Electronic supplementary information (ESI)

Molecularly Imprinted Nanotubes for Enantioselective Drug Delivery and Controlled Release

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1. Preparation of MIP nanotubes by ATRP grafting

Propranolol-imprinted nanotubes were fabricated by using a porous AAO template with atom transfer radical polymerization (ATRP). ATRP allows the synthesis of a wide range of (co)polymers ATRP is notably used to grow well-defined polymers with predictable molecular weight and low polydispersity, as well as well-defined topology.

A commercial AAO membrane from Whatman (quoted pore diameter, 200 nm; thickness, 50 μm) was first modified with 2 mL of 3-aminopropyl-trimethoxysilane (APTMS) in 10 mL mixture of sodium acetate solution (50 mM, pH 5.12): ethanol (1: 9, v/v). The silanization reaction was terminated after 2 h by repeatedly rinsing the membrane with ethanol and then was cured in an oven at 120°C for 2 h. The ATRP initiator was grafted onto the silanized AAO template by dipping the membrane into 10 mL of dry dichloromethane containing 0.2 mL of triethylamine, followed by dropping 50 μL of 2-bromoisobutyryl bromide in to this mixture at 0°C. This mixture was kept at room temperature (25 ± 2°C) for 12 h, and then the AAO membrane was repeatedly rinsed with acetone and toluene before being dried under vacuum at 60°C. ATRP grafting was carried out in a conical flask, in which the initiator-modified AAO membrane was immersed into a degassed methanol-toluene (9:1, v/v, 10-20 ml) solution containing a mixture of *S*-propranolol: methacrylic acid (MAA): ethylene glycol dimethacrylate (EDMA) (0.2: 0.8: 4.0 mmol). To this solution, 4 μmol of CuBr and 8 μmol of *N, N, N'', N''*-pentamethyldiethylenetriamine (PMDETA) were added as catalysts. The conical flask was then put into a 65°C water bath for 6-24 h.

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After the polymerization, the AAO template was rinsed with methanol and DI water, and then dried under vacuum. Usually, the sides of AAO membrane were also grafted with MIP. To remove the polymer layers on both sides, the AAO membrane was carefully polished with abrasive paper.

For SEM and TEM measurements, MIP-grafted AAO membrane was dissolved in 1.0 M NaOH solution with ultrasonication. The resulting solution was then centrifuged at 10000 rpm for 30 min, while the MIP nanotubes were collected at the bottom of centrifugal vial. Repeatedly rinsing the nanotubes with DI water to remove the aqueous soluble impurities, ca. 65% MIP grafted onto the membrane could be recovered. The weights of the AAO membrane in each step were: crude membrane, 35.6 ± 0.4 mg; MIP grafted AAO membrane before removal of propranolol, 38.7 ± 0.7 mg; MIP grafted AAO membrane after removal of propranolol, 38.4 ± 0.5 mg (n=5).

