Supporting Information

A strategy using mesoporous polymer nanospheres as nanocarriers of Bcl-2 siRNA towards the therapy of breast cancer

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Supplementary methods and materials

General

Phenol, formalin (37wt%), triblock copolymer Pluronic F127 (Mw=12600, PEO_{106} $PPO_{70}PEO_{106}$), NaOH, HCl, sulfuric acid (H_2SO_4), nitric acid (HNO_3), polyethyl eneimine (PEI, Mw=600 and Mw=13000), arginine (Arg, Mw=174.2), 2-morpho linoethanesulfonic acid (MES), oligo DNA (DNA), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), trypsin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's phosphate-buffered saline (P BS), acridine orange (AO), propidium iodide (PI), 4',6-diamidino-2-phenylindole (DAPI), dimethyl sulfoxide (DMSO), diethyl pyrocarbonate (DEPC), FAM label ed siRNA ($siRNA^{FAM}$)，Bcl-siRNA (siRNA), Annexin V-FITC apoptosis assay kit (Absin, Shanghai).

Synthesis of mesoporous polymer nanospheres

The nanospheres were synthesized according to soft templating synthetic methods. Briefly, phenol (0.6 g) was added in formalin aqueous solution (2.1 mL, 37 wt%), then the solution was mixed with NaOH aqueous solution (15 mL, 0.1 M) and stirred at 70°C for 0.5 h to obtain the phenolic resols precursor. After that, 0.96 g of F127 dissolved in

15 ml of H_2O was added. The mixture was stirred at a speed of 340 ± 40 rpm at 70°C for 2 h, and subsequently 50 ml water was added to dilute the mixture solution. The reaction was stopped when the deposit was observed, then 17.7 ml of the obtained solution was transferred into an autoclave and diluted with 56 ml of H_2O and heated at 130°C for 8 h. The products were collected and washed with deionized water and ethanol for three times and then were resuspended in deionized water in the autoclave and heated at 200°C for 12h to obtain mesoporous polymer nanospheres (MPNs).

Surface functionalization of mesoporous polymer nanospheres

In order to improve the loading ability of nanospheres with siRNA, MPNs were modified by following two steps. First, MPNs were oxidized by the mixture solution of dilute sulfuric acid of 9% and dilute nitric acid of 3% with the ratio of 3:1 (v/v) and heated in water bath for 4h at 60°C.The products (nitrificated MPNs, as OMPNs) were washed with deionized water by vacuum filtration until the pH reached neutral. Then OMPNs were dried under cryodesiccation. Second, PEI was grafted onto OMPNs through electrostatic interaction. Briefly, OMPNs (40 mg) were dissolved in MES buffer solution (20 mL). Then PEI (600 mg) was added. The mixture was kept stirring overnight, and then washed with deionized water three times. The OMPNs-PEI conjugate was collected by cryodesiccation.

Loading of siRNA

We performed loading capacity study by using oligo DNA as a model for siRNA. The dosage of carrier and DNA used for the study of loading capacity was shown in Table S1. First, the PEI-OMPNs and oligo DNA were mixed in varying ratio from 1 to 3 (1:1,

1.5:1, 2:1, 3:1) in MES buffer solution (pH=5). Then，the solution was stirred overnight at room temperature . After the loading step，the solution was centrifuged at 10000 rpm for 5 min and the supernatant was collected to calculate the loaded oligo DNA amount by UV spectrometer at a wavelength of 260 nm.

Table S1 the dosage of carrier and DNA used for the study of loading capacity

Ratio of carrier to DNA	Carrier: OMPNs-PEI	DNA
1:1	1mg	1mg
1.5：1	1mg	660μg
2:1	1mg	500μg
3:1	1mg	330μg

Synthesis of OMPN-PEI^1@siRNA@PEI^2@FA

After activation of carboxyl groups by mixing FA with EDC and NHS for half an hour, PEI (Mw=13000) was added. After overnight dialysis, PEI^2@FA was obtained. Then, PEI^2@FA was mixed with OMPN-PEI^1@siRNA. The unabsorbed PEI^2@FA (supernatant) was removed by centrifugation, and then OMPN-PEI^1@siRNA@PEI^2@FA was obtained.

Release of siRNA from OMPNs-PEI

The release profiles of siRNA from the OMPNs-PEI were investigated in MES buffer solutions (pH 7.4 and 5.0) with and without PEI (Mw=13000) wrapped onto the surface of the nanospheres. Oligo DNA was also used as a model for siRNA. At predetermined time intervals, release media was collected and replaced with equal amount of fresh media. The released oligo DNA amount was also calculated as mentioned above.

The size and morphology of the nanospheres were examined by transmission electron microscopy (TEM, JEOL2100F, Japan) and scanning electron microscopy (SEM, Quanta 200, Philips). Fourier transform infrared (FTIR) spectra were measured in the

range of 500–4000 cm^{-1} using a 5700 spectrometer (Nicolet, USA). The zeta potential of the nanospheres was measured at 20°C by a Nanosizer ZS-90. The porosity of the mesoporous products was measured by a pore size distribution analyzer (ASAP-2020M, USA).

Supplementary Schemes and Figures

The temperature for template removal was determined by DSC thermal analysis and was based on the fact that hydrogen bonding is the basis for the formation of supramolecular structures. Therefore, it is possible to detect supramolecular structures by detecting hydrogen bonds. As shown in Figure S1a, an endothermic phenomenon appeared in the DSC curve of the mesoporous polymer nanospheres obtained at 130°C, below the carbonization temperature of 350°C, and TG did not show any weight change in the material. This phenomenon was caused by the destruction of hydrogen bonds that form the supramolecular structure. For the mesoporous polymer nanospheres obtained at 200°C (Figure S1b), there was no obvious change in the DSC curve or the TG curve below the carbonization temperature of 350°C, indicating that the template had been removed and thus the supramolecular structure did not exist. These results suggested that 200°C can be one of the optimal temperatures for removing the template.

The removal of the template was semiquantitatively analyzed by infrared spectroscopy (Figure S2). In the infrared spectrum, hydrogen bonds are characterized in the broad range of 3570 cm^{-1} to 3050 cm^{-1}; hence, the peak area can be used to semiquantitatively analyze the strength of the hydrogen bonds. It can be seen that the peak area for the hydrogen bonds in the mesoporous polymer nanospheres obtained at 200°C was smaller

than that obtained at 130°C, demonstrating the removal of the template.

From the results of the XPS analysis, it can be seen that the N element appeared in the full spectrum, and the peaks of the C-N and N-O groups appeared in the individual C1s, O1s and N1s spectra, thus further confirming the presence of the $-NO_2$ group in the nanospheres and the success of nitrification.