRI detection, several limitations should be considered. The present work was restricted to *in vitro* experiments; therefore, the *in vivo* biodistribution, targeting efficiency, and MRI performance of the labeled exosomes remain to be established. Furthermore, although the physicochemical characteristics of the labeled exosomes were comprehensively evaluated,

the potential influence of nanoparticle labeling on exosomal biological activity was not investigated. Another important consideration is the long-term stability of the nanoparticle-exosome association. Following exosome degradation or intercellular transfer, magnetic nanoparticles may dissociate from the vesicles or be redistributed to surrounding cells, meaning that persistent MRI signal may not necessarily represent the continued presence of intact labeled exosomes (59). In addition, nanoparticle labeling may potentially influence exosome colloidal stability, aggregation behavior, and size distribution. A previous study has shown that SPION labeling can be associated with exosome aggregation under certain experimental conditions, although optimized processing conditions can substantially minimize these effects and preserve vesicle size distribution (36).

Collectively, these limitations underscore the need for future studies to assess the *in vivo* stability, biodistribution, magnetic targeting efficiency, biological functionality, and therapeutic performance of USPIONs-exosomes.

Conclusion

In conclusion, we demonstrated that USPIONs can efficiently label OE-MSCs without cytotoxicity, and the resulting exosome fraction contained sufficient iron to enable MRI tracking. This approach is also promising for therapeutic applications such as targeted delivery. Future studies should optimize labeling agents and methods to achieve more stable and stronger MRI signals for exosome tracking and biodistribution studies.

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Authors' Contributions

Z A, R S, Z B, JS M, and M M designed the experiments; Z A performed the experiments and collected the data; R S, Z B, JS M, and M M discussed the results and strategy; M M supervised, directed, and managed the study; Z A, R S, Z B, JS M, and M M approved the final version for publication.

Conflicts of Interest

The authors declare no competing interests.

Declaration

The authors made limited use of ChatGPT (OpenAI) during manuscript preparation, primarily to address occasional scientific inquiries and clarify certain concepts. All output was carefully reviewed, edited, and verified by the authors.

References

1. Rui K, Zhang Z, Tian J, Lin X, Wang X, Ma J, *et al.* Olfactory ectomesenchymal stem cells possess immunoregulatory function and suppress autoimmune arthritis. *Cell Mol Immunol* 2016;13:401–408.
2. Simorgh S, Milan PB, Saadatmand M, Bagher Z, Gholipourmalekabadi M, Alizadeh R, *et al.* Human olfactory mucosa stem cells delivery using a collagen hydrogel: As a potential candidate for bone tissue engineering. *Materials (Basel)* 2021;14:3909.

