Science Advances
AAAS

Supplementary Materials for

Engineered MgO nanoparticles for cartilage-bone synergistic therapy

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Sci. Adv. **10**, eadk6084 (2024)
DOI: 10.1126/sciadv.adk6084

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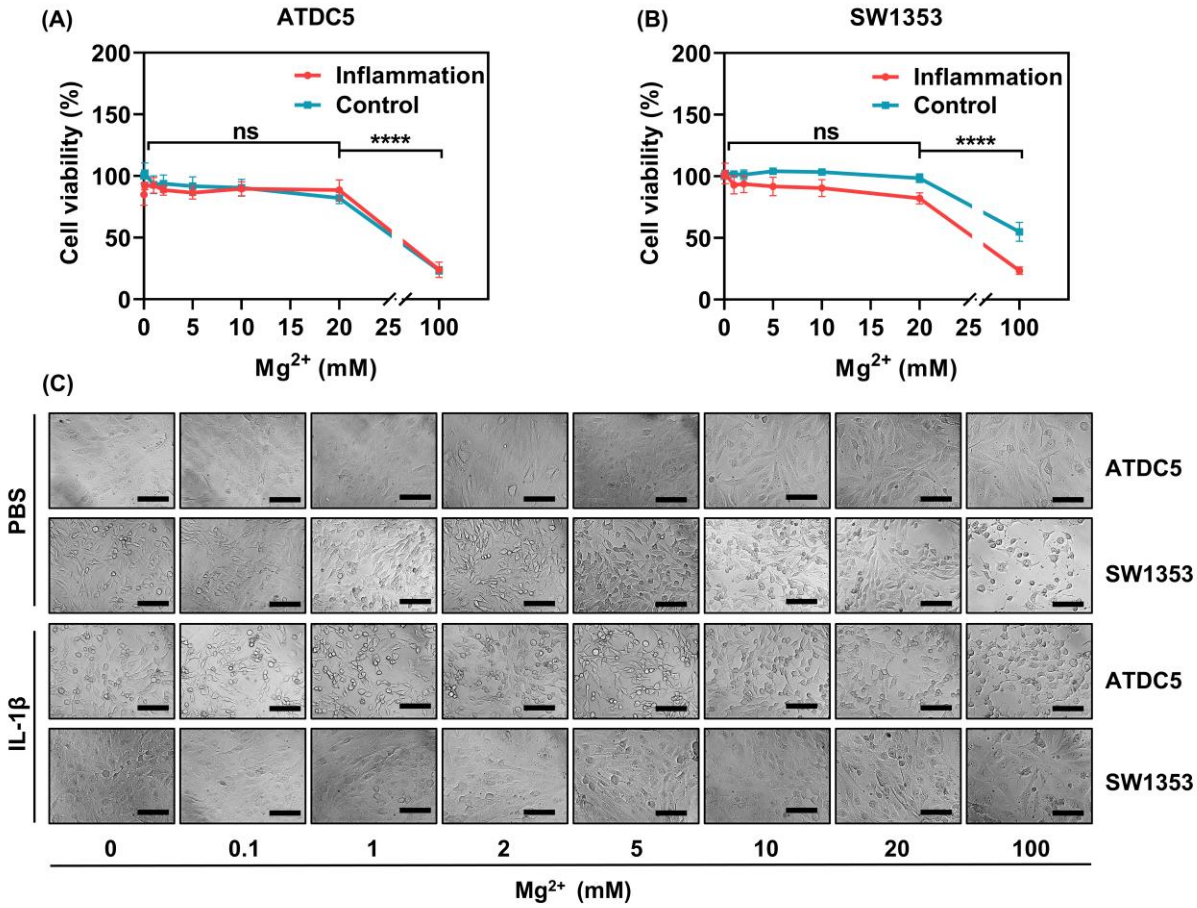

Fig. S1. Cell viability of Mg^{2+} in different kinds of cell lines.

Cell viability of ATDC5 (A) and SW1353 (B) cells after treatment with gradient concentrations of Mg^{2+} with/without inflammatory stimulation. (C) Representative microscopy images of ATDC5 and SW1353 cells after treatment with gradient concentrations of Mg^{2+} with/without inflammatory stimulation.

Notes: Scale Bar: 100 μm. All data are the mean ± s.d. Statistical differences between groups were determined by one-way ANOVA analysis. ****P<0.0001, ns P>0.05. n=3.

