# Controlled Release of Chemotherapeutic Agents from Targeted Nanoparticles for Prostate Cancer Therapy

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## Introduction

Over the past decades, there has been increasing interest in developing smart targeted cancer therapy to deliver chemotherapeutic drugs directly to cancer cells while minimizing the killing of healthy noncancerous cells[1,2,3]. The success of such development lies in the design of nanoparticles that are capable of being taken up by the targeted cancer cells and release their drug payload intracellularly over an extended period to achieve a clinical response. In this study, we developed drug encapsulated nanoparticles that can be specifically taken up by the prostate cancer cells and release their encapsulated Taxotere® drugs over an extended period in vitro. The targeted nanoparticles were developed using an amphiphilic block copolymer containing the following three segments 1) a targeting molecule made of a ribonucleic acid (RNA) aptamer that can bind specifically to the human prostate cancer cells, 2) a segment of poly(ethylene glycol) (PEG) to maximize the nanoparticle circulation half life, 3) a segment of poly(D,L-lactide-co-glycolide) (PLGA) for encapsulation and sustained release of chemotherapeutic drugs. By systemically optimizing the nanoparticle composition, we identified the parameters that are important to achieve sustained drug release while maintaining efficient cancer targeting capability.

## Experimental methods:

Targeted nanoparticle preparation: The PLGA-COOH and NH2-PEG3400-COOH polymers were used to synthesize PLGA--PEG copolymer with terminal carboxylic acid groups (PLGA-PEG-COOH). PLGA-COOH was preactivated to its succinimide by using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS) and then reacted with NH2-PEG-COOH4. The same reaction was performed to conjugate 5′-NH2 modified A10 PSMA Aptamers to synthesis PLGA-PEG-aptamer triblock copolymer.

Docetaxel encapsulation and release: Docetaxel and 14C-paclitaxel encapsulated nanoparticles were prepared using the nanoprecipitation method. We measured the rate of docetaxel release using HPLC. Briefly, PLGA-PEG-aptamer and docetaxel were dissolved in acetonitrile and together mixed dropwise into RNAase and DNAase free water. The nanoparticles were stirred for 2 h, and were then centrifuged and washed with deionized water. The size and surface charge of NPs were evaluated by using a ZetaPALS dynamic light-scattering detector. The drug release study was set up as followed: formulations of docetaxel encapsulated nanoparticles were added into a semi-permeable dialysis tube which was then placed in a beaker containing 100 mL of water. At a predetermined time, the water in the container was sampled, and replaced with fresh water to mimic infinite sink condition. The concentration of docetaxel in the release media was quantified by HPLC. To determine the encapsulation efficiency, nanoparticles were dissolved in acetonitrile and measured by HPLC. The docetaxel peak was measured at a wavelength of 227 nm and quantitatively determined by comparing with a standard curve.

Targeted nanoparticle cell binding and uptake studies: The prostate LNCaP and PC3 cell lines were grown in 6-well plates in RPMI 1640 and Ham's F12K medium, supplement with 10% fetal bovine serum at concentrations to allow 70% confluence in 24 h. On the day of experiments, cells were

washed with prewarmed PBS and incubated with prewarmed phenol-red-reduced OptiMEM media for 30 minutes before the addition of nanoparticles. The percentage of nanoparticle uptake was traced by encapsulating 14C-paclitaxel in the nanoparticles. Cells were incubated for 4 hours at 37°C, washed with PBS three times.

