Supporting Information

A Traceable and Bone-targeted Nanoassembly Based on Defect-related Luminescent Mesoporous Silica for Enhanced Osteogenic Differentiation

Huihui Ren,[a,#] Shizhu Chen, [a,#] Yanan Jin,[a] Cuimiao Zhang,[a] Xinjian Yang,[a] Kun Ge,[a] Xing-Jie Liang,[b] Zhenhua Li[a,] and Jinchao Zhang[a,*]*

[a] College of Chemistry & Environmental Science, Chemical Biology Key Laboratory of Hebei Province, Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of the Ministry of Education, Hebei University, Baoding 071002, P. R. China *Email: zhenhuali1013@163.com; jczhang6970@163.com

[b] Chinese Academy of Sciences (CAS) Center for Excellence in Nanoscience and CAS Key Laboratory for Biological Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology, Beijing 100190, P. R. China *Email: liangxj@nanoctr.cn

[#] These authors contributed equally to the manuscript.

Synthesis of defect-related luminescent MSNs

The MSNs were prepared based on the previous method with some modifications.[1] In briefly, 0.9614 g of CTATos and 0.14 mL of $TEAH_3$ were dispersed into 50 mL of deionized water under vigorous stirring for 1 h at 80°C to get transparent solution and 8 mL of TEOS was added. Then the mixture was stirred for another 2 h at the same temperature. After that, the precipitates were washed with deionized water and anhydrous ethanol respectively and dried in a vacuum oven to obtain MSNs. Finally, the MSNs were calcined at 230°C for 2 h in air to form the defect-related luminescent MSNs.

For the NH_2 modification, 500 mg as synthesised MSNs were dried for 12 h in a vacuum oven to remove residual water molecules on the surface. All MSNs were dispersed to 20 mL anhydrous toluene and 4 mL APTES was added at 110°C for 24 h under reflux condition. Then the products

were centrifuged and washed with an absolute alcohol and deionized water for three times. Finally, the amine-functionalized MSNs were lyophilized.

For the COOH modification, the product MSNs-NH_2 was dispersed into 15 mL anhydrous DMF in a 50 mL round-bottom flask. Then 5mL of DMF solution containing 1.72 g succinic anhydride (molar ratio of APTES/succinic anhydride=1:1) was added under vigorous stirring and reacted for 24 h at room temperature with nitrogen protection. The product was purified by centrifugation at 13000 rpm for 5 min and washed for several times with deionized water and anhydrous ethanol respectively. Subsequently, carboxyl-functionalized MSNs were lyophilized.

Characterization

The nanoparticles' morphology was observed with a SEM (JSM-7600F, JEOL, JPN) and the nanostructure was observed with a TEM (Tecnai G2 S-Twin, FEI, USA). The N_2 adsorption/desorption isotherms, BET surface area and pore volume were obtained by a surface area and porosimetry system (ASAP 2010N, Micromeritics, USA). The photoluminescence spectra were recorded by a spectrophotometer (F-7000, Hitachi, JPN). FT-IR spectra were obtained by infrared spectrometer (580B, PE, USA). The Gd element of MSN-ALN-Gd was tested using EDX. The content of Gd element in MSN-ALN-Gd was measured by an ICP-MS (XSeriesII, Thermo, USA). The T_1-weighted and T_2-weighted images were measured using an animal magnetic resonance imaging (MRI) scanner (Biospec7T/20, Bruker, GER). DEX loading was determined by UV-vis spectrophotometry (Bluestart A, Labtech, CHN). The sizes and zeta potential was tested using a laser particle size analyzer (Zetasizer Nano ZS, Malvern, UK). All measurements were performed at room temperature.

Cell culture

All animal procedures were in accord with the guidelines of the Institutional Animal Care and Use Committee. The BMSCs were obtained from ICR female mice at 4 to 6 week using a modified method previously described.[2,3] Briefly, BMSCs were obtained from femora and tibia of mice. The whole bone marrow was flushed by a 1 mL syringe using DMEM supplemented with 10% FBS. Human umbilical vein endothelial cells (HUVEC) were cultured in DMEM, osteoblast cell line (MC3T3-E1) were cultured in α-MEM. The three kinds of cells were cultured at 37°C, 5% CO_2 humidified incubator. The medium was changed every 3 d to remove the unattached cells.