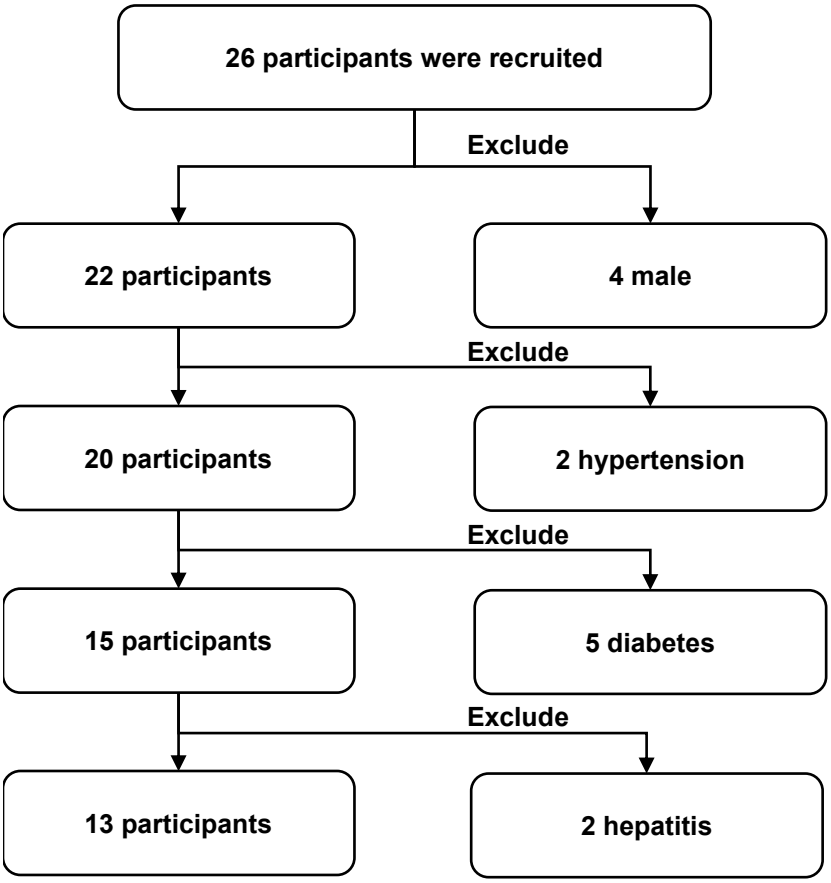

Fig. S2. Flow chart of the inclusion and exclusion criteria.

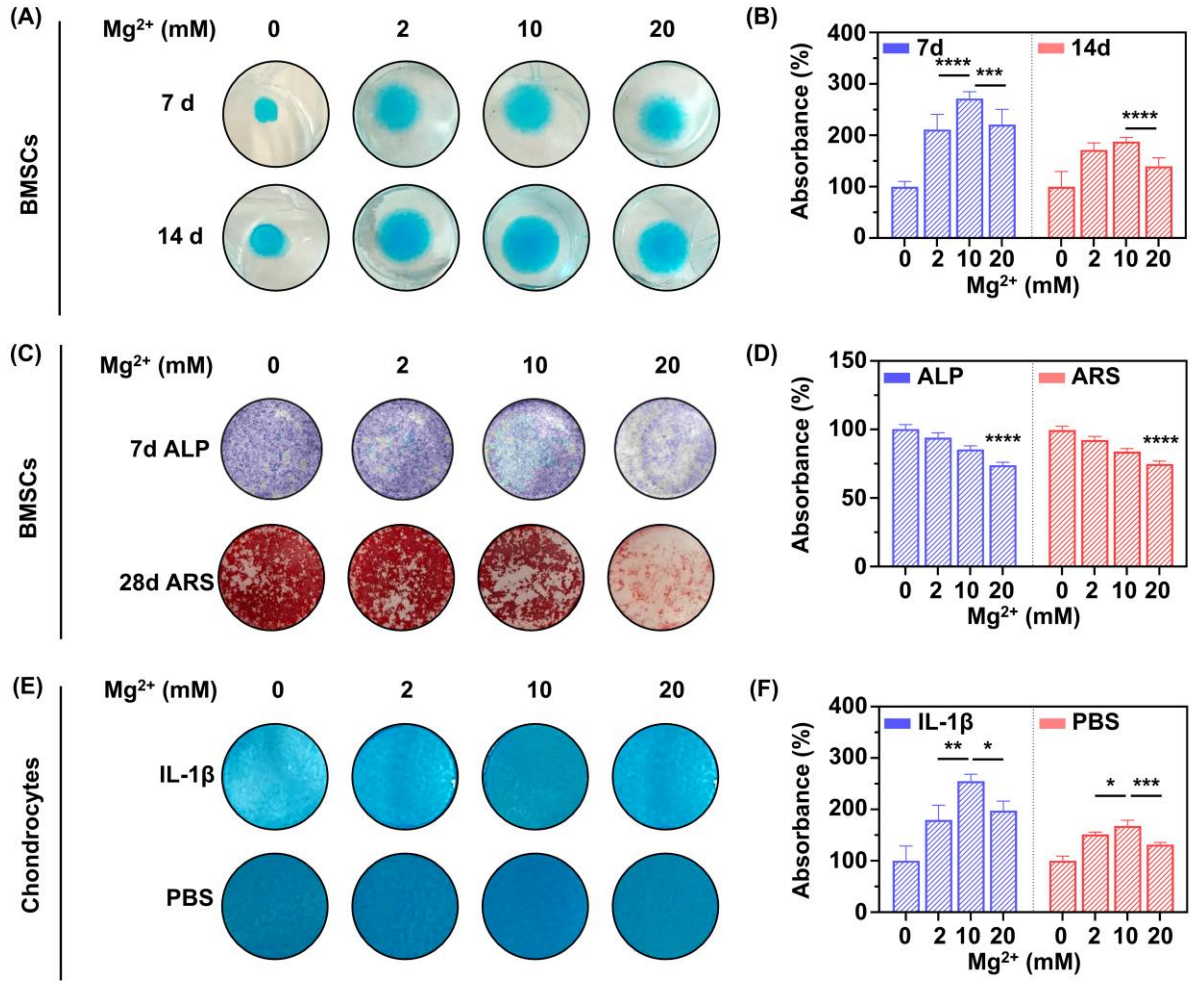

Fig. S3. Gradient Mg^{2+} regulated chondrogenic and osteogenic differentiation of BMSCs and protected inflammatory damaged chondrocytes *in vitro*.

Alcian blue staining of BMSCs treated with gradient Mg^{2+} (A) and quantitative results (B). ALP and ARS staining of BMSCs treated with gradient Mg^{2+} (C) and quantitative results (D). Alcian blue staining of chondrocytes treated with gradient Mg^{2+} with/without supplementation with 10 ng/mL IL-1β (E) and quantitative results (F).

