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Citation	The 18th Joint Annual Meeting of ISMRM-ESMRMB, Stockholm, Sweden, 1-7 May 2010.
Issued Date	2010
URL	http://hdl.handle.net/10722/126136
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Cellular Uptake and Imaging Studies of Gadolinium-loaded Single-walled Carbon Nanotubes

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Introduction

Single-walled carbon nanotubes (SWCNTs) have recently been proposed as a carrier for efficient delivery of biomolecules such as drugs and genes into targeting sites for therapeutic purposes (1-2). In order to monitor the delivery location and efficiency, visualization of these SWCNTs is crucial. Studies showed that gadolinium loaded ultra-short single-walled carbon nanotubes (gadonanotubes) can be used as high relaxivities r_1 and r_2 agents (3-4) and can be functionalized for targeted delivery (5). In this study, we investigate the intracellular uptake of gadonanotubes with MRI and demonstrated single cell visualization in a sparsely distributed cell agarose phantom.

Methods

Gadonanotubes Labeling: J774 mouse macrophages cell line (5×10^5 cells/well) was maintained in DMEM culture media with 10% FBS and 1% S/P in a 6-well plate to allow cell adhering. The cells were then co-cultured with gadonanotubes solution, at a final concentration of (27.25μM Gd, 185.5 mg C/L) for 24 hours. Labeled cells were then re-suspended in equal volume of 2× culture media and 2% agarose gel after washing with PBS, and transferred to 1cc syringe (3.5 cm long) for MRI measurements. Five different concentrations of labeled cells phantoms (2.3×10^6, 1.15×10^6, 0.75×10^6, 0.57×10^6, and 5,500 cells/ml) were prepared. Another four phantoms with unlabeled cells (2.3×10^6, 1.50×10^6, 0.75×10^6, 0.57×10^6 cells/ml) were also prepared as controls. The mean cellular uptake of gadonanotubes was quantified using Inductively-coupled Plasma (ICP) analysis. Cytotoxicity of the gadonanotubes was tested in a 96-well plate (triplet) at various gadonanotubes concentrations (n=4) using MTS assay prior to experiment.

MRI Imaging: MRI was performed at a 3T system (General Electric Milwaukee, WI) using a 35mm I.D. research quadrature coil for relaxivities measurement and at a 9.4T system (Bruker Biospec 94/20 USR) for cell visualization. For 3T, R_2 and R_2* measurements of the phantoms were acquired with spin echo and gradient echo sequences (TR=1500 ms, TE=15, 30, 45, 60, 75, 100, 125 ms, FOV=5.0cm, matrix=128×128, NEX=1, thickness=1 mm) respectively. Circular ROIs were drawn and the R_2 and R_2* were computed based on the mean intensity of each ROI in the phantoms. ΔR_2 and ΔR_2* of the labeled cell phantoms were calculated by subtracting the R_2 and R_2* values with that of the unlabeled cell phantoms controls. Sparsely distributed labeled cell phantom (5,500 cells/ml) was imaged at 9.4T with a 3D spoiled gradient echo sequence (TR/TE=3000/40ms, alpha=28.6˚, FOV=0.64×2.56 cm, resolution = 50 μm isotropic, NEX=12). Reconstruction was done using susceptibility weighted imaging (SWI) method to enhance the contrast effect for better visualization (6).

Results

Fig. 1 shows the microscopic image of gadonanotubes labeled J774 cells. The cells appear black in the intracellular space, showing efficient gadonanotubes internalization. MTS assay shows no significant cytoxicity effect at the concentration of gadonanotubes used. ICP results show an average cellular uptake of 0.44±0.09 pg Gd/cell, corresponding to an uptake of ~19.3±3.8 pg C/cell. Fig. 2 shows ΔR_2 and ΔR_2* measurements of the gel phantoms with gadonanotubes labeled cells at different concentration at 3T. The ΔR_2 and ΔR_2* values increase linearly with an increasing number of labeled cells. This matches well to the ICP results showing increasing concentration of gadonanotubes (reflected as Gd^{3+} ions) in the phantoms (Table 1). Fig. 3 shows a 9.4T T_2*-weighted image of sparsely distributed cells (5,500 cells/ml) in agarose gel within a 1cc syringe showing single cell visualization.

Discussions

In this study, we demonstrated the intracellular uptake of gadonanotubes exhibits a linear change of transverse relaxivities (R_2 and R_2*) with concentration with R_2* being the dominant relaxation mechanism at 3T. Therefore, monitoring of drug delivery dose encapsulated in the gadonanotubes can be done by quantification of R_2* (7). Single cell visualization of gadonanotubes is possible with high performance gradient to achieve 50 μm isotropic resolution, which is not possible with the gradient performance on clinical system. Although gadonanotubes is not as effective as a molecular contrast agent alone compared to iron oxide, the possibility of using gadonanotubes as drug and gene delivery carriers has immense potential to visualize the molecular imaging target and quantify the amount of drug and gene biomolecules being delivered.

References 1. J Am Chem Soc 2008;130(49):16778-85, 2. Cancer Res 2008;68(16):6652-60, 3.Chem Commun 2005;(31):3915-7, 4. WMIC 2009 p604, 5. J Am Chem Soc 2009;131(24):8342-3, 6. MRM 2004 52:612-18, 7. MRM 2009 61:196-204

cells/ml	[Gd] μg/ml
2.30×10^6	0.85
1.50×10^6	0.61
0.75×10^6	0.43
0.57×10^6	0.25

Table 1 Total amount of Gd^{3+} ions in phantoms.

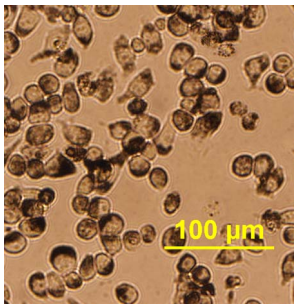

Fig. 1 Microscopic image of gadonanotubes labeled J774 cells. Gadonanotubes accumulated in the cytoplasm (black).

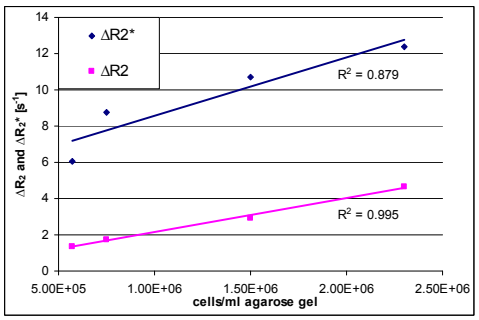

Fig. 2 ΔR_2 and ΔR_2* relaxation rates at increasing number of Gadonanotubes labeled cells in 1.0% agarose gel at 3T.

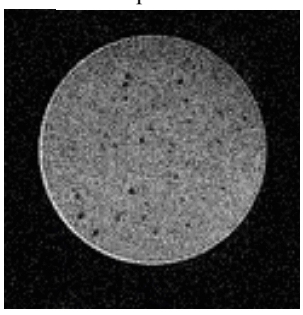

Fig. 3 T_2*-weighted image of Gadonanotubes labeled J774 cells (5,500 cells/ml) at 9.4T, using SWI reconstruction. Dark spots are clearly seen.