Supplementary materials for:

Polyelectrolyte multilayer functionalized mesoporous silica nanoparticles for pH-responsive and layer thickness-dependent drug delivery

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Supplementary materials and methods

Synthesis of MCM-41 type MSNs and FITC-labeled MSNs

MCM-41 type MSNs were prepared by modifications of previously reported methods [1]. Briefly, 1.0 g of CTAB and 0.28 g of NaOH were dissolved and mixed in 480 ml of double-distilled water under vigorous magnetic stirring (600 rpm) at 80 °C for 2 h. Then, 5.0 ml of TEOS was added dropwise to the surfactant solution under vigorous stirring at 80 °C for 20 h. The product was collected by filtration through a filter of 0.22 μm, washed with water and methanol, and then air dried. As-synthesized nanoparticles were suspended in a mixture of 150 ml methanol and 3 ml HCl (37.4%) and refluxed at 80 °C for 12 h to remove the surfactant CTAB, then washed three times with water and methanol, respectively. The surfactant-free MSNs were dried under vacuum for further use.

To obtain FITC-labeled MSNs, FITC-conjugated APTES (FITC-APTES) was firstly synthesized by reacting of FITC (4 mg) with APTES (44 μl) in 1.0 ml of ethanol under dark conditions for 24 h, and the prepared FITC-APTES stock solution was kept at 4 °C. Separately, 1.0 g of CTAB and 0.28 g of NaOH were successively added to 480 ml of double-distilled water. After stirring at 80 °C for 2 h, 0.5 ml of FITC-APTES and 5 ml of TEOS were added to the mixture. The reaction mixture was further stirred at 80 °C for 20 h in the dark. Then, the surfactant-free FITC-labeled MSNs was treated and collected in the same way as that described above for the preparation of MSNs.

Hemolysis assay

Fresh human blood stabilized with heparin was kindly supplied by the Shanghai First People's Hospital (Shanghai, China). Human red blood cells (RBCs) were isolated via centrifugation at 3000 rpm for 10 min at 4 °C and further washed 5 times with PBS. The purified RBCs were then diluted by 20% with PBS buffer. The diluted RBCs suspension was then mixed with bare MSNs or PEM-MSNs suspensions in PBS (1.2 ml) at various concentrations. 1.2 ml of PBS and water were used instead of MSNs suspensions as negative and positive control, respectively. The mixture was gently vortexed and incubated at 37 °C for 3 h, followed by centrifugation at 3000 rpm for 3 min, and the absorbance values of the supernatants at 570 nm

were measured by using a Multiskan MK3 microplate reader with 630 nm as absorption reference. The percent hemolysis of RBCs was calculated as:

Hemolysis % = [(Sample absorbance - negative control) / (positive control - negative control)] × 100%.

For FESEM observation, 0.3 ml of the diluted RBCs suspension was incubated with bare MSNs or PEM-MSNs suspensions in PBS (1.2 ml) at various concentrations for 3 h. The treated samples were then fixed in 2.5% glutaraldehyde in PBS buffer for 2 h at room temperature, and dehydrated in an ethanol series for 30 min each. Finally, the samples were air dried, sputter-coated with gold and observed under a Hitachi S-4800 FESEM.

Platelet Aggregation Analysis

Fresh human full blood was mixed with a 1/9 volume of 3.2 wt% trisodium citrate solution. Platelet rich plasma (PRP) was obtained by centrifugation of citrate blood at 900 rpm for 10 min at room temperature, and platelet poor plasma (PPP) for subsequent coagulation assay was obtained by centrifuging PRP at 4000 rpm for 10 min. For platelet aggregation analysis, 450 μl of PRP was treated with 50 μl of bare MSNs or PEM-MSNs solution with various concentrations at 37 °C for 30 min and platelet counting was carried out with a ABX Pentra 60 hematology analyzer (Horiba ABX, France). PBS solution and 50 μM ADP (Sigma) were used as negative and positive controls, respectively.

