Design of Mesoporous Silica Nanoparticles for Crossing the Blood-Brain-Barrier by Modulating Surface Properties

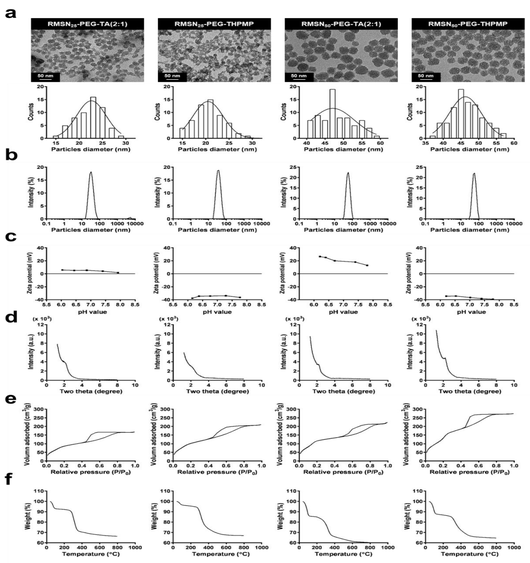
Zih-An Chen,[a,c,] Cheng-Hsun Wu,[b,] Chung-Yuan Mou,[a,b] Si-Han Wu,[c,d,*] Yi-Ping Chen,[c,d,*] Peilin Chen,[e*]

[a]Department of Chemistry, National Taiwan University,Taiwan
[b]Nano Targeting & Therapy Biopharma Inc.
[c]Graduate Institute of Nanomedicine and Medical Engineering, Taipei Medical University Taiwan
[d]International Ph.D. Program in Biomedical Engineering, College of Biomedical Engineering, Taipei Medical University, Taiwan
[e]Reseach Center for Applied Sciences, Academia Sinica, Taiwan

