Communication

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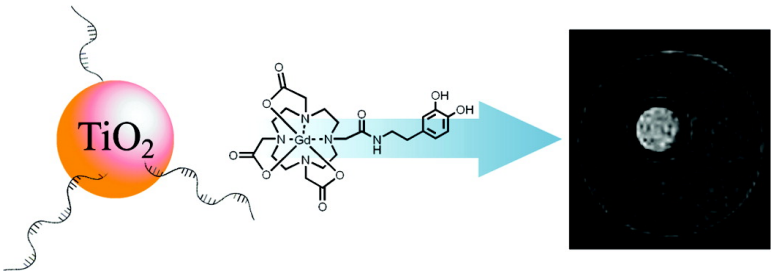

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DNA−TiO_2 Nanoconjugates Labeled with Magnetic Resonance Contrast Agents

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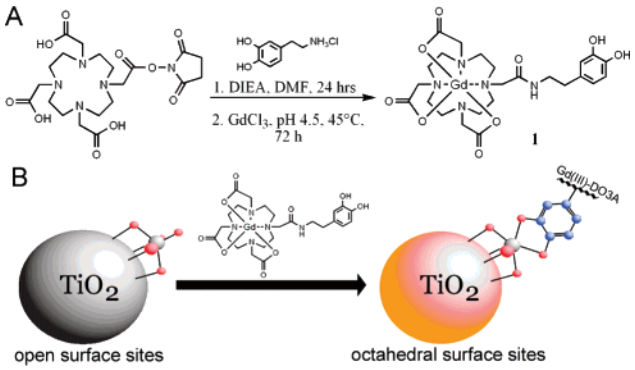
Recent efforts to develop nanoscale materials for use as biocompatible delivery scaffolds for drugs and imaging agents have produced significant advances in the area of multifunctional probes.[1] These new materials provide the means to chaperone and concentrate both drugs and contrast agents in specific organs, tissues, and cells.[2] Primarily, superparamagnetic (SPIO) nanoparticles, known for their ability to enhance signal contrast in T_2-weighted MR images, have dominated reports employing this approach.[3,4] These agents typically result in negative contrast enhancement, and occasionally, their inherent magnetic susceptibility leads to background artifacts.[3] Conversely, T_1 contrast agents do not suffer from this phenomenon. However, there are very few examples of paramagnetic (T_1)-labeled nanoparticles that combine therapeutics with an MR reporter.[5]

In conjunction, our recent work shows that TiO_2 nanoparticles decorated with DNA oligonucleotides function in a targeted and therapeutic capacity.[6,7] Targeting is accomplished via oligonucleotide hybridization to an intracellular organelle's matching DNA sequence, and therapeutic activity is elicited by light-induced scission of the nanoparticle-bound DNA. Considering these properties, attachment of a T_1 contrast agent to such a targeted nanoparticle would allow the visualization of certain genomic sequences in cells and tissues via MRI. Knowing that cancer is a result of DNA mutations, one can envision that a therapeutic MR probe targeting only mutant oncogene sequences would have a significant impact on the therapeutic and diagnostic communities. As a result, we have functionalized DNA-labeled nanoconjugates with a Gd(III)-based contrast agent to produce a biocompatible, therapeutically active delivery scaffold that is detectable by routine T_1-weighted MR imaging.[7]

The Gd(III)-labeled nanoparticles were prepared from previously reported TiO_2−oligonucleotide nanoconjugates.[6,7] The TiO_2 nanoparticles (3−5 nm) are surface modified by exploiting their selective reactivity to ortho-substituted enediol ligands (such as dopamine).[8] These reactions form semiconducting, photocatalytically cleavable bonds which allow for the surface conjugation of dopamine-modified DNA. This nanoparticle-bound DNA retains its subcellular targeting capabilities while remaining stable enough to allow in vivo compartmental accumulation before photocatalytic cleavage is induced.[7]

The synthesis of a dopamine-modified contrast agent was accomplished by coupling the commercially available succinimidyl ester activated 1,4,7,10-tetraazacyclododecane-1,4,7-tetraacetic acid to dopamine hydrochloride (Scheme 1). Metalation (with $GdCl_3$) and purification were carried out under strict pH control as the catechol in the metalated complex (**1**) is easily oxidized at pH >5.