Coagulation assay

Coagulation assays were performed on a Sysmex CA-1500 automated blood coagulation analyzer (Kobe, Japan) using the reagents provided by the same manufacturer (Sysmex Shanghai Ltd, China). The reagents used for partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), and fibrinogen time (Fib) tests were Dade Actin Activated Cephaloplastin Reagent, Thromborel S, Test Thrombin Reagent, and Dade Thrombin Reagents, accordingly. Briefly, 50 μl PBS solutions of bare MSNs or PEM-MSNs at different concentrations were respectively incubated with 450 μl PPP at 37 °C for 1 h. The upper clear solutions obtained after centrifugation were used to measure APTT, PT, TT, and Fib values according to the manufacturer's instructions.

HPLC analysis

A Waters C18 column (4.6 × 250 mm, 5 μm) was used. The mobile phase A was 2.28 g/L sodium dodecyl sulfate (SDS) (adjust pH to 6.0 with phosphoric acid), while the mobile phase B was acetonitrile. The mixing ratio of the mobile phase A and B was 25:75 (v/v) at a flow rate of 1 ml/min, and the column temperature was 40 °C. The column effluent was monitored with a fluorescence detector set at excitation and emission wavelengths of 480 nm and 580 nm, respectively.

Supplementary Figures

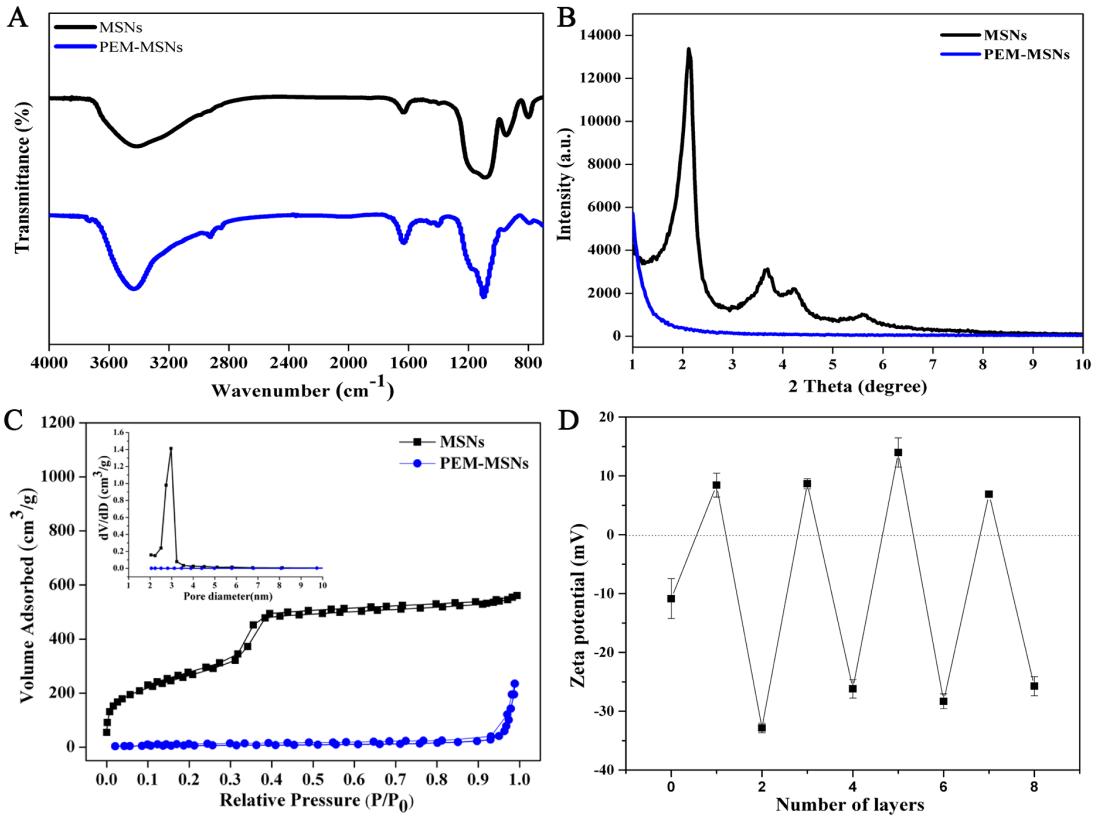

Fig. S1. Characterization of the synthesized MSNs and PEM-MSNs (8 layers). (A) FT-IR spectra, (B) XRD patterns, and (C) Nitrogen sorption isotherms (insets are the pore size distributions) of MSNs and PEM-MSNs (8 layers). (D) Zeta potential changes in the process of coating polyelectrolyte layers.