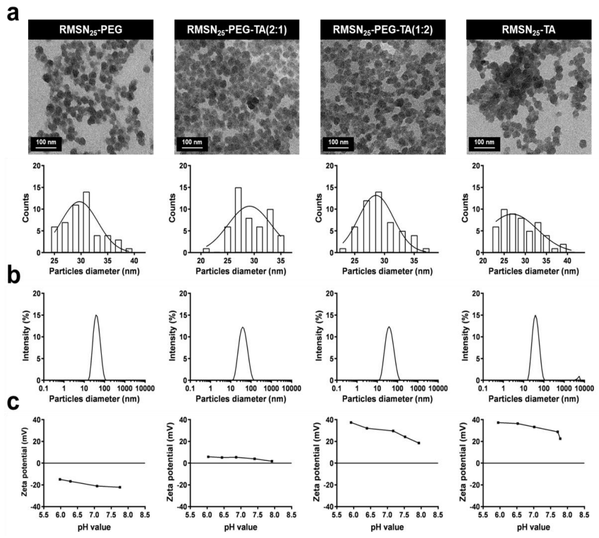
Abstract

Mesoporous silica nanoparticles (MSNs) hold great potential as a drug delivery carrier to tumors via the enhanced permeability and retention (EPR) effect. However, delivering chemotherapeutics and bio-therapeutics in adequate quantities to reach brain tumors remain challenging due to the presence of the blood−brain barrier (BBB). Here, we propose and execute a simple strategy to target MSNs to the brain tumor by crossing BBB. We investigated the transport of PEGylated MSNs with distinct sizes and surface charges in BBB penetration. Our in vitro BBB model study indicates that the small size of MSN with near neutral charged ($RMSN_{25}$-PEG-TA) possesses higher transport efficiency across the BBB-mimicking endothelial cell layer. Based on two-photon imaging, MSNs remained in the circulation system for over 24 hours, and their fluorescence signal was detected outside of the cerebrovascular during brain imaging. Also, we examine the EPR effect with various functionalized MSNs. A particular surface modification on MSNs led to their abundant accumulation in tumor tissue. In a drug delivery study, we explored the potential of using MSN to deliver drugs (DOX@MSN) into the glioma tumors. The in vitro BBB model kit showed that the functionalized MSNs could support DOX@MSN penetration of the model. Moreover, in vivo study shows that the functionalized MSN with DOX (DOX@MSN) significantly suppresses the growth of orthotopic glioma tumors and improves DOX's severe side effects. Using DOX@MSN against a spontaneous brain tumor model, to simulate the clinical cases, extended the overall survival of mice by more than 28% increase in lifespan. Biosafety results indicate that the given dose of the functionalized MSN appeared safe and decreased severe side effects caused by DOX in animals. Experimental results suggest that this functionalized MSN lead to a higher accumulation of DOX in brain tumors. Finally, LC-MS analysis of the protein corona in the plasma of the $RMSN_{25}$-PEG-TA, the nanoparticle most capable of crossing BBB, shows that the particle selectively recruit Apolipoprotein E (Apo-E), the principal cholesterol carrier in the brain. This suggests that BBB penetration capability is due to strongly adsorbed (hard corona) Apo-E on the surface of drug-carrying MSNs via targeting the cholesterol receptor on the blood vessels. This approach simplified the manufacturing process of the drug-nanoparticle complex by avoiding chemical functionalization of the BBB-targeting moiety.

Mesoporous Silica Nanoparticles

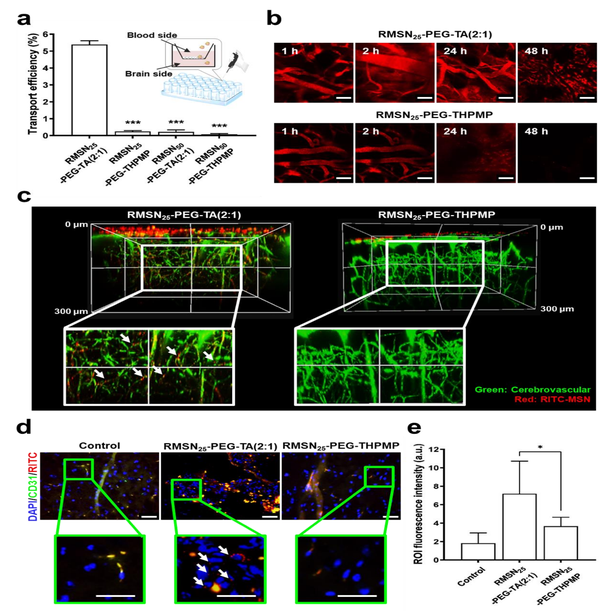


Physical Characterizations of MSNs (a) TEM images and particle size distribution histograms from left to right were $RMSN_{25}$-PEG-TA(2:1), $RMSN_{25}$-PEG-THPMP, $RMSN_{50}$-PEG-TA(2:1), and $RMSN_{50}$-PEG-THPMP. (b) Hydrodynamic diameter and (c) zeta potentials of RMSNs. (d-f) X-ray diffraction patterns, nitrogen sorption isotherms, and thermogravimetric analyses from left to right were $RMSN_{25}$-PEG-TA(2:1), $RMSN_{25}$-PEG-THPMP, $RMSN_{50}$-PEG-TA(2:1), and $RMSN_{50}$-PEG-THPMP.