Cell viability assay

The viability was assessed by MTT assay. Briefly, cells (5×10^5 cells per well) were seeded in 96-well culture plates and incubated for 4 d. MSN-ALN-Gd was added to wells at serial concentrations. After incubation for 12, 24 and 48 h, 10 μL MTT (5 mg/mL) was added every well and cultured for another 4 h at 37°C. Then the medium was removed and 100 μL DMSO was added to dissolve the crystal. Then the absorbance was measured at 570 nm using a microplate reader (Versamax, Molecular Devices, USA).

Hemolysis assay

Blood compatibility was evaluated by hemolysis assay using a method previously reported with some modifications.[4] Fresh blood was obtained from a female mice and stabilized with EDTA. The blood was centrifuged and washed with cold PBS for several times at 5000 rpm for 5 min to ensure without any released hemoglobin. Then the supernatant was removed carefully, and the cells were suspended in cold PBS to obtain red blood cells (RBC) solution (2% w/w). 0.2 mL RBC suspension was dispersed in 0.8 mL of MSN-ALN-Gd at the final concentrations of 50, 100, 200,

400 and 600 μg/mL. PBS was used as negative controls (NC), while deionized water was used as positive controls (PC). After 2 h incubation at 37°C, the samples were centrifuged at 6000 rpm for 5 min, and then 100 μL supernatant was removed and the optical density (OD) was measured at 545 nm by a microplate reader. Each group was represented for four parallels. The hemolytic ratio (%) was calculated based on the formula:

Hemolysis ratio (%) = $(OD_{samples}-OD_{NC})/(OD_{PC}-OD_{NC})\times 100$.

Toxicity of MSN-ALN-Gd in vivo

The mice were randomly divided into two groups (n=8): control group (PBS) and MSN-ALN-Gd group (10 mg/kg^{-1}). Blood samples were obtained from ophthalmic veins mice at 14 days after administration. The blood-element was analyzed by assay kits (Randox Laboratories Ltd. UK) by an automatic hematology analyzer (COULTER LH 750, Beckman, American). The blood-element included white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean cell hemoglobin concentration (MCHC), blood platelet (PLT), red cell distribution width (RDW).

The serum was obtained by centrifugation for the whole blood (3000 rpm, 15 min). Total bilirubin levels (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phos-phatase (ALP) in serum were measured using assay kits (Beijing Capital Medical University Clinical Science Center) for evaluating liver function. Uric acid (UA), blood urea nitrogen (BUN), and creatinine (Cr) in serum were measured using assay kits (Randox Laboratories Ltd. UK) for evaluating nephrotoxicity. The enzymes of creatine kinase (CK), lactate dehydrogenase (LDH), and alpha-hydroxybutyrate dehydrogenase (HBDH) in serum were measured using assay kits (Merit Choice Bioengineering (Beijing) Co,. Ltd.) for evaluating cardiac

damage by means of an automatic biochemical analyzer (7600-110, Hitachi, Tokyo).

After blood collection, the main tissues including the heart, spleen, liver, lung, and kidney were collected from the above two groups and fixed in 10% neutral buffered formalin. Then, the collected tissues were embedded in paraffin, sectioned (4 mm thick), as well as stained with hematoxylin and eosin (H&E). The histological sections were observed under an optical microscope.

DEX loading and release in vitro

For DEX loading, 5 mg of MSN-ALN-Gd and 5 mg of DEX was added in 1 mL ethanol and the mixture was stirred for 24 h at room temperature under a magnetic stirring apparatus. The DEX@MSN-ALN-Gd was collected by centrifugation and washed with PBS. The supernatant and the washing solutions were collected together to determine the DEX loading by UV-vis spectrophotometry at 242 nm.

