Manganese(II)-Block Copolymer Complexes and Their Use for MRI of Biological Processes

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Purpose: The purpose of this work is to investigate the use of ionic block copolymers to form nanocomplexes with paramagnetic ions and enhance overall ionic and molecular relaxivities. We also exploit the release behavior of Mn^{2+} ions for in vivo imaging of biological transport and activities.

Methods: Nanocoplexes (MnBCs) were first synthesized in aqueous solution using electrostatic self-assembly between poly(ethylene oxide)-poly(methacrylate) block copolymer and Mn^{2+} ions (Fig. 1). A carbodiimide-mediated crosslinking reaction was used to enhance stability of the complexes. The complexes were purified by dialysis. To obtain slow release and pH-sensitive nanocomplexes (iMnBCs), MnBCs were reacted with 1 N NaOH in which pH was raised to ~ 10. The mixtures were allowed to react at room temperature for 1 hour before adding 1 N HCl to adjust pH to 7. The complexes were centrifuged through membrane (Millipore, MWCO 30,000 g/mol), washed with DI water, and recovered by freeze-drying. The Mn concentrations were based on the molar concentration of manganese atom measured by ICP-AES. To assess in vivo release properties of Mn ions and subsequent transport, intracortical injection of particles for Mn^{2+} Thalamo-cortical tracing was performed. Three male Sprague-Dawley rats received brain injections of MnBCs, iMnBCs, and $MnCl_2$ (10 mM Mn^{2+}). Injections were aimed to the S1 cortex in the forepaw area (Fig. 3 upper panel). Brain images were acquired on an 11.7T/31 cm horizontal bore magnet (Magnex Scientific Ltd., Abingdon, UK), A Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence was used. Sixteen coronal slices with FOV= 2.56 x 2.56 cm, matrix 256 x 256, thickness = 0.5 mm (TR = 4000 ms, Echo TR/TE = 15/5 ms, TI = 1000 ms, number of segments = 4, and averages = 8)

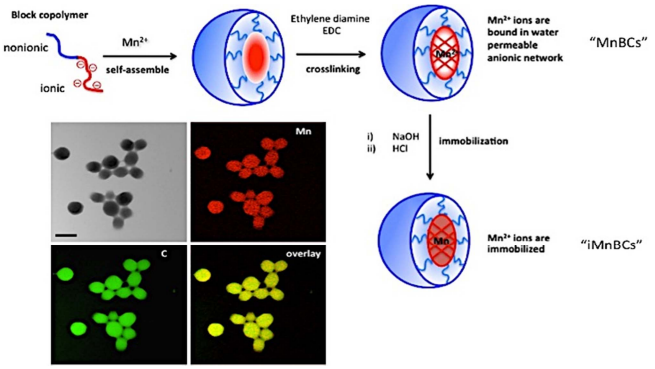

Figure 1. Synthesis of MnBCs and subsequent formation of iMnBCs. TEM and electron energy loss spectroscopy analysis show Mn elements bound within polymeric particles (scale bar = 200 nm).

Results: Longitudinal relaxivities (r_1) of MnBCs were 40.2, 21.7, and 16.1 $mM^{-1}s^{-1}$ at 4.7, 7.0, and 11.7T, respectively. Upon transformation to iMnBCs, the relaxivities were reduced to 8.55, 6.60, and 3.10 $mM^{-1}s^{-1}$, respectively. Phantom MRI (Fig.2) showed the bright contrast signals obtained from aqueous dispersions of the MnBCs were most prominent. Contrast signal generated from iMnBCs was shown to be comparable to that of $MnCl_2$, but better than MnDPDP as expected. In vivo transports of Mn^{2+} along axon from sensory S1 region of the brain to thalamus were clearly detectable. Figure 3 shows that the increase of signal intensity in thalamus area (bottom panel) on the side that was injected with iMnBCs was slower than the side injected with MnBCs demonstrating slow in vivo release of Mn^{2+} by iMnBCs.

