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Molecularly imprinted nanotubes for enantioselective drug delivery and controlled release†

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Molecularly imprinted nanotubes for enantioselective drug delivery and controlled release are fabricated by the combination of template synthesis and ATRP grafting. The release of *R*-propranolol from the imprinted nanotubes in rats is restricted while the release of pharmacologically active *S*-enantiomer is greatly promoted.

The application of nanomaterials for biomedical purposes promotes a burgeoning new field, nanomedicine.¹ Nanoparticles and nanotubes are expected to generate innovations in drug delivery/release and therapeutic approaches by taking advantage of their unique properties in drug transport across biological barriers and intracellular delivery.² Targeted drug delivery towards specific tissues or organs is a fascinating aspect of this newly developing field. A major challenge in selective and targeted drug delivery is to design appropriate vector systems which can both deliver and release the therapeutic drugs efficiently and specially towards the target sites.³ Molecularly imprinted polymers (MIPs) have been suggested as promising candidates for targeted drug delivery and controlled release, relying on their excellent selectivity.⁴ Traditional MIP bulks have to be triturated to the desired particles with appropriate sizes, and this tedious process often produces particles that are irregular in size and shape, resulting in low capacity and poor site accessibility to the target. Thus the drug delivery and controlled release usually suffered from the large/irregular sizes of the vectors, the unexpected constraint release and low drug-released concentration relative to a therapeutically effective level.

MIP nanomaterials are proposed as a pain reliever for headaches by improving the accessibility and the homogeneity of the binding sites. Herein, we report the unique fabrication of MIP nanotubes by atom transfer radical polymerization (ATRP) and their applications in enantioselective drug delivery and controlled release. We found that *S*-propranolol imprinted nanotubes provided differential release of enantiomers, whereby the release of the more therapeutically active *S*-propranolol (eutomer) is greatly promoted whilst the release of the less active *R*-enantiomer (distomer) is retarded. As *S*-

approximately 100 times more pharmacologically active than *R*-enantiomer in the treatment of hypertension and cardiovascular disorders,⁵ the enantioselective release of *S*- and *R*-propranolol via MIP nanotubes may offer considerable therapeutic advantages.

The synthesis of *S*-propranolol-MIP nanotubes was performed in the pores of anodic aluminium oxide (AAO) membranes by the combination of template synthesis⁶ and surface-initiated ATRP strategy (Scheme 1).^{7,8} After the polymerization, the polymer grafted AAO membrane was placed into a syringe filter holder, which was connected to a high-performance liquid chromatography (HPLC) pump with a PTFE pipe. The imprinted propranolol was removed by pumping methanol–acetic acid (4 : 1, 20 mL) through the membrane. MIP nanotubes were collected by dissolving the polymer grafted AAO in 1 M NaOH aqueous solution. Approximately 2.3 mg nanotubes could be obtained, which was about 65% of the polymer grafted onto AAO.

MIP nanotubes were then characterized by SEM, TEM and XPS, providing evidence concerning the quantity and quality of the MIP nanotubes (Fig. 1). The SEM image reveals that the diameters of the prepared nanotubes range from 140 to 210 nm with a length of $25 \pm 10 \mu\text{m}$. TEM images (Fig. 1b–d) visualize the particular morphological properties of these nanotubes. The wall thickness ranges from 6 nm to 60 nm, which can be varied by controlling the polymerization temperature and time during the ATRP procedure. For instance, thinner-wall nanotubes (Fig. 1c) were prepared by controlling ATRP grafting via reducing the polymerization time (3 h) or decreasing the reaction temperature (45°C). However, the obtained thinner nanotubes ($0.6 \pm 0.3 \text{ mg}$) are not adequate for quantitatively investigating their properties including binding capacity and selectivity. Thus, MIP nanotubes with an average thickness of 22 nm were employed for further investigations.

MIP nanotubes show a selectivity factor (*S*) of 3.18 ± 0.26 over the non-imprinted nanotubes. The binding properties of the *S*-propranolol imprinted nanotubes were investigated by a

Scheme 1 Schematic illustration of the fabrication of propranolol imprinted nanotubes in a porous AAO membrane.

