

Immunotargeted Nanoshells for Integrated Cancer Imaging and Therapy

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ABSTRACT

Nanoshells are a novel class of optically tunable nanoparticles that consist of a dielectric core surrounded by a thin gold shell. Based on the relative dimensions of the shell thickness and core radius, nanoshells may be designed to scatter and/or absorb light over a broad spectral range including the near-infrared (NIR), a wavelength region that provides maximal penetration of light through tissue. The ability to control both wavelength-dependent scattering and absorption of nanoshells offers the opportunity to design nanoshells which provide, in a single nanoparticle, both diagnostic and therapeutic capabilities. Here, we demonstrate a novel nanoshell-based all-optical platform technology for integrating cancer imaging and therapy applications. Immunotargeted nanoshells are engineered to both scatter light in the NIR enabling optical molecular cancer imaging and to absorb light, allowing selective destruction of targeted carcinoma cells through photothermal therapy. In a proof of principle experiment, dual imaging/therapy immunotargeted nanoshells are used to detect and destroy breast carcinoma cells that overexpress HER2, a clinically relevant cancer biomarker.

Background. Nanoshells are composed of a dielectric silica core covered by a thin metal shell which is typically gold. Based on the relative dimensions of the core radius and shell thickness, nanoshell optical resonances may be continuously tuned through wavelengths ranging from the ultraviolet to the infrared,¹ including the NIR region where tissue transmissivity is highest due to low scattering and absorption from intrinsic chromophores.² Gold nanoshells offer appealing properties for biomedical sensing and therapeutic applications including large optical cross-sections exceeding conventional NIR dyes, such as indocyanine green, by many orders of magnitude as well as significantly improved photostability resulting from the rigid metallic structure of a nanoshell.³ Furthermore, antibodies and other targeting moieties can be readily conjugated to the gold surface of nanoshells.⁴ Although the gold surfaces of nanoshells are generally considered to be biocompatible, stealthing polymers such as poly(ethylene glycol) (PEG) may be attached to nanoshell surfaces to further enhance biocompatibility and improve blood circulation times.⁵

Nanoshells designed to have a high scattering optical cross-section are potentially valuable contrast agents for photonics-based imaging modalities such as reflectance confocal microscopy (RCM)⁶ and optical coherence tomography (OCT),⁷ which offer high-resolution approaches to early

cancer detection. To image deeper tumors, other methods such as frequency-domain photon migration may be used to enable optical visualization of scatter-based contrast.⁸ Alternatively, nanoshells can be designed to strongly absorb NIR light providing a novel means to mediate photothermal ablation of cancer cells.^{9,10} Of particular interest is the possibility of engineering nanoshells with optical properties suitable for combined imaging and therapy. Past attempts to develop combined approaches to imaging and therapy have relied on methods such as the use of radio-immunoconjugates¹¹ whose clinical effectiveness is limited by factors including low tumor uptake, dose-limiting toxicity, and the necessity to expose patients to ionizing radiation.¹² Nanoshells provide an alternative means to enable dual imaging/therapy applications as they can be engineered to simultaneously provide both scattering and absorption properties at specific frequencies. Selective accumulation of nanoshells may be achieved via passive extravasation based on the enhanced permeability and retention (EPR) of small particles (<400 nm) associated with the leaky tumor vasculature,¹³ with further targeting possible using antibodies targeted against oncoproteins overexpressed on cell surfaces. In principle, upon accumulation within tumors, nanoshells may provide both molecular-specific image contrast and, when clinically indicated, mediate cancer treatment based on NIR thermal ablation therapy. Here we provide an in vitro demonstration of the dual imaging/therapy approach, first detecting and then thermally ablating human breast cancer cells that overexpress HER2 using immunotargeted nanoshells that have been designed to both scatter and absorb light within the NIR.

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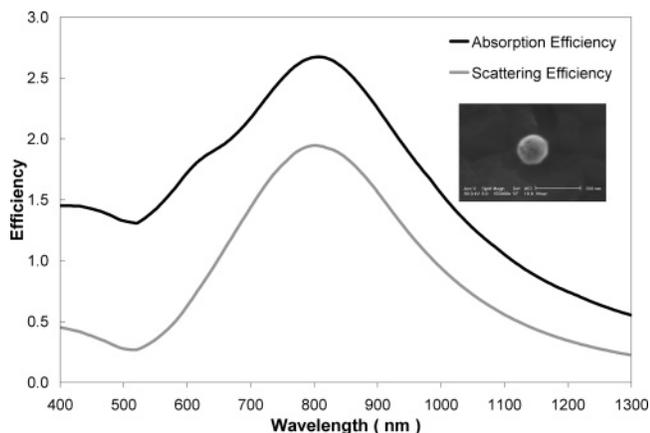


Figure 1. Spectral characteristics and SEM image of NIR scattering/absorbing nanoshells. Spectrum obtained by Mie scattering theory shows the dual scattering/absorbing NIR nature (~800 nm) of nanoshells with dimensions consisting of a 120 nm diameter silica core and 10 nm thick shell. Predicted optical extinction, the sum of the combined contributions of absorption and scattering, was confirmed with a UV-vis spectrophotometer (data not shown). SEM image of nanoshells with an overall diameter of 140 nm is included. Scale bar = 200 nm.

