# NANOSHELL-MEDIATED NEAR INFRARED PHOTOTHERMAL TUMOR THERAPY

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## INTRODUCTION

Complications associated with invasive malignant tumor excision have led to alternative treatment methods including chemotherapy, photodynamic therapy, and thermal coagulation. Metal nanoshells, which are a new class of optically active nanoparticles, may provide a novel means of targeted photothermal therapy in tumor tissue, minimizing damage to surrounding healthy tissue. Silica gold nanoshells posses a silica core encapsulated by a thin gold shell. These particles possess a strong tunable absorption, which is determined by the relative core and shell thickness [1], permitting the synthesis of a particle with strong absorbance in the near IR where maximal penetration of light through tissue is achieved. When conjugated with a tumor-specific protein, these nanoshells could be systemically injected, but preferentially bound to the tumor site. Near IR light administered at the site would heat the localized nanoshells, killing the tumor. We have successfully conjugated antibodies against oncoproteins to nanoshells. Furthermore, we have demonstrated photothermally-induced death of nanoshell-bound carcinoma cells *in vitro*, as well as *in vivo*. Cell death was limited to the laser spot, and under control conditions (no nanoshells or no light), no cell death or tissue damage was observed.

## MATERIALS AND METHODS

Gold nanoshells were manufactured as previously described [1,2]. Briefly, the silica cores were fabricated by the Stöber method [3] in which tetraethoxysilane is reduced in ethanol. The surfaces of the silica particles were aminated by reacting with aminopropyl triethoxysilane. Small gold colloid (~ 3 nm) was then adsorbed onto the aminated silica nanoparticle surface. More gold was then reduced onto these colloid nucleation sites by chemical reduction in solution. Nanoshells were evaluated by their optical absorption profiles and SEM,. The nanoshells used in the following study had a 128 nm core diameter with a 14 nm thick gold shell and a peak absorbance at 820 nm.

To test the feasibility of nanoshell photothermal therapy *in vitro*, metal nanoshells were adsorbed directly onto cells followed by NIR treatment. 821 nm resonant nanoshells were incubated over the HTB-30 cells in serum-free McCoy's 5a growth medium ($2.9 \times 10^9$ particles/ml) for 1 hr followed by 3 saline rinses to remove unbound nanoshells. Control cells were incubated with medium only. The cells were then irradiated with a diode laser at 821 nm, 44 W/cm$^2$ for 10 min. The cells were incubated for an additional 2 hr at 37 $^{o}$C, then treated with calcein AM viability stain, which causes viable cells to fluoresce green.

For *in vivo* studies, female NOD CB17-Prkd c SCID/J mice were innoculated subcutaneously with the canine transmissible venereal tumor line in each hind leg.

Tumors were grown to ~1 cm in diameter. Nanoshell solutions were passivated with a thiolated-polyethylene glycol (SH-PEG), which self assembles on the gold nanoshell surface, providing steric stabilization of the nanoparticles. SH-PEG was synthesized by reacting PEG-Amine (Shearwater) with 2-iminothiolane (Sigma) for 1 hour. The product was then dialyzed against DI $H_2O$ for at least 1 hour to remove excess reagent.

The tumor site of anesthetized mice was shaved and swabbed with 600 MW PEG index matching agent. Nanoshell were injected 2 – 5 mm beneath the tumor surface. Tumors were irradiated at 821 nm, 4 W/cm$^2$ for 4 minutes. Temperature profiles were monitored using a fast phase gradient echo images in a 1.5 Tesla GE Signa Echospeed LX MRI. Following treatment, animals were sacrificed and tissue damage was examined by histology. Nanoshell distribution in histological sections was assessed using silver enhancement staining.

## RESULTS AND DISCUSSION

In the *in vitro* studies, it was shown that neither exposure to nanoshells nor exposure to the NIR light source induced cell death. In contrast, the combination of laser and nanoshells created circular regions of cell death corresponding to the laser spot (demonstrated by the absence of calcein AM fluorescence in Fig. 1, B).

*In vivo* photothermal nanoshell studies are in good agreement with the above results. MRI analysis revealed that control tumors receiving saline injections (no nanoshells) demonstrated minimal tissue heating (6 ± 4 $^0$C) over 6 minutes of heating. However, significant termperature increases (35 ± 10 $^0$C, n=5) were observed in nanoshell-treated tumors after only 4 min of NIR irradiation. Tissue was successfully heated above the 55 $^o$C damage threshold, resulting in thermal coagulation that was visible at the surface and in histological sections. Furthermore, histological analysis identified common markers of thermal damage, such as cellular vacuolization and coagulation. The regions with identifiable thermal damage correspond with the regions where nanoshells were located.

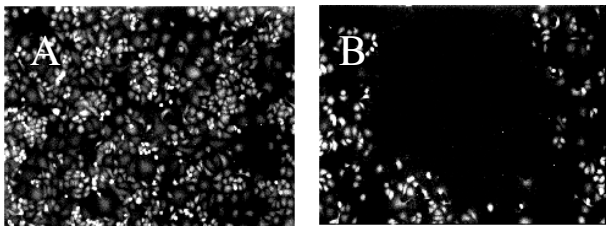


Fig. 1: A) Cells treated with laser in the absence of nanoshells maintain viability, while cells treated with both nanoshells and laser, B) create confined circular zones of cell death.

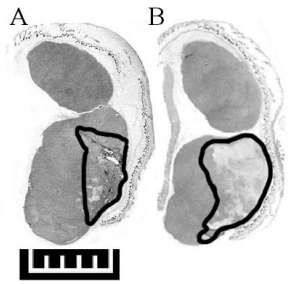


Fig. 2: A) Silver enhancement staining indicates the region of nanoshell treatment while B) staining with hematoxylin and esoin allows visualization of tissue damage.

## CONCLUSIONS

The lack of effective treatments for tumor malignancies demands new approaches that target tumors on the molecular level. Nanoshells show promise as a new therapy in this regime. The gold nanoparticle is biocompatible, and does not demonstrate cytotoxicity to healthy cells/tissue. The material is capable of inducing selective photothermal destruction of cancer cells within the laser/nanoshell treatment area. Likewise, *in vivo* studies of tumors pretreated with nanoshells demonstrated selective photothermal destruction of tissue, while nanoshell-free controls demonstrated minimal heating.

## REFERENCES

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