**Results and discussion**:

As a proof of concept, we synthesized an aptamer-PEG-PLGA triblock copolymer using PLGA with carboxylic acid terminal group, a heterobifunctional PEG, (amine-PEG-carboxylic acid) and an amine modified A10 prostate specific membrane antigen (PSMA) aptamer. The synthesis reaction was carried out in two steps. 1) synthesis of PLGA-PEG diblock polymer by conjugating of the carboxylic end of PLGA to the amine terminal of a bifunctional PEG; and 2) conjugation of the carboxylic acid end of the bifunctional PEG to the amine modified aptamer. The nanoparticles were prepared by precipitation of triblock copolymer in aqueous solution. The size and surface charge of the nanoparticles were found to be 185 nm and -26, respectively. In order to readily examine the presence of aptamer on nanoparticle surface, we used A10 aptamers with a 5'-FITC-labeled and a 3'-$NH_2$ modification and performed a conjugation reaction to yield fluorescent nanoparticle-aptamer bioconjugates. The carboxylic acid group on the nanoparticle surface was first converted to NHS ester in the presence of 1-(3-Dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (EDC) and covalently coupled to the amine modified aptamer. To assess the specificity of aptamer interaction with the particle surface, we incubated the aptamers and nanoparticles without the conversion of carboxylic acid to NHS ester (i.e. absence of EDC); thus any interaction would be non-specific (i.e. charge or hydrogen bond interaction). The nanoparticle-aptamer bioconjugates were characterized by flow cytometry which confirmed the specificity of our conjugation reaction and lack of non-specific interactions (Figure 1).

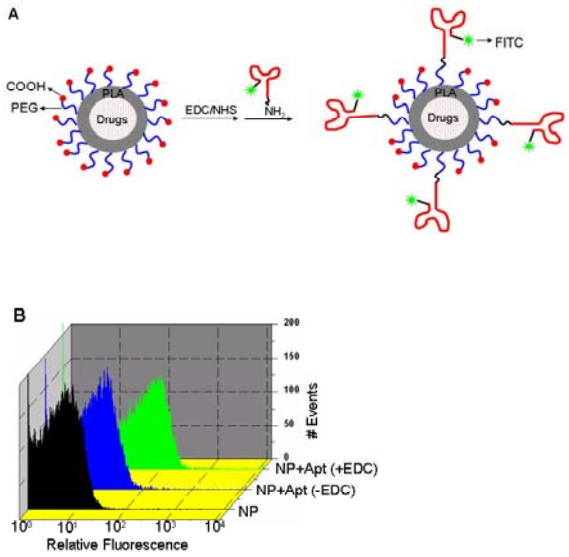


Figure 1. A) Schematic representation of aptamer conjugation strategy. PLA-PEG-COOH nanoparticles have 3 favorable characteristics. 1) Negative surface charge attributed to COOH (red), 2) Presence of PEG (blue) enhances circulating half-life while decreasing passive non-specific uptake of particles, 3) The COOH group can be readily converted to NHS ester in the presence of EDC for conjugation to amine modified aptamers. Using 5'-FITC-labeled and 3'-NH2 modified aptamers, fluorescent nanoparticle-aptamer bioconjugates were generated. B) Flow Cytometry to evaluate specificity of conjugation strategy. The acid group on the surface of PLA-PEG-COOH nanoparticles were left untreated (black and blue) or converted to NHS ester in the presence of EDC (green), and particles were examined without aptamers (black) or incubated with 5'-FITC-labeled and 3'-NH2 modified aptamers (blue and green). The bioconjugates resulting from the covalent linkage of aptamers and nanoparticles (green) demonstrated an approximate 7 fold increase in fluorescence when compared to nanoparticles that were incubated but had no covalent linkage to aptamers (blue).

Through time-course studies, we next demonstrated that the binding of pegylated nanoparticle-aptamer bioconjugates to LNCaP cells was significantly enhanced when compared to control pegylated nanoparticles lacking the A10 aptamer (Figure 2). In the case of PC3 prostate epithelial cells, which do not express the PSMA protein, no measurable difference in binding was observed between the bioconjugate and the control group. The number of nanoparticles attaching to

representative cells after 75 min incubation of bioconjugates or control nanoparticles with LNCaP or PC3 cells was quantified by fluorescent microscopy. The data demonstrates a 77 fold enhancement in the binding of bioconjugates vs. the control group in LNCaP cells (13.25 ± 7.56 vs. 0.17 ± 0.45 nanoparticles / cell for bioconjugates vs. control group, respectively; Mean ± SD, N=150, P>0.001). A notable observation was a remarkably low binding efficiency of nanoparticles in non-targeted PC3 cells (0.03 ± 0.18 vs. 0.30 ± 0.62 nanoparticles/cell for bioconjugates vs. control group; Mean ± SD, N=150), presumably attributed to the presence of PEG group.

