The functional effects of the clustered SPIO on the physical and biological properties of multifunctional polymeric micelles for MR imaging and drug delivery

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Statement of Purpose: Recently, superparamagnetic iron oxide nanoparticles (SPIO) have gained much attention in nanomedicine research because of its powerful applications in magnetic resonance imaging and in vivo cell tracking [1]. In this study, we investigated the effect of clustered SPIO on characteristics of micelles such as size, doxorubicin (Doxo) loading content (DLC), in vitro cellular uptake and therapeutic efficacy on lung cancer cells, pharmacokinetics behaviors and MR sensitivity etc

Methods: SPIO- and Doxo-loaded polymeric micelles were fabricated according to a published procedure [2]. The physical properties of Doxo-SPIO-micelles such as particles size, morphology, DLC and MR sensitivity were evaluated. At first, Transmission electron microscopy (TEM) was used to characterize the size and morphology of SPIO-Doxo-polymeric micelles with negative staining of micelles. DLC was quantified by determining the absorbance at 485 nm using a UV-Vis spectrophotometer. To examine the clustering effects of SPIO on MR sensitivity, MR relaxation rates ($1/T_2$, s^{-1}) were measured on a 4.7 T Varian INOVA MRI scanner

The effect of the clustered SPIO on biological properties of micelles was evaluated. H1299 lung cancer cells were used for in vitro study. The cellular uptake efficiency of SPIO-Doxo-polymeric micelles was evaluated by flow cytometry and confocal microscopy. In vitro cell viability also estimated by estimation of cellular ATP. The pharmacokinetic of SPIO-Doxo-polymeric micelles in BALB/C mice was estimated after i.v. injections. Doxo in plasma was extracted and analyzed by fluorescence.

Results: TEM images of SPIO-Doxo-loaded micelles were shown in Fig. 1. As shown in the data, SPIO nanoparticles were clustered in the hydrophobic cores of the micelles, affecting the size of micelles. Interestingly, Doxo loading content (DLC) was also increased by adding SPIO in the micelles. At first, we found that DLC was increased by incorporating SPIO into micelle cores. For example, DLC of Doxo in PEG-PLA micelles without SPIO was 3.3 ± 2.0 %. The DLC value increased to 8.7 ± 1.4 % when 7nm SPIO was added at the ratio of 10:5:2. As reported by previous studies, the clustered SPIO dramatic increase by 2~8 folds in the T_2 relaxivity (per Fe) that allows the detection of micelles at nanomolar concentrations. In this way, clustered SPIO turned out to affect not only physical properties but also biological properties such as the cell viability, cellular uptake efficiency and pharmacokinetics. Generally, the uptake efficiency decreased with increased loading amount of SPIO. And cell viability increased by adding SPIO in the micelles. Figure 3 shows pharmacokinetic behavior of Doxo from SPIO-Doxo-polymeric micelles is highly affect by the SPIO loading in the micelles, showing the more increased AUC and biological half life than those of SPIO free micelles and drug.

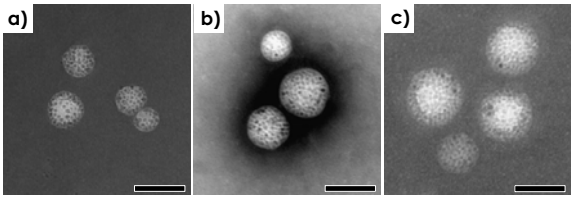

Figure 1. TEM images of 7nm SPIO and Doxo loaded micelles with different loading ratio of SPIO (2.5, 5 and 10) to polymer, respectively.

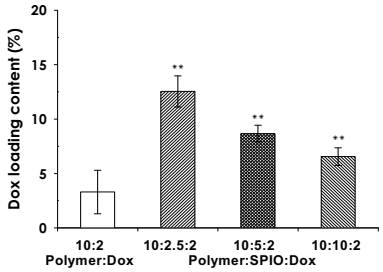

Figure 2. Doxo loading content (%) in SPIO-loaded polymeric micelles with different loading amount of SPIO.

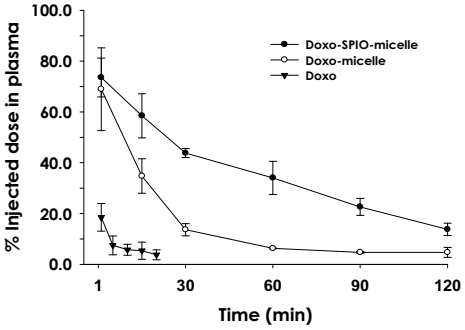

Figure 3. Plasma concentration time profiles after i.v. injection at a 2.5mg/kg doxo dose.

Conclusions: In summary, clustering of SPIO in the micelle proved to affect the physical and biological properties such as size, Doxo loading, MR sensitivity, cellular behaviors and pharmacokinetics. Hopefully, these results could be a good guidance for the design of multifunctional polymeric micelles for MR imaging and drug delivery

References: [1] B. Sumer, J. Gao, Theranostic nanomedicine for cancer. Nanomedicine (London, England) 3(2) (2008) 137-140. [2] N. Nasongkla, E. Bey, J. Ren, H. Ai, C. Khemtong, J.S. Guthi, S.F. Chin, A.D. Sherry, D.A. Boothman, J. Gao, Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems. Nano letters 6(11) (2006) 2427-2430.