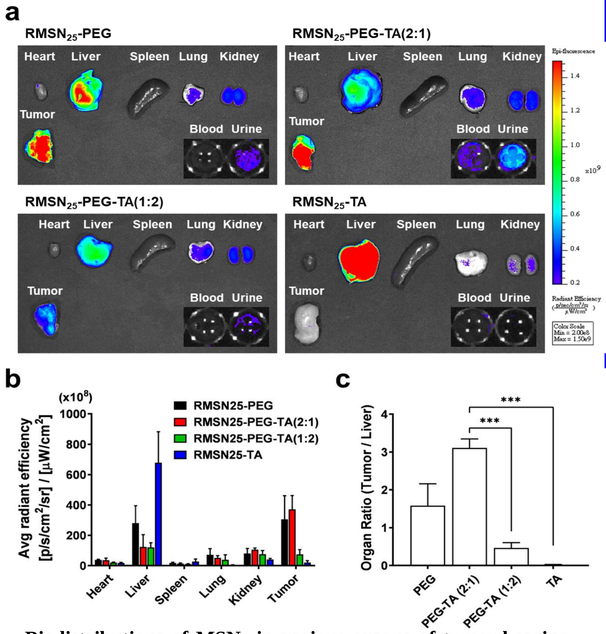


Sizes and charges of the various MSN_{25} (a) TEM images and particle size distribution histograms from left to right were $RMSN_{25}$-PEG, $RMSN_{25}$-PEG-TA(2:1), $RMSN_{25}$-PEG-TA(1:2), and $RMSN_{25}$-TA. (b) Hydrodynamic diameter and (c) zeta potential of various MSNs at different pH values measured by dynamic light scattering. We then investigate the bio-distribution of the four types of MSNs in tumor-bearing mice (Figure 4). In vivo imaging system (IVIS) imaging showed MSNs with PEGylation could promote nanoparticles accumulation in tumor tissue, and the quaternary ammonium group only ($RMSN_{25}$-TA, without PEG), highly positively charged, was mainly trapped in the liver. Intriguingly, $RMSN_{25}$-PEG-TA(2:1) abundantly accumulated in tumors to give a tumor-to-liver ratio of 3.10 with an excellent EPR effect, whereas a stronger positive charge of $RMSN_{25}$-PEG-TA(1:2) gives a little worse tumor accumulation. In this study, we will select $RMSN_{25}$-PEG-TA(2:1) as a drug carrier for further study, which we will refer to as MSN.