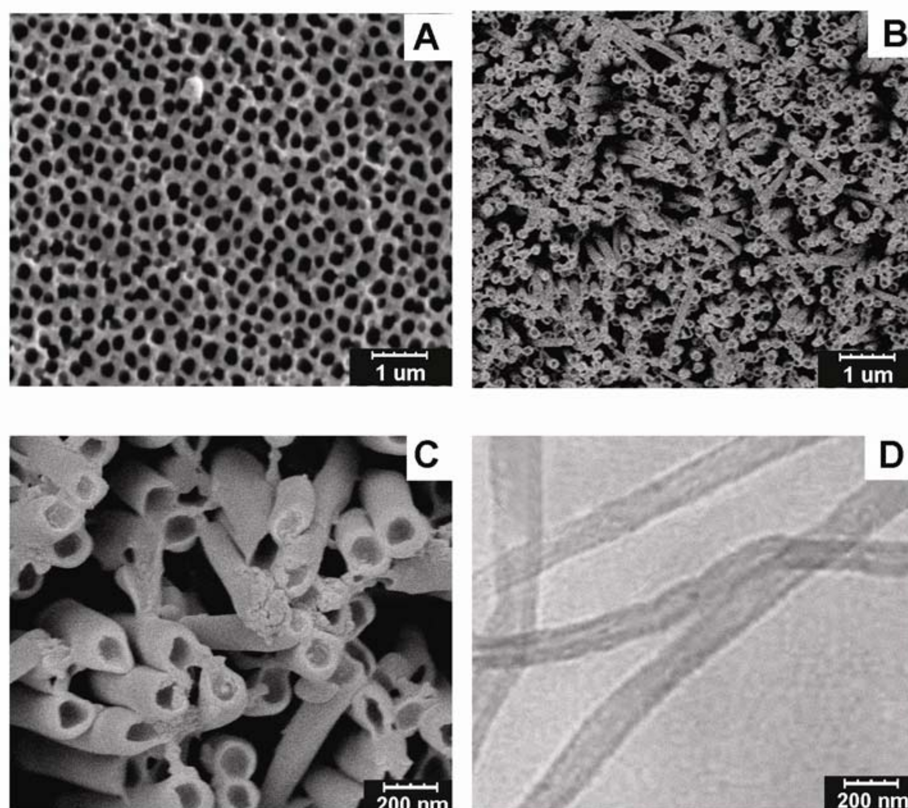

Figure S1. (A) SEM image of crude AAO template, (B and C) SEM images of MIP nanotubes and (D) TEM image of MIP nanotubes.

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2. Preparation of MIP granules

MIP granules were prepared by a typical bulk polymerization. The template *S*-propranolol (0.5 mmol), functional monomer MAA (2 mmol), and cross-linker EDMA (10 mmol) were dissolved in 10 mL acetonitrile. AIBN (40 mg) was added to this solution in steps, as initiator of the radical polymerization reaction. The mixture was saturated with nitrogen for 10 min, degassed under vacuum for 5 min, and then sealed. Polymerization was performed by heating the mixture at 60°C for 24 h. The obtained bulk polymer was subsequently ground to small particles and sieved through 320- and 400-mesh sieve, leaving 25– 40 μm polymer granules. These MIP granules were packed in to a steel column, and then washed on-line with 25% acetic acid in methanol until no propranolol was found in the rinses (UV detection, 225 nm). The NIP, blank polymer for comparison experiments was prepared similarly to the process described above, except that the polymerization mixture did not contain the imprinted molecule.

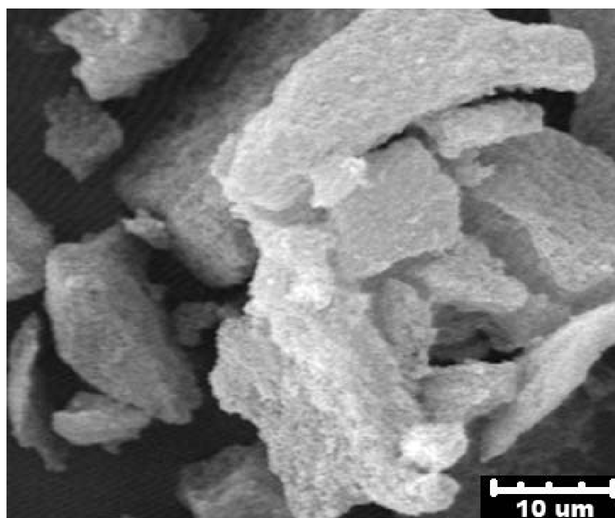

Figure S2. SEM image of *S*-propranolol-MIP granules (25- 40 μm).

3. Rebinding assay and binding kinetic profiles

Rebinding experiment was carried out for investigation of binding properties of MIP nanotubes. MIP-grafted AAO membranes were immersed into 2.0 mL of a known concentration of *S*-propranolol solution (in methanol) in a wide-mouth container. The container was discontinuously ultrasonicated at 25°C for 12 h. The mixture was filtrated through another crude AAO membrane filter. The absorbance of free propranolol in the filtrate was determined by HPLC. The amount of propranolol bound to the MIP, Q, was calculated by subtracting the concentration of the free substrate from the initial concentration. Binding isotherms were determined in a propranolol concentration range of 0.05- 1.0 mM. Data were processed with Langmuir-Freundlich fitting and Scatchard plotting (based on Langmuir model).

Langmuir-Freundlich model (Eq.1) has been regarded as the very mathematical model for addressing the heterogeneity in MIPs.

$$Q = \frac{N_t a C^m}{1 + a C^m} \qquad \text{(Eq. 1)}$$

where N_t is the total number of binding sites, a is related to the median binding affinity constant K_0 ($K_0 = a^{1/m}$), and m is the heterogeneity index (0-1, which is equal to 1 for a homogeneous material). Figure S3a shows the experimental adsorption isotherm for *S*-propranolol-imprinted nanotubes and granules (25- 40 μm) according to Langmuir-Freundlich fitting.