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Fig. 1 (a) SEM images of MIP nanotubes, (b–d) TEM images of MIP nanotubes whose wall thickness is 22 nm (b), 10 nm (c) and 66 nm (d), and (e) XPS spectra of MIP nanotubes. Scale bars are 200 nm.

rebinding experiment. Data processed with Scatchard plot⁹ shows only one straight line in the linear region (Fig. S3, ESI†). This is different from the findings reported in the previous study.⁸ The experimental data were also fitted to the Langmuir–Freundlich model, as it is capable of describing the heterogeneous binding surfaces.¹⁰ The total number of binding sites is calculated as $91.6 \mu\text{mol g}^{-1}$ for MIP nanotubes, which means almost a half of the imprinted sites (to theoretical maximum capacity of $200 \mu\text{mol g}^{-1}$) are available in the rebinding process with high affinity. By contrast, for the bulk MIP granules made in a conventional approach, only $\sim 20\%$ of the imprinted sites ($38.8 \mu\text{mol g}^{-1}$) are valid for the target molecule. Moreover, the heterogeneity index (m) is equal to 0.983 for MIP nanotubes, indicating a quite homogeneous binding sites distribution (ESI†).

For the sake of their further use in drug delivery and enantioselective controlled-release, the properties of MIP nanotubes in both drug-uploading and *in vivo* drug-release were investigated. Interestingly, when racemic propranolol was used in the formulation, enantiomer discrimination would be observed on MIP nanotubes. Up to $53.1 \mu\text{mol g}^{-1}$ of *S*-enantiomer (61%) and $33.4 \mu\text{mol g}^{-1}$ *R*-enantiomer (39%) were found in the nanotubes which had been saturated with 0.5 mM racemic propranolol. In contrast, non-imprinted nanotubes did not show discrimination between the two enantiomers under the same conditions. Fig. S4a (ESI†) shows the binding isotherms of *S*- and *R*-propranolol on MIP and non-imprinted nanotubes grafted AAO membranes. It is evident that MIP nanotubes have higher selectivity to *S*- than *R*-enantiomer. Fig. S4b shows the binding kinetic profile of propranolol on MIP nanotubes, indicating a fast targeted solute-uptaking with significant specific binding after 5 min and a saturation time within 30 min. This implies that the nm-sized nanotubes and the porosity of the AAO membrane have a favorable effect on the diffusion times of the target into the recognition sites. In this experiment, to load propranolol for a single dose (200 μg) for pharmacokinetics study, only 1/3 MIP nanotubes in quantity (13 mg) is required, as compared to 40 mg MIP granules.

A MIP nanotubes based controlled-release system offers the possibility of generating differential release of enantiomers,

whereby the release of the more therapeutically active enantiomer is promoted, whilst the release of the less or non-active enantiomer is retarded. This effect was demonstrated by *in vivo* drug-release assay in Wistar rats. MIP nanotubes (14.2 mg) uploaded with 250 μg of propranolol (152 μg *S*- and 98 μg *R*-enantiomer) were compressed to a 40 mg tablet by blending with the excipients. Twelve-hour pharmacokinetic profiles of propranolol enantiomers for Wistar rats (280 ± 20 g) receiving an oral dosing of 40-mg tablet were investigated.¹¹ The release rate of *S*-propranolol loaded on MIP nanotubes was lower than that of *S*-propranolol powder, and comparable to that of MIP granules, which was reflected by the time to maximum plasma concentration (T_{max}). The maximum plasma concentration (C_{max}) of *S*-propranolol, which is relative to drug action and usually used to assess the bioequivalence, was achieved within 4 h, in contrast to the instant-release which reached C_{max} in 2 h (Fig. 2a). Drug release rate has implications for the therapeutic effects of the delivery system. Propranolol releases from the carriers too late (not in a timely manner) may lead to a reduced therapeutic effect, and too fast, may result in a therapeutic effect that is similar to those seen for direct administration of the drug powder, and failure to control the drug release *in vivo*. In a wide range of wall thickness from 20 to 66 nm, MIP nanotubes exhibited a desirable drug release rate and an appropriate T_{max} (4 h). This highlights the potential of MIP nanotubes to be used as a drug-controlled release carrier.

