EphrinA1-Conjugated Nanoshells for Prostate Cancer Cell Therapy

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Statement of Purpose: While many cancer therapeutics do not discriminate between healthy and diseased tissue, nanotechnology offers the potential to more specifically treat tumors through targeting mechanisms. Herein, we report the use of ephrinA1 to target metal nanoshells to the EphA2 receptor, a tyrosine kinase receptor overexpressed on many prostate cancer cell surfaces.[1] Gold nanoshells, composed of a gold shell over a dielectric core, can interact with near-infrared (NIR) light, where transmission through human tissue is maximal, either by scattering for diagnostic purposes or absorption for photothermal ablation.[2,3] In this study, we characterize both gold-silica and gold-gold sulfide nanoshells for targeted photothermal ablation of prostate cancer cells.

Methods:

Particle Synthesis

Gold-silica and gold-gold sulfide shell-core nanoshells were fabricated as previously described.[4,5] Silica cores 120 nm in diameter were functionalized with amine groups, upon which gold colloid (~3 nm) was adsorbed and nucleated the growth of a complete gold shell in the presence of reduced $HAuCl_4$ (Alfa Aesar). Gold-gold sulfide nanoshells were synthesized by mixing Na_2S (Sigma-Aldrich) and $HAuCl_4$, followed by removal of the sulfur source via centrifugation to cease particle growth. Particles were characterized with transmission electron microscopy (TEM) and UV-Vis spectroscopy.

Particle Functionalization

Mouse ephrinA1/Fc chimera (R&D Systems) was conjugated to nanoshells with a hetero-bifunctional poly(ethylene glycol) (PEG) linker (Creative PEGWorks), containing an N-hydroxysuccinimide end-group for protein conjugation and a disulfide end-group for attachment to gold. Particle surfaces were further coated with PEG-SH (MW = 5000 Da, Laysan) to prevent non-specific cell binding. As a control, nanoshells were conjugated to PEG-SH only.

Photothermal Ablation

EphrinA1-conjugated nanoshells or PEG-SH coated nanoshells were incubated with PC3 cells, which overexpress the EphA2 receptor.[6] The cells were washed with PBS to remove unbound nanoshells and then irradiated (808 nm, 80 W/cm^2, 5-7 min). Calcein AM (live) and ethidium homodimer-1 (dead) staining (Invitrogen) were used to characterize cell viability under fluorescent microscopy.

Results: Both gold-silica and gold-gold sulfide nanoshells, with approximate diameters of 150 and 45 nm respectively, were designed to absorb NIR light with peak absorption near 800 nm (Figure 1). EphrinA1 loading on nanoshell surfaces successfully targeted the particles to PC3 cells for photothermal ablation as shown in Figure 2. PEG-SH coated nanoshells did not bind PC3 cells, and as a result, cell death did not occur after laser irradiation.

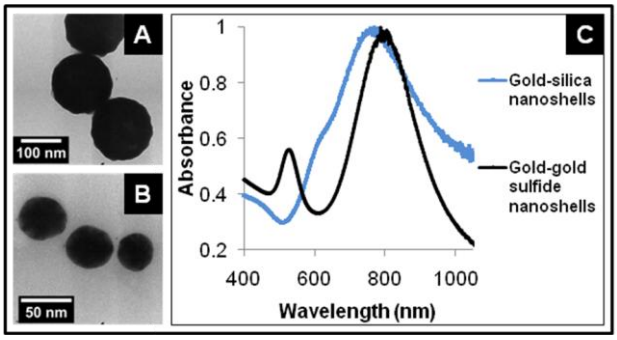

Figure 1: TEM images of (A) gold-silica and (B) gold-gold sulfide nanoshells. (C) The particles are designed to maximally absorb NIR light.

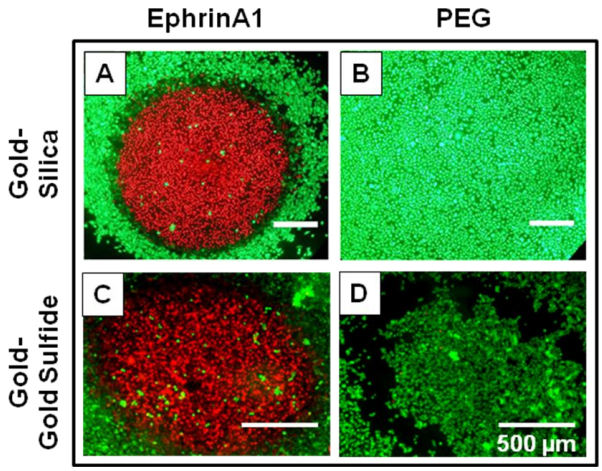

Figure 2: Live/dead (green/red) staining after NIR ablation of PC3 cells incubated with (A and B) gold-silica and (C and D) gold-gold sulfide nanoshells. (A and C) Area of laser exposure after cells incubated with targeted ephrinA1-conjugated and (B and D) control PEG-conjugated nanoshells.

Conclusions: NIR absorbing particles can induce cancer cell death as light is converted to heat. EphrinA1 targeting offers an alternative to antibody targeting for cancer cell types overexpressing the associated eph receptor. Further work will include combined imaging and therapy studies *in vitro* and *in vivo*, including characterization of particle biodistribution, which may vary with nanoshell diameter.

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