To measure the drug release behavior, DEX@MSN-ALN-Gd was re-dispersed in 2 mL PBS (pH=7.4) at 37°C on magnetic stirring apparatus for continuous 22 d. At predetermined time intervals, 1 mL of release medium was collected and replaced with same volume of fresh PBS into the release system. The amount of DEX released in the medium at various times was determined at 242 nm by the formula:

The accumulative release rate of DEX (%) = (total amount of DEX released/total loaded DEX) × 100.

ALP activity

BMSCs (2.5×10^6cells per well) were seeded and cultured in 48-well culture plates. The cells were treated with PBS, DEX, MSN-ALN-Gd, and DEX@MSN-ALN-Gd respectively. Then 5.0 mM β-

glycerophosphate and 50 μg /mL ascorbic acid were added to the medium. After being exposed to samples for 14 d, the cells were washed with ice-cold PBS and lysed. ALP activity of BMSCs was evaluated by an ALP activity kit according to the manufacturer's instructions. All results were normalized by protein content.

ARS staining

The formation of mineralized matrix nodules was measured as a marker at the later time points of osteogenesis. The mineralized extracellular matrix was stained with ARS to determine bone mineralization.[5] Briefly, BMSCs (5×10^6 cells per well) were seeded in a 24-well culture plates. After being exposed to samples for 21 d, the cells were fixed with 95% ethanol for 10 min at 37°C, washed with PBS, stained with 40 mM ARS for 30 min, and washed with deionized water. Quantitation of mineralized matrix nodules was carried out by dissolving matrix nodules with 10% (w/v) cetylpyridium chloride and measuring the absorbance values at 570 nm.

Table S1. BET specific surface area and pore volumes calculated from the N_2 adsorption-desporption isotherms

	Surface Area (m^2/g)	**Pore Volume (cm^3/g)**
MSN	**490.5495**	**0.884998**
MSN-ALN-Gd	**11.5145**	**0.043714**
DEX@MSN-ALN-Gd	**2.2169**	**0.025201**

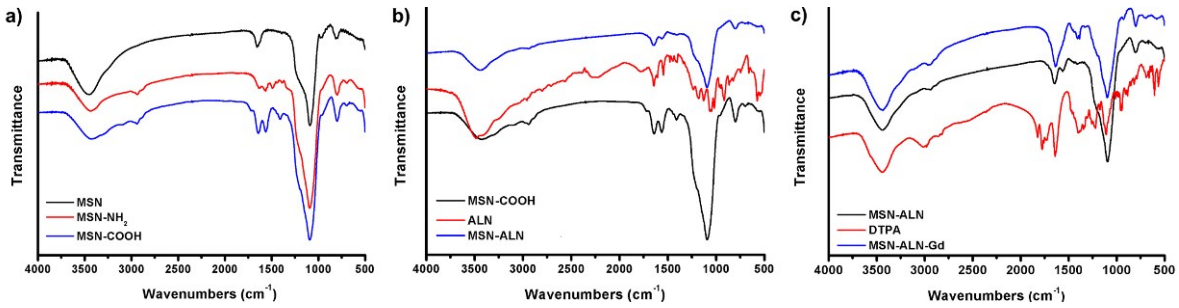

Fig. S1. FTIR spectra of the MSN of different functionalization.