Notes: All data are the mean ± s.d. Statistical differences between groups were determined by one-way ANOVA analysis. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. n=3.

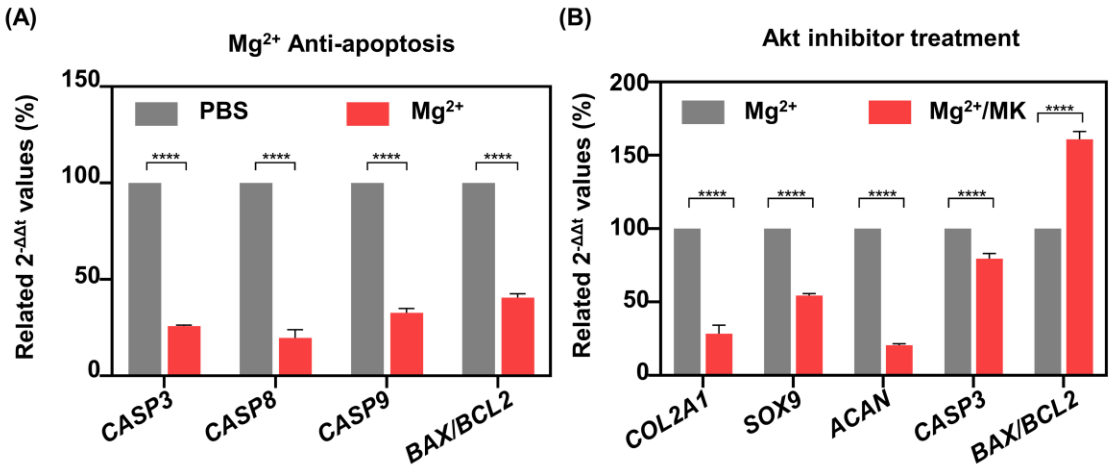

Fig. S4. Mg^{2+} inhibited apoptosis of chondrocytes via phosphorylation of Akt.
(A) Relative mRNA levels of apoptosis-related genes (*CASP3*, *CASP8*, *CASP9*, and *BAX*/*BCL2*) in SW1353 cells after treatment with 10 mM Mg^{2+} for 24 hours. (B) Relative mRNA levels (*COL2A1*, *SOX9*, *ACAN*, *CASP3*, *BAX*/*BCL2*) of apoptosis-related genes in SW1353 cells after treatment with 10 mM Mg^{2+} and MK2206 for 24 hours.
Notes: All data are the mean ± s.d. Statistical differences between groups were determined by t test. ***P<0.0001. n = 4.

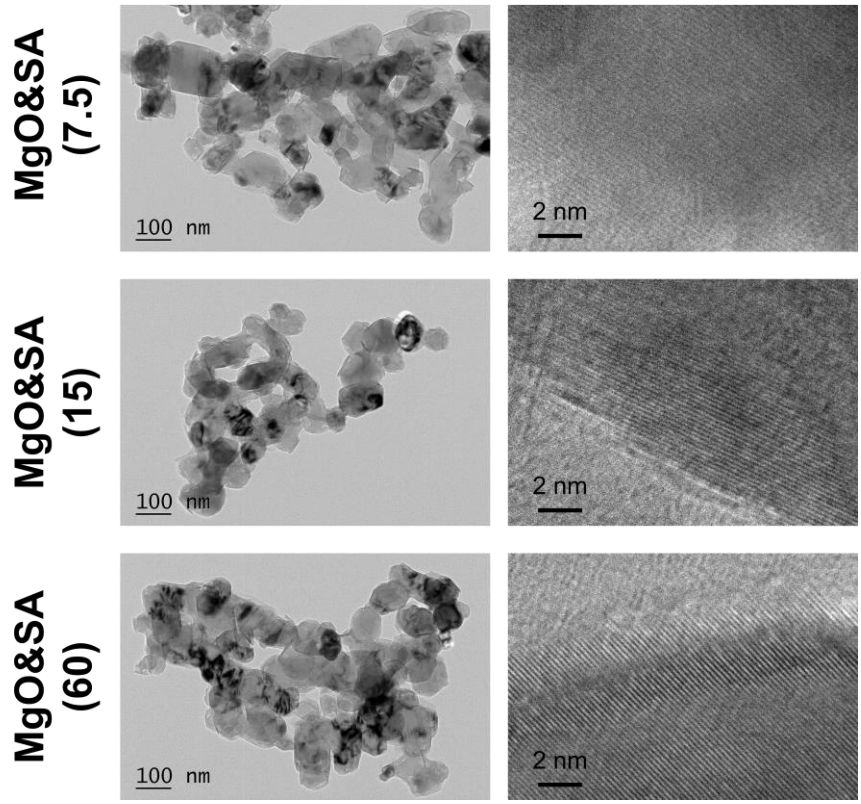

Fig. S5. TEM images of MgO nanoparticles modified with different concentrations of SA.

