Supplementary Information

Magnetic Nanocomposites for Magneto-Promoted Osteogenesis: From Simulation Auxiliary Design to Experimental Validation

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Synthesis of APS-Modified IO NPs

APS-modified IO (IO-APS) NPs was prepared based on our previous study with some modifications. Briefly, 0.44 g of APS was added to 200 mL of alcohol-water solution ($V_{ethanol}$: V_{water} = 9:1), and the solution was stirred for 30 min at room temperature. After 2 g of IO was added into the above solution, the mixture was dispersed by ultrasonic treatment for 20 min and then stirred continuously for 3 h. Subsequently, the pH of the solution was adjusted to 9-10, and the reaction system was stirred at 25°C for 3 h again. The product was washed with ethanol and water for 3 times, respectively. After that, the sample was collected with a magnet, placed at -80°C for 2 h and then dried in a freeze drier under vacuum.

Synthesis of BLG-NCA

The synthesis of γ-benzyl-L-glutamate N-carboxyanhydride (BLG-NCA) was performed based on previous reports. Briefly, BLG (20 g, 0.084 mol) and triphosgene (14 g, 0.047 mol) were mixed in a flame-dried three-neck round bottom flask, and anhydrous THF (180 mL) was added to the flask. Then, the reaction system was heated to 55 °C under N_2 protection and stirred constantly for about 40 min until the solution became transparent. Subsequently, the reaction solution was injected slowly into petroleum ether (1500 mL) and white precipitate appeared immediately. The white product was filtered through a filter funnel and then dissolved in ethyl acetate (100 mL). The product solution was gently washed with cooled distilled water for 3 times, and then dried with anhydrous $MgSO_4$ for 6 h. After that, the solution was concentrated with a rotary evaporator and dried with a vacuum pump to remove the ethyl acetate. The obtained product was recrystallized 3 times with anhydrous ethyl acetate for dissolving and anhydrous petroleum ether for precipitating. Finally, white precipitate was filtered and vacuum-dried to obtain the product BLG-NCA.

Dispersibility

The dispersibility of magnetic nanoparticles in chloroform was determined by sedimentation experiment at different times with a concentration of 30 mg/mL.

Contact angle test

The wettability of magnetic composites with different NPs was determined through the

test of the contact angle. The static contact angle measurements were carried out by using a Kru¨ss DSA 10 instrument (Germany) following the standard sessile drop method with ultrapure water. At least five droplets were dropped onto the same material. Subsequently, the contact angles of different samples were analyzed using software provided by the manufacturer.

Cell adhesion assays

The cover slides coated with different materials were placed on 24-well culture plates (Costar), and then sterilized by 75% alcohol along with UV for 2 h. After PBS (pH = 7.4) washing for 3 times, 2×10^4 MC3T3-E1 cells were seeded into each well and cultured with DMEM for 24 h with and without SMF. Then, the cells cultured on composite membranes were fixed with 4% paraformaldehyde (PFA) solution for 20 min at room temperature. After that, the F-actins of cells were stained by Acti-stain^{TM} 555 phalloidin (Beyotime, Shanghai, China), and the cell nuclei were stained by 4, 6-diamidino-2-phenylindole (DAPI, Sigma, USA) according to the manufacturer's protocols. After staining was completed, the morphology of the cells was observed and visualized by the fluorescent inverted microscope.

Cell morphology observation and proliferation assays

Cell morphology was observed by fluorescence isothiocyanate (FITC, Sigma) and DAPI staining after the cells were incubated for 3 days. The cells were fixed with 4% PFA solution for 20 min, and washed 3 times with PBS. Then, the cells were stained with FITC for 10 min at 37°C and washed with PBS again. Subsequently, the cell nuclei were stained with DAPI for 1 min at room temperature, and then washed repeatedly with PBS. Finally, the morphology of the cells was observed using a fluorescence reverse microscope (TE-2000U, Nikon, Japan). Moreover, the proliferation viability of MC3T3-E1 cells incubated on the different substrates was determined by the Cell Counting Kit-8 (CCK-8) assay. When the cells were incubated for 1 days, 3 days, and 7 days with and without SMF, the medium was replaced by fresh culture medium with 10% CCK-8. Then, the cells were incubated for another 2 h. Subsequently, 200 μL solution was transferred to 96-well culture plate, and measured at 450 nm by a multifunction microplate scanner.