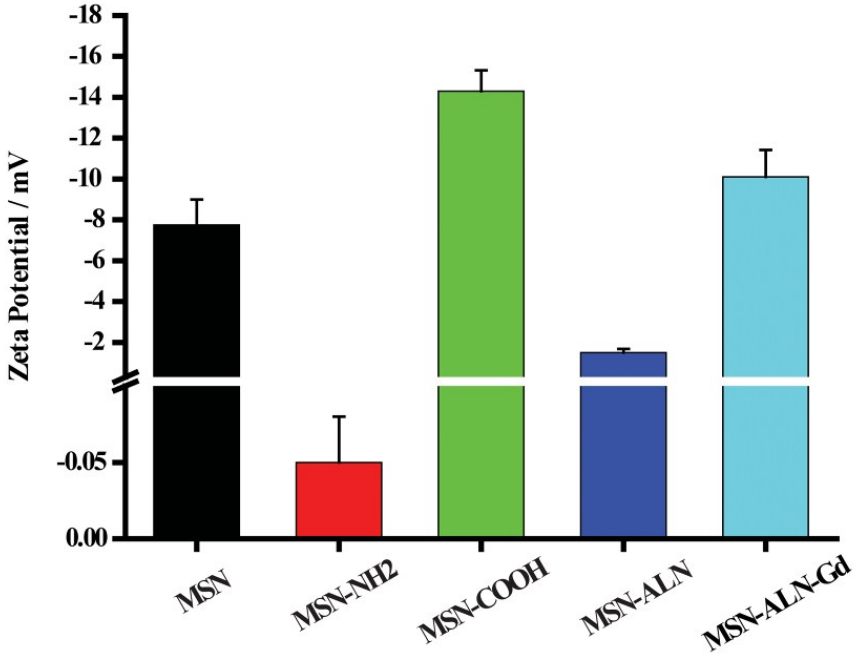

Fig. S2. Zeta potential of MSN and the functionalized MSN.

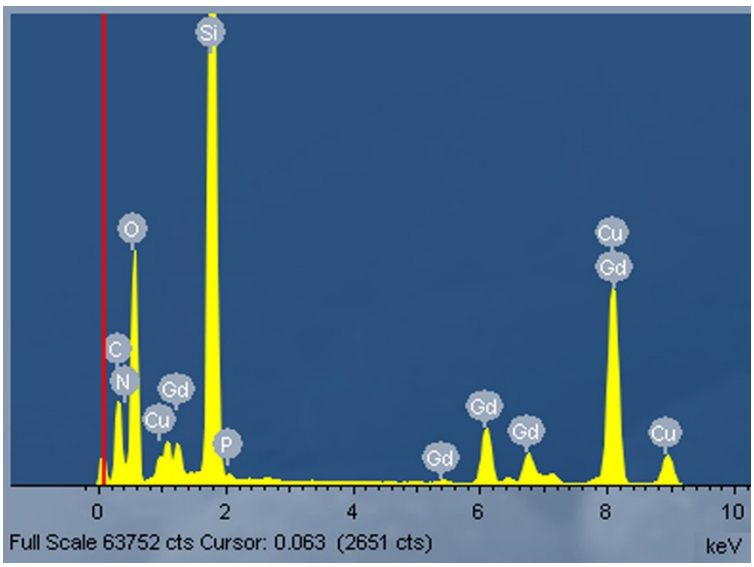

Fig. S3. EDX analysis of MSN-ALN-Gd.

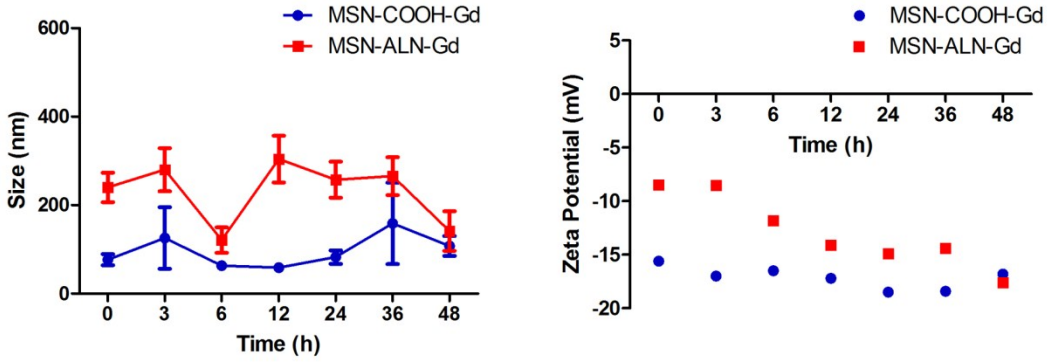

Fig. S4. Hydrodynamic size and zeta potential of MSN-COOH-Gd and MSN-ALN-Gd in 10% FBS media.