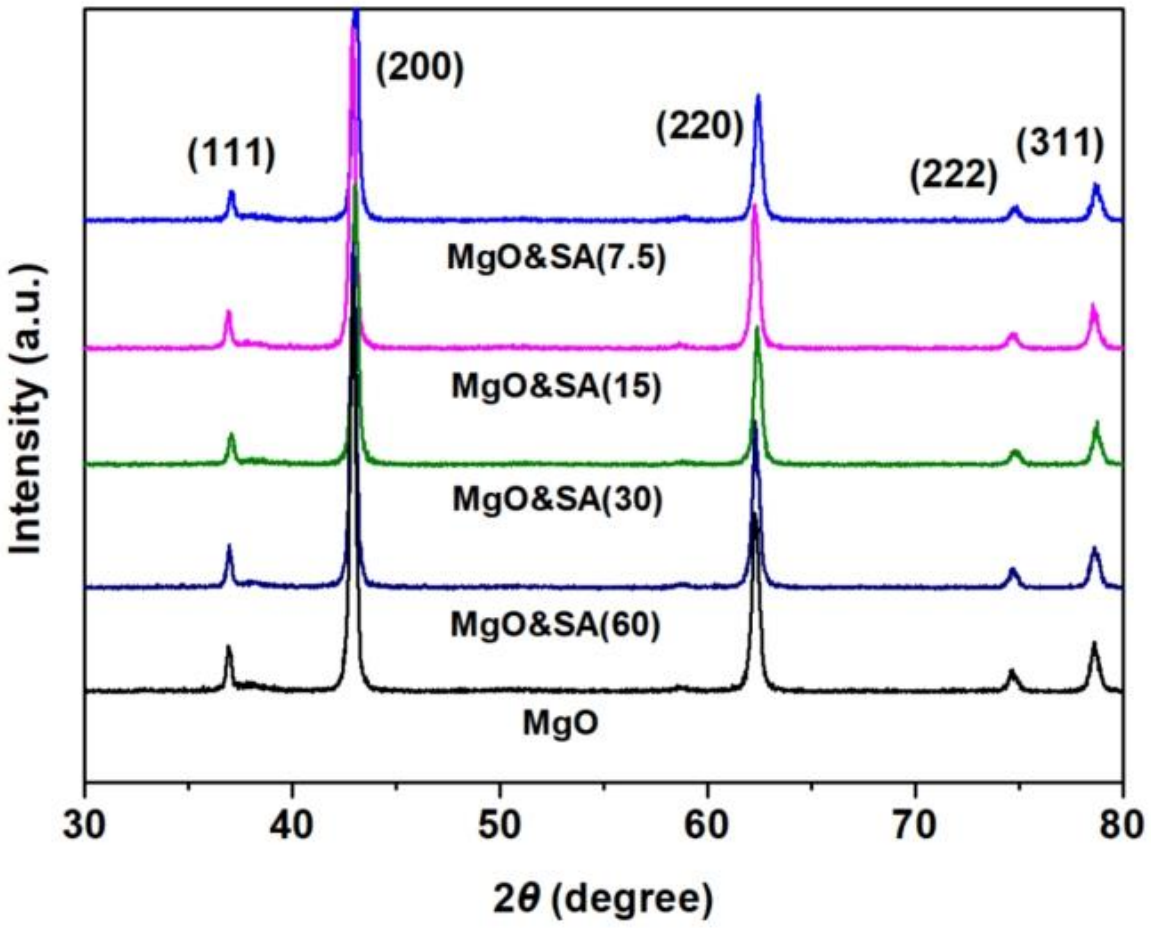

Fig. S6. XRD patterns of MgO nanoparticles modified with different concentrations of SA.

