Minimally Invasive Multifunctional Nanoscale System for Selective Targeting, Imaging and NIR Photothermal Therapy of Malignant Tumors

Center for Optical Sensors and Spectroscopies
http://www.coos.snu.uab.edu
Hadiyah Nicole Green®, Dmitri V. Martyshkin®, Eben L. Rosenthal®, Sergey B. Mirov®
University of Alabama at Birmingham, Departments of Physics, 310 Campbell Hall, 1300 University Blvd, Birmingham AL 35294
Department of Surgery, Division of Otolaryngology–Head and Neck Surgery, University of Alabama at Birmingham, Birmingham, Alabama, USA®

Abstract
The mastery of active tumor targeting has been a great challenge in near infrared (NIR) photothermal therapy (PTT). By conjugating gold nanoparticles to tumor-specific fluorescent antibodies using covalent bonds and exciting with an NIR laser, improved efficiency for targeted treatment and imaging of malignant tumors can be achieved. Polyethylene glycol coated (PEGylated) gold nanorods (GNPs) with aspect ratio of ~4 and plasmon resonance peak at ~785 nm were fabricated and conjugated to cetuximab, an antibody to the epidermal growth factor receptor (EGFR). We compare the efficiency of antibody binding and the photothermal effect of nanorods before and after conjugation.

Introduction
One of the greatest obstacles with cancer treatments, past and present, is the ability to actively target and selectively treat only the malignant cells while leaving normal cells unharmed. NIR PTT using gold nanoparticles (GNPs) and NIR light to treat malignant tumors in vitro and vivo have demonstrated promise as treatments for cancer but further development of the active targeting and imaging components are necessary. A durable conjugation process using covalent bonds is needed to protect the conjugation in the body, reduce non-specific binding, ensure active delivery of the nanoparticles to the tumor site and increase the efficacy of NIR PTT. While utilization of GNPs as contrast agents in vitro yielded good imaging results, their use in vivo was severely limited. Using fluorescently labeled antibodies can provide tumor imaging in real time in vivo. Incorporating this method of active targeting and fluorescence imaging will improve the impact of PTT making it a viable approach for a variety of carcinomas that over-express the epidermal growth factor including head and neck, colorectal, ovarian, skin, cervical, breast, bladder, pancreatic, and prostate cancers.

Results
By improving the conjugation efficiency of nanorods to tumor targeted antibodies, we can consequently improve the delivery of the nanorods. We modify the order of conjugation and PEGylation of an existing protocol taking advantage of covalent bonds, amide and thiol group chemistry. We compare the order of conjugation and PEGylation and its effects on the nanorods forming clusters, aggregating, and the percentage of binding efficiency of the antibody in both cases. We observe the effect of modifying the crosslinker to antibody molar ratio.

Conjugation Method

Figure 2. Illustration of gold nanorod to antibody conjugation and PEGylation order. A) conjugation to antibody followed by PEGylation and B) conjugation of antibody to PEGylated nanorod.

Figure 3. TEM images of nanorod conjugation to antibody before PEGylation vs. PEGylated nanorod conjugation to antibody. A) Nanorods conjugated to anti-EGFR antibody then PEGylated; B) Nanorods first PEGylated then conjugated to anti-EGFR antibody; C) PEGylated nanorods without antibody or crosslinker present. All scale bars shown are 100nm.

Figure 4. A comparative antibody binding assay based on the sequential order of conjugation and PEGylation, summarizing the binding efficiency of the antibody when: A) conjugated to nanorods then PEGylated and B) conjugated to PEGylated nanorods. (INSET) The average US-VIS absorption spectra of gold nanorods immediately after fabrication with original CTAB surfactant (blue) and after PEGylation (red). The maximum absorption has red shifted 10 nm after the PEGylation process.

Summary and Conclusion
We have evaluated several parameters for the covariant conjugation of gold nanorods to fluorescent anti-EGFR antibody and demonstrated the following:
(1) The percentage of antibody binding efficiency is 15 times greater when conjugating the antibody to a PEGylated nanorod versus PEGylating the nanorod-antibody conjugation.
(2) Modifying the antibody-crosslinker molar ratio tunes the space between nanorods.
(3) The binding ability of nanorod conjugated fluorescent antibody is not compromised: further • 3.93 before conjugation and 4.4 after conjugation on a scale from 0 to 5.
(4) Cell death by NIR PTT with tumor-targeted fluorescent antibody conjugated gold nanorods is not compromised: • Only a 7% difference in the therapeutic effect for nanorods before and after the conjugation process: both killed nearly 92% of a 4 tumor cells.

Future Work & Broader Impact
This combination of active targeting and subsequent photothermal treatment of malignant cells is a viable approach for future in vivo and translational studies for a variety of cancer types, potentially providing an alternative to chemotherapy and radiation cancer treatments.

Acknowledgments
This work has been funded by the National Physical Science Consortium, NSF grant: EPS-0814103, and NIH/NIDCR R21 grant: 1R21DE019232-01A2. We thank the UAB High Resolution Imaging Facility, Melissa Foley and Cynthia M. Redenburg. We also appreciate support from NSBS ’11 UIUC and NSF grant CMMI 1136287.

References