Potential of MRI-based sensing of cargo release from mesoporous silica for tracking drug delivery capabilities

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Abstract

Novel research results on application of multi-step surface functionalized mesoporous silica nanoparticles (MSNs) in targeted cancer therapy and MRI imaging are demonstrated. The external surface of MSN is functionalized with Chlorotoxin which targets the most aggressive type of brain cancer-Glioblastoma multiforme (GBM). Entrapping drugs (e.g. Paclitaxel) and the FDA-approved MRI contrast agent Gadobutrol within the pores is achieved through binding of cyclodextrin to the pore entrances through an acidification-cleavable hydrazone linkage. This further enables pH-responsive drug or contrast agent release from the MSNs. The tests on cancer cells reveal the capabilities of the prepared materials to deliver drugs and kill cancer cells. On the other hand, the tests involving MRI measurements revealed the capabilities of real time tracking of the release of contrast agents by MRI, which evidences the promising potential for the development of MRI-based methodology for monitoring and tracking the release of therapeutic content.

Introduction

Stimuli-responsive drug delivery systems (DDSs) show a great potential for the safely delivering drugs to specific target areas while avoiding the adverse effects of cytotoxic drugs [1]. However, even in the case of nanomedicines containing cancer-targeting modalities (e.g. presence of tumor-specific ligands with stimuli-responsive triggering of drug release), the cancer-targeted therapy may still not be successful on all patients [2]. Recently, the release of the MRI contrast agent from the pores of mesoporous silica nanoparticles (MSNs) was studied by MRI upon exposure to high-intensity focused ultrasound [3]. In our work, we describe a concept for novel MRI-based methodology that could allow real-time tracking of the capabilities of MSNs to deliver their therapeutic cargo upon acidification, at the site of disease though monitoring the changes in MRI intensity.

The pH-responsive DDSs were synthesized by loading the pores of MSNs either with the anticancer drug paclitaxel (PTX) or with the MRI-CA gadobutrol (GdB) and entrapped by pore-blocking with β-cyclodextrin (CD) moieties, which were covalently attached to MSN through acidification-cleavable hydrazone linkage (Figure 1). Further functionalization of nanoparticles was conducted with cancer-targeting oligopeptide Chlorotoxin (CHX).

The newly developed materials were characterized by different methods such as SEM, TEM, XRD, nitrogen sorption porosimetry, DLS, zeta potential, TGA, DSC and FTIR spectroscopy. The pH-responsive release kinetics of GdB was monitored by fluorescence spectroscopy while the release of PTX was determined by UV/VIS spectroscopy. *In vitro* studies, including confocal microscopy, flow cytometry and toxicity measurements were performed on the human U87 GBM cell line. Finally, the changes in MRI relaxation occurring in response to the release of GdB were measured from the buffered suspensions at pH 5.0 and 7.4.

Results and Discussion

Mercaptopropyl-MSN (MPMSN) was synthesized by co-condensation methodology and further functionalization with N-beta-maleimidopropionic acid hydrazide (BMPH) was used as a crosslinker between MPMSNs and the pore blocking agent – β-CD-CHO, which yields in entrapment of the cargo molecules in the process. Further functionalization with CHX was achieved by its covalent attachment to adamantane-amine and strong host-guest interactions between adamantane and the pore-capping β-cyclodextrin moieties.

The SEM images evidence spherical morphology of the particles with a diameter in the range of 100-250 nm, while TEM images confirm the structured particle porosity. The low angle XRD measurements revealed that the MSN material exhibits 2D-hexagonal pore structure, which is not significantly influenced after functionalization with BMPH. The presence of the BMPH functional group on the MSNs is evident from the Fourier-transform infrared spectroscopy (FTIR) spectrum of the BMPH-MPMSN material, with the band at 1701 cm^{-1} ascribed to the vibrations of the carbonyl groups of BMPH. The TGA and DSC curves confirm the successful functionalization of BMPH. The hydrodynamic diameter of the particles (ca. 250 nm) and their zeta potential (ca. -24 mV) do not change significantly after functionalization. Nitrogen sorption measurements confirmed the functionalization of MPMSN with BMPH, as the surface area, calculated using the Brunauer, Emmett and Teller (BET) method, is reduced from 864 m^2/g to 773 m^2/g and the pore volume from 0.53 cm^3 to 0.47 cm^3 after functionalization. Both materials show the same predominant pore size peak at 1.9 nm and the average pore size at 2.4 nm, determined following the Barrett, Joyner and Halenda (BJH) method. The isotherms of both materials are clearly type IV before loading,

without evident hystereses. However, after the loading of GdB and PTX and capping with CD-CHO, the BET isotherm changes to types I and II, which is typically observed in the case of MSNs with the capped mesopores.

The release kinetics measurements (Figure 2) were performed on supernatants at designated time points. In case of GdB, the fluorescence was measured with excitation at 280 nm and the emission at 312 nm while PTX determination was performed by UV/VIS measurements in buffer (acetate pH 5 or PBS pH 7.4)/methanol mixtures.