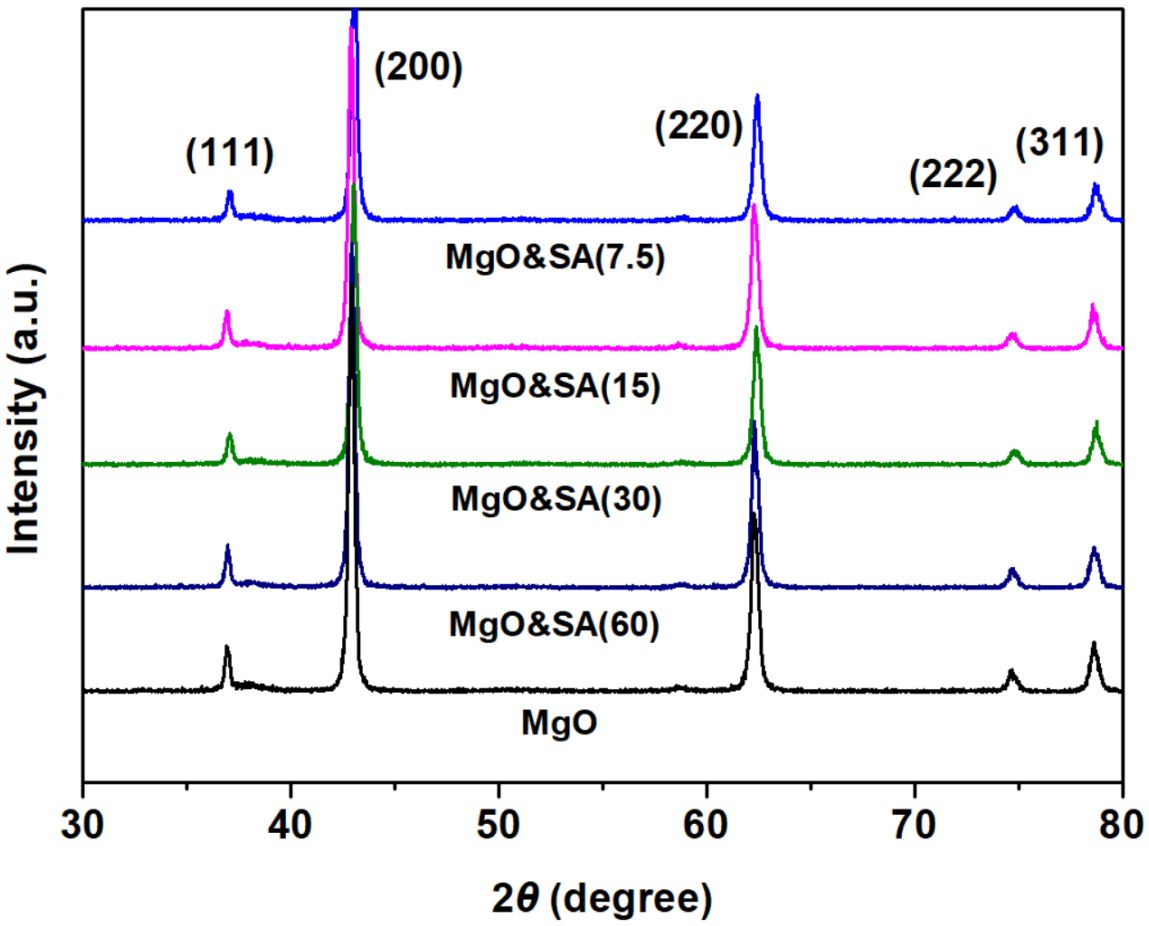

Fig. S7. FTIR spectra of MgO nanoparticles modified with different concentrations of SA.

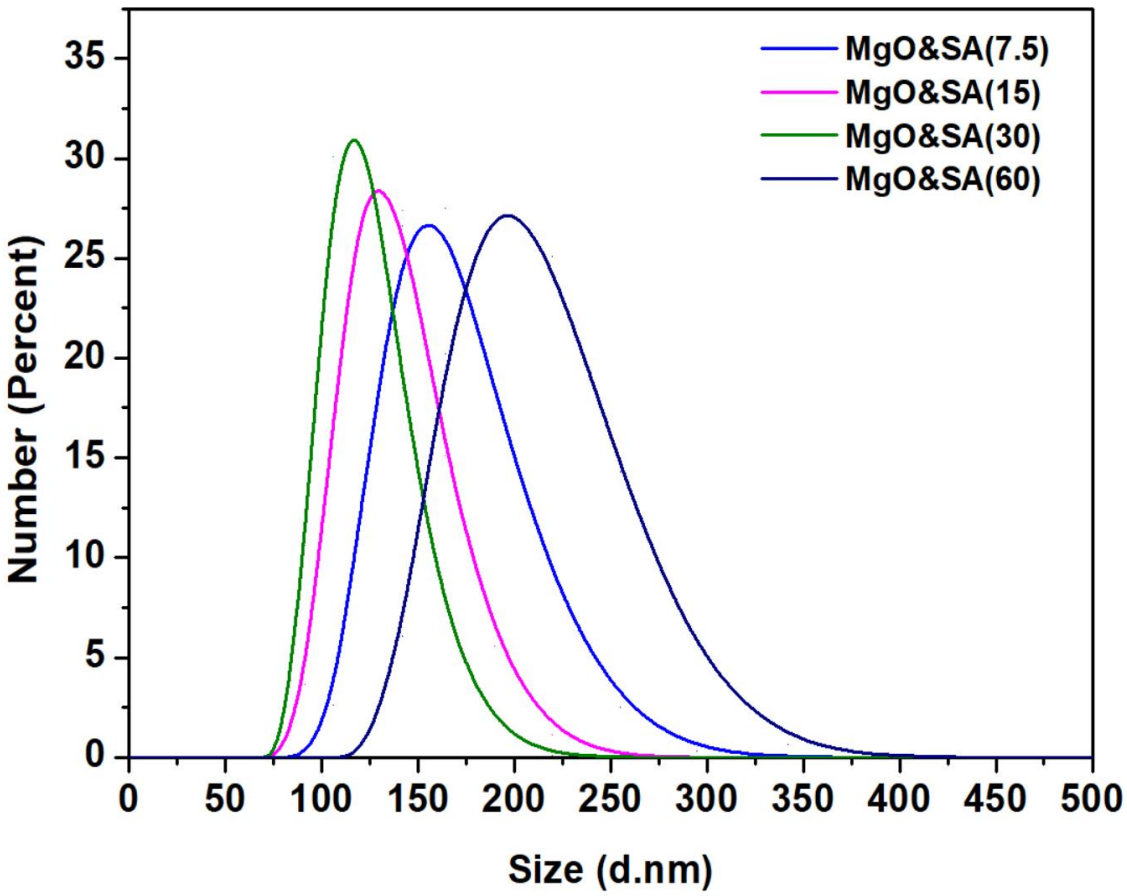

Fig. S8. DLS results of MgO nanoparticles modified with different concentrations of SA in DCM.

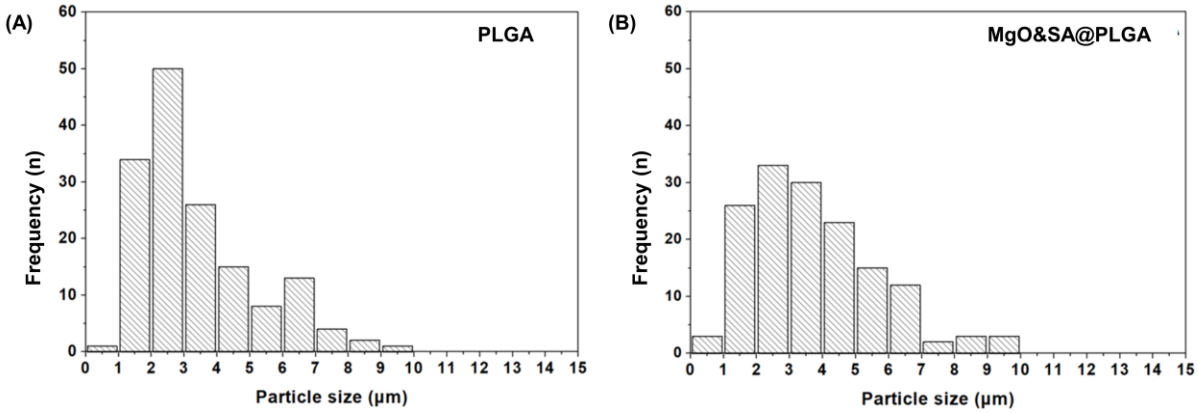

Fig. S9. Size distribution of PLGA (A) and MgO&SA@PLGA microspheres (B).