Table S2. Blood-element test and blood coagulation examination of mice after intraperitoneal injection with MSN-ALN-Gd.

Parameters	Control	MSN-ALN-Gd
WBC (10^9/L)	4.27±1.22	3.55±0.43
RBC (10^{12}/L)	9.98±0.61	10.30±0.75
HGB (g/L)	149.33±12.34	156.00±12.29
HCT (L/L)	0.44±0.02	0.46±0.04
MCV (10^{-15}/L)	45.26±3.85	45.43±2.20
MCHC (g/L)	315.33±11.01	333.66±17.61
RDW (%)	22.26±1.90	24.76±2.30
PLT (10^9/L)	723.00±83.21	781.00±89.01

Table S3. Measuring biochemical parameters in the serum of mice after treating with samples.

Parameters	Control	MSN-ALN-Gd
Urea (mmol/L)	8.99±0.74	9.18±0.67
Cr (μmol/L)	7.98±1.26	8.33±1.01
UA (μmol/L)	104.18±8.05	107.82±11.80
CK (U/L)	1410.65±93.12	1409.60±172.40
HBDH (U/L)	228.20±39.70	230.53±62.13
LDH (U/L)	710.40±72.21	712.55±98.01
AST (U/L)	152.00±12.16	143.00±33.20
ALP (U/L)	79.53±8.89	72.53±5.59
ALT (U/L)	43.56±4.97	42.26±4.45
TBIL (μmol/L)	0.91±0.62	1.19±0.48

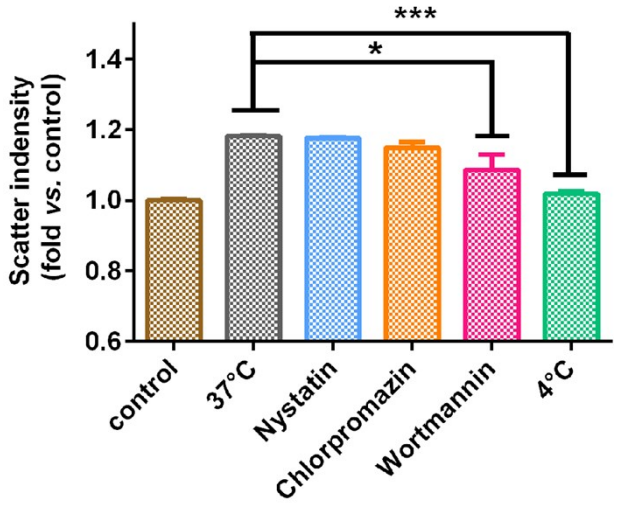

Fig. S5. Uptake of MSN-ALN-Gd by BMSCs in the presence of different endocytosis inhibitors and analyze energy-dependent processes of endocytosis by flow cytometric light scatter. *$p<0.05$ and ***$p<0.001$.

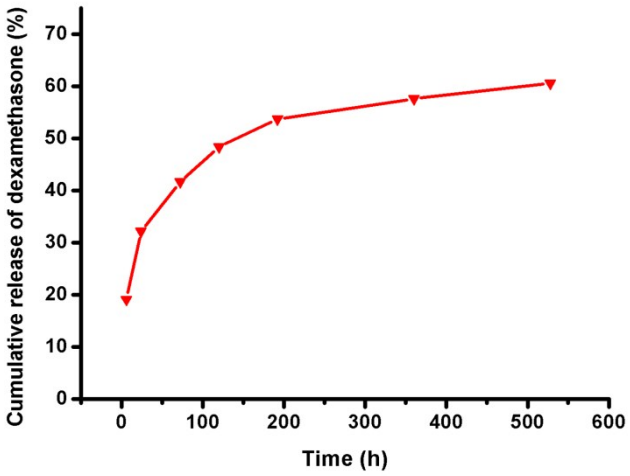

Fig. S6. Cumulative release of DEX in phosphate buffer solution at 7.4.

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