Multifunctional Plasmonic Nanoparticles for Photothermal Therapy of Cancer with MRI Monitoring

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Introduction

Photothermal therapy of cancer *in vitro* and *in vivo* has recently been demonstrated with a variety of metal nanoparticles, allowing the localized destruction of the tumor while causing only minimal damage to surrounding tissue [1, 2, 3]. However, the inability to monitor and track the accumulation of these particles at target sites remains a limitation. Improvements in determining the dose of nanoparticles delivered to the tumor will help to increase the specificity of treatment, reducing damage to healthy tissues surrounding the tumor. Plasmonic nanoparticles with a paramagnetic core can exhibit both magnetic and optical properties that make them ideal for determining nanoparticle distribution within the tumor prior to laser therapy, potentially increasing the efficacy and specificity of treatment as well as providing a platform for attaching biomolecules to target cancer biomarkers. Here we demonstrate MR imaging and selective photothermal therapy in the near-infrared (NIR) region using iron oxide core gold shell nanoparticles targeted to epidermal growth factor receptor (EGFR). EGFR is overexpressed in a variety of tumors including oral cavity, cervix and lung.

Methods and Materials

Ten nanometer diameter iron oxide nanoparticles were synthesized via reduction of $FeCl_2$ and $FeCl_3$ in a 2:1 molar ratio. Gold ions were reduced onto the surface of the iron via an iterative hydroxylamine seeding technique resulting in *ca*. 50 nm diameter particles [4]. Following synthesis the particles were functionalized with anti-EGFR Ab (Neomarker c225).

MDA-MB-468 breast cancer cells overexpressing EGFR were grown to confluence on coverslips for photothermal treatment. Some cells were incubated with EGFR targeted magnetic gold nanoparticles for 30 minutes. Labeled and unlabeled coverslips were irradiated with 5 ns pulsed lasers at 532 and 680 nm at different doses. Following irradiation the coverslips were treated with 1 uM calceinAM, a live cell fluorescent dye, to determine the outcome of laser treatment. Fluorescent imaging was done on a Leica 6000 DM using a 10x objective and a 470 nm band-pass excitation and 515 nm long-pass emission fluorescent filter cube.

The nanoparticles were also injected into a mouse to demonstrate *in vivo* MR contrast. T1-, T2-, and T2*-weighted images were collected before and after injection of 100 uL, 10^{10} particles/ml into the abdominal fat pad of a normal mouse. Imaging was done using a 4.7 T Biospec experimental MR system (Bruker Biospin MRI, Billerica, MA, USA).

Results and Discussion

Molecular targeted iron/gold core/shell nanoparticles greatly improve the efficacy of photothermal treatment as well as provide MRI contrast. Cell death was observed following 1s of irradiation for labeled cells while no effects were seen until 15s for unlabeled cells using 680 nm irradiation, as shown in figure 1. It is important to note that despite the fact that anti-EGFR gold nanoparticles have an extinction maximum at 540 nm the difference in the photothermal effect for unlabeled and labeled cells is more pronounced in the red and near-infrared region. This shift in the photothermal response is attributed to plasmon resonance coupling between hybrid nanoparticles when they interact with closely spaced EGFR molecules on cytoplasmic membrane of cancer cells. Nanoparticles were also clearly distinguished in a mouse *in vivo*, providing negative T2 and T2* contrast. Representative T2-weighted images are shown in Figure 2).

References

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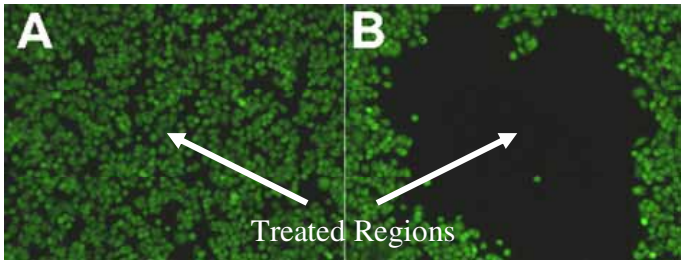

Figure 1: Phothermal treatment of MDA-MB-468 cells after 5s exposure to 5 ns pulsed 680nm laser at 10 Hz repetition rate and 2 mJ/pulse: A) unlabeled cells; B) cells labeled with multifunctional nanoparticles targeted to EGFR using C225 monoclonal antibodies.

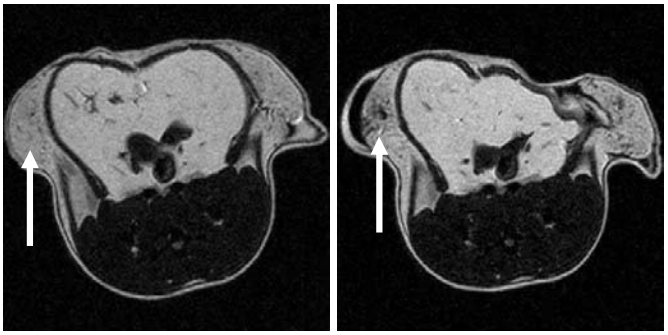

Figure 2: T2*-weighted images of normal mouse before (left) and after (right) injection of 100 uL, 10^{10} 1/ml of iron/gold nanoparticles.