SPIO-Loaded Nano Test Tubes as Ultra-Sensitive MRI Contrast Agents

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Introduction

Molecular imaging is becoming an important discipline that investigates disease-specific molecular information through diagnostic imaging methods.[1] Among various imaging modalities, magnetic resonance imaging (MRI) provides superb *in vivo* imaging capability with high resolution (<1 mm), excellent soft tissue contrast, and sensitivity to blood flow. The primary limitation of MRI has been its lower sensitivity for the detection of targeted agents over other imaging modalities (e.g. nuclear imaging).[1] Recently, we reported the development of superparamagnetic iron oxide (SPIO)-loaded polymeric micelles as an effective strategy to enhance MRI sensitivity of detection.[2] MRI detection limit at nanomolar (~nM) concentrations of micelles were achieved as a result of the high loading of SPIO inside the micelles. In this application, we report the development of a nanotubular design of MR imaging probes to further increase the MR sensitivity of detection based on increased SPIO loading and assymetrical tubular design.

Experimental Methods

Silica nano test tubes (NTTs) were synthesized from home-grown alumina templates using a reported method.[3] The NTT dimensions in these proof-of-principle experiments were 100 nm in diameter and 500 nm in length. The inner pores of the tubes were loaded with 11 nm SPIO particles. SPIO-loaded NTT samples were prepared by suspending the tubes in 1% agarose gel. All MRI experiments were conducted using a Litz coil (diameter 4 cm, length 5 cm, DOTY Scientific INC, NC) on a 4.7 T horizontal scanner (Varian, CA, USA) at room temperature (~20°C). T_2-weighted imaging of the phantom samples were collected using a spin-echo pulse sequence with a repetition time of 6.0 s and varying echo times of 9, 20, and 65 ms. The MRI images were processed using the ImageJ software (a freeware from the NIH).

Results and Discussion

SPIO-loaded NTTs were produced as illustrated in Figure 1A. First, layer-by-layer deposition was used to synthesize silica NTTs (membrane thickness ~500 nm). SPIO particles were loaded in the NTTs when the NTTs were membrane bound. Upon membrane dissolution, the NTTs were collected and examined by TEM to verify SPIO loading (Fig. 1B). Due to the hydrophobic surface coating of the SPIO particles,[2] the loaded SPIO did not leak out of the NTTs even after suspension in water for 7 days as determined by TEM examination.

T_2-weighted imaging was carried out to evaluate the MR sensitivity of detection. Images were processed and mean gray value intensity was measured and normalized to the agarose gel control without NTTs. Figure 1C shows the MR intensity as a function of SPIO-loaded NTT concentrations. SPIO-free NTT samples were used as a control. For all the samples, MR intensity decreased when the SPIO-loaded NTT concentration was increased. Moreover, increasing TE time also led to a considerable decrease of MR intensity at low NTT concentration. The sensitivity limits were 1.1 pM, 4.3 pM and 8.6 pM for TE values at 65, 20 and 9 ms, respectively. Here the sensitivity limit of detection is defined as the NTT concentration where the MR intensity is decreased to 50% of the control sample. These results suggest an approximately 1000 fold increase in sensitivity over our previously published micellar systems (5 nM).[2] This dramatic increase in sensitivity promises to expand the use of MR probes in imaging specific markers in molecular imaging applications.

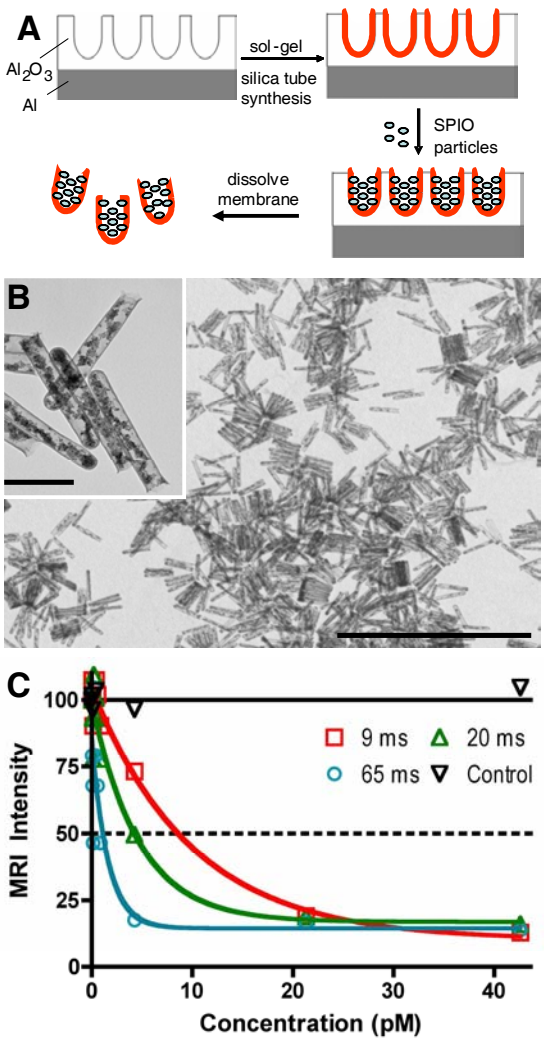

Figure 1. (A) Schematic of SPIO-NTTs synthesis. (B) TEM image of SPIO–NTTs (scale bar = 5 µm), inset: high magnification image, scale bar = 200 nm. (C) MRI intensity as a function of SPIO-NTT concentrations in 1% agarose gel by T_2-w imaging using a spin-echo sequence (TE = 9, 20 and 65 ms). The control sample represents empty NTTs without SPIO loading with TE = 65 ms.

Conclusion

Here we demonstrate the feasibility of SPIO-loaded NTTs as a novel ultra-sensitive platform with a picomolar (pM) detection limit. For proof-of-concept studies, tubes of dimension 100 nm x 500 nm were used. Future studies are in progress to decrease the size of NTTs (e.g., 50 nm x 200 nm) and fuctionalize the NTT surface with cell targeting ligands for molecular imaging applications in cancer.

References

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