**Nanoshells targeted with anti-PSMA for prostate cancer therapy.**

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**Introduction:**

Metal nanoshells, are a class of optically tunable nanoparticle that consist of a dielectric core and a metal shell [1, 2]. We have previously used gold nanoshells with a silica core for photothermal tumor ablation [3]. Prostate specific membrane antigen (PSMA) has been identified [4] and has subsequently been used as a target in the development of therapies for prostate cancers [5]. PSMA represents an ideal target for prostate cancer since the expression level has been quantified and found to be 100 to 1000 fold greater in the prostate compared to other tissue [5]. In these studies we conjugate anti-PSMA to nanoshells. We have quantified the number of antibodies bound to the surface and demonstrated preferential binding to prostate cancer cell lines over-expressing the surface marker. We believe that the targeted delivery of nanoshells to prostate cancer cells will allow the development of a more potent therapy for this mode of treatment of prostate cancer. Furthermore, it could allow for treatment of prostate tumors that have metastasized to distant location, a primary reason for death by prostate cancer.

**Methods and Materials**:

Nanoshell Fabrication:

Nanoshells were made as previously described [1]. Briefly, silica cores are grown using the Stöber process. Silica nanoparticles were reacted with (3-aminopropyl) triethoxysilane. Gold colloid was prepared to a size of 2-4 nm using the method of Duff. Gold colloid was mixed with the aminated silica particles to adsorb the very small colloid act as nucleation sites for reduction of additional gold. The gold shell was then grown by the reduction of gold from $HAuCl_4$ in the presence of formaldehyde. NIR absorption characteristics of the nanoshells were determined using a UV-Vis spectrophotometer (Carey 50 Varian, Walnut Creek, CA).

Conjugation of anti-PSMA to nanoshells:

Mouse anti-huPSMA was reacted in a 1:2 molar ratio with OPSS-PEG-NHS mw 2000, (Nektar, Alabama) for 2 hours. Conjugated antibodies were attached to washed nanoshells by incubation for 4 hours and PEG-SH, MW 5000 (Nektar, Alabama) was added to block any remaining gold surface to prevent protein adsorption. Control nanoshells were made with PEG-SH only to act as non-binding nanoshells or left bare as a positive control.

Antibody concentration determination on nanoshells:

Nanoshells with antibodies or PEG only were incubated with a secondary antibody labeled with horse radish peroxidase (HRP). Nanoshells were spun down twice to remove excess unbound secondary antibodies and incubated with Tetramethylbenzidine (TMB) substrate (Sigma, Milwaukee) to quantify HRP concentration.

*In vitro* cell binding:

Prostate cancer cells, LNCaP cells which overexpress the PSMA surface protein and PC-3 cells which express normal levels of PSMA were used for the targeting studies. Nanoshells were incubated with cells in media for 2 hours and gently rinsed 2X with PBS. Binding of nanoshells was assessed by silver enhancement using a silver stain kit (Amersham, Buckinghamshire, England)

**Results/Discussion**:

We obtain ~150-175 Ab binding per nanoshell. Figure 1 shows good binding of the targeted nanoshells using PSMA to the LNCaP cell line while there is minimal binding to the PC-3 cell line. PEG only nanoshells show no binding to either cell line while bare nanoshells show indiscriminant binding to both cell types.

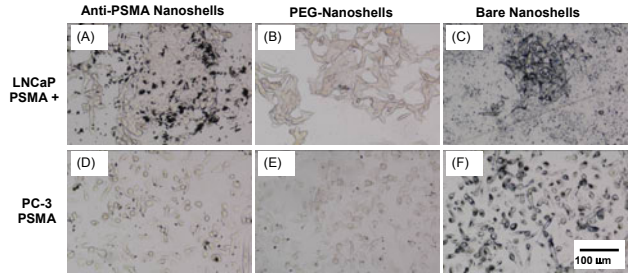


**Figure 1: Binding of anti-PSMA targeted nanoshells to LNCaP (A) and minimal binding to PC-3 (D), there is no binding with PEG only nanoshells (B) & (E), and complete binding with bare nanoshells (C) and (F).**

**Conclusions & Future Plans:**

We have thus far demonstrated the ability to conjugate antibodies to PSMA to nanoshells surfaces for a targeted prostate cancer therapy application. We have seen good binding of anti-PSMA nanoshells to cell lines over-expressing the PSMA surface membrane protein and we expect to be able to translate this into an *in vivo* model to verify improvements in targeted delivery compared to non-targeted delivery of nanoshells. We are currently working a mouse model with both over-expressing and non-expressing PSMA tumors cell lines.

**References**

1. S. J. Oldenburg*, et al.* Ch Phys Lett 1998;**288:***243-247*
2. R. Averitt*, et al.* J. Opt. Soc. Am. B 1999;**6:***1824-1832*
3. D. P. O'Neal*, et al.* Cancer Lett 2004;**209:**
4. R. S. Israeli*, et al.* Cancer research 1994;**54:***1807-11*
5. N. H. Bander*, et al.* Seminars in oncology 2003; **30:***667-76*