# Gadolinium oxide nanoparticles for positive contrast MRI of human aortic endothelial cells at 7 Tesla

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**TARGET AUDIENCE:** cellular/molecular imager, contrast agent/drug developer

**PURPOSE:** Positive-contrast MRI provides improved delineation and specificity over conventional methods based on negative-contrast iron oxides. A major limitation to advancing positive-contrast MRI is the low signal enhancement provided by agents used for labeling. The gadolinium (Gd) ion is a candidate positive-contrast agent but performs poorly in its chelated form due to low cell uptake. Recently, gadolinium oxides ($Gd_2O_3$) have been introduced [1,2]. These small nanoparticles are ingested efficiently by cells and contain a high fraction of superficial Gd for considerably higher relaxivity [3,4]. However, their ability to generate positive contrast has not been demonstrated at high field strengths, where relaxivity is known to decline. In this study, we investigate the potential of $Gd_2O_3$ for positive-contrast MRI at 7 Tesla.

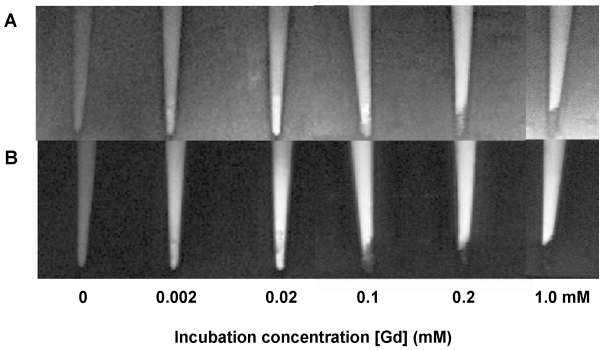


**Fig 1.** A) T1-weighted and B) T2-weighted MRI of $Gd_2O_3$-labeled endothelial cells at 7 Tesla. Hypointensity on T1-weighted images stems from competing T2 effects.

**METHODS:** Normal human aortic endothelial cells (Lifeline Cell Technology, Walkersville, MD) were cultured and labeled by incubating in a mixture of Gado CELLTrack (-) (BioPAL, Worcester, MA), a $Gd_2O_3$ nanoparticle, and poly-L-lysine at a 5:2 ratio for 24 hours. The following $Gd_2O_3$ concentrations were prepared: 0 (control), 0.002, 0.02, 0.1, 0.2, and 1.0 mM of Gd ion.

MRI was performed at 7 Tesla (BioSpec 70/30, Bruker, Ettlinger, Germany) using a 4-channel phased-array receive coil. About 250,000 cells were used to create cell pellets. T1 was measured using 2D saturation recovery RARE: RARE factor=2, 10 TRs from 125 to 5000 ms, TE=7.43 ms, FOV=2.56 cm, 4 mm slices, 100x100 µm in-plane. T2 was measured using CPMG: TR=4000 ms, 128 TEs from 4.19 to 536.5 ms, 5 mm slices, 200x200 µm in-plane, NEX=2. T1 and T2 were also measured for $Gd_2O_3$ in water. Data was analysed in-house (Matlab, v.7.8, Natick, MA).

Gd content in cells was determined using ICP-AES on a PerkinElmer spectrometer. Detection limit was 0.01 µg/mL. Cell viability and proliferation were measured (Promega, Madison, WI) on fluorescence (560/590 nm for viability, 490 nm for proliferation).

**RESULTS:** Relaxivity constants of $Gd_2O_3$ in water were $r_1$=4.7 $mM^{-1}s^{-1}$ and $r_2$=29.3 $mM^{-1}s^{-1}$. Cells showed progressive signal enhancement with increasing [Gd] until competing T2 effects began to offset T1 signal increase at 0.1 mM (Fig 1). Fig 2 shows both T1 and T2 decrease. Positive contrast was maintained over 7 days post-cell labeling, as relaxation times remained relatively stable (data not shown). Viability and proliferation were unaffected up to day 7, when both were reduced, *$P$ < 0.05 (Fig 3). Table 1 shows linear and high Gd uptake with no saturation effect.

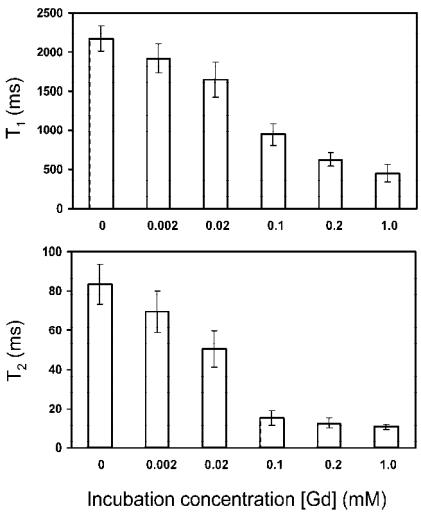


**Fig 2.** Relaxation times in labeled cells.

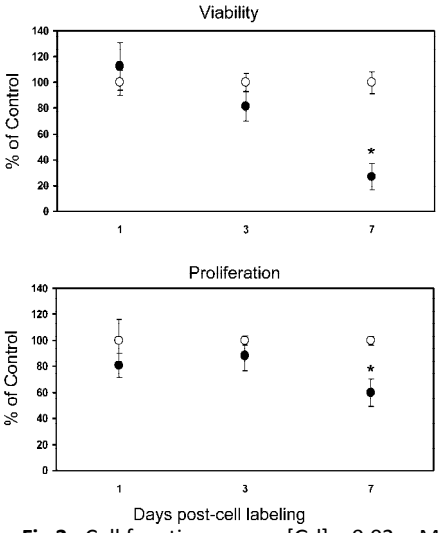


**Fig 3.** Cell function assays. [Gd] = 0.02 mM.

**Table 1.** Cell uptake of $Gd_2O_3$ measured on ICP-AES

| Incubation medium [Gd] (mM) | Mass of Gd (pg/cell) |
|---|---|
| 0 | 0 |
| 0.002 | 0.71 |
| 0.02 | 7.26 |
| 0.1 | 34.1 |
| 0.2 | 84.9 |
| 1.0 | 289 |

**DISCUSSION:** $Gd_2O_3$ provides positive contrast at 7 Tesla, is efficiently and spontaneously ingested by cells, and provides stable labeling for at least 7 days.

**CONCLUSION:** $Gd_2O_3$ nanoparticles are a promising contrast agent for positive-contrast cell-tracking and molecular MRI applications at high-field strengths.

**REFERENCES:** 1. Engstrom M et al. MAGMA 2006; 19:180-6. 2. Klasson A et al. Contrast Media Mol Imaging 2008; 3:106-11. 3. Ahren M et al. Langmuir 2010; 26:5753-62. 4. Faucher L et al. Contrast Media Mol Imaging 2011; 6:209-18.