

# 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 ( $5 \times 10^5$  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 (27.25  $\mu$ M Gd, 185.5 mg C/L) for 24 hours. Labeled cells were then re-suspended in equal volume of  $2 \times$  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 ( $2.3 \times 10^6$ ,  $1.15 \times 10^6$ ,  $0.75 \times 10^6$ ,  $0.57 \times 10^6$ , and 5,500 cells/ml) were prepared. Another four phantoms with unlabeled cells ( $2.3 \times 10^6$ ,  $1.50 \times 10^6$ ,  $0.75 \times 10^6$ ,  $0.57 \times 10^6$  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 UR) 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 $\times$ 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.  $\Delta R_2$  and  $\Delta R_2^*$  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,  $\alpha=28.6^\circ$ , FOV=0.64 $\times$ 2.56 cm, resolution = 50  $\mu$ m 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 cytotoxicity effect at the concentration of gadonanotubes used. ICP results show an average cellular uptake of  $0.44 \pm 0.09$  pg Gd/cell, corresponding to an uptake of  $\sim 19.3 \pm 3.8$  pg C/cell. Fig. 2 shows  $\Delta R_2$  and  $\Delta R_2^*$  measurements of the gel phantoms with gadonanotubes labeled cells at different concentration at 3T. The  $\Delta R_2$  and  $\Delta R_2^*$  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  $\mu$ m 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] $\mu$ g/ml
$2.30 \times 10^6$	0.85
$1.50 \times 10^6$	0.61
$0.75 \times 10^6$	0.43
$0.57 \times 10^6$	0.25

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

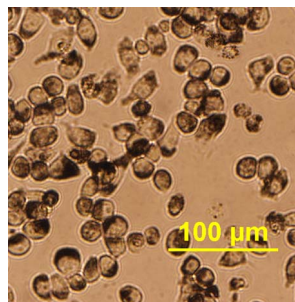


Fig. 1 Microscopic image of gadonanotubes labeled J774 cells. Gadonanotubes accumulated in the cytoplasm (black).

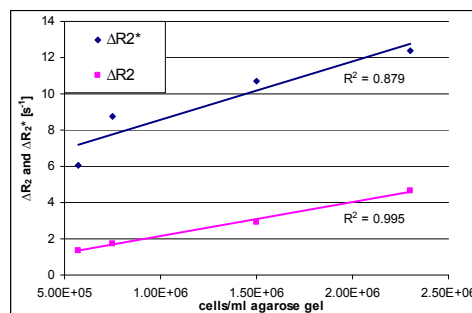


Fig. 2  $\Delta R_2$  and  $\Delta R_2^*$  relaxation rates at increasing number of Gadonanotubes labeled cells in 1.0% agarose gel at 3T.

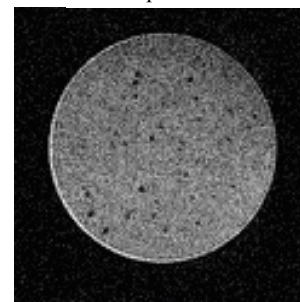


Fig. 3  $T_2^*$ -weighted image of Gadonanotubes labeled J774 cells (5,500 cells/ml) at 9.4T, using SWI reconstruction. Dark spots are clearly seen.