Data were also processed with Scatchard plot (Eq. 2) to estimate the binding properties.

$$\frac{Q}{C_e} = \frac{Q_{\max} - Q}{K_D} \qquad \text{(Eq. 2)}$$

where Q is the amount of propranolol bound to MIP, $Q_{\max}$ the apparent maximum number of binding sites, C_e the free concentration of propranolol at equilibrium, and K_D the dissociation constant. Scatchard plot (Figure S3b) for MIP nanotubes shows only one straight line in the linear region with a linear regression equation Q/C_e=3072-41.56Q (r=0.968). From the slope and intercept of the Scatchard plot, the equilibrium dissociation constant (K_D) and the apparent maximum number ($Q_{\max}$) of the high affinity binding sites were 2.41×10^{-5} mol/L and 73.86 μmol/g, respectively.

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Scatchard plot for MIP granules (Figure S3c) showed two distinct sections which can be regarded as straight lines. The linear regression equations for two linear regions are Q/C_e = 158.5 -7.83Q (r=0.979) and Q/C_e =86.64- 2.28Q (r=0.945), respectively. This indicates that the binding sites in the imprinted polymer are heterogeneous in respect to *S*-propranolol, and those binding sites could be classified into two distinct groups: the high- and the low-affinity binding sites. From the slope and intercept of the Scatchard plot, the equilibrium dissociation constant (K_D) and the apparent maximum number (Q_{max}) were 1.27×10^{-4} mol/L and 20.11 μmol/g for the high affinity binding sites, and 5.35×10^{-4} mol/L and 33.96 μmol/g for the low affinity binding sites, respectively.

Binding kinetic profiles were investigated by determination of concentrations of *S*- and *R*- propranolol solutions (initial concentration, 0.2 mM) which were extracted with 4 mg MIP nanotubes at different intervals (Figure S4). Propranolol enantiomers in binding solutions were assayed by chiral HPLC method with fluorometric detection (Ex 290 nm, Em 340 nm). The detection limit was 1.0 ng/mL for each enanotimer.