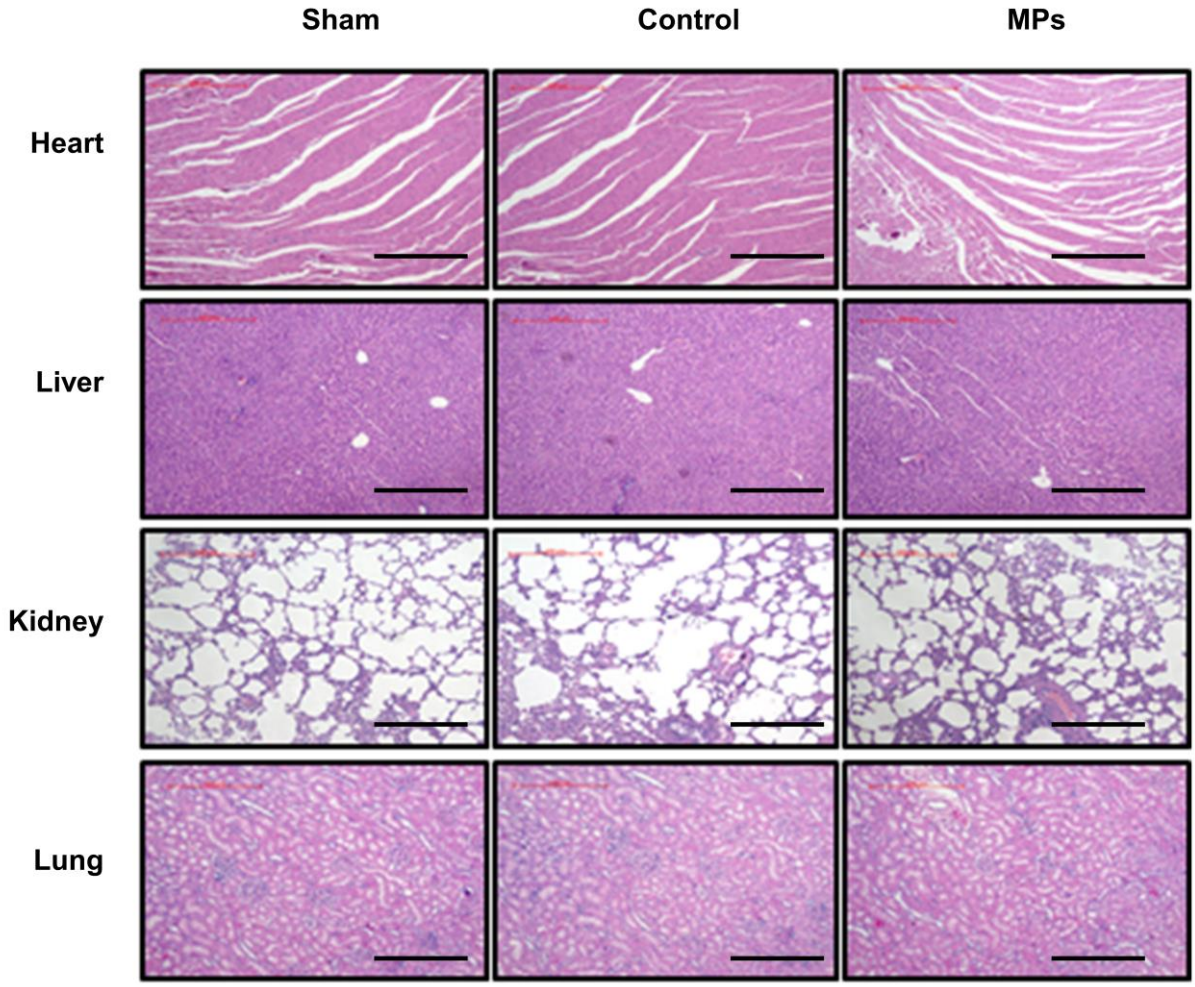

Fig. S10. Mg^{2+}-releasing MPs showed no toxicity *in vivo*.
H&E staining of sections of heart, liver, kidney, and lung of rats conditionally treated for 4 weeks.
Scale bar: 100 μm.

