Evaluation of Nanoparticle Contrast Agent Uptake in Murine Microglia (Bv-2) and Human Teracarcinoma (NT2) for Cell Tracking in Neurodegenerative Disease at 21.1 T

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Introduction: We previously have reported a successful bimodal contrast agent providing both MRI and fluorescent properties [1]. This nanoparticle agent, consisting of a fluorescent Quantum Dot (Qdot) conjugated through peptides (CAAKA) with a Dy-DOTA chelate for paramagnetic contrast (Fig 1), has shown increased enhancement at 21.1 T when transfected into Chinese Hamster Ovary Cells (CHO) (Fig 2). Our long-term goal is to develop a multimodal contrast agent for *in vivo* tracking of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Alzheimer's. In this report, we investigate the uptake efficiency of the Dy-labeled Qdot in two new cell lines pertinent to the study of neurodegenerative disease, human neuronal stem cells (NT2) and murine microglia cells (Bv-2). The efficiency of the Dy-labeled Qdot nanoparticle will be compared to an existing contrast agent, namely superparamagnetic iron oxide (SPIO), for these two cell lines.

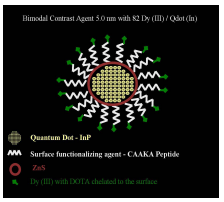

Fig 1: Bimodal MRI contrast agent

Background: MRI contrast agents that are currently commercially available are limited in their effectiveness at high magnetic fields. For example, the effectiveness of Gd-based agents drops drastically with field strength. Meanwhile, other lanthanides (*e.g.* Dy) improve at higher fields due to different relaxation mechanisms [3]. Preliminary studies on commercial agents and Qdots demonstrate this improvement for fields between 4.7 and 17.6 T. However, these novel high field agents need to be manipulated to increase their contrast efficiency for bimodal intracellular operation. The two cell lines used in this study are both associated with neurodegenerative diseases. Bv-2 cells are murine microglia that plays an important role in the brain's immune system. Bv-2 cells can be activated *in vitro* by LPS (*E. coli* lipopolysaccharide) [4]. NT2, which is a human teratocarcinoma cell line, has been proven to have therapeutic effects in stroke models [5]. Theoretically, this human cell line can be used to restore some function in areas of neuronal deficit. Consequently, these two cell lines are prime candidates for animal studies that seek to track implanted cells in models of neurodegeneration.

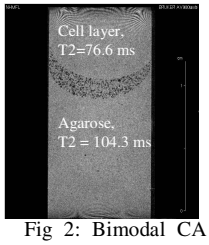

Fig 2: Bimodal CA with Qdot-CAAKA-DOTA-Dy showing T_2* contrast.

Methods: Cells were maintained with standard cell culture methods (DMEM supplemented with bovine serum and antibiotics). Cells were grown in a 5% CO_2, 37°C incubator. For Bv-2 cells, SPIOs (Feridex, Bayer, Inc.) or Dy-Qdots were transfected into cells by adding LPS 24 hours before introduction of the contrast agent. NT2 cells require an additional transfecting agent (Lipofectamine 2000, Sigma, Inc.), which was used in accordance with the manufacturer's instructions. Cells were transfected by co-incubation of Lipofectamine 2000 with either SPIOs, Dy-Qdots or isolated Dy-DOTA/DTPA conjugates for 6 hours; NT2 cells were harvested after an additional 24 hours of incubation. Three washes were performed with TBS (tris-buffered saline) before trypsination to ensure that no agents were attached to the cell surface. Cells were immobilized in 1% agarose layers and imaged to quantify T_1, T_2 and T_2^* relaxation. A high resolution 3D gradient-recalled echo (GRE) also was performed with TE/TR=7.5/150 ms to achieve an isotropic resolution of 50 µm.

Results:

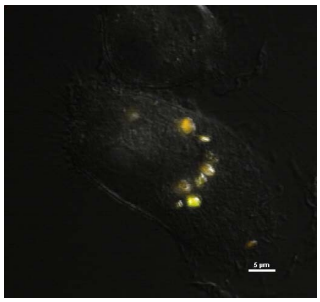

Fig 3: Fluorescent image (550-nm emission) image of a Bv2 cell incubated with Qdots for 4 hrs. Qdots are believed to be inside perinuclear endosomes.

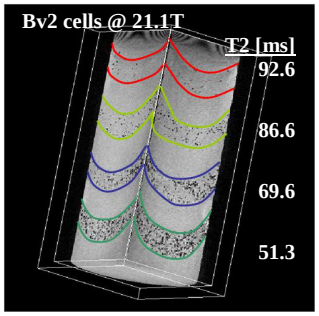

Fig 4: 3D GRE image of Bv2s incubated with 56-µg SPIO. Bv2s are in layers with different cell counts (25k, 50k, 100k and 200k).

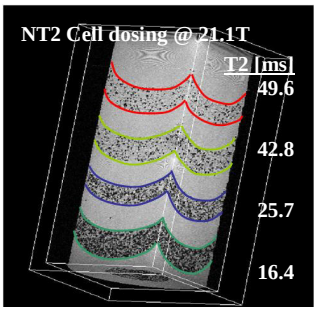

Fig 5: 3D GRE image of NT2 transfected with 56-µg SPIOs. NT2s are in layers with different cell counts (25k, 50k, 100k and 200k).

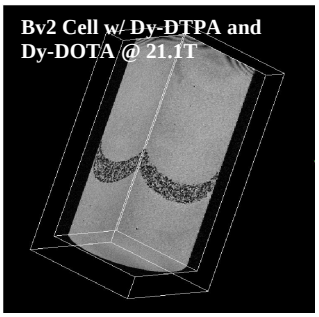

Fig 6: 3D GRE image of Bv2s incubated with Dy chelates. Only Dy-DOTA shows contrast: Dy-DOTA T2=28 ms; Agarose T2=145 ms

Discussion: We have shown that Bv-2 and NT2 cells show good uptake properties with different MR contrast agents. It is believed that agents are accumulated inside endosomes in close proximity to the nucleus for re-packing into lysosomes or exocytosed (Fig 3). There seems to be a more efficient uptake with NT2 cells as seen by the lower T2. This increased efficiency is most likely due to the transfection agent that induces CA uptake by significantly affecting cell membrane permeability rather than by activating phagocytosis. Fig 6 displays the enhanced contrast generated by Bv-2 cells incubated with Dy-DOTA, indicating that the more positively charged DOTA molecule may stimulate uptake in Bv-2s. In an extension of this work, we also have shown improved nanoparticle uptake with the incorporation of the Tat peptide (from HIV) on the Dy-Qdot. Currently, we are incubating the two cell lines with Qdot-CAAKA-Tat-Dy-DOTA particles to evaluate uptake and contrast efficiency. In future work, these agents will be used for *in vivo* studies to label implanted cells instituted at different points of disease progression to generate a timeline of neurodegeneration.

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