Intravital Images of MSNs

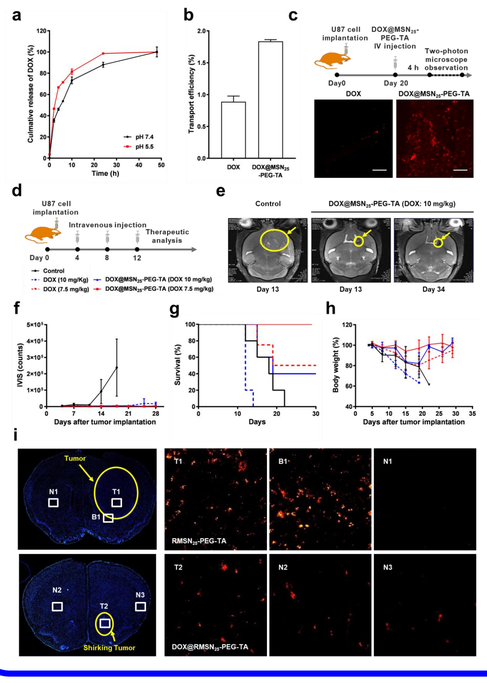


Penetration ability of $RMSN_{25}$ as studied by in vitro BBB model and Two-photon in vivo images of mouse brain (a) The transport efficiency of in vitro BBB model incubated with 0.1 mg/mL of $RMSN_{25}$-PEG-TA(2:1), $RMSN_{25}$-PEG-THPMP, $RMSN_{50}$-PEG-TA(2:1), and $RMSN_{50}$-PEG-THPMP for 6 h and was quantified by ICP-OES analysis. (b) Multi-photon laser scanning microscopy imaged the circulation of $RMSN_{25}$-PEG-TA(2:1) and $RMSN_{25}$-PEG-THPMP inside the blood vessels. Scale bar = 70 μm. (c) The depth images (up to 300 μM) of mice cerebrum were observed by using multi-photon laser scanning microscopy with the cerebrovascular (green signals) stained with dextran-conjugated FITC dye. The white arrowhead pointed out the red signals of the $RMSN_{25}$-PEG-TA(2:1) outside of the cerebrovascular. (d) Immunofluorescence (IF) staining images of the mice cerebrum. Red, green, and blue signals represent RITC-conjugated MSN, FAM Fluor 488-stained CD31 (cerebrovascular), and DAPI-stained cell nuclei, respectively. The white arrowhead indicated the red signal of the $RMSN_{25}$-PEG-TA was not colocalized with green signals of blood vessels but presented surrounding or nearby the cell nucleus. Scale bar = 40 μm. (e) Quantitative fluorescence image analysis based on intensities of regions of interest (ROI) at three different regions was calculated by Image J software, shown in Figure S2. Data are presented as mean ± SD (n = 3). *p < 0.05.

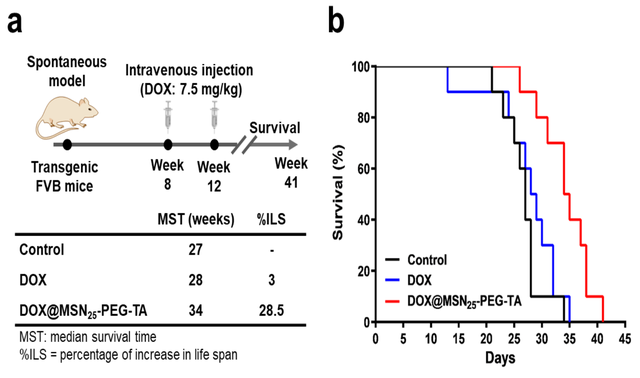


Biodistributions of MSNs in various organs of tumor-bearing mice (a) Biodistribution images of various MSNs obtained from in vivo imaging system (IVIS) imaging system. (b) Quantitative biodistribution of tumor-bearing mice with these four types of MSNs determined by IVIS imaging. (c) Qualitative analysis for organ ratio of tumor to liver (tumor/liver) based on IVIS.

Drug Release Profile and Efficacy



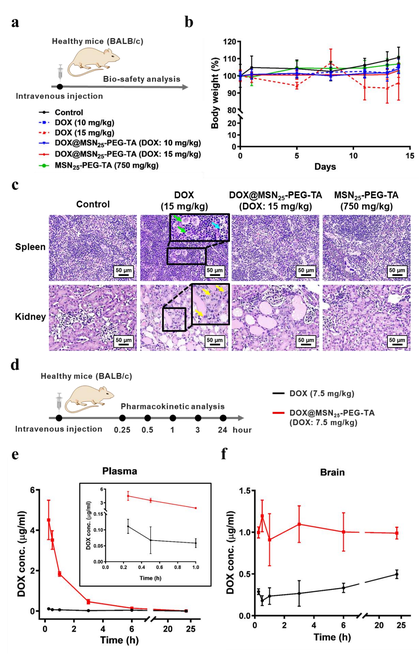
Drug release profile and Therapeutics effects on mice orthotopically implanted with U87 brain tumor (a) The in vitro DOX release of $DOX@MSN_{25}$-PEG-TA at different pH values (pH=7.4 and 5.5). (b) The transport efficiency of in vitro BBB model incubated with DOX (10 μM) and $DOX@MSN_{25}$-PEG-TA (an equivalent dose of 10 μM DOX) for 6 h was quantified by fluorescence spectrometry. (c) Diagrams of the experimental design (upper panel). Representative multi-photon microscopy images of the U87 orthotopic xenograft tumor mice (BALB/c) in the brain tumor region. Scale bar = 70 μm. (d) Detailed experimental procedure of therapeutic efficacy on the mice xenograft orthotopic glioma (n = 5). (e) Magnetic resonance imaging (MRI) images of a control mouse brain and a brain with U87 orthotopic glioma xenograft that was administrated with $DOX@MSN_{25}$-PEG-TA. (f) Tumor size quantification based on luciferase intensity measured using an IVIS imaging system. (g) Overall survival. (h) Body weight variations. (i) Histological image of the brain in the U87 orthotopic xenograft tumor mice treated with $RMSN_{25}$-PEG-TA (200 mg/kg) and $DOX@RMSN_{25}$-PEG-TA. The yellow arrowhead indicated the location of the tumor in the brain. Blue: nuclei (DAPI). Red: DOX/RITC. The upper left ($RMSN_{25}$-PEG-TA treatment) and upper right ($DOX@RMSN_{25}$-PEG-TA treatment) responded to before and present tumor shrinkage, respectively.