Confocal microscopy and flow cytometry measurements revealed that all tested materials are endocytosed by the U87 cells. Furthermore, it was demonstrated that GdB- and PTX-loaded materials exhibit a cytotoxic effect on these cells, with the highest toxicity observed for the CHX-functionalized PTX-loaded material. Finally, the MRI measurements on buffered suspensions of the materials confirmed the capabilities of this methodology to identify enhanced release of CAs at weakly acidic conditions. The obtained proof-of-concept results are promising toward future applications of this MRI-based methodology for possible testing of different CA-loaded nanotheranostics, which might enable identification of optimized drug delivery systems in terms of their targeted drug delivery capabilities to specific tissues and diminished adverse effects, toward a more personalized treatment of cancer.

References

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Figures

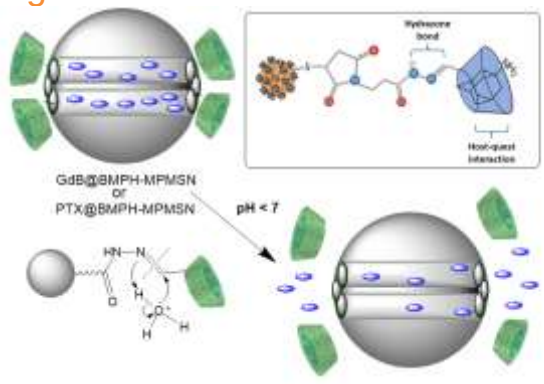

Figure 1. Release of Gadobutrol (GdB) or Paclitaxel (PTX) by hydrazone hydrolysis in acidic conditions

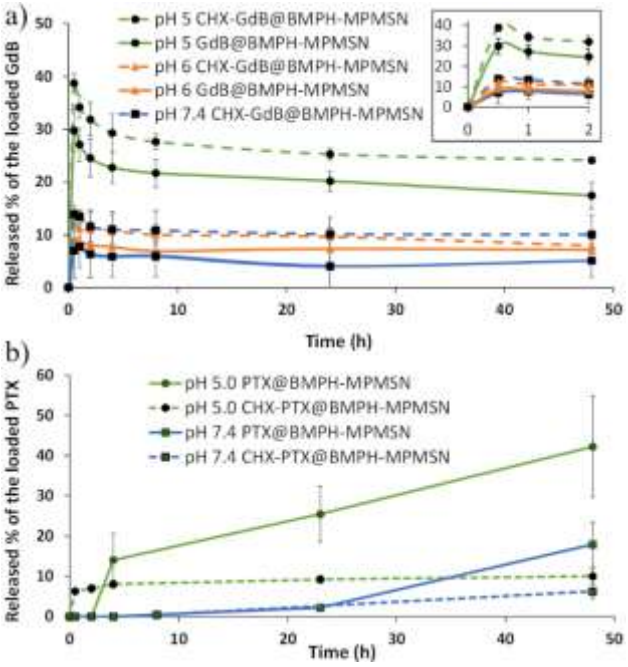

Figure 2. a) Release of GdB from GdB@BMPH-MPMSN and CHX-GdB@BMPH-MPMSN at pH 5.0, 6.0, and 7.4. Inset shows the first two hours of GdB release; b) Release of PTX from PTX@BMPH-MPMSN and CHX-PTX@BMPH-MPMSN at pH 5.0 and 7.4.

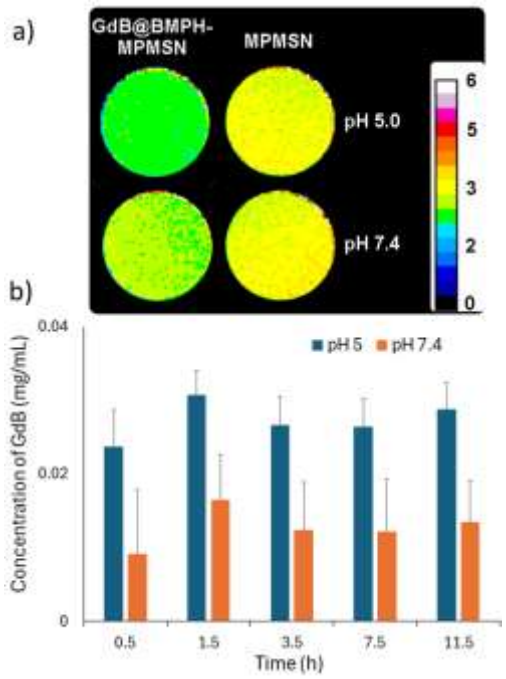

Figure 1. a) T_1-maps (pseudo-colored) of supernatants after 48 of incubation of GdB@BMPH-MPMSN and MPMSN suspensions at pH 7.4 and pH 5; **b)** Gadobutrol concentrations [mg/mL] ± SD in GdB@BMPH-MPMSN in the bulk solution calculated based on linear fitting of GdB known concentrations vs. longitudinal relaxation rate ($R_1=1/T_1$) for pH 5.0 and pH 7.4

Acknowledgments

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