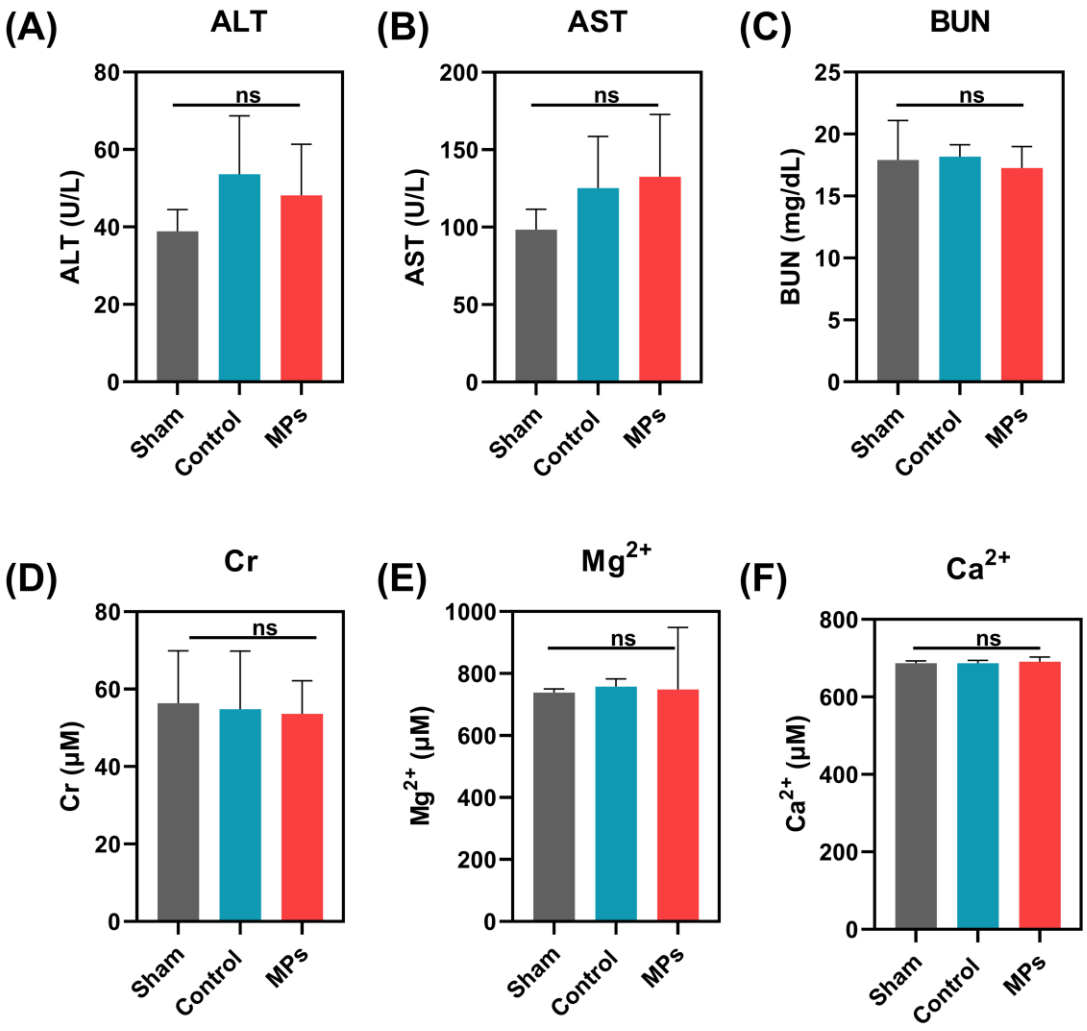

Fig. S11. Mg^{2+}-releasing MPs showed no toxicity *in vivo*.
Serum indexes, including ALT (A), AST (B), BUN (C), Cr (D), Mg^{2+} (E), and Ca^{2+} (F), were tested.
Note: All data are the mean ± s.d. Statistical differences between groups were determined by one-way ANOVA analysis. ns, P>0.05. n=6.

Table S1. Demographic data of the participants involved.

Participants	1	2	3	4	5	6	7
Age	71	75	71	72	74	73	74
Sex	Female	Female	Female	Female	Female	Female	Female
Diagnosis	Left Knee OA	Left Knee OA	Left Knee OA	Right Knee OA	Left Knee OA	Right Knee OA	Left Knee OA
Medical History	10 years	2 years	3 years	15 years	10 years	1 years	10 years
Height (cm)	150	159	153	160	160	156	152
Weight (kg)	70	90	57	71	83	65	70
BMI (kg/m^2)	31.1	36.5	24.3	27.7	32.4	26.7	30.3
Hypertension	N	N	N	N	N	N	N
Diabetes Mellitus	N	N	N	N	N	N	N
Mental State	Good	Good	Normal	Normal	Normal	Normal	Good
Exercise	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced
Usage	Fig 2 b-d	Fig 2 b-d	Fig 2 b-d	Fig 2 b-d e, f	Fig 2 b-d e, f	Fig 2 b-d e, f	Fig 2 e, f

Participants	8	9	10	11	12	13
Age	70	72	71	68	68	73
Sex	Female	Female	Female	Female	Female	Female
Diagnosis	Left Knee OA	Left Knee OA	Left Knee OA	Left Knee OA	Left Knee OA	Left Knee OA
Medical History	8 years	7 years	9 years	9 years	8 years	9 years
Height (cm)	163	162	157	161	162	155
Weight (kg)	80	75	79	84	73	71
BMI (kg/m^2)	30.1	28.6	32.0	32.4	27.8	29.6
Hypertension	N	N	N	N	N	N
Diabetes Mellitus	N	N	N	N	N	N
Mental State	Good	Normal	Good	Good	Normal	Normal
Exercise	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced
Usage	Fig 2g, h	Fig 2g, h	Fig 2g, h	Fig 2g, h	Fig 2g, h	Fig 2g, h

Table S2. Bone morphological parameters of subchondral bone at 2 and 4 weeks after the OA model was established.

