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Supporting Information

Nanocapsules engineered from polyhedral ZIF-8 templates for bone-targeted hydrophobic drug delivery

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1. Materials

Alendronate (Aln, 98.0%), simvastatin (SIM) and hydroxyapatite (HA) were purchased from the National Institute of Control of Pharmaceutical and Biological Products (China). Gelatin (Type B from bovine skin, ~225 bloom), fluorescein isothiocyanate (FITC), coumarin-6 (its excitation wavelength was 466 nm, emission wavelength was 504 nm), IR-780 iodide (98.0%, its excitation wavelength was 683 nm, emission wavelength was 750 nm) and tris(hydroxymethyl)amiomethane (Tris) were purchased from Sigma-Aldrich Chemical Company(USA). Dopamine hydrochloride was purchased from Yuancheng Technology Development Co. Ltd (China). $Zn(NO_3)_2 \cdot 6H_2O$ (99.0%) and 2-methylimidazole (Hmim, 99.0%) were purchased from Aldrich (China). 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC·HCl) and N-hydroxysuccinimide (NHS) were purchased from Shanghai Medpep Co. Ltd (China). Tannic acid (TA, Mw=1701.23 Da), methanol (99.0%) and ethylenediaminetetra acetic acid disodium (Na_2EDTA) were purchased from Tianjin Guangfu Fine Chemical Research Institute (China). Phosphate Buffered Saline (PBS) was procured from the Shijiazhuang biotechnology Co., Ltd (China). Mouse myoblasts cells (C2C12 cells) and human osteoblast-like cells (MG-63) were provided by Institute of Basic Medical Sciences, Peking Union Medical College (China).

2. Synthesis and characterization of catechol modified gelatin (GelC)

The synthesis and characterizationof GelC polymer has been discussed in detail in our previous literature,[1] and the synthesis process was as illustrated in Fig.S1. 4 g of gelatin was dissolved in PBS (100 mL, pH 5.5) at 40 °C. EDC (4 mmol), NHS (4 mmol) and dopamine (8 mmol) were added to the gelatin solution, the reaction was conducted under N_2 protection at 40 °C for 12 h. The product was obtained after precipitating with cold alcohol followed by lyophilization.

UV−vis spectra of gelatin and GelC solution were monitored as shown in Fig. S2a. The peak at 280 nm was ascribed to the stretching of the catechol groups from GelC. According to the calibration curve of dopamine (See Fig. S2b), the concentration of the conjugated catechol groups were calculated. Therefore, the graft ratio and catechol content in GelC were calculated as 21.3% and 213 μmol g^{-1} GelC according to the concentration of the conjugated catechol groups.

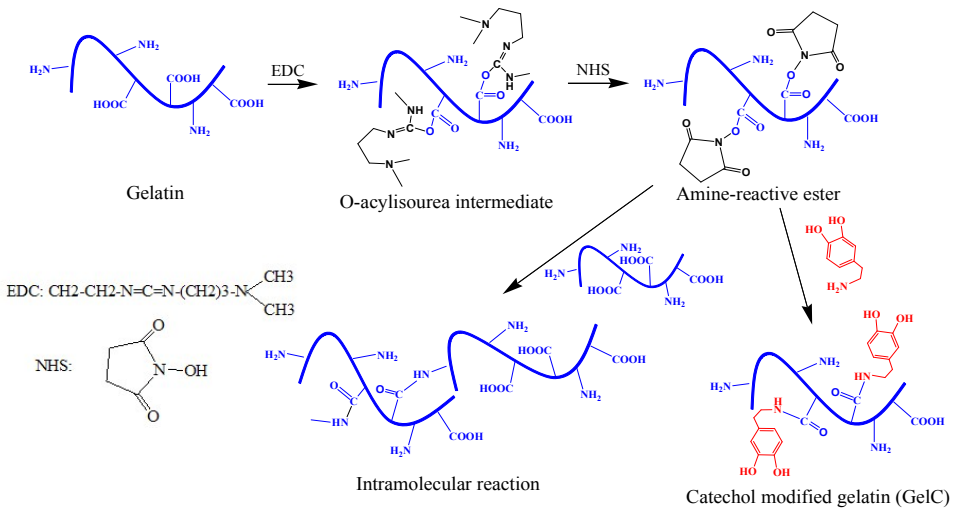

Fig. S1 Schematic representation of the synthesis process of catechol modified gelatin (GelC).[1]