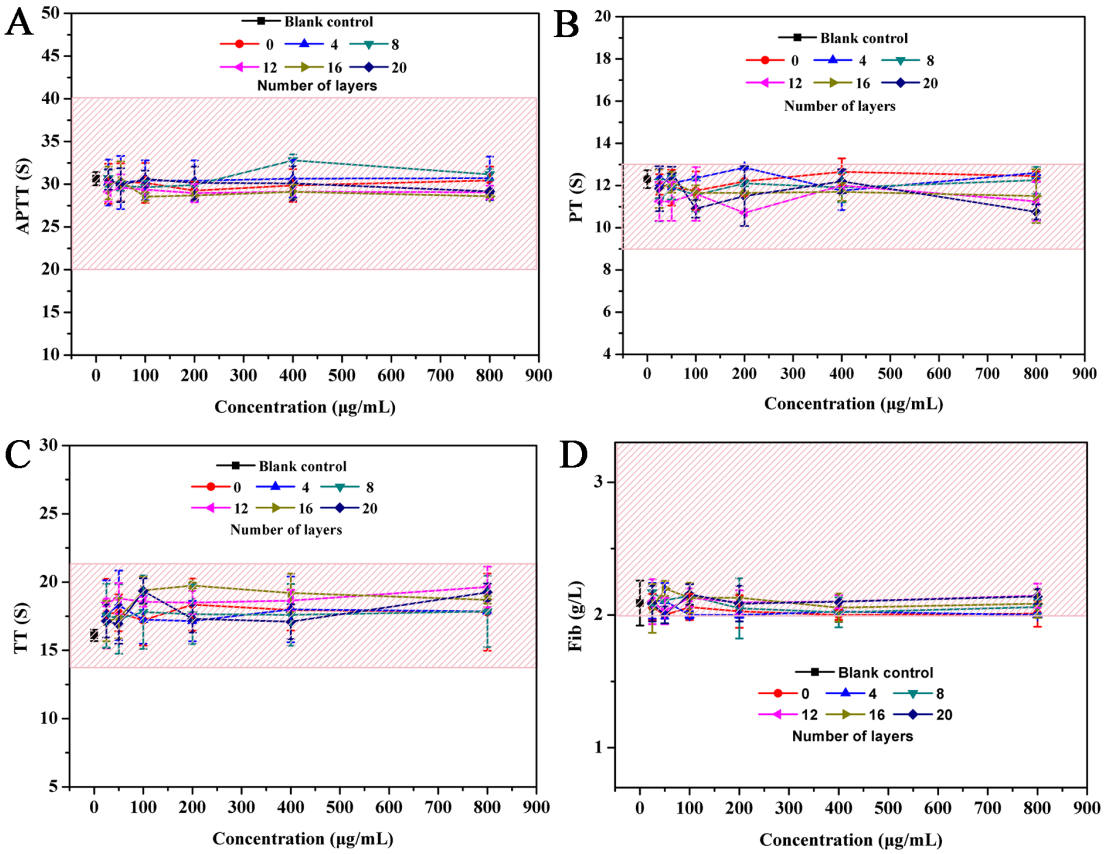

Fig. S2. Blood coagulation properties of bare MSNs and PEM-MSNs. (A) APTT, (B) PT, (C) TT, and (D) Fib levels of bare MSNs and PEM-MSNs treated platelet poor plasma samples, which show no significant variation from the normal range (depicted as a shaded region).

Supplementary Reference

[1] D.R. Radu, C.Y. Lai, K. Jeftinija, E.W. Rowe, S. Jeftinija, V.S.Y. Lin, A polyamidoamine dendrimer-capped mesoporous silica nanosphere-based gene transfection reagent, J. Am. Chem. Soc. 126 (2004) 13216-13217.