

Multimodal Optical and MRI Studies with Multifunctional Spinel Nanoparticles

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Abstract:

Magnetic resonance imaging (MRI) and optical imaging are commonly used medical imaging techniques for the visualization of the internal structures of the body and molecular events in tissues. Together, the exceptionally high spatial resolution provided by MRI complements the high detection sensitivity of optical methods. Thus, a multimodal imaging approach that combines both reporting strategies would allow accurate localization of signal source and assessment of molecular processes in tissue by MRI and optical methods, respectively. To attain this goal, we are developing a tissue-specific multimodal nanoprobe with a superparamagnetic spinel metal ferrite core and fluorescent dye.

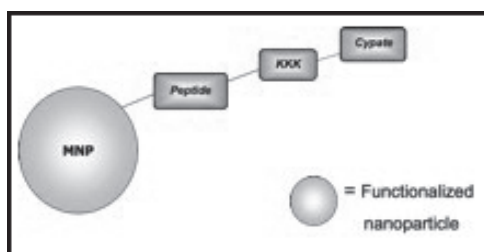


Figure 1: Schematic of synthetic pathway.

Introduction:

MRI contrast can be enhanced with positive or negative contrast agents resulting in brighter (T1-weighted) or darker (T2-weighted) images, respectively. Spinel ferrite magnetic nanoparticles (MNPs) are proven negative contrast agents [1]. The normal spinel structure, AFe_2O_4 , creates a non-compensated magnetic moment and offers the greatest possible magnetic susceptibility due to the higher number of Fe sites compared to metal cation, A [2].

Tissue specificity directly resulted from our chosen peptide sequence. The cleavable linker, lys-lys-lys (KKK), was activated by the enzyme, cathepsin B (Figure 1). Cathepsin B plays a significant role in the detection of inflammatory breast cancer (IBC) [3]. IBC is an especially aggressive, locally advanced breast cancer, and is therefore usually diagnosed at a late stage. However, an enzyme specific nanoprobe such as ours would enable early disease detection and subsequently more accurate diagnosis.

Optical imaging easily detects fluorophores. To take advantage of this high sensitivity, our design included the near infrared fluorescent dye, cypate. The novelty of our experimental nanoprobe was based upon its action of self-quenching. While the dye remained conjugated to the nanoparticle surface, its fluorescent activity was quenched. However, once the specific enzyme, cathepsin B, cleaved the conjugated cypate dye from the MNP, the probe regained its fluorescence.

This special property can be powerfully used to our advantage to supplement enhanced MRI images with clearer molecular visualization. In addition, this particular nanoprobe can act as a cathepsin B indicator for IBC diagnosis.

Methods:

Surface Coating. It has been reported that coating the MNP surface is integral to biocompatibility and increased circulation lifetime in the body [4]. For our research, we used meso-2,3-dimercaptosuccinic acid (DMSA) as the hydrophilic surfactant for aqueous solubility. Coating had the added advantage of providing the necessary functional group for amine peptide conjugation, $-\text{COOH}$. To prepare DMSA coated spinel MNPs, various paths were taken. The experiment was conducted at several pH levels ranging from 3-10 and in an assortment of solvents. DMSA was pre-dissolved, then added to the MNP mixture. The reaction mixture was then sonicated for 2-3 hours under argon to minimize disulfide cross-linking. The nanoparticles were washed with methanol and water.

	<i>T1</i> (ms)	<i>T2</i> (ms)
<i>Water</i>	3460	400
<i>Fe₃O₄</i>	2137.03 $\Delta = -1322.9$	1750.03 $\Delta = +1350.73$
<i>NiFe₂O₄</i>	759.81 $\Delta = -2700.2$	13.40 $\Delta = -386.6$

Table 1: MR contrast of spinel ferrite nanoparticles.

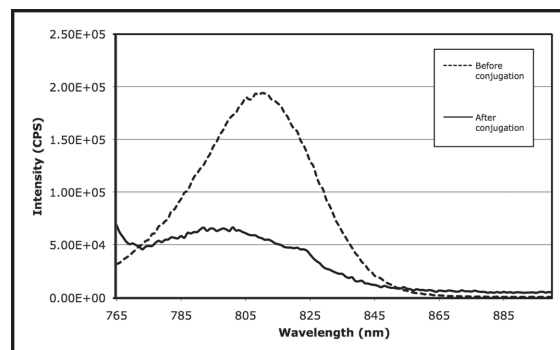


Figure 2: Fluorescence data before and after conjugation.

Peptide Dye Conjugation. The peptides used in this study possess an amine functional group at the N-terminus. The conjugation of the peptide amine ($-\text{NH}_2$) and surface functionalized carboxyl groups ($-\text{COOH}$) creates a strong amide bond. The soluble MNP solution was isolated and a ratio of MNP:peptide conjugate was established. The reaction mixture was left overnight in a 4°C refrigerator and away from the light to forestall photobleaching. Spinel ferrite nanoparticles possess enhanced magnetic properties and have already been reported as effective negative contrast agents for improved MRI sensitivity. Negative contrast agents are typified by lower *T1* and *T2* values. As such, spinel structures show greater magnetization than current ferrite (Fe_3O_4) contrast agents (Table 1).

Surface Coating. The MNPs were successfully coated and functionalized. FT-IR (Table 2) shows evidence for the necessary carboxylic acid group. Functionalized MNPs experience significant shifts in $\text{C}=\text{O}$ stretches and $\text{C}-\text{O}$ stretches as compared to free DMSA. Presence of doublet peaks around 2553.89 nm and 2556.27 nm on the coated MNP suggest free unconjugated thiol groups. From previous knowledge, the surface of gold nanoparticles have shown to have a very strong interaction with thiols [5]. However, if spinel MNPs displayed the same bonding interaction as gold nanoparticles, we would have expected both thiols of DMSA to conjugate to the particle surface in a cis configuration, leaving only the two $-\text{COOH}$ functionalities free (Table 2). Coating was accomplished in two ways: one using a double ligand exchange method, the other being directly in DI water. Double ligand exchange offered gentler reaction conditions and required fewer washes.

Substance	Peak Locations (Wavelength)			
	-CH	-SH	C=O	C-O
DMSA		2561.68	1687.24	1310.68
		2537.19		1179.03
Coated	2923.30	2553.89	1698.25	1379.98
MNP		2556.27		1157.31

Table 2: FT-IR data.

Peptide Dye Conjugation. The extent of successful peptide conjugation was measured by fluorescence and UV-vis. By UV-vis, we found the excitation wavelength for fluorescence to be 780 nm. Normal fluorescence for unconjugated peptide dye amounted to about 2×10^5 counts per second (CPS), while conjugated peptide dye had a lower intensity at 5×10^4 CPS—a 4-fold decrease (Figure 2). Although we expected a complete loss of fluorescent signal, the observed intensity decrease hints to a partial quenching. This can be a result of incomplete surface coating, which thereby provided an insufficient number of available $-\text{COOH}$ functional groups for amide bond formation.

Conclusion:

Molecular imaging techniques are pivotal for the *in vivo* assessment of biological processes in a wide spectrum of diagnostic roles. However, since every technique carries inherent advantages and disadvantages, it is necessary to formulate imaging strategies that will capitalize on the increasingly sophisticated capabilities of molecular imaging.

In a two-step process, a nanoprobe was created that harnesses the individual strengths of MRI and optical imaging, while simultaneously compensating for their weaknesses.

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References:

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