Strangely, we found that *S*-propranolol was released faster than *R*-propranolol. Fig. 2b shows the concentration of *S*- and *R*-enantiomer in rat plasma *versus* time following the reception of a 40-mg tablet dose. The maximum concentration of *S*-propranolol (29.8 ng mL^{-1}) was about two times as high

Fig. 2 (a) Pharmacokinetic profiles of *S*-propranolol in rats receiving an oral dosing of 250 μg *S*-propranolol powder (■), drug-loaded granules (●) and nanotube-encapsulated tablet (▲) containing 250 μg *S*-propranolol. (b) Pharmacokinetic profiles of released *S*- (■) and *R*-propranolol (□) in rats receiving 40 mg of nanotube-encapsulated tablet.

Fig. 3 (a) MTT assay: effect of MIP nanotubes concentrations on the viability of HepG2 cells. HepG2 cells were incubated with propranolol-MIP nanotube (1, 5, 10 and 50 $\mu\text{g mL}^{-1}$) for 24 h, MTT reduction was determined. * $P < 0.05$ (vs. none). (b, c) Fluorescent image of live (green) and dead (red) HepG2 cells after 24 h incubation with (b) 1 $\mu\text{g mL}^{-1}$ and (c) 50 $\mu\text{g mL}^{-1}$ MIP nanotubes.

as *R*-enantiomer (14.6 ng mL^{-1}), and area under the curve (AUC_{0-12}) for *S*-propranolol (237.6 ng h mL^{-1}) is about 2.8 times as great as that for *R*-enantiomer (84.5 ng h mL^{-1}). However, when it comes to non-imprinted nanotubes, no apparent differences were observed in both the loading amount and release concentration for *S*- and *R*-propranolol. It seems the enantiomer is “easy to bind, easy to release”. On the one hand, this effect should be attributed to the disparity in the enantiomer uploading, in which 61% *S*- and 39% *R*-propranolol were loaded on the MIP tablet. Even taking into account this disparity in drug uploading, the AUC_{0-12} for *S*-propranolol is also much higher than that for *R*-propranolol, indicating there must be another contributor. We assume that MIP nanotubes can regulate the release of *S*-propranolol via a selective transport route through the formation of drug–receptor complexes, whereas the release of *R*-enantiomer is via a nonselective pathway. The release of *S*-propranolol undergoes a process that involves reversible rebinding and exchange between the target substrate (*S*-propranolol) and the receptor (binding sites). *S*-propranolol, owing to its accordance with the recognition sites in stereo structure and the functional groups arrangement, tends to occupy the binding sites and otherwise exchange binding sites during the migration. It migrates along these binding sites in the nanotube wall, thus forming a special route for its transfer and release. In this process, the fast mass transfer of *S*-propranolol in nanotubes also greatly facilitates this enantioselective release.

The potential cytotoxicity of these MIP nanotubes was also investigated by MTT assay (ESI†). Fig. 3a indicates more than 94% viability of HepG2 cells in the presence of 1.0, 5.0 and 10 $\mu\text{g mL}^{-1}$ of MIP nanotubes (22 nm), as compared to 100% viability in the control experiment. The live (green) and dead cells (red) were also visualized by inverted fluorescence microscopy with FITC and Rhod filters. The combined fluorescent image is shown in Fig. 3b–c. Results suggest that, in a relatively wide range, MIP nanotubes based on poly(methacrylic acid-co-ethylene glycol dimethacrylate) matrices are low- or non-toxic to cells and biocompatible.

In summary, this study presents a promising approach for fabrication of imprinted polymer nanotubes using ATRP grafting. Compared with traditional MIP particles, nanotubes exhibit unique properties: homogenous binding sites distribution, high yield of high-affinity sites, and good site accessibility which enables a fast mass transfer. By virtue of these merits, MIP nanotubes are proposed as promising candidates for enantioselective drug delivery and controlled release. The pharmacokinetic studies revealed that the release of *R*-propranolol in rats was largely restricted while the release of *S*-enantiomer was well modulated. This enantioselectivity of MIP nanotubes would offer therapeutic advantages when considering the different pharmacological activities of the two enantiomers. Polymeric nanotubes provide an improvement over nanoparticles and liposome delivery systems, allowing the amount of drug loaded onto the nanotube to be regulated. The results reported here may open up new perspectives in enantioselective drug delivery and controlled release.

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