Prostate Cancer-Specific Drug Delivery and Imaging System: Design, Synthesis and Characterization of Multifunctional AuNPs

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ABSTRACT

Early stage prostate cancer is usually treated with radiation and surgical excision of the cancer. This treatment is often accompanied by adjuvant anti-hormonal therapy that blocks the effects of testosterone in the prostate, thereby reducing the progression of the disease[1]. Anti-hormonal therapy typically loses its effectiveness as the cancer cells become androgen-independent[2]. At that point chemotherapy must be used, however, aggressive cytotoxic chemotherapy produces undesirable side effects. A prostate cancer-specific drug delivery system, such as multifunctional nanoparticles, that can effectively eradicate prostate–cancer cells, including those associated with recurrence and resistance, would prolong the disease free interval for the patient, reduce morbidity, and possibly eradicate the disease.

INTRODUCTION

In this paper, a strategy based on the design and synthesis of a multifunctional gold nanoparticulate (MfAuNPs) drug delivery system that is specific for prostate cancer therapy will be discussed. We have employed a modular assembly approach to prepare a series of individual target components functionalized with an azido-tetraethylene glycol moiety that can be coupled to complementary lipoic acid units functionalized with a propargyl tetraethylene glycol moiety. Huisgen [3+2] "click" cyclo-addition chemistry gives the final target system bearing terminal units such as-a) an inhibitor of the over express prostate specific membrane antigen (PSMA) prostate cancer cells; b) a pH-sensitive doxorubicin for inhibiting the cell proliferation via the hydrolysis of hydrazone bond to release the parent doxorubicin only inside the cytoplasm at lower pH; c) a radiolabeled chelate for binding radionuclides, for use for in imaging or radiotherapy. The overall assembly process is illustrated in Figure 1.

METHODOLOGY

Each individual component that comprises the MfAuNPs, such as the peptidomimetic PSMA, the hydrazone Doxorubicin conjugate, and the dithiolated PEG chains of varying length with modified terminal functionalities - azido, alkyne, and maleimide, can be prepared from readily available starting materials. The surface of AuNPs is prepared with the appropriate array of terminally modified linkers and subsequently can be chemo-orthogonally ligated with the corresponding modified targeting units (Figure 1). A series of MfAuNP formulations will be initially characterized and evaluated *in vitro* with LNCaP and PC-3 prostate cancer cells to determine uptake and localization properties. Our convergent method allows for the facile modifications of the individual components to improve biological activity and pharmacokinetics. Ultimate analysis of the data will provide the basis for selecting nanoparticulate composition for future *in vivo* studies.

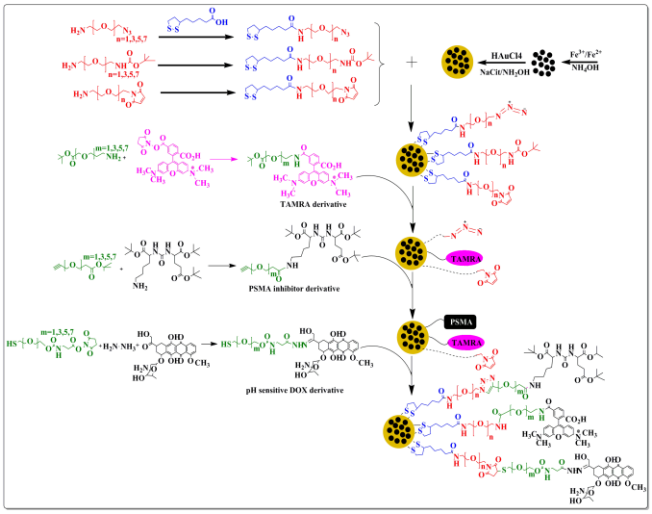

Figure 1: Convergent synthesis of MfAuNPs

INTITIAL RESULTS

Herein we report our initial results for the synthesis of a series lipoamide functionalized targets including peptidometic-PSMA, hydrazinobenzoyl- Doxorubicin (Dox), and bis-quinolinyl module for Re/^{99}Tc chelation, as indicated in Figure 2, which can be bound to AuNPs, in a top-down approach, to yield monofunctional AuNPs. Alternatively, we can utilize a fixed ratio-mixture of these individual target moieties to prepare MfAuNPs that are specifically functionalized with PSMA, Dox and radio-labeling targets, via the convergent method. The initial primer, an azido-tetraethylene glycol (TEG) lipoamide, serves as a linker to activate the AuNP surface. Targeting or imaging moieties, prepared with the complementary propargyloxy oligoethylene glycols or terminal alkyl

carboxylate, are then ligated to the primed AuNPs via "Huisgen [3+2] click" chemistry. Our initial results suggest that the AuNP surface can be manipulated via this strategy, leading to a variety of monofunctional AuNPs, and ultimately to our designed prostate cancer-specific MfAuNPs.

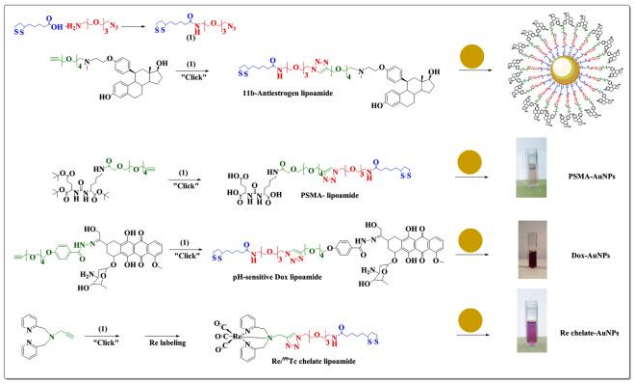

Figure 2: Lipoamide funtional targets

CHARACTERIZATION

Initial characterization, using 1D and 2D NMR, ^{13}C NMR, LC-MS, UV, and TEM, confirmed that our modular approach for synthesizing individual targets was successful. This is indicated by the signature triazole proton at 8.6ppm when the target molecule is coupled with the lipoamide (as shown in the ^{1}H NMR of alkynylnated 11β-subtitituted antiestrogen "clicked" with azidolipoamide). Furthermore, the ^{13}C and 2D NMR of the Re chelation ligand complex indicated three distinguished peaks of C≡O in lower field of the spectra, revealing the chelation $Re(CO)_3$ in the bis-picolyl ligand complex. This modified moiety could be further appended on AuNPs surface, allowing the NPs to have a radioimaging modality.

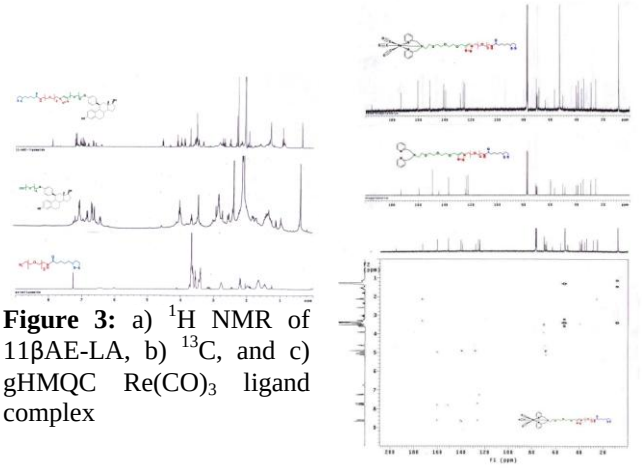

Figure 3: a) ^{1}H NMR of 11βAE-LA, b) ^{13}C, and c) gHMQC $Re(CO)_3$ ligand complex

In addtion, UV spectroscopy showed that the monofunctionalined AuNPs still retained the surface plasmon characteristic of gold nanopheres (λ =520nm). TEM revealed the average diameter of AuNPs ranged from 80 to 120nm, and with no clustering formation when functionlized with theapeutic moieties.

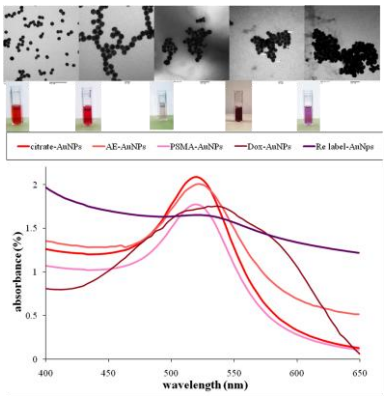

Figure 4: UV and TEM of different monofunctionalized AuNPs.

CONCLUSION

The preliminary results of this study suggest that our convergent approach for assembling MfAuNPs for prostate cancer-specific drug delivery and imaging is feasible. To further support our strategy, several of our monofunctionalized AuNPs are undergoing *in vitro* biological studies to the evaluate therapeutic enhancement of cytotoxic agents when attached to AuNPs. We are also currently developing methods specifically to quantitate the number of each individual target attached on AuNP surface when a fixed ratio-mixture of targets is used to assemble the MfAuNPs.

The successful completion of this project will provide a new class of MfAuNPs that can potentially deliver anticancer drugs at lower doses and with greater selectivity than current therapeutic agents. Such treatment may not just treat prostate cancer, but also prevent its recurrence. This would improve the outcome and quality of life in patients with prostate cancer.

ACKNOWLEDGEMENT

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