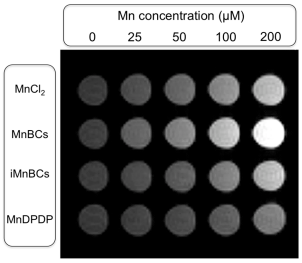

Figure 2. Phantom images from at 7T.

Discussion: Relaxivity values of nanocomplexes decrease with increasing field strengths due to slow tumbling of large macromolecules. Yet, the results obtained from three different high-field MRI scanners point out that both MnBCs and iMnBCs have excellent r_1 values, exceeding those of previously reported manganese oxide and dendritic or polymeric manganese chelates.[1,2,3] In vivo Mn tracing experiments, there was no difference between MnBCs and $MnCl_2$ because fast release nature of MnBCs. However, there was ~ 2 hour before any appreciable increase in signal intensity was observed in thalamus area after injection of iMnBCs. Due to pH-sensitive nature of the particles (Fig. 3A-B), we hypothesize that this delay was caused by the slow uptake of particles by brain cells followed by accumulation of particles within acidic compartment, which triggered release of Mn^{2+} ions and therefore allow Mn^{2+} to be transported.

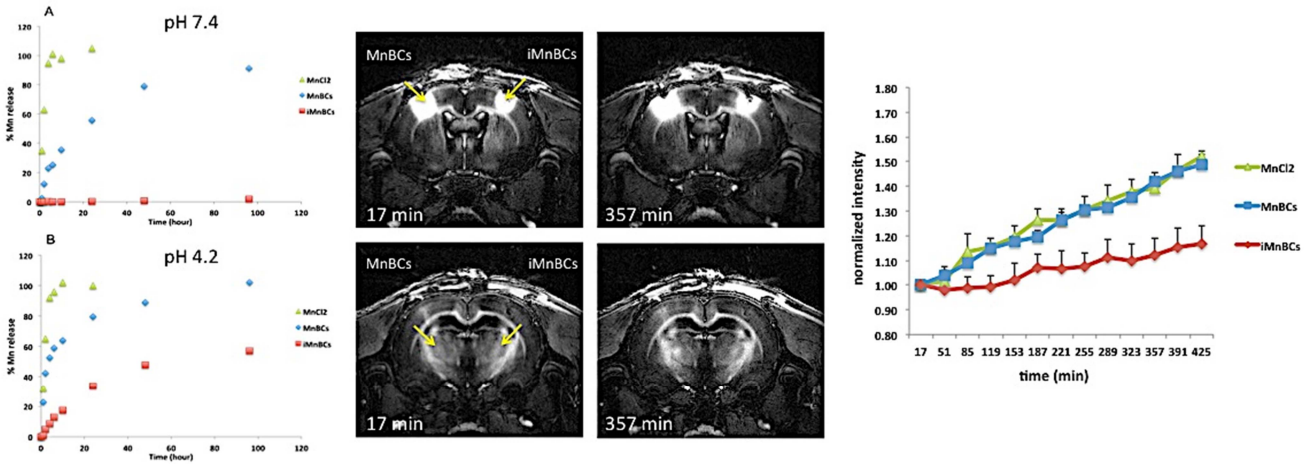

Figure 3. pH-dependent release of Mn (A-B) and MP-RAGE images of thalamocortical tract-tracing with. The transport of Mn tracing from S1 (arrows in upper panel) region to thalamus (arrows in lower panel.) Normalized signal intensities was plot as function of scan time (left)

Conclusion: Mn-block copolymer complexes were prepared and exhibited very high r1 relaxivities per Mn ion. We have exploited release properties of these complexes in response to biological processes of Mn^{2+}-neuronal tracing in the brain .The slow release of MRI contrast triggered by the low pH environment of the endosomal/lysosomal pathway should be very useful for development of molecular imaging agents with improved sensitivity and specificity.

References: [1] Tan M. et al. *Bioconjugate Chem* 2011, 22, 931-937. [2] Kim T. et al. *J. Am. Chem. Soc.* 2011, 133, 2955-2961. [3] Ye Z. et al. *JMRI* 2012, 35, 737-744.