3. Delorme B, Nivet E, Gaillard J, Häupl T, Ringe J, Devèze A, *et al.* The human nose harbors a niche of olfactory ectomesenchymal stem cells displaying neurogenic and osteogenic properties. *Stem Cells Dev* 2010;19:853–866.
4. Hauser S, Widera D, Qunneis F, Müller J, Zander C, Greiner J, *et al.* Isolation of novel multipotent neural crest-derived stem cells from adult human inferior turbinate. *Stem Cells Dev* 2012;21:742–756.
5. Saïd Z, Pauline C, Claire B, Celia D, Jean-Paul M, Nicolas BM. Olfactory ecto-mesenchymal stem cells in laryngeal nerve regeneration in rats. *J Voice* 2021;35:349–359.
6. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: Pathological and therapeutic implications. *Nat Immunol* 2014;15:1009–1016.
7. Tian J, Zhu Q, Zhang Y, Bian Q, Hong Y, Shen Z, *et al.* Olfactory ecto-mesenchymal stem cell-derived exosomes ameliorate experimental colitis via modulating Th1/Th17 and Treg cell responses. *Front Immunol* 2020;11:598322.
8. Riazifar M, Mohammadi MR, Pone EJ, Yeri A, Lässer C, Segaliny AI, *et al.* Stem cell-derived exosomes as nanotherapeutics for autoimmune and neurodegenerative disorders. *ACS Nano* 2019;13:6670–6688.
9. Zhang B, Yin Y, Lai RC, Tan SS, Choo ABH, Lim SK. Mesenchymal stem cells secrete immunologically active exosomes. *Stem Cells Dev* 2014;23:1233–1244.
10. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, *et al.* Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018;7:1535750.
11. Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells* 2019;8:727.
12. van Niel G, Carter DR, Clayton A, Lambert DW, Raposo G, Vader P. Challenges and directions in studying cell–cell communication by extracellular vesicles. *Nat Rev Mol Cell Biol* 2022;23:369–382.
13. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem* 2019;88:487–514.
14. Busato A, Bonafede R, Bontempi P, Scambi I, Sciaffaro L, Benati D, *et al.* Magnetic resonance imaging of ultrasmall superparamagnetic iron oxide-labeled exosomes from stem cells: A new method to obtain labeled exosomes. *Int J Nanomedicine* 2016;11:2481–2490.
15. Hu L, Wickline SA, Hood JL. Magnetic resonance imaging of melanoma exosomes in lymph nodes. *Magn Reson Med* 2015;74:266–271.
16. Bao C, Xiang H, Chen C, Zhao Y, Gao Q, Huang F, *et al.* A review of labeling approaches used in small extracellular vesicles tracing and imaging. *Int J Nanomedicine* 2023;18:4567–4588.
17. Abdulrehman T, Al-Haik MY, Haik Y. Metal nanoparticle-loaded exosomes for cancer theranostics. *BioNanoSci* 2026;16:1.
18. Hohnholt MC, Geppert M, Dringen R. Treatment with iron oxide nanoparticles induces ferritin synthesis but not oxidative stress in oligodendroglial cells. *Acta Biomater* 2011;7:3946–3954.
19. Cores J, Caranasos TG, Cheng K. Magnetically targeted stem cell delivery for regenerative medicine. *J Funct Biomater* 2015;6:526–546.
20. Sosnovik DE, Nahrendorf M, Weissleder R. Magnetic nanoparticles for MR imaging: Agents, techniques and cardiovascular applications. *Basic Res Cardiol* 2008;103:122–130.
21. Mahmoudi M, Sant S, Wang B, Laurent S, Sen T. Superparamagnetic iron oxide nanoparticles (SPIONs): Development, surface modification and applications in chemotherapy. *Adv Drug Deliv Rev* 2011;63:24–46.
22. Andreas K, Georgieva R, Ladwig M, Mueller S, Nötter M, Sittlinger M, *et al.* Highly efficient magnetic stem cell labeling with citrate-coated superparamagnetic iron oxide nanoparticles for MRI tracking. *Biomaterials* 2012;33:4515–4525.
23. Zhuo Z, Wang J, Luo Y, Zeng R, Zhang C, Zhou W, *et al.* Targeted extracellular vesicle delivery systems employing superparamagnetic iron oxide nanoparticles. *Acta Biomater* 2021;134:13–31.
24. Helsper S, Yuan X, Jeske R, Bhagu J, Esmonde C, Duke L, *et al.* Superparamagnetic iron oxide nanoparticle-labeled extracellular vesicles for magnetic resonance imaging of ischemic stroke. *ACS Appl Nano Mater* 2024;7:24160–24171.
25. Helsper S, Sun L, Jeske R, Liu C, Athey J, Yuan X, *et al.* Cargo analysis and MRI-based therapeutic assessment of iron oxide labelled extracellular vesicles of hypoxia human stem cells in ischemic stroke. *J Extracell Biol* 2025;4:e70063.
26. Wu R, Jin L, Zeng A, Xu K, Shen W, Ni J, *et al.* Ultrasmall iron oxide as an imaging nanoenzyme loaded autologous exosomes for targeted imaging and chemotherapy promotion of pancreatic cancer. *Mol Pharm* 2026;23:1831–1844.
27. Sarikhani A, Alamzadeh Z, Beik J, Irajirad R, Mirrahimi M, Pirhajati Mahabadi V, *et al.* Ultrasmall Fe₃O₄ and Gd₂O₃ hybrid nanoparticles for T₁-weighted MR imaging of cancer. *Cancer Nanotechnol* 2022;13:43.
28. Simorgh S, Alizadeh R, Eftekharzadeh M, Haramshahi SMA, Milan PB, Doshmanziari M, *et al.* Olfactory mucosa stem cells: An available candidate for the treatment of the Parkinson's disease. *J Cell Physiol* 2019;234:23763–23773.
29. Bagher Z, Kamrava SK, Alizadeh R, Farhadi M, Absalan M, Falah M, *et al.* Differentiation of neural crest stem cells from nasal mucosa into motor neuron-like cells. *J Chem Neuroanat* 2018;92:35–40.
30. Hour FQ, Moghadan AJ, Shakeri-Zadeh A, Bakhtiyari M, Shabani P, Mordizadeh M. Magnetic targeted delivery of the SPION-labeled mesenchymal stem cells derived from human Wharton's jelly in Alzheimer's rat models. *J Control Release* 2020;321:420–441.
31. Li X, Wang Y, Shi L, Li B, Li J, Wei Z, *et al.* Magnetic targeting enhances the cutaneous wound healing effects of human mesenchymal stem cell-derived iron oxide exosomes. *J Nanobiotechnology* 2020;18:113.
32. Yang L, Zhai Y, Hao Y, Zhu Z, Cheng G. The regulatory functionality of exosomes derived from hUMSCs in 3D culture for Alzheimer's disease therapy. *Small* 2020;16:1906273.
33. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, *et al.* Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018;7:1535750.
34. Babaei H, Alibabrdel M, Asadian S, Siavashi V, Jabarpour M, Nassiri SM. Increased circulation mobilization of endothelial progenitor cells in preterm infants with retinopathy of prematurity. *J Cell Biochem* 2018;119:6575–6583.
35. Jabarpour M, Siavashi V, Asadian S, Babaei H, Jafari SM, Nassiri SM. Hyperbilirubinemia-induced pro-angiogenic activity of infantile endothelial progenitor cells. *Microvasc Res* 2018;118:49–56.
36. Hood JL, Scott MJ, Wickline SA. Maximizing exosome colloidal stability following electroporation. *Anal Biochem* 2014;448:41–49.
37. Srivastava S, Awasthi R, Gajbhiye NS, Agarwal V, Singh A, Yadav A, *et al.* Innovative synthesis of citrate-coated superparamagnetic Fe₃O₄ nanoparticles and its preliminary applications. *J Colloid Interface Sci* 2011;359:104–111.
38. Nigam S, Barick K, Bahadur D. Development of citrate-stabilized Fe₃O₄ nanoparticles: Conjugation and release of doxorubicin for therapeutic applications. *J Magn Magn Mater* 2011;323:237–243.
39. Wang H, Dwamena A. Olfactory ecto-mesenchymal stem cells in modeling and treating Alzheimer's disease. *Int J Mol Sci* 2024;25:8492.
40. Farhadi M, Boroujeni ME, Kamrava SK, Bagher Z, Tehrani AM, Aghajanpour F, *et al.* Implantation of human olfactory ecto-mesenchymal stem cells restores locomotion in a rat model of Parkinson's disease. *J Chem Neuroanat* 2021;114:101961.
41. Alizadeh R, Bagher Z, Kamrava SK, Falah M, Hamidabadi HG,