ALP activity assay

Alkaline phosphatase (ALP) staining was applied to evaluate the ALP activity of MC3T3-E1 cells incubated on different substrates. When cultured for 3 days and 7 days with and without SMF, the cells were washed 3 times with PBS, fixed with 4% PFA for 20 min, and washed with PBS again. Then, the cells were stained with 500 μL ALP dye (Beyotime Biotechnology, Inc) for at least 12 h at 25 °C under dark conditions. Thereafter, the excess ALP dye was washed away with PBS and then the purple color intensity was observed using a light microscope (TE2000U, Nikon). Moreover, the ALP relative activity was detected by Alkaline phosphatase detection kit (Beyotime, China). After cultured for 3 days and 7 days under different conditions, MC3T3-E1 cells were washed 3 times with PBS and immersed in 200 μL RIPA cell lysis solution. Then, the cells were freezed at 80 °C and thawed at 37 °C for 3 times. After that, p-nitrophenyl phosphate disodium salt and BCA solution were added and incubated at 37 °C for 30 min. The absorbances at 405 nm (OD_{405}) and 562 nm (OD_{562}) were read using a multifunction microplate scanner, and the corresponding ALP expression was calculated according to the formula OD_{405}/OD_{562}.

Alizarin red staining for mineralization

Alizarin red staining was used to assess mineral deposition capacity of MC3T3-E1 cells cultured on various substrates with and without SMF. When incubated for 14 days and 21 days, the cells were washed 3 times with PBS and fixed with 4% PFA for 20 min. Then, the cells were stained with 1% (w/v) Alizarin Red S (ARS, Sigma) solution for 30 min at 37°C. After that, the extra ARS dye was washed away with PBS, and the content of calcium-rich deposits was qualitatively evaluated according to the red color intensity observed by an inverted microscope (TE2000U, Nikon). Calcium quantification was tested using 10% cetylpyridinium chloride (CPC) solution. ARS-stained membranes were washed with PBS and then treated with 1 mL CPC solution for 1 h to desorb calcium ions. Absorbance was measured at 540 nm by a multifunction microplate scanner.

Quantitative real-time polymerase chain reaction

MC3T3-E1 cells cultured on different materials were incubated for 7 days with and

without SMF. The expression of osteogenesis-related genes and osteogenesis-related force-gene was quantitatively evaluated via real-time PCR. The total RNA of the cells cultured on different substrates was extracted using TRIzol Reagent (Invitrogen, Thermo Fisher, USA) on the basis of the manufacturer's manual. The purity and concentration of RNA were assessed by Nanodrop Plates (Infinite M200, Tecan, Switzerland). The mRNAs of all the samples were reversely transcribed according to the description in Prime Script RT Reagent Kit with gDNA Eraser RR047A (TaKaRa, Japan). The expression of osteogenesis-related genes was quantified using SYBR Premix Ex Taq RR420A (TaKaRa, Japan). Gene-specific primers containing glyceraldehyde-3-phosphate dehydrogenase (GAPDH), bone morphogenetic protein 2 (BMP2), runt-related gene 2 (Runx2), osteopontin (OPN), osteocalcin (OCN), collagen type I (Col- I), and Piezo type mechanosensitive ion channel component 1 (Piezo1) were designed by the primer design software of beacon 5.0 as shown in Table S1. Real-time PCR analysis was implemented using Stratagene Mx3005P Real-time PCR System (Agilent Technologies Inc., USA) and the gene expression levels were acquired by the threshold cycles (Ct). Relative transcript quantities were calculated through using the ΔΔCt method. GAPDH was used as a reference gene and was amplified as well as the target genes from the same cDNA samples. The difference of the Ct value between the sample and GAPDH was defined as the ΔCt. The difference in the ΔCt of the cells grown on the experimental groups relative to the control group cells was defined as the ΔΔCt. The fold change in gene expression was expressed as 2-ΔΔCt.

