Customized Nanoparticle Probes for Biomedical Imaging, Diagnostics and Therapeutics Applications

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ABSTRACT

The modular synthetic approach that was developed to design a spectral library of core-shell nanoparticle contrast agents, which will have broad applications in biomedical imaging due to the potential for multi-modal imaging (e.g., fluorescence, MRI, X-ray) and active targeting through molecular surface functionalization. Gadolinium oxide, hafnium oxide, and gold core compositions were prepared at a common size (12-15 nm) using sol-gel and microemulsion syntheses. Nanoparticle cores were encapsulated in a silica shell with a controlled thickness of 1-15 nm using polymer shells or microemulsions. Controlled silica shell formation enabled the incorporation of fluorescent molecules and provided a common platform for molecular surface functionalization using silane chemistry. Antibodies and other small molecules were efficiently conjugated to the nanoparticles using carbodiimide chemistry. We anticipate that this modular approach will provide a platform for facile customization of multi-component nanoparticles with tailored surface functionality that will have applications beyond the biomedical field due to the ability to customize electromagnetic properties in size, shape, and elemental composition-dependent manner.

Keywords: modular assembly, biomedical imaging, bifunctional nanoprobes, cancer theranostics, metastatic cancer

1 INTRODUCTION

The high mortality and poor prognosis for cancer patients are mainly due to late diagnosis. Improved detection of primary tumors and recurring tumors after chemotherapy is, therefore, crucial to reduce cancer mortality and improve progression-free survival. However, current clinical screening and diagnostic imaging methods are limited by low sensitivity and specificity. Contrast-enhanced computed tomography (CT), and spectral (color) X-ray CT has the potential to enable targeted molecular imaging with CT as a lower cost and higher resolution alternative to PET and MRI.[1] Therefore, we have developed a modular approach for the design and scalable synthesis of core-shell nanoparticle (NPs) imaging probes enabling multi-modal imaging (e.g., fluorescence, MRI, X-ray, plasmonic), and active targeting through bioconjugation.

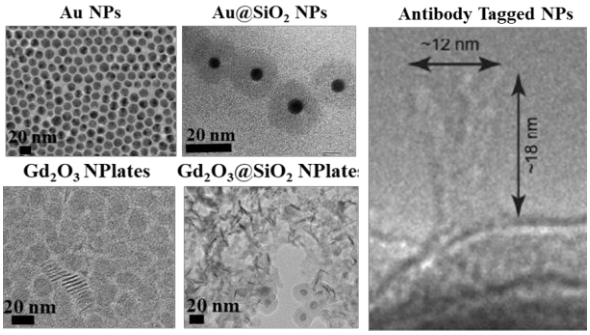

Figure 1. A limited snapshot of our NPs toolbox comprised of Au NPs, Gd_2O_3 NPs and their corresponding core-silica shell NPs. Negatively stained TEM images of antibodies on the surface of core shell NPs, Scale bar = 5 nm

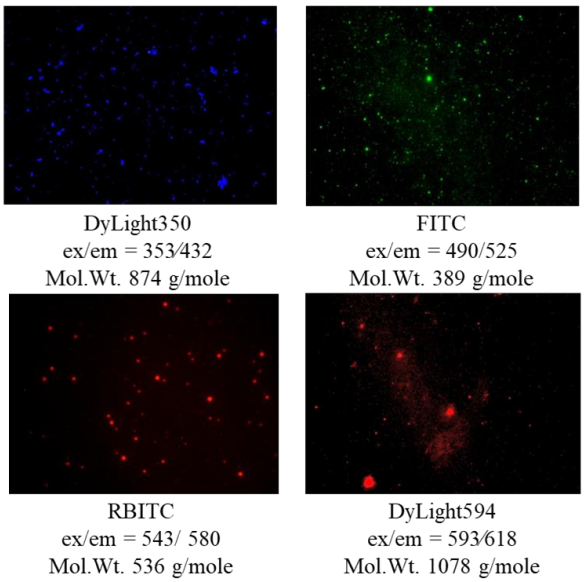

Figure 2. An overview of the fluorescent gold nanoparticles being synthesized in-house.

2 METHODS

As demonstrated in **Fig.1-2**, We modularly assembled $Au@SiO_2$ core-shell NPs (~10 nm Au core, 1-15 nm shell thickness), which enabled controlled loading of fluorophores and bimodal imaging by fluorescence and CT. [2] Antibodies were efficiently conjugated to the silica shell

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using appropriate CLICK chemistry to enable cell surface receptor targeting.[3] For *in vitro* proof-of-concept, CD133(+) SKOV3-IP cells were used as a model system. The targeted binding of $Au@SiO_2$ core-shell NPs to the *in vitro* model was characterized quantitatively and qualitatively over 24 h, using confocal fluorescence microscopy and flow cytometry.[3] A murine xenograft model[4] of ovarian cancer was developed using CD133(+) SKOV3-IP cells. *In vivo,* CT and fluorescence imaging of the tumor site was used to assess targeted binding of $Au@SiO_2$ core-shell NPs to the tumor site for 48 h. Histology and immunohistochemistry (IHC) were used to assess tumor structure and qualitatively assess CD133 expression levels in the tumor.