Nanoshell Fabrication. Nanoshells with dimensions providing peak optical scattering and absorption efficiencies in the NIR (~800 nm) were designed and fabricated as described in previous studies.¹⁴ Using the Stöber method,¹⁵ 120 nm diameter silica nanoparticles were fabricated. The particle surface was then terminated with amine groups by reaction with (aminopropyl)triethoxysilane (APTES). Small gold colloid was grown using the method of Duff¹⁶ and then adsorbed onto the aminated silica nanoparticle surface. More gold was reduced onto these colloid nucleation sites using potassium carbonate and HAuCl₄ in the presence of formaldehyde. Gold nanoshell formation was assessed with a UV-vis spectrophotometer. Nanoshell dimensions were assessed using scanning electron microscopy (SEM). Shell thickness was mathematically corroborated by Mie scattering theory with good agreement with SEM. Figure 1 shows the spectral characteristics and an SEM image of nanoshells possessing a 10 nm thick shell that were used in this study.

Antibody Conjugation. Either anti-HER2 or a nonspecific antibody (anti-IgG) was first attached to a PEG linker (OPSS-PEG-NHS, MW = 2 K) through an amidohydroxysuccinimide group (NHS). The antibody-PEG linker complex was then attached to the nanoshell surface through a sulfur-containing group located at the distal end of the PEG linker. The antibody-PEG complex was reacted with nanoshells for 1 h. Additional PEG-thiol (MW = 5 K, 50 nM) was later added to the nanoshell suspension in order to block nonspecific adsorption sites. Nanoshells were centrifuged to remove excess PEG and antibody and resuspended in water at a final concentration of 3×10^9 nanoshells/mL.

Cell Culture and Incubation of Nanoshell Bioconjugates with Cells. HER2-positive SKBr3 breast adenocarcinoma cells were grown in McCoy's 5A growth media containing 10% FBS and 1% antibiotics at 37 °C and 5% CO₂. Concentrated 10× McCoy's media (free of FBS and

antibiotics to eliminate nonspecific interactions with nanoshells) was quickly added to the nanoshells at a volumetric ratio of 1:9. Next, 500 μL of this McCoy's nanoshell suspension was placed on cells, followed by 1 h incubation. McCoy's media containing FBS and antibiotics was added following rinsing of unbound nanoshells.

Molecular Imaging of HER2 Expression and in Vitro Photothermal Therapy. Cells were imaged under a darkfield microscope sensitive to scattered light. Images were taken with a Zeiss AxioScope2 microscope equipped with a black/white CCD camera. All images were taken at the same magnification under the same lighting conditions. Immediately following imaging, cells were exposed to NIR irradiation (820 nm, 0.008 W/m² for 7 min). The overlap of peak nanoshell absorbance with the emission wavelength of the laser source promoted optimal laser-induced nanoshell heating. Cells were stained for viability using calcein AM. Stained cells were examined under fluorescence and phase contrast microscopy with a Zeiss Axiovert 135 microscope. Silver staining was then performed to assess the presence of nanoshell binding on cell surfaces.

Results. Figure 2 presents results from combined imaging and therapy of SKBr3 breast cancer cells using nanoshells targeted against HER2 (right column). In addition, control images of cells taken without nanoshells (left column) and of cells incubated with nonspecifically labeled nanoshells (middle column) are presented. Significantly increased scatter-based optical contrast due to nanoshell binding was observed in cells incubated with anti-HER2 nanoshells (top row, right column) as compared to the two control cell groups (top row, left and middle columns). After photothermal therapy, cell death was observed only in cells treated with NIR laser following exposure to anti-HER2 nanoshells (middle row, right column). This effect was not observed in cells treated with either nanoshells conjugated to a nonspecific antibody or NIR light alone (middle row, left and middle columns). Greater silver staining intensity was seen in cells exposed to anti-HER2 nanoshells (bottom row, right column) compared to controls (bottom row, left and middle columns), suggesting enhanced nanoshell binding to cell surfaces overexpressing HER2. To establish that anti-HER2 nanoshells alone do not induce cytotoxicity, we incubated SKBr3 cells with anti-HER2 nanoshells over a range of concentrations and incubation times. Figure 3 shows calcein fluorescence of SKBr3 cells that were exposed to HER2-targeted nanoshells (3×10^9 nanoshells/mL). In statistical analysis comparing cells incubated with nanoshells and control cells not exposed to nanoshells, no differences in viability were observed.