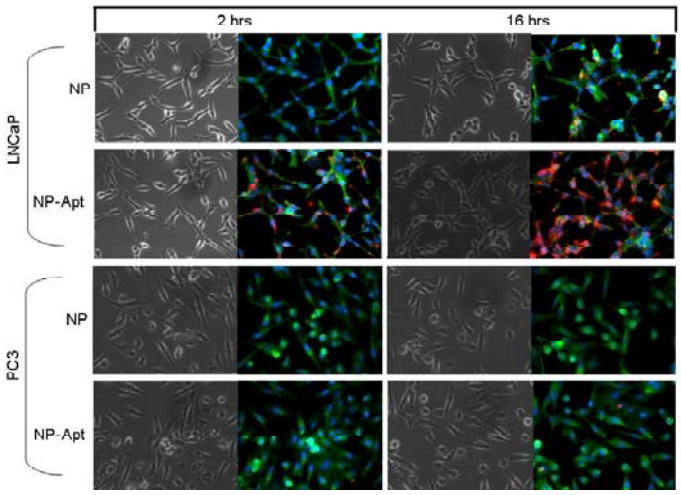


Figure 2. Binding of nanoparticle-aptamer bioconjugates to prostate epithelial cells. LNCaP cells and PC3 cells were grown on chamber slides and incubated in culture medium supplemented with 50 mg rhodamine-labeled dextran encapsulated pegylated nanoparticles (NP), or 50 mg rhodamine-labeled dextran encapsulated pegylated nanoparticle-aptamer bioconjugates (NP-Apt), for 2 hrs (left panel) or 16 hrs (right panel). Cells were washed in PBS x 3, fixed and permeabilized, stained with DAPI (nuclei) and Alexa-Flour Phalloidin (cytoskeleton), washed and analyzed by light transmission or fluorescent microscopy. The rhodamine-dextran encapsulated nanoparticles or nanoparticle-aptamer bioconjugates are shown in red.

To quantify prostate cancer cell specific uptake, nanoparticles were encapsulated with 14C radiolabeled paclitaxel. The percentage of nanoparticles endocytosed by the cells was quantified by the amount of radioactive paclitaxel detected (Figure 3). In the case of PC3 prostate epithelial cells, which do not express the PSMA protein, no measurable difference in binding was observed between the targeted aptamer-PEG-PLGA and untargeted PEG-PLGA nanoparticles. In LNCap cells, which do express PSMA protein, the data demonstrate significant enhancement in the binding of targeted nanoparticles vs. the non-targeted nanoparticles, and the percentage of nanoparticle endocytosed into the cells was directly related to the amount of aptamer conjugated onto the nanoparticle surface.

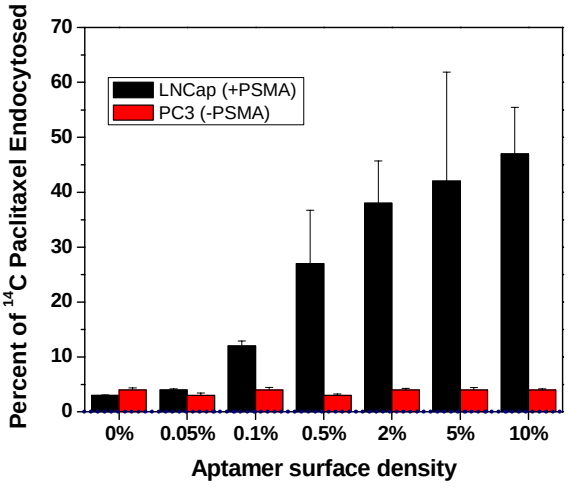


Figure 3. The effect of aptamer concentration on the nanoparticle surface on the rate of nanoparticle endocytosed by the PCa cells in vitro. LNCap cells (cells express PSMA protein, black bars) and PC3 cells (cells don't express PSMA, red bars) were grown in 6 well tissue culture plates containing nanoparticles with different aptamer concentration. Each formulation was obtained by mixing PLGA-PEG-Aptamer triblock with different amount of PLGA-PEG diblock copolymer prior to particle formation. The PSMA cell specific uptake was demonstrated by amount of radioