Supporting Information

Bioinspired Zwitterionic Polyphosphoester Modified Porous Silicon Nanoparticles for Efficient Oral Insulin Delivery

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1. Synthesis of 2-(2-pyridyldithio)-ethanol.

2, 2'-dithiodipyridine (15 g) was dissolved in methanol (60 mL) and ethanoic acid (1 mL). Mercaptoethanol (2.65 g) in 30 mL methanol was added in above solution drop by drop and the mixture was stirred overnight. After the reaction, it was evaporated and purified by silica gel column chromatography. The eluent was the mixture of ethyl acetate / petroleum ether (initial at 10%). Then the polarity of the eluent was gradually increased. 2-(2-pyridyldithio)-ethanol came out in 15% ethyl acetate / petroleum ether as eluent. The products were evaporated and dried under vacuum overnight. The 2-(2-pyridyldithio)-ethanol with the pyridine protons peak at 7.26 (a), 7.64 (b), 7.82 (c) and 8.46 (d) ppm was confirmed by 1H NMR.

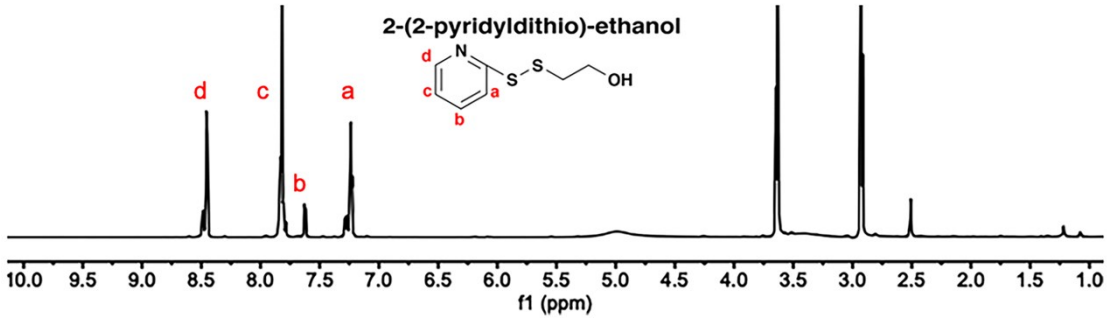

Figure S1. [1]H NMR spectra of 2-(2-pyridyldithio)-ethanol in d_6-DMSO.

2. Fluorescence modification on PSiNPs.

To prepare the Alexa Fluor 488-conjugated AmPSiNPs, the AmPSiNPs was added to 50 μL of Alexa Fluor 488-NHS ester solution (5 mg/mL in DMSO), and kept stirring for 2 h.[1] Then, the reaction solution was centrifuged and subsequently washed to remove the unconjugated Alexa Fluor 488. Then the Alexa Fluor 488-conjugated AmPSiNPs were redispersed in anhydrous DMSO followed by adding CDI for further P(PyEP-g-SB) modification. The corresponding fluorescence intensities (FI) of Alexa Fluor 488-conjugated NPs were detected by the fluorescence spectrophotometer (F-4500, Hitachi, Japan) at $\lambda_{Ex/Em}$ of 494 / 517 nm.

3. Insulin detection with High-performance liquid chromatography (HPLC).

Insulin loaded NPs were centrifuged at 18000 rpm for 3 min with Milli-Q water and the amount of insulin from supernatants was measured by HPLC assay (Agilent 1200).[2] It was used by an Agilent C_{18} reversed phase column (4.6 × 250 mm; pore size 5 μm). The mobile phase consisted of acetonitrile and 0.1% trifluoroacetic acid (TFA, pH 2.0). It was conducted in gradient mode using a mobile phase of acetonitrile / TFA (20:80, v/v) for an initial 5 min, followed by a change to 60:40 v/v of the mobile phase for 10 min, and then changed back to the initial ratio. The flow rate was 0.5 mL/min and the injection volume was 50 μL. The detection wavelength and temperature was set at 214 nm and 25°C.

4. Insulin ELISA assay.

ELISA method was selected to test the activity of insulin in cells and an organized incubation system. The cells were incubated with various insulin suspensions at 37°C for 3 h. Then, the cells were rinsed three times by cold PBS. The active insulin of cell extracts was obtained by several freeze-thaw cycles and was measured by Human Insulin Elisa Kit (0 - 40 mIU/L). The cell associate proteins was calibrated by the BCA assay kit (KeyGen Biotech Co., Ltd., Nanjing, China).