Indexes	Sham	Control	MPs	P-value
BMC (mg/cm^3)	571.3 ± 149.1	421.1 ± 35.7	512.4 ± 39.0	**0.0048**
BMD (mg/cm^3)	929.1 ± 37.3	953.1 ± 18.7	957.2 ± 21.9	0.7596
BV/TV (A.U.)	0.61 ± 0.19	0.43 ± 0.05	0.53 ± 0.05	**0.0099**
BS/BV (1/mm)	14.78 ± 4.79	19.32 ± 1.95	16.50 ± 1.75	**0.0423**
Conn.D ($1/mm^3$)	84.46 ± 29.21	80.92 ± 20.8	93.44 ± 14.89	0.3054
SMI (A.U.)	-3.41 ± 3.89	-0.38 ±0.58	-1.16 ± 0.78	0.1113
Tb.N (1/mm)	4.05 ± 0.40	3.96 ± 0.36	4.20 ± 0.36	0.3258
Tb.Th (μm)	0.15 ± 0.06	0.10 ± 0.01	0.12 ± 0.01	**0.0344**
Tb.Sp (μm)	0.10 ± 0.05	0.15 ± 0.02	0.12 ± 0.02	**0.0267**

Note: P value showed the difference between Control and MPs groups and the P value was bold when $P < 0.05$. A.U. Arbitrary Units.

Table S3. Culture medium used to induce differentiation and indexes evaluated in different cells.

Cells	Medium for differentiation	Indexes evaluated
ATDC5	BM + ITS	Expression of Sox9 and Col2α1 separately at 7 and 14 days.
BMSCs (chondrogenic differentiation)	BM + Insulin (6.25 μg/mL) + TGF-β1 (10 ng/mL) + ascorbic acid (50 μg/mL)	Alcian blue staining at 7 and 14 days.
hBMSCs (osteogenic differentiation)	BM + β-sodium glycerophosphate (10 mM) + dexamethasone (0.1 μM) + ascorbic acid (50 μg/mL)	ALP and ARS staining at 7 and 28 days. Expression of osteogenic related genes at specific period.
Mice monocytes	BM + MCSF (30 ng/mL) + RANKL (50 ng/mL)	The number and area of osteoclasts at 7 days.

Table S4. List of sequences of primers used in this study.

Gene	Primer sequences (5'-3')	Annealing temperature	Source	Species
ACTB	F: TCACCCACACTGTGCCCATCTACGA	64.1	Thermo	Human
	R: CAGCGGAACCGCTCATTGCCAATGG	70.1	Fisher	
RUNX2	F: TGGTTACTGTCATGGCGGGTA	62.9	Thermo	Human
	R: TCTCAGATCGTTGAACCTTGCTA	61.1	Fisher	
OPN	F: CTCCATTGACTCGAACGACTC	60.2	Thermo	Human
	R: CAGGTCTGCGAAACTTCTTAGAT	60.0	Fisher	
OCN	F: CACTCCTCGCCCTATTGGC	62.2	Thermo	Human
	R: CCCTCCTGCTTGGACACAAAG	62.9	Fisher	
COL1A2	F: GAGGGCCAAGACGAAGACATC	55.0	Thermo	Human
	R: CAGATCACGTCATCGCACAAC	53.8	Fisher	
CASP3	F: CATGGAAGCGAATCAATGGACT	60.7	Thermo	Human
	R: CTGTACCAGACCGAGATGTCA	60.6	Fisher	
CASP8	F: AGAGTCTGTGCCCAAATCAAC	51.3	Thermo	Human
	R: GCTGCTTCTCTCTTTGCTGAA	52.6	Fisher	
CASP9	F: CTGTCTACGGCACAGATGGAT	61.3	Thermo	Human
	R: GGGACTCGTCTTCAGGGGAA	62.8	Fisher	
BAX	F: CCCGAGAGGTCTTTTTCCGAG	62.1	Thermo	Human
	R: CCAGCCCATGATGGTTCTGAT	61.9	Fisher	
BCL2	F: GGTGGGGTCATGTGTGTGG	62.6	Thermo	Human
	R: CGGTTCAGGTACTCAGTCATCC	61.8	Fisher	
SOX9	F: AGCGAACGCACATCAAGAC	50.9	Thermo	Human
	R: CTGTAGGCGATCTGTTGGGG	54.4	Fisher	
ACAN	F: ACTCTGGGTTTTCGTGACTCT	49.5	Thermo	Human
	R: ACACTCAGCGAGTTGTCATGG	52.5	Fisher	
COL2A1	F: TGGACGATCAGGCGAAACC	55.6	Thermo	Human
	R: GCTGCGGATGCTCTCAATCT	54.4	Fisher	

Table S5. List of antibodies used in this study.

Name	Source	Cat.NO	Species
Anti-GAPDH	Abcam	ab8245	Mouse
Anti-CASPASE-3	Abcam	ab184787	Rabbit
Anti-B-ACTIN	Abcam	Ab115777	Mouse
Anti-SOX9	Abcam	ab185966	Rabbit
Anti-AKT1(phospho S473)	Abcam	ab81283	Rabbit
Anti-AKT3+AKT2+AKT1	Abcam	ab32505	Rabbit
Anti-COL2A1	Abcam	Ab34712	Rabbit
Anti-COL1A2	Abcam	AB308455	Rabbit
Anti-COL10A1	Abcam	AB182563	Rabbit
Anti-PI3KCA	CST	4249S	Rabbit
Anti-BAX	Abcam	Ab32503	Rabbit
Anti-BCL2	Abcam	Ab182858	Rabbit
Anti-INTEGRIN-B1	CST	4706	Rabbit
Anti-ERK1/2	CST	4695	Rabbit
Anti-ERK1/2(phosphor Thr202/Tyr204)	CST	4370	Rabbit