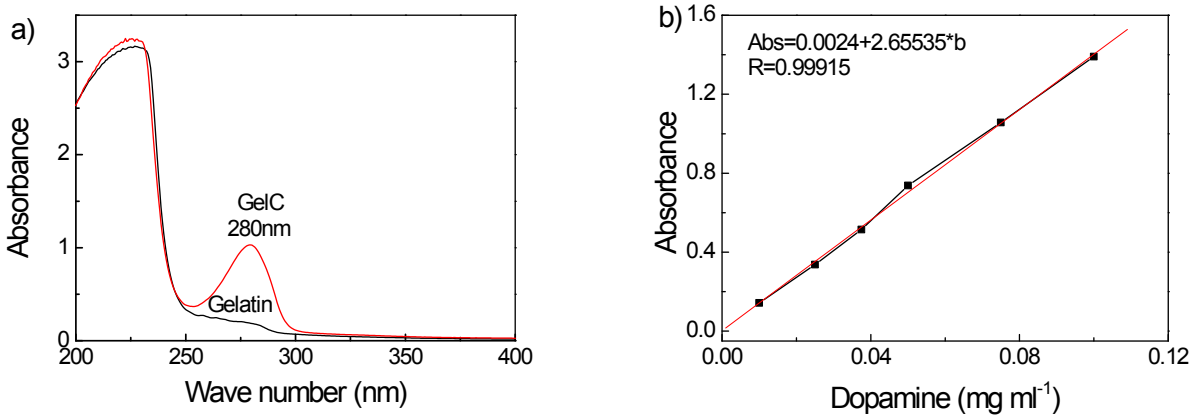

Fig. S2 (a) UV−vis spectra of GelC aqueous solutions (2 mg mL^{-1}) and (b) a calibration curve given by monitoring the absorbance of different concentration of dopamine at 280 nm.

3. Fabrication of bone-targeting nanocapsules (Aln-SIM@GelC NCs)

3.1 Synthesis of the ZIF-8 nanoparticles

The ZIF-8 particles were fabricated according to the method reported by Cravillon et al.[2] The methanol sollution of $Zn(NO_3)_2 \cdot 6H_2O$ (3 g, 100 mL) was rapidly poured into an equal volume methanol solution of Hmim (6.6 g) with stirring at ambient temperature. After 1 h vigorous stirring, the ZIF-8 particles were collected by centrifuging (9000 rpm, 7 min) and washing with methanol, and then lyophilized prior to utilization.

3.2 Fabrication of the GelC nanocapsules (GelC NCs)

200 mg of ZIF-8 particles were suspended in 20 mL of enthanol by ultrasonic dispersion, and an equal volume TA aqueous solution (4 mg mL^{-1}) was added and stirred for 30 min. Then, TA-coated ZIF-8 particles were centrifuged and washed with water until the supernatant was colorless. Subsequently, TA-coated ZIF-8 were dispersed in GelC aqueous solution (25 mg mL^{-1}, Tris-Hcl, pH 7.5) with stirring at 40 °C for 4 hours. Finally, the GelC nanocapsules (GelC NCs) were obtained by dissolution of the ZIF-8 templates in an EDTA solution (50 mM, pH 8.0) for two times followed by centrifuging and washing (20000 rpm, 15 min).