	MST (weeks)	%ILS
Control	27	-
DOX	28	3
$DOX@MSN_{25}$-PEG-TA	34	28.5

MST: median survival time
%ILS = percentage of increase in life span

Therapeutic effects on transgenic FAB mice with spontaneous brain tumor (a) Schematic diagram of experimental design in transgenic FAB mice with spontaneous brain tumors receiving an intravenous injection with DOX (7.5 mg/kg) and an equivalent DOX dose of $DOX@MSN_{25}$-PEG-TA for 3 times at 4-days interval at weeks 8 and 12. Saline alone was used as the control group. Kaplan–Meier plots of overall survival. (b) Graph showing the median survival time (MST) and percent increase in life span (%ILS) of spontaneous brain tumors in mice.



Toxicity and Pharmacokinetics studies of $DOX@MSN_{25}$-PEG-TA on healthy mice (a) Schedule of single-dose toxicity study. The healthy BALB/c mice were injected intravenously with different concentrations of DOX alone, $DOX@MSN_{25}$-PEG-TA, and MSN_{25}-PEG-TA, respectively. (b) Body weight variations of mice in the different treatments. (c) Representative histopathology analysis of spleen and kidney in healthy BALB/c mice.). Splenic extramedullary hemopoiesis (green arrowhead), lymphocytic apoptosis (cyan arrowhead), and renal hyaline cast and tubular regeneration (yellow arrowhead) were presented, respectively. Scale bar = 50 μm. (d) Schedule of pharmacokinetic study. The BALB/c healthy mice were intravenously injected with a single dose of DOX alone (7.5 mg/kg) or $DOX@MSN_{25}$-PEG-TA (DOX: 7.5 mg/kg) for one time. The concentration-time curves of DOX in the plasma (e) and brain (f) of mice were determined with a fluorescence spectrophotometer at the indicated times.

Conclusion

The drug delivery efficiency and biological barriers (intra/extracellular barrier and serum protein corona effect) are still critical issues that restrict the therapeutic development of cancer nanomedicine. The present work demonstrates a simple strategy to specifically target MSNs to the brain tumor via the EPR effect and BBB penetration by regulating the diameter and the ratio of polyethylene glycol (PEG) molecules (short PEG with MW 500) and surface charge molecules (positively charged TA-silanol). The results suggested that small size (25 nm), near neutral charge (+4 mV), and a specific ratio of PEG and TA-silanol groups modification (2:1) could favor MSNs crossing the BBB both in vitro and in vivo, as well as promote the ability of tumor targeting based on EPR effect. After DOX loading, the therapeutic MSNs could significantly enhance the pharmacokinetics change of DOX in the plasma and the brain, enabling the delivery of DOX to the brain through BBB penetration, accompanied by the suppression of brain tumor growth with the improvement of DOX-induced severe side effects. Although DOX is not approved for brain tumor treatment, U87 orthotopic glioma xenograft and transgenic FAB mice with spontaneous brain tumors models have shown prolonged survival rates, indicating such DOX-loaded MSNs may open a new window and improve therapeutic outcomes with the potential of being a clinical brain tumor drug.