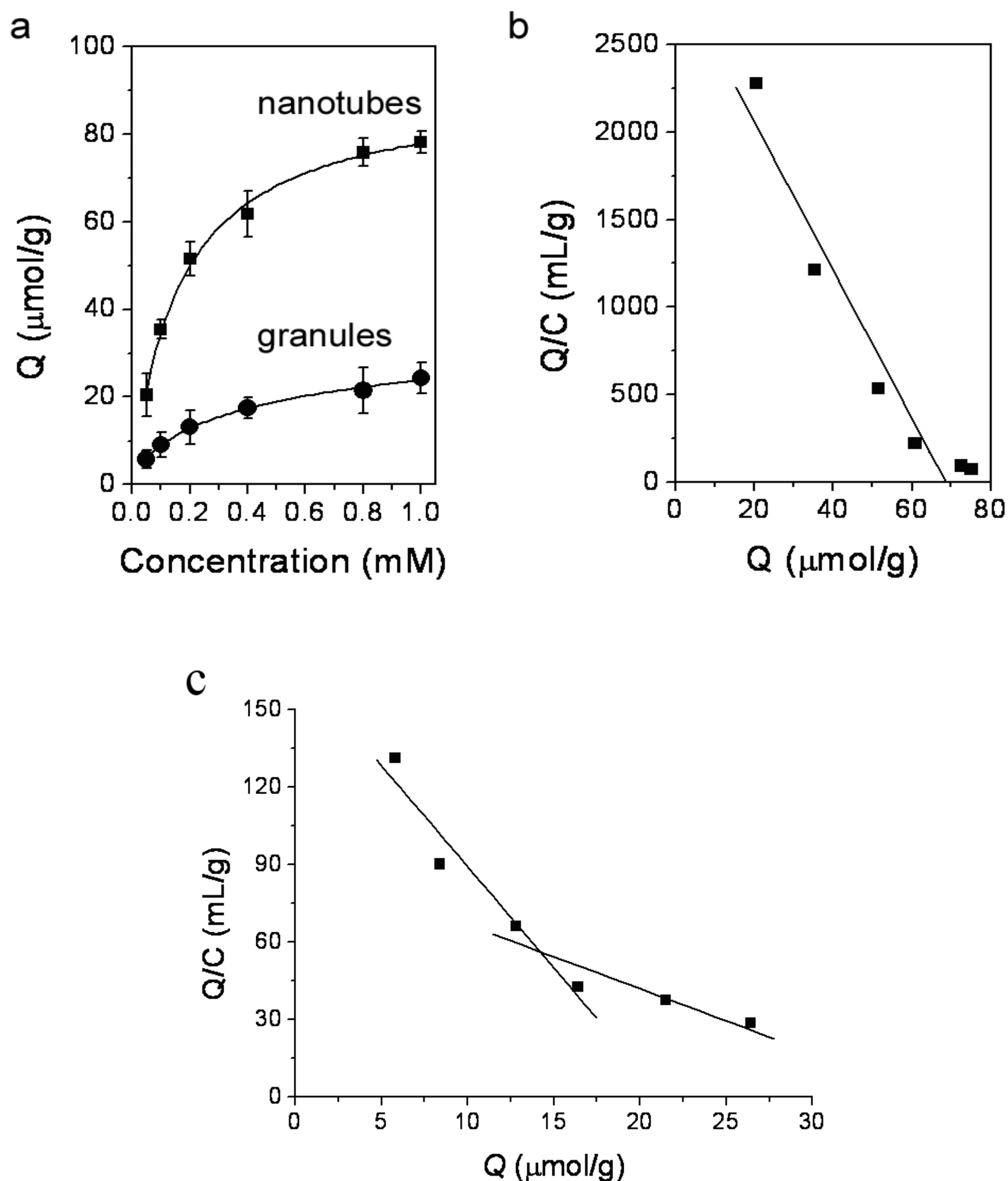

Figure S3. (a) Langmuir-Freundlich fitting for *S*-propranolol-imprinted nanotubes and granules. For MIP nanotubes, N_t= 91.6 μmol/g, m= 0.983, correlation coefficient r= 0.995; for granules, N_t= 39.1 μmol/g, m= 0.726, r= 0.989. (b) Scatchard plot for MIP nanotubes, K_D= 2.41×10^{-5} mol/L and Q_{max}= 73.86 μmol/g. (c) Scatchard plot for 25-μm MIP granules, K_D= 1.27×10^{-4} mol/L and Q_{max}= 20.11 μmol/g for the high affinity binding sites (the upper line), and K_D= 5.35×10^{-4} mol/L and Q_{max}= 33.96 μmol/g for the low affinity binding sites (the lower line).

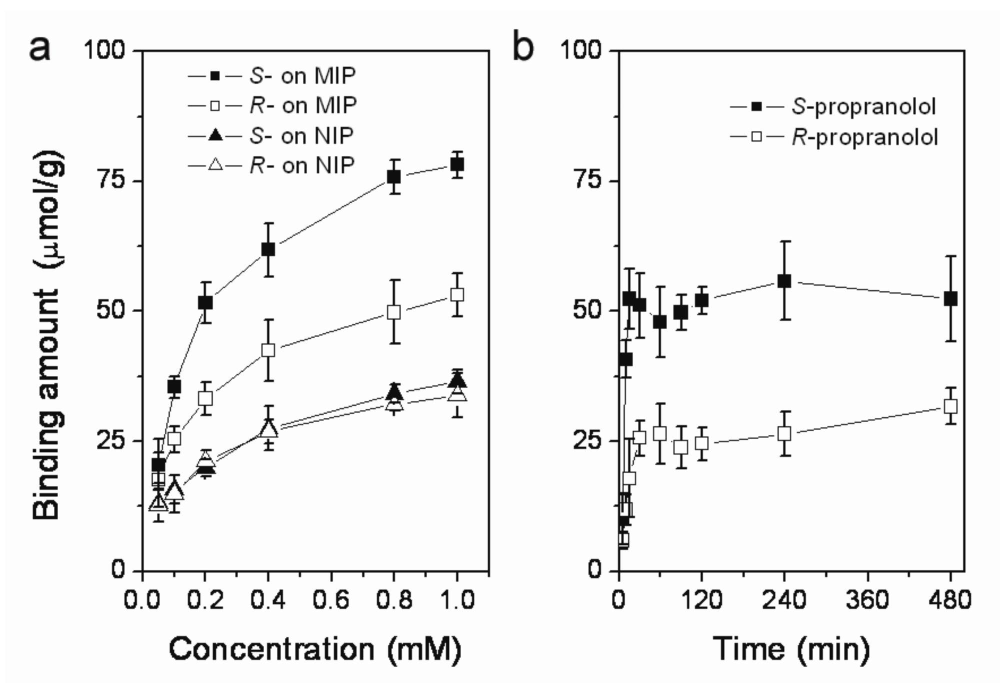

Figure S4. (a) Binding isotherm of *S*-propranolol on MIP (■), *R*-propranolol on MIP (□), *S*-propranolol on NIP (▲) and *R*-propranolol on NIP (Δ). (b) Binding kinetic profiles of *S*-(■) and R-propranolol (□) on MIP nanotubes ($C = 0.2$ mM).