3.3 Encapsulation of SIM inside GelC nanocapsules (SIM@GelC NCs)

50 mg of SIM and 6.6 g of Hmim were dissolved in 100 mL of methanol solution, and the methanol sollution of $Zn(NO_3)_2 \cdot 6H_2O$ (3 g, 100 mL) was then rapidly poured and stirred for 1 hours at room temperature. The SIM-doped ZIF-8 particles (SIM@ZIF-8) were obtained after centrifuging and washing with methanol to remove residual SIM, Hmim and salts. Subsequently, 200 mg of SIM-doped ZIF-8 were suspended in 20 mL of enthanol by ultrasonic dispersion, and the SIM-encapsulated GelC nanocapsules (SIM@GelC NCs) were prepared following the same procedure as described in section 3.2.

The supernatants were collected and filtered, then the concentration of SIM in the supernatants were measured by HPLC with the C18 reverse-phase column. The column temperature was at 25 °C, the mobile phase was 25 mM, pH 4.5 NaH_2PO_4:acetonitrile (35:65) with a flow rate of 1.0 mL min^{-1}, the detection wavelength was 238 nm, the injection volume was 20 μL.

The SIM encapsulation efficiency and loading efficiency in ZIF-8 (or GelC NCs) were determined according to eq.1−2 (3−4):

$$\text{SIM encapsulation efficiency in ZIF-8 } (EE_{ZIF\text{-}8,}\%) = \frac{(m - C_1V_1)}{m} \times 100\% \quad (1)$$

$$\text{SIM loading efficiency in ZIF-8 } (LE_{\text{ZIF-8,}}\%) = \frac{(m - C_1V_1)}{W_{ZIF\text{-}8}} \times 100\% \quad (2)$$

$$\text{SIM encapsulation efficiency in GelC NCs (\%)} = \frac{\left(200 \times EE_{ZIF\text{-}8} - C_2V_2\right)}{m} \times 100\% \quad (3)$$

$$\text{SIM loading efficiency in GelC NCs (\%)} = \frac{\left(200 \times EE_{ZIF\text{-}8} - C_2V_2\right)}{W_{GelC}} \times 100\% \quad (4)$$

where m (mg) was the amount of SIM introduced into the immobilization medium; C_1 (mg mL^{-1}) and V_1 (mL) were the SIM concentration and supernatant volume when preparing SIM-doped ZIF-8 particles; C_1V_1 (mg) was the residual amount of SIM in the immobilization medium; C_2 (mg mL^{-1}) and V_2 (mL) were the SIM concentration and volume of the supernatant, respectively; C_2V_2 (mg) was the amount of SIM leaked out during removal of ZIF-8; $W_{ZIF\text{-}8}$ (g) was the weight of the ZIF-8 particles, W_{GelC} (g) was the weight of the GelC NCs.

3.4 Covalent attachment of Aln onto the GelC nanocapsules

The SIM@GelC NCs and Aln (1:1 w/w) were dispersed separately in 500 μL PBS. Subsequently, the two solutions were mixed and reacted for 1 hours for Aln conjugation, and the reaction was terminated by adding 100 μL of Tris-HCl (pH 7.4) for 15 min. Alendronate was attached on the surface of SIM@GelC NCs via the Michael addition and Schiff base reactions, and the Aln-SIM@GelC NCs were obtained after centrifuging and washing to remove unconjugated Aln (20000 rpm, 15 min). The amount of Aln in the supernatant was measured by using TNBS assay for detection of primary amino group according to the method reported in literature. [3, 4] The amount of Aln attached on the surface of nanocapsules was determined according to the introduced Aln and the quantity of Aln in the supernatant. The reaction without Aln was as a controlled experiment.

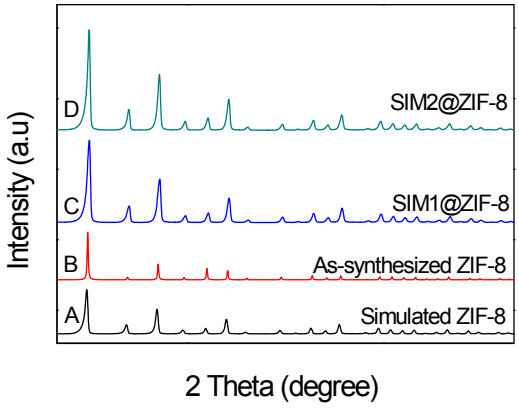

Fig. S3 XRD patterns of the simulated data from ZIF-8 (A), the as-synthesized ZIF-8 (B), ZIF-8 encapsulating with 50 mg SIM (C, SIM1@ZIF-8) and 100 mg SIM (D, SIM2@ZIF-8).