Immunofluorescence staining

The expression of BMP2 and OPN in cells cultured on various substrates for 7 days was investigated by immunofluorescence staining. The cells were fixed with 4% PFA for 20 min at room temperature and washed 3 times with PBS. Then, the cells were permeabilized with Triton X-100 (0.25 %, v/v) in PBS for 5 min and blocked with 1% BSA in TBST (0.2% Tween 20) for 30 min. Subsequently, the cells were incubated with primary antibodies against BMP2 and OPN (1:1000; Abcam, Cambridge, MA) for 1 h at room temperature, and then washed repeatedly with PBS. After that, Alexa Fluor-488 IgG (1:1000; Abcam) was added as a secondary antibody, and the cells were

incubated for another 1 h. Finally, the cell nuclei were stained with DAPI and observed using upright fluorescence microscope (Nikon Eclipse C1, Japan). The mean fluorescence intensities of BMP2 and OPN were calculated by Image J.

2.14 Measurement of intracellular Ca^{2+}

The intracellular Ca^{2+} levels of MC3T3-E1 cells incubated on different substrates were tested by Fluo4-AM calcium indicator (Beyotime, China). When the cells were cultured for 2 days and 7 days with and without SMF, 500 μL of Fluo4-AM working solution (2μM in PBS) was added to each well and the plates were incubated at 37 °C for 30 min. After washed 3 times with PBS, the cells were detached with 0.5 mL trypsin, and collected by centrifugation. The mean fluorescence intensity of cell suspension was measured by a multifunctional microplate scanner (Tecan Infinite M200) with setting the exaction wavelength at 488 nm and the emission wavelength at 518 nm. Moreover, after the cells were cultured for 2 days and treated with Fluo4-AM working solution, the fluorescent images of calcium signal were taken by a fluorescence reverse microscope (TE-2000U, Nikon, Japan).