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4. The enantioselectivity of MIP nanotubes

The enantioselectivity of the imprinted nanotubes can be evaluated by the saturation binding experiment. Selectivity factor (*S*) that describes the specific recognition ability of MIP to propranolol, can be calculated by comparing the partition coefficient (*K*) of MIP nanotubes with that of NIP nanotubes.

$$K = \frac{(C_0 - C_e)w_s}{C_e w_n} \qquad \text{(Eq. 3)}$$

$$S = \frac{K(MIP)}{K(NIP)} \qquad \text{(Eq. 4)}$$

where C_0 is the concentration of the initial propranolol solution, C_e the concentration of the equilibrium solution (which can be quantitatively determined by HPLC analysis), w_s the mass of propranolol solution, and w_n the mass of nanotubes. Results showed that the molecularly imprinted polymer nanotubes had a selectivity factor of 3.18 ± 0.26 over the NIP nanotubes (control).

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5. In vivo propranolol-release study.

Blood samples (200 μL) were withdrawn at different time intervals from the femoral vein of Wistar rats (290 g, male) administered a single oral dose of MIP nanotube-doped tablet. Then they were centrifuged (3000 × g, 10 min) to produce plasma samples, and stored in vials at -20°C if not used immediately.

Plasma samples (75 μL) were spiked with of 50 ng/mL *S*-phenylephrine (75 μL, in acetonitrile/water = 2/3, v/v), followed by a solid-phase extraction. The analytes were eluted by 1 mL acetonitrile and dried by nitrogen. Residues were reconstituted by 50 μL mobile phase, and then subjected to HPLC. Propranolol enantiomers in rat plasma were assayed by chiral HPLC method: column, Chiralcel OD-R (4.6 × 250 mm, Daicel, Japan); mobile phase, 50 mM $NaPF_6$ (pH=5.6):CH_3CN = 60:40; flow-rate, 1.0 mL/min; temperature, 25°C; fluorometric detection, Ex 290 nm and Em 340 nm. The detection limit was 1.0 ng/mL for *R*- and *S*-enanotimer.

6. MTT assay and fluorescent imaging

MTT assay has been widely used as a colorimetric approach based on the activity of mitochondrial dehydrogenase enzymes in cells. Human hepatoma HepG2 cells (~ 8000/ well) were inoculated in 96-well plate and treated with different concentration of MIP nanotube (10, 50, 100 and 500 μg/mL, containing 0.8, 4, 8 and 40 nmol *S*-propranolol) for 24 h and then subjected to MTT assay. Control experiments were carried out through the incubation of cells in *S*-propranolol solutions in the absence of nanotubes. Water soluble yellow MTT was metabolized by the metabolically active cells to the water insoluble purple formazan, which was further dissolved in 200 μL DMSO and 20 μL buffer (glycine 0.1M, NaCl 0.1M, pH 10.5). The resultant product can be quantified by spectrophotometry using a plate reader at 570 nm.

HepG2 cells were cultured on sterile coverslips with MIP nanotubes (10- 500 μg/mL) for 24 h when acceptable cell densities were obtained. The coverslips were gently washed with 500 volumes of D-PBS buffer (KCl, 200 mg/L, KH_2PO_4, 200 mg/L, NaCl, 8.0 g/L and Na_2HPO_4 1.15 g/L) to remove the serum-supplemented growth media. Then the cells were incubated with LIVE/DEAD® reagent kit (2 μM calcein AM, 4 μM EthD-1) in PBS buffer (containing 0.1% DMSO) for 2 h. Live cells have intracellular esterases that can convert nonfluorescent, cell-permeable calcein AM to the intensely fluorescent calcein (green). Dead cells have damaged membranes; therefore EthD-1 can enter the damaged cells and produce a red fluorescence when it binds to nucleic acids. The labeled live and dead cells were visualized under the fluorescence microscope. The fluorescence from calcein AM and EthD-1 were observed separately: calcein AM was viewed with a FITC bandpass filter and EthD-1 was viewed with a Rhod filter.