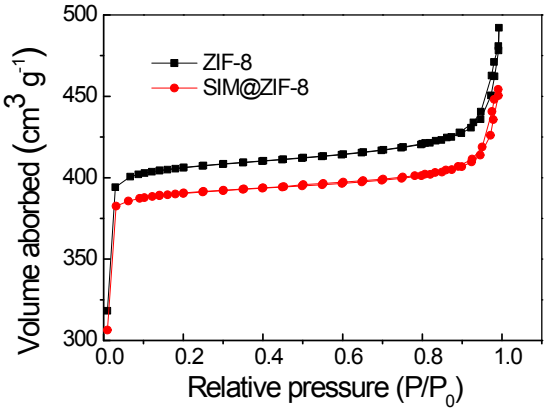

Fig. S4 Nitrogen adsorption–desorption isotherms of ZIF-8 and SIM@ZIF-8

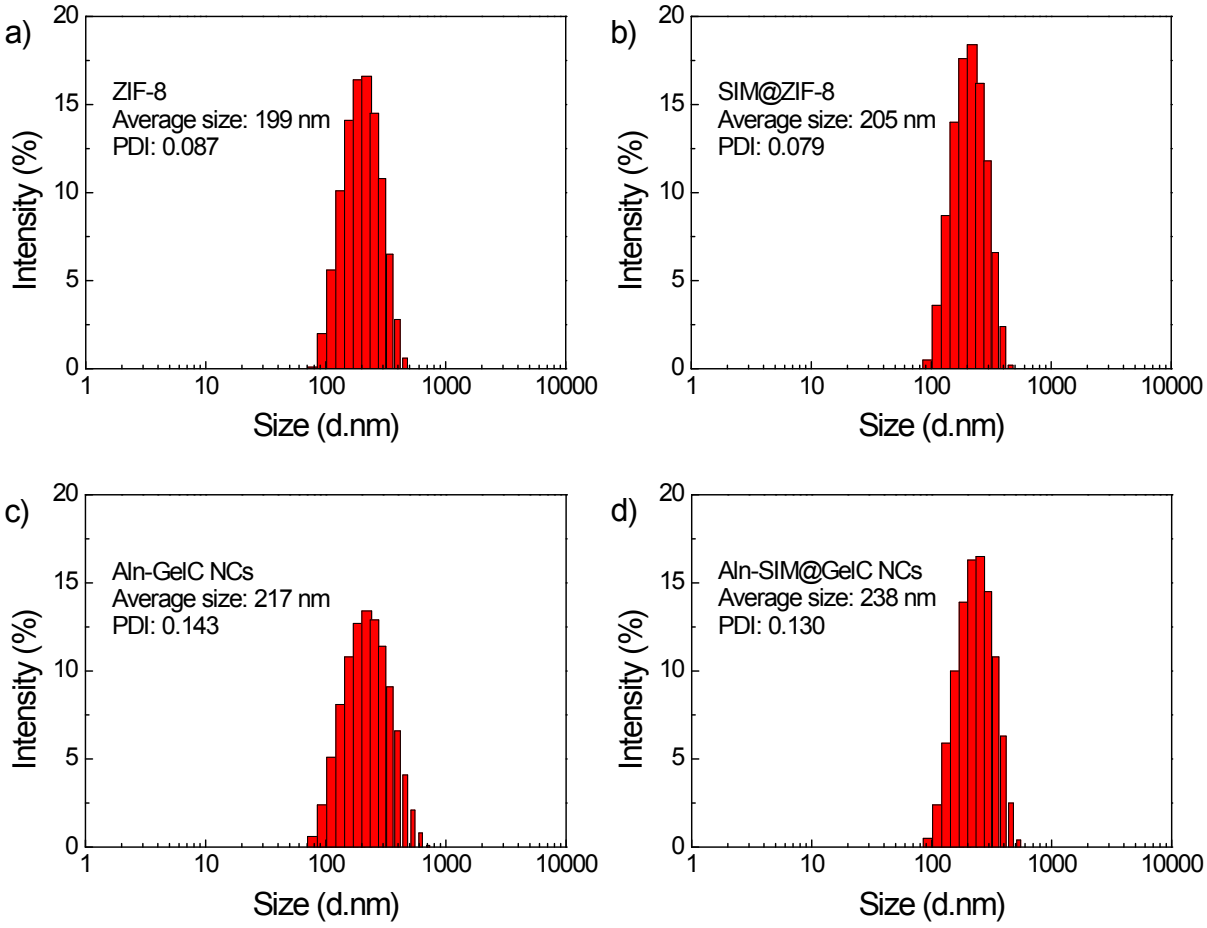

Fig. S5 DLS size distributions of different formulations

To assess the stability of Aln-SIM@GelC NCs under physiological conditions, the size of the Aln-SIM@GelC NCs was measured by DLS after incubation in cell culture media (DMEM, Gibco, without serum) at 37 °C with a speed of 150 rpm for extended periods of time. Despite some

aggregation was observed over the period of 4 days, the main population of the NCs remained intact with a slight reduction in average particle size (Fig S6.).

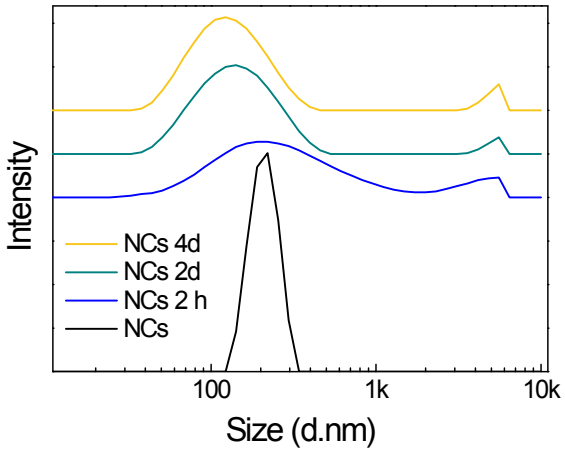

Fig. S6 DLS size distribution of Aln-SIM@GelC NCs prior to and after incubation in Dulbecco's modied Eagle medium (DMEM, Gibco, without serum) at 37 °C for extended periods of time.

4. Properties of bone targeting nanocapsules (Aln-SIM@GelC NCs)

4.1 *In vitro* drug release

The release of SIM from SIM@GelC and Aln-SIM@GelC NCs was performed in a shaking incubator at 37 °C with a shaking frequency of 100 rpm. 1 mg of SIM@GelC and Aln-SIM@GelC NCs were suspended in 6 mL of PBS (pH 7.4). At each time point, samples were centrifuged, 3 mL of the supernatant was taken out and measured by spectrophotometry at 238 nm. Equal volume of fresh PBS was replenished. The cumulative release of SIM was calculated using a calibration curve of SIM obtained in incubation medium and the results were presented in terms of cumulative release as a function of time.

4.2 Cell culture and cytotoxicity assessment

Mouse myoblasts cells (C2C12 cells) were grown at 37 °C under humidied air (5 vol% of CO_2) in Dulbecco's modied Eagle medium (DMEM, Gibco), which was supplemented with 10 vol% of FBS, 100 units mL^{-1} of penicillin and 100 units mL^{-1} of streptomycin.