- Boroujeni ME, *et al.* Differentiation of human mesenchymal stem cells (MSC) to dopaminergic neurons: A comparison between Wharton's jelly and olfactory mucosa as sources of MSCs. *J Chem Neuroanat* 2019;96:126–133.
42. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: Toward cell-free therapeutic applications. *Mol Ther* 2015;23:812–823.
43. Lou G, Chen Z, Zheng M, Liu Y. Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases. *Exp Mol Med* 2017;49:e346.
44. Bull E, Madani SY, Sheth R, Seifalian A, Green M, Seifalian AM. Stem cell tracking using iron oxide nanoparticles. *Int J Nanomedicine* 2014;9:1641–1653.
45. Laurent S, Saei AA, Behzadi S, Panahifar A, Mahmoudi M. Superparamagnetic iron oxide nanoparticles for delivery of therapeutic agents: Opportunities and challenges. *Expert Opin Drug Deliv* 2014;11:1449–1470.
46. Santhosh PB, Ulrih NP. Multifunctional superparamagnetic iron oxide nanoparticles: Promising tools in cancer theranostics. *Cancer Lett* 2013;336:8–17.
47. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, *et al.* A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. *Biomaterials* 2014;35:2383–2390.
48. Kourembanas S. Exosomes: Vehicles of intercellular signaling, biomarkers, and vectors of cell therapy. *Annu Rev Physiol* 2015;77:13–27.
49. Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. *J Cell Biol* 2013;200:373–383.
50. Tada Y, Yang PC. Iron oxide labeling and tracking of extracellular vesicles. *Magnetochemistry* 2019;5:60.
51. Reimer P, Balzer T. Ferucarbotran (Resovist): A new clinically approved RES-specific contrast agent for contrast-enhanced MRI of the liver: Properties, clinical development, and applications. *Eur Radiol* 2003;13:1266–1276.
52. Matuszewski L, Persigehl T, Wall A, Schwindt W, Tombach B, Fobker M, *et al.* Cell tagging with clinically approved iron oxides: Feasibility and effect of lipofection, particle size, and surface coating on labeling efficiency. *Radiology* 2005;235:155–161.
53. Malatesta M, Giagnacovo M, Costanzo M, Conti B, Genta I, Dorati R, *et al.* Diaminobenzidine photoconversion is a suitable tool for tracking the intracellular location of fluorescently labelled nanoparticles at transmission electron microscopy. *Eur J Histochem* 2012;56:e20.
54. Kim HY, Kumar H, Jo M-J, Kim J, Yoon J-K, Lee J-R, *et al.* Therapeutic efficacy-potentiating and diseased organ-targeting nanovesicles derived from mesenchymal stem cells for spinal cord injury treatment. *Nano Lett* 2018;18:4965–4975.
55. Safi M, Sarrouj H, Sandre O, Mignet N, Berret JF. Interactions between sub-10-nm iron and cerium oxide nanoparticles and 3T3 fibroblasts: The role of the coating and aggregation state. *Nanotechnology* 2010;21:145103.
56. Fleige G, Seeberger F, Laux D, Kresse M, Taupitz M, Pilgrimm H, *et al.* *In vitro* characterization of two different ultrasmall iron oxide particles for magnetic resonance cell tracking. *Invest Radiol* 2002;37:482–488.
57. Tombácz E, Turcu R, Juculescu V, Vékás L. Magnetic iron oxide nanoparticles: Recent trends in design and synthesis of magnetoresponsive nanosystems. *Biochem Biophys Res Commun* 2015;468:442–453.
58. Dadfar SM, Roemhild K, Drude NI, von Stillfried S, Knüchel R, Kiessling F, *et al.* Iron oxide nanoparticles: Diagnostic, therapeutic and therapeutic applications. *Adv Drug Deliv Rev* 2019;138:302–325.
59. Chen H, Hou S. Recent progress in the effect of magnetic iron oxide nanoparticles on cells and extracellular vesicles. *Cell Death Discov* 2023;9:195.