5. Cell culture and cell viability.

Caco-2 and HT29-MTX cells were cultured in 75 cm^2 culture flasks (Corning Inc. Life Sciences, USA). The growth medium was changed every other day until the time of use. Before each test, the cells were harvested using 0.25% (v/v) trypsin - ethylenediamine tetraacetic acid (EDTA) - phosphate buffer solution (PBS) and seeded in the desired density. For the cytoxicity assessment, 100 μL of a 2×10^5 cells/mL solution in DMEM were seeded in 96-well plates and incubated overnight. The medium was removed and replaced with 100 μL of NPs suspensions with the NPs concentrations of 400, 250, 100, 50, and 25 μg/mL. After 12 h of incubation, the wells were washed twice with PBS and were added with 20 μL/well of the MTT (5 mg/mL) reagent assay. The plate was measured for luminescence using a microplate reader (Thermo Fisher Scientific, USA). All assays were carried out at least in triplicate.

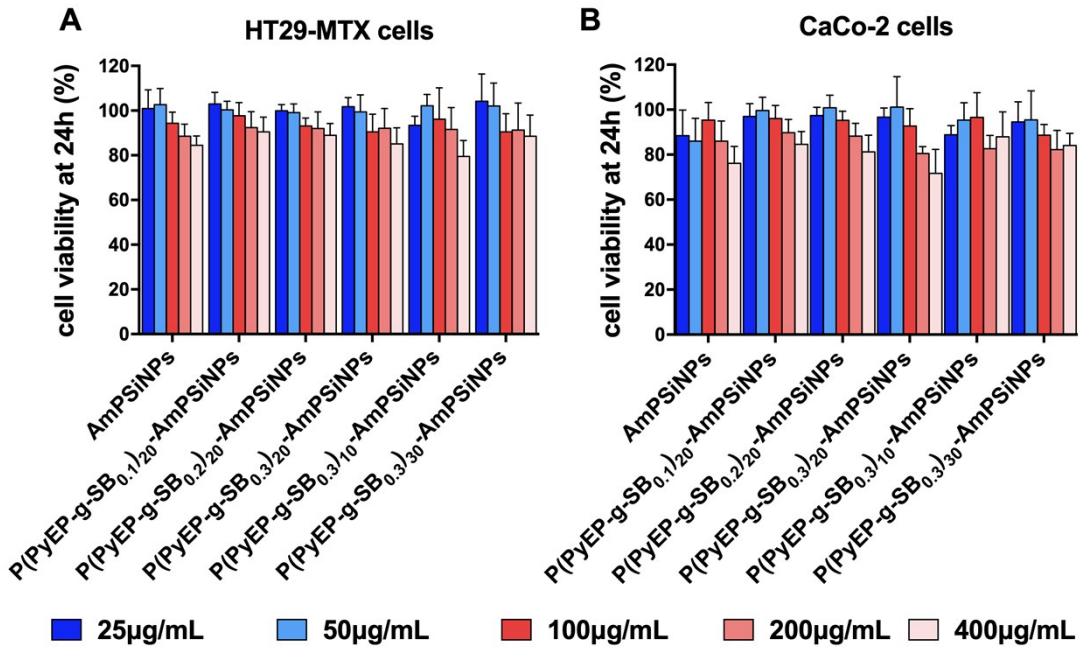

Figure S2. Cell viability of (A) HT29-MTX cells and (B) Caco-2 cells after 12 h incubation at 37°C with different NPs.

6. Transepithelial Transport.

The penetration capacity of NPs to across through Caco-2 and HT29-MTX cells monolayer was assessed using a Transwell system as manuscripts method 2.12 described. Alexa Fluor 488-conjugated NPs (0.6 $mg \cdot mL^{-1}$) were carefully added to the donor compartment. The amount of penetrated NPs was measured using fluorescence spectrophotometer. The intracellular NPs results of various groups were presented as the percentage of the control group (denoted as 100% uptake).

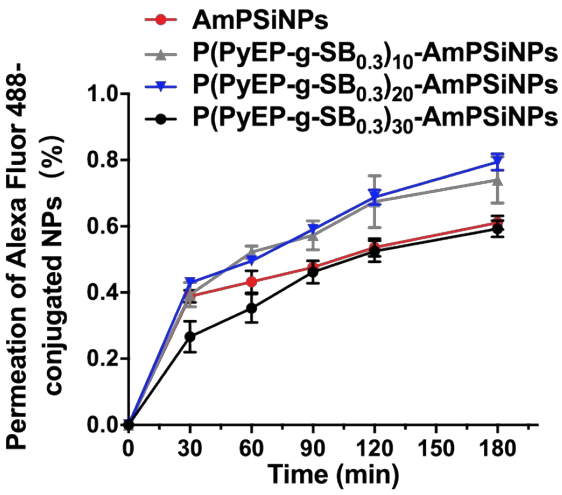

Figure S3. The permeability profiles of Alexa Fluor 488-conjugated P(PyEP-g-$SB_{0.3}$)$_n$-AmPSiNPs (n = 10, 20, 30) across Caco-2/HT29-MTX cocultured cells layers for 3 h.

References

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2. Y. B. Shen, Z. Du, C. Tang, Y. X. Guan and S. J. Yao, *International Journal of Pharmaceutics*, 2016, **505**, 223-233.