Discussion. Currently, distinct diagnostic and therapeutic modalities are employed for the diagnosis and treatment of cancer. Furthermore, in most cases, standard of care treatment requires invasive surgical procedures or other therapies associated with significant side effect profiles, high cost, and poor clinical outcome. A single technology providing both diagnostic and therapeutic capabilities would potentially yield significant savings in time, cost, and patient discomfort associated with diagnosing and treating many cancers today. Nanoshells offer unique properties that facilitate an integrated

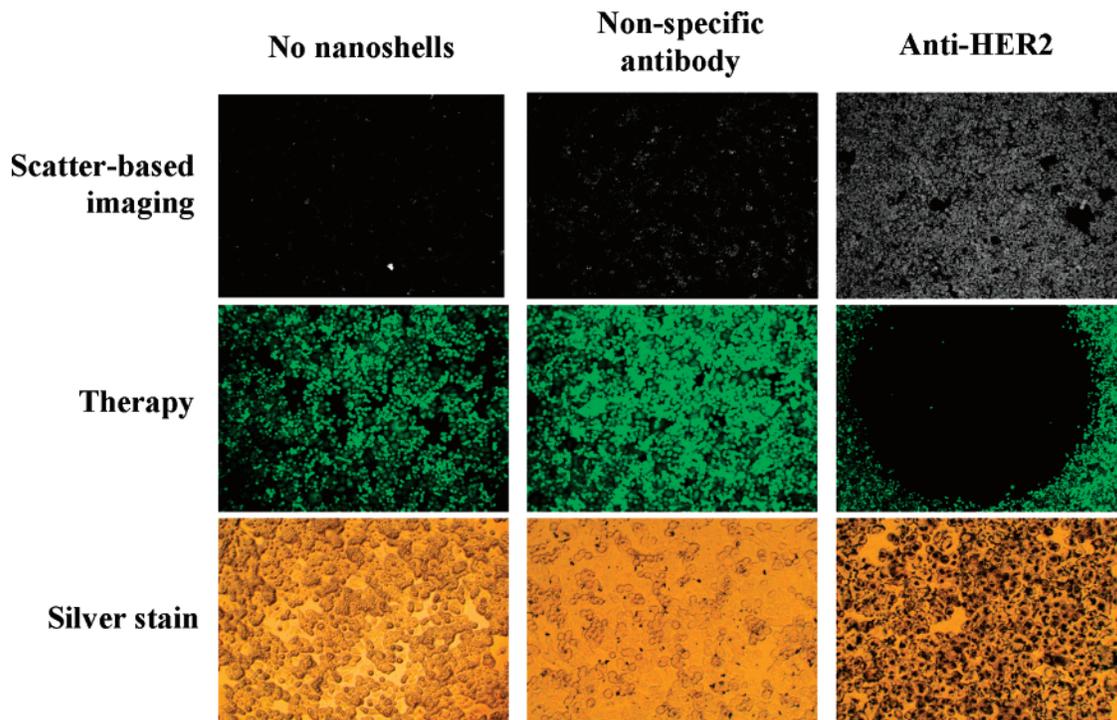


Figure 2. Combined imaging and therapy of SKBr3 breast cancer cells using HER2-targeted nanoshells. Scatter-based darkfield imaging of HER2 expression (top row), cell viability assessed via calcein staining (middle row), and silver stain assessment of nanoshell binding (bottom row). Cytotoxicity was observed in cells treated with a NIR-emitting laser following exposure and imaging of cells targeted with anti-HER2 nanoshells only. Note increased contrast (top row, right column) and cytotoxicity (dark spot) in cells treated with a NIR-emitting laser following nanoshell exposure (middle row, right column) compared to controls (left and middle columns).

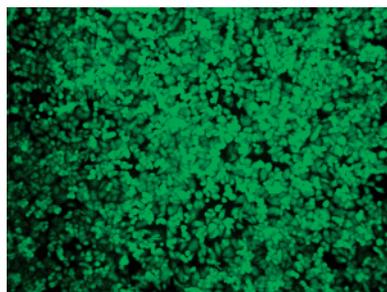


Figure 3. Calcein fluorescence of HER2-positive SKBr3 cells incubated with anti-HER2 nanoshells without NIR photothermal therapy. No cytotoxicity is observed.

imaging/therapy approach including systematic control of both optical scattering and absorption, tunability throughout the NIR where tissue penetration is highest, and a particle size conducive to passive extravasation from the tumor vasculature. We showed that immunotargeted nanoshells can provide scattering contrast for imaging while also exhibiting sufficient absorption to enable effective photothermal therapy. This is the first demonstration of coupling a bioimaging application to a cancer therapy application using nanoshells targeted against a clinically relevant biomarker. Future studies will extend the in vitro concept demonstrated here to in vivo animal experiments.

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