Scheme 1. (A) Synthetic Route to **1**; A Dopamine-Modified MR Contrast Agent (DOPA−DO3A) and (B) Functionalization of TiO_2 Nanoparticles with **1**



Following characterization (HPLC, ESI-MS, and EA), the dopamine-modified contrast agent (**1**) was reacted with TiO_2 nanoparticles in the presence and absence of surface-conjugated oligonucleotides (see Supporting Information, S6−S9). Coupling **1** to the nanoparticle was initially detected by a distinct color change resulting from a red shift in nanoparticle absorption between the unmodified nanoparticles (white) to the modified nanoparticles (orange/brown).[8] In addition, formation of the DOPA−DO3A nanoparticles was confirmed by UV and IR spectroscopy (see Supporting Information, S10 and S11). The modified nanoparticles revealed a characteristic rise in UV light absorption toward higher energies (200−360 nm) that has been attributed to semiconducting nanocomposites.[8,9] Similarly, the modified nanoconjugates showed significant (20% increase) IR absorption over control nanoparticles in the carbon−carbon double bond and aromatic stretching frequencies of 1540−1680 cm^{-1}.

As expected, conjugation of **1** to the DNA-modified nanoconjugates occurs in a linear relationship to the equivalents added. The success of the conjugation reaction (assessed by the ICP-MS of Ti and Gd in purified trials of varying [**1**]:[TiO_2 active site] equivalents) increases linearly until the ratio of [**1**]:[TiO_2 active sites] reaches 1.0. At a 1:1 equivalence ratio, the conjugation reaction plateaus, giving a value of 4.6 ± 0.2% of functionalized TiO_2 active sites at ratios extending to 5:1. This is most likely due to steric effects as the available TiO_2 active sites may be blocked by the DNA oligonucleotides and the freely rotating, surface-conjugated DOPA−DO3A contrast agents.[10]

To determine the relaxivity of the DOPA−DO3A−TiO_2 nanoconjugates, the slope of the line generated by plotting the inverse of the T_1 relaxation time versus Gd(III) concentration was measured (see Supporting Information, S12). The nanoconjugates yielded a relaxivity of 3.5 ± 0.1 mM^{-1} s^{-1} per Gd(III) ion, similar to clinical small molecule contrast agents.[11] On the basis of the Ti:Gd ratio acquired from ICP-MS, each individual nanoparticle has an average

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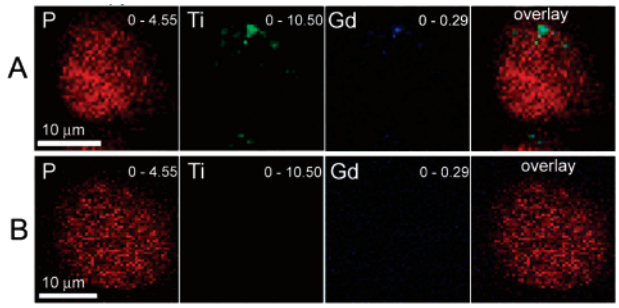

Figure 1. Two-dimensional XRF maps of (A) transfection of PC12 cells with DNA−DOPA−DO3A-modified nanoconjugates; (B) control, non-transfected PC12 cells. Phosphorus is red, titanium is green, and gadolinium is blue. The scale bars represent 10 μm. Note that each image is scaled to its respective maximum value (displayed at the upper right corner and given in $\mu g/cm^2$).

