Cell Labeling with a Gd-Fullerenol Contrast Agent Complexed to Protamine Sulfate

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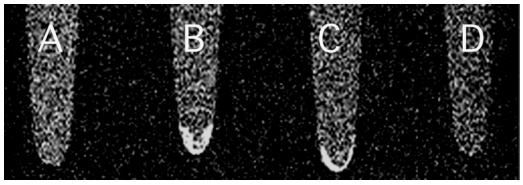
Paramagnetic C(82) gadolinium metallofullerenes have been prepared with polyhydroxyl functionality on the carbon cage to confer water solubility. Their solubility, high R1 relaxivities, small particle size and high stability(1) make them attractive candidates for paramagnetic cell labeling for cellular MRI. These agents have R1 relaxivities of 81 mM^{-1} s^{-1} at 1.0T and 31 at 4.7T (1). They display rapidly increasing R2 at increasing field strength and may act as a T2 agent in certain conditions. Previous reports have shown predominant T2 effects for cells incubated with Gd-fullerenol and imaged at 9.4T (communication, American Hightech Materials, Davis, CA). We have examined labeling methods for cells in culture with C(82) Gd-fullerenol and demonstrate increased cell uptake with protamine sulfate transfection and strong T1 effect for labeled cells at 7T which have not been previously demonstrated.

Methods: HeLa cells were cultured in DMEM media (Biofluids, Rockville, MD) at 4M ml and plated in a 6-well plate at 2M/well. Cells were incubated 10 hours with equal volume 69µg/mL Gd, Gd-fullerenol (American Hightech Materials, Davis, CA) alone or with Gd-fullerenol and Protamine sulfate () at 35:2 or 35:6 weight ratios. Unlabeled HeLa cells were used as controls. Cells were incubated overnight at 37C, then washed with PBS and 7 units/mL heparin in PBS. Cells were fixed in formalin and imaged with a 7.0T, 20 cm horizontal bore Bruker MRI system (Bruker, Billerica, Mass). T1W spin echoes (TR=500ms, TE=7.7ms), gradient echoes (300/4.5 ms), and T1 maps were performed. Relaxometry was performed on the supernatants using a relaxometer at 1.0T. Cytospins were prepared for microscopy and imaged with a Zeiss Axioplan2 imaging microscope (Carl Zeiss, Germany).

Results: Cells cultured with 35:2 and 35:6 Gd-fullerenol:protamine sulfate were visibly dark in culture and showed a >200% SI increase vs background on T1W SE (**Fig. 1**). Cells were also detectable with positive SI on T2*W gradient echoes. Cells incubated with Gd-fullerenol alone were slightly dark and enhanced minimally on MRI (~10% vs. background). Control cells were not detectable in these images. Relaxometry showed no difference between control and the labeled cell supernatants indicating excess fullerenol can be efficiently washed and the visible material was adherent to the cells. Microscopy indicates a darker cytoplasm and rim in labeled cells as well as clumps of cells with a dark appearance (**Fig. 2**). **Conclusions:** We were able to label cells with significantly higher uptake of Gd by complexing the Gd-fullerenol with protamine sulfate for transfection than with Gd-fullerenol alone. While a common problem with Gd cell labeling is compartmentalization and T2 and T2* shortening, this combination for labeling results in strong T1 enhancement at 7T and suggests further investigation of this material in cell tracking studies.

1



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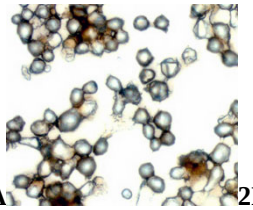

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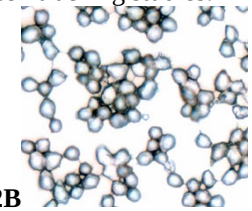

Fig. 1. Spin echo image of HeLa cells. A, no label, B, 35:6 ratio Gd:PS, C, 35:2 ratio Gd:PS, D, cells with Gd Fullerenol alone. Fig. 2. Cytospin at 20x of A. 35:2 labeled HeLa cells, B. control unlabeled cells.

References: 1. Mikawa M et al. Bioconj. Chem. 2001, 12(4):510-14 .