Firstly, C2C12 cells were seeded in 96-well plate at a density of 5×10^3 cells per well and grown in DMEM complete medium for 24 h. Subsequently, cells were incubated with different concentrations (1, 10 and 100 μg mL^{-1}) of Aln-SIM@GelC NCs and SIM@GelC NCs for 24 h. Afterwards, the medium was removed, 20 μL of MTT (5 g L^{-1}) and 180 μL of medium were added. After 4 h of incubation, MTT-containing medium was removed and 200 μL of DMSO was added and incubated

for another 10 min. The absorbance at 490 nm was measured by using a micro-plate reader (Tecan M200 PRO). Cell viabilities were determined relative to the untreated cells, which was taken as a control (100% viability). For each concentration, 6 parallel measurements were carried out at the same time.

4.3 HA affinity test

Aln-SIM@GelC NCs and SIM@GelC NCs were dispersed in ultrapure water with a concentration of 1 mg mL^{-1}, and the initial absorbance was measured by an UV spectrophotometer at 280 nm. Then, 25 mg of HA was added into 4 mL of Aln-SIM@GelC NCs and SIM@GelC NCs, respectively. Furthermore, a control group was performed. Specifically, excess amount of free Aln (100 mg) was incubated with HA (25 mg) for 1 h, then the obtained Aln-HA was incubated with 4 mL of Aln-SIM@GelC NCs. After slowly stirring for a period of time, the suspension was centrifuged for 15 min at 3000 rpm, the supernatant was collected and analyzed by an UV spectrophotometer at 280 nm. And the binding kinetic of Aln-SIM@GelC NCs to HA, Aln-SIM@GelC NCs to Aln-HA and SIM@GelC NCs to HA was obtained.

The relative HA binding was determined according to eq.5:

$$\text{Relative HA binding (\%)} = \frac{absorbance_{initial} - absorbance_{supernatant}}{absorbance_{initial}} \times 100\% \quad (5)$$

4.4 Cellular uptake investigation *in vitro*

The cellular uptake of the nanocapsules formulations was evaluated by confocal microscopy. Specifically, the human osteoblast-like cells (MG-63) were plated in a confocal dish at a density of 8×10^4 cells and grown at 37 °C under humidied air (5 vol% of CO_2) in α-MEM, which was supplemented with 10 vol% of FBS, 100 units mL^{-1} of penicillin and 100 units mL^{-1} of streptomycin. After culture for 24 h, cells were treated with coumarin-6 encapsulated Aln-GelC NCs and GelC NCs for another 3 h. Subsequently, the cells were washed twice times with PBS and fixated with 300 μL of 4% paraformaldehyde for 20 min. Finally, the cells were washed three times with PBS and stained with 300 μL of DAPI for 8 min. Prior to imaging with CLSM (CarlZeiss LSM710), the cells were washed three times with PBS.

For flow cytometry, MG-63 cells were seeded in 6-well plates at a density of 2×10^5 cells per well and cultured for 24 h. Subsequently, coumarin-6 encapsulated Aln-GelC NCs and GelC NCs were added and the cells were cultured for another 3 h. MG-63 cells without any theatment were used as a negative control. Finally, the cells were detached from the wells and washed with PBS, then measured on a BD FACSCalibur flow cytometer. Data processing was done in FlowJo.

4.5 Evaluation of the bone targeting nanocapsules in rats *in vivo*

The bone targeting abilities of Aln-GelC NCs and GelC NCs encapsulated with IR-780 iodide (near-infrared lipophilicity fluorescence dye) were evaluated *in vivo* using SD rats (purchased from Institute of Health and Environmental Medicine of AMMS, Tianjin 300050, China). All procedures were in compliance with the regulations of the Tianjin Committee of Use and Care of Laboratory Animals and the overall project protocol was approved by the Animal Ethics Committee of the Chinese Academy of Medical Science. 3-month-old female SD rats were randomly divided into two groups (n=6) and injected from the tail vein of each rat with 0.5 mg/kg of NCs. After 48 h, the rats were sacrificed, and the major organs (heart, liver, spleen, lung, kidney, bilateral femur/tibia of hind limb bone) were collected. The fluorescence signals (450–950 nm) in these organs were detected using Maestro EX *in vivo* imaging system (CRI Maestro, USA). The retention of NCs was confirmed by quantifying the average fluorescence intensities of these organs image.

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