Gadolinium-based "Smart" MRI Probes for Enzyme-targeted Cancer Imaging

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Introduction: MRI is a widely used diagnostic tool due to its completely noninvasive/nonionizing nature and excellent spatial and temporal resolution. Contrast agents further increase sensitivity, though the effectiveness of generic contrast agents is limited owing to their non-specificity, rapid clearance and low relaxivity. "Smart" contrast agents overcome these limitations by modulating their properties upon interaction with specific molecular targets.

Our group has devised a novel platform for the development of such "smart" probes [1-2]. This technique is based on biocompatible chemical reactions where Gd-containing molecules, under the control of disulfide reduction and/or enzymatic cleavage, get activated intracellularly and undergo polymerization. These polymeric products further aggregate and assemble into nanoparticles through hydrophobic interactions, resulting in enhanced relaxivity and retention. The activation schematic is shown in Fig. 1. Here we present two generations of these "smart" probes; the first generation has a single trigger (disulfide reduction) enabling intracellular activation, while the second generation is dual-triggered (both disulfide reduction and enzymatic cleavage) enabling the sensing of a specific enzyme in the intracellular space and thereby increasing the specificity of the probe. Caspase-3, which is an apoptosis marker, was the targeted enzyme used in this work.

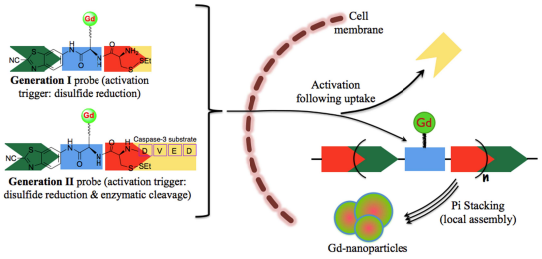

Figure 1: Intracellular activation followed by polymerization reaction between the two functional groups, aminothiol and cyano benzo thiazole of two different molecules. Hydrophobic reactions between the oligomers result in the formation of Gd-nanoparticles.

Methods:

Probe characterization – Control and active forms of the probe for both generations were designed and synthesized. For the control compounds, replacing the functional group with a methylated thiol prevented activation. Nano-characterization of the condensation and self-assembly products of the probes was carried out using High Performance Liquid Chromatography (HPLC), Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM). MTS cytotoxicity assay was carried out on MDA-MB-468 breast cancer cells to determine the optimum, safe incubation concentration for cell loading.

MR characterization – Activation and MR relaxivity of the probes was determined in three different systems with progressively increasing biological relevance; (a) incubated in PBS solution and activated by reducing agent TCEP and/or caspase-3, (b) incubated in cell lysate obtained from MDA-MB-468 breast cancer cells, and (c) incubated in live MDA-MB-468 breast cancer cells, which were then fixed and pelleted before imaging. Relaxivity measurements were carried out at 0.5T, 1.5T and 3T at 35ºC with a series of inversion-prepared FSE scans. Signal intensity vs TI relationships were fit to the exponential T_1 recovery model by non-linear least square regression. Longitudinal relaxivities were calculated as the slope of $1/T_1$ vs [Gd], where ICP-MS was used to determine true Gadolinium concentration.

Results and Discussion:

(1) Activation by reduction and/or enzymatic cleavage produced polymeric condensation products (oligomers, from dimers upto 6-mers), which further self-assembled into Gd-nanoparticles with diameters ranging from 200-350 nm, as revealed by HPLC, DLS and TEM.

(2) Results of the MTS assay after 24 hours of incubation with various concentrations of the probes showed that percent viability for MDA-MB-468 cells starts decreasing for concentration > 250 µM (Fig. 2). Thus an incubation concentration of 250 µM was chosen for cell loading.

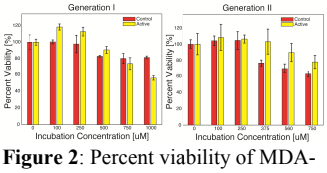

Figure 2: Percent viability of MDA-MB-468 cells as determined by MTS assay after 24 hours of incubation.

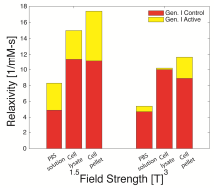

Figure 3: MR relaxivity of Gen. I control and active probes for the 3 systems.

(3) Figure 3 shows the results for Gen. I probes at 1.5T and 3T. Relaxivity of the active "smart" probe is ~50% higher than that of GdF-M, a commonly used Gd-based contrast agent for cell uptake studies. After activation, there is an increase in the relaxivity of the active compound as compared to the control compound, which is greater at lower fields (125% at 0.5T (not shown here) and 57% at 1.5T in MDA-MB-468 cell pellets).

(4) Relaxivity results for Gen. II probes are shown in Fig. 4. Preliminary measurements in PBS solution show 100% and 70% increase in relaxivity after activation for the active compound at 0.5T and 1.5T, respectively. For the control compound, the change is negligible. Absolute relaxivity of Gen. II probes in PBS is higher than that of Gen. I probes. Based on the trend seen in the Gen. 1 results, we predict the *in cellulo* relaxivity would be even higher than that in PBS solution.

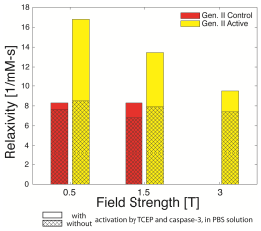

Figure 4: MR relaxivity of Gen. II control and active probes in PBS solution, before and after activation.

Conclusions: We have developed a Gd-based molecular template that can be selectively activated to assemble into nanoparticles to provide enhanced relaxivity. These results successfully demonstrate the *in cellulo* behavior of our Gen. I (reduction triggered) probes. Introduction of the dual mechanism (Gen. II), of both enzyme and reduction triggered activation, further increased the specificity of the probes. Both generations of the probe show higher percent relaxivity enhancement at lower field strengths.

References: [1] *Liang, et al, Nature Chemistry, (2010) 2: 54-60.* [2] *Ye, et al, Angew Chem. Int. Ed., (2011) 50: 2275-2279.*

This research is supported by the National Cancer Institute (NCI) Center for Cancer Nanotechnology Excellence (1U54A151459-01) and the NCI In vivo Cellular and Molecular Imaging Center (1P50A114747-06) at Stanford University, and the Stanford Molecular Imaging Scholars Program (NIH R25 CA118681).