3 RESULTS

We achieved ~77% efficiency of conjugation of $Au@SiO_2$ NPs to anti-CD133. Bioconjugated, $Au@SiO_2$ NPs were successfully targeted to CD133(+) SKOV3-IP cells, which are known to be over-expressed in chemoresistant ovarian cancer tumors (**Fig.3**).[5] Flow cytometry determined that ~15-16% of SKOV-3-IP cells, overexpressed CD133. Quantitative fluorescence imaging confirmed that the CD133(+) cell lines were targeted with a specificity of an order of magnitude higher than control cells *in vitro*. The intracellular distribution of NPs was characterized spatiotemporally at single NP sensitivity using confocal microscopy. *In vivo,* CT consistently showed clinically significant $\Delta HU > 30$ during the first 12-24h post-injection of nanoprobes **(Fig.4)**. Histopathology showed no metastatic sites. IHC confirmed overexpression of CD133 at select clusters in the tumor site.

4 CONCLUSIONS

Using the modular approach, we successfully created CD133 targeting, CY5 fluorescent, $Au@SiO_2$ core-shell NPs with >12h of persistence in the blood pool of mice. The nanoprobes successfully targeted CD133(+) ovarian cancer cells *in vitro,* and more importantly, the *in vivo* tumor location and margin could be determined noninvasively by CT and was correlated with *in vivo* fluorescence imaging. The modular approach described here can be used to easily vary the targeting molecules, the therapeutic molecules and the imaging modalities used, for the broad detection of multiple diseases. We have in fact demonstrated the quick turnaround time of our modular synthetic approach by delivering custom synthesized fluorescent and luminescent nanoprobes within a week of the order. Our collaborators used these probes in a novel super-resolution fluorescent imaging technique titled "Super-resolution fluorescence microscopy by stepwise optical saturation"[6]

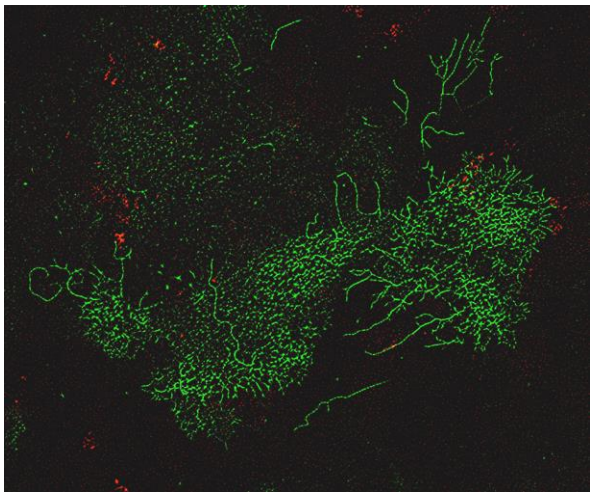

Figure 3. A super-resolution radial fluctuations (ImageJ) analyzed image of GFP-tagged CD133 expressing SKOV3-IP cells in the green channel. CY5 tagged $Au@SiO_2$ NPs conjugated to anti-CD133 are in the red channel. Super-resolution image analysis clearly showed highly specific targeting of the GFP-CD133 receptors on the live cell surface by the bioconjugated nanoprobes. The cells are on average 10 μm in length along their short axis.

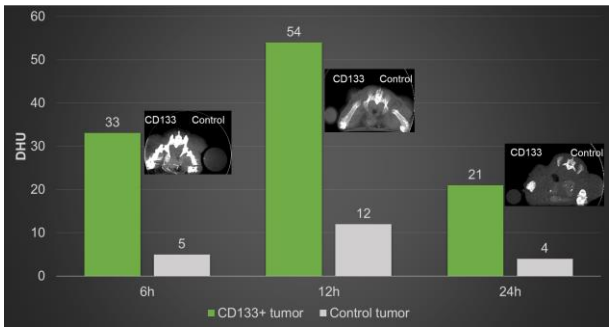

Figure 4. X-ray microCT scans of tumor bearing mice. Cy5 tagged $Au@SiO_2$ NPs conjugated to anti-CD133 were successfully targeted to CD133(+) tumors in fox nu/nu mice and detected using a scanco microCT at 45 kVp and 11.6 micron voxel size. A delta Hounsfeld unit (DHU) $\geq$ 30 is considered clinically significant.

5 REFERENCES

[1] Roeder RK, et al., Proc. SPIE, 2016;10132:101320X.
[2] Nallathamby, PD et al. JMC-B 2016, 4, 5418-5428.
[3] Huang, T et al. Anal. Chem. 2007, 79, 7708-7718.
[4] L. E. Cole et al. ACS Nano 2014, 8, 7486-7496.
[5] Sahlberg, SH et al. PLoS One, 2014, 9, e9462.1
[6] Zhang, Y et al. Biomedical Optics Express, 2018, 9, 1613-1629

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