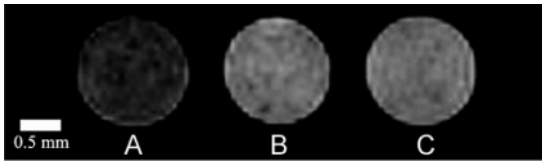

Figure 2. T_1-weighted MR images of (A) control PC3M cells ($T_1 = 3527 \pm 48$ ms); (B) PC3M cells incubated with 0.001 mM DNA−DOPA−DO3A nanoparticles with 1.8% **1**:TiO_2 active site coverage ($T_1 = 2178 \pm 88$ ms); (C) PC3M cells incubated with 0.001 mM DNA−DOPA−DO3A nanoparticles with 4.4% **1**:TiO_2 active site coverage ($T_1 = 2356 \pm 100$ ms). The scale bar represents 0.5 mm (at 14.1 T within a FOV of 20 mm and a slice thickness of 0.5 mm). T_1 values were confirmed to be statistically different via student t tests at a 95% confidence level (Supporting Information Table S4).

relaxivity of 61.0 ± 1.7 mM^{-1} s^{-1}. In order to assess the viability of **1** to enhance the MR image contrast of the TiO_2 nanoparticles, DOPA−DO3A−TiO_2 nanoconjugates at varying [**1**]:[TiO_2 active site] ratios were gravity packed in glass capillary tubes and imaged. As expected, the TiO_2 nanoconjugates functionalized with **1** display a brighter signal than the control nanoparticles (see Supporting Information Figure S4).

The cellular distribution and single cell association of the DOPA−DO3A−TiO_2 nanoconjugates were evaluated by X-ray fluorescence (XRF).[12] XRF data, when deconvoluted and standardized, provides high-resolution (0.3×0.3 μm), two-dimensional images that can be employed to map locations and total elemental concentrations in a desired subcellular region of interest.[6] Therefore, this imaging modality allows direct visualization of the optically undetectable nanoconjugates while simultaneously providing cellular outlines and contrast agent location (phosphorus and gadolinium, respectively).[12,13]

A DNA oligonucleotide targeted to the mitochondrial genome was used to functionalize the nanoparticles. The targeting sequence was specific for the sense strand of a NADH dehydrogenase 2 (ND2) mitochondrial gene present in the rat PC12 cell line: 5′-carboxy dT-cacgacaccttagcaccaacttac (ND2s).[7] Presence of nanoconjugates in the cytoplasmic but not the nuclear regions suggests localization in either the targeted organelle, mitochondria, or possibly endosomes (this oligonucleotide has previously shown sequence specificity in this cell line).[7] Colocalization of the Ti and Gd fluorescence signals reveals that the DOPA−DO3A−TiO_2 nanoconjugates are biologically stable and that their presence inside cells is responsible for the increase in MR image intensity versus control untreated cells (Figure 1).[12]

To assess the biocompatibility and MR image contrast of the modified nanoparticles in vitro, PC3M cells were imaged after incubation with the nanoconjugates (Figure 2). Comparison of the images from Figure 2 reveals that the cells incubated with the DOPA−DO3A nanoconjugates display a greater contrast enhancement over control cells. Corroborating the nanoconjugate phantom images (Supporting Information Figure S4) and XRF images (Figure 1), we see that Figure 2 demonstrates the utility of quantitative T_1 analysis and XRF to predict viable contrast enhancement via MR imaging.

In conclusion, we have prepared a Gd(III)-modified DNA−TiO_2 semiconducting nanoparticle that is detectable in cells by MR imaging. The labeled particles appear to be retained at specific locations inside cells by the conjugated DNA oligonucleotides hybridizing to intracellular targets, hence creating the first nanoparticle system capable of targeting specific DNA sequences while being simultaneously detected by MR imaging.[7] As a result, we anticipate that any dopamine-functionalized molecule (e.g., cell-penetrating peptides for passive cell membrane transport) can be linked to this modified TiO_2 nanoparticle scaffold, allowing noninvasive monitoring of cells containing target molecules and removal of target gene sequences pending TiO_2 excitation.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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