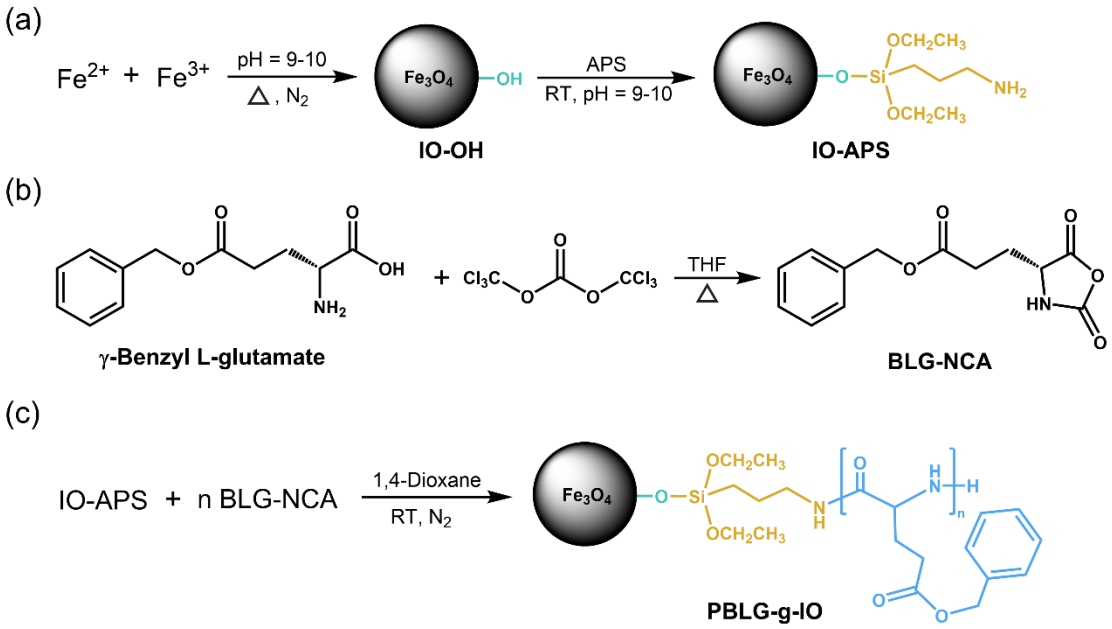

Fig. S1. The synthetic route of (a) IO-APS, (b) BLG-NCA and (c) PBLG-g-IO.

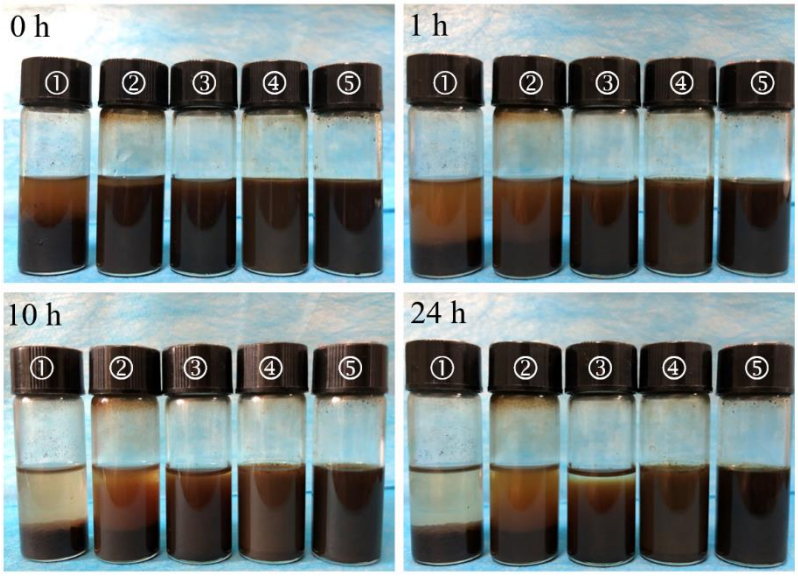

Fig. S2. Images of IO (①), IO-APS (②), PBLG-g-IO-1 (③), PBLG-g-IO-2 (④), and PBLG-g-IO-3 (⑤) in chloroform at different times after ultrasonication.

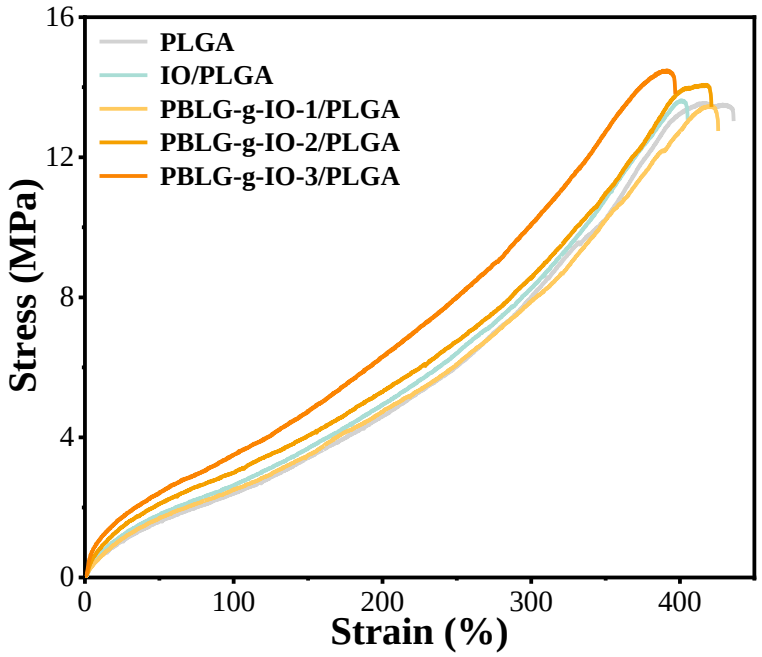

Fig. S3. The typical stress–strain curves of the magnetic composites.

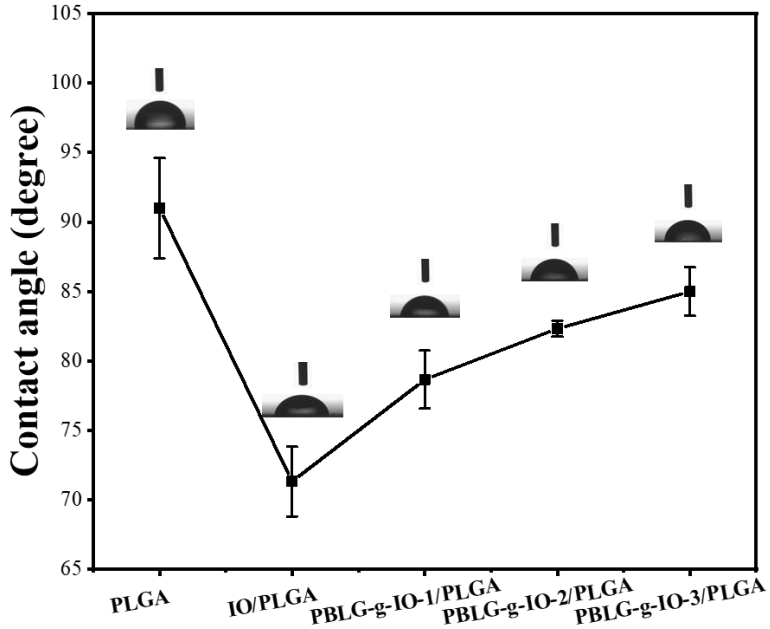

Fig. S4. Contact angle analysis of the magnetic composites.

Table S1 Graft amount and reaction efficiency of IO-APS and PBLG-g-IO related to reaction conditions analyzed by TGA

Samples	Feed ratio (w/w) IO-APS : NCA	Reaction time (h)	Graft amount[a] (%)	Graft efficiency[b] (%)
IO-APS	-	-	0.96	
PBLG-g-IO-1	1 : 0.3	32	13.21	52.76
PBLG-g-IO-2	1 : 0.6	32	21.46	49.52
PBLG-g-IO-3	1 : 1	32	32.34	47.61

[a] Graft amount of APS (GA%): GA (%) = $W_{(IO\text{-}APS)}$ - $W_{(IO)}$ (%). Graft amount of PBLG (GA%): GA (%) = $W_{(PBLG\text{-}g\text{-}IO)}$ - $W_{(IO\text{-}APS)}$ (%). [b] Graft efficiency (GE%): GE (%) = $M_{(PBLG)}/M_{(NCA)}$ (%).

Table S2 Primary sequences of GAPDH, BMP2, Runx2, OPN, OCN, Col-I and Piezo1

Gene	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')
GAPDH	TGAACTAACACAGAGGAGGATCAG	GCTTAGGGCATGAGCTTGAC
BMP2	GAAGCAAGGTGTCTCCAAG	TCCGCTGTTTGTGTTTCG
Runx2	GCCCTCATCCTTCACTCCAAG	GGTCAGTGCCTTTCCTC
OPN	TCAGGACAACAACGGAAAGGG	GGAACTTGCTTGACTATCGATCAC
OCN	AAGCAGGAGGGCAATAAGGT	TTTGTAGGCGGTCTTCAAGC
Col-1	CGCTGGCAAGAATGGCGATC	ATGCCTCTGTCACCTTGTTCG
Piezo1	ATCGCCATCATCTGGTTCCC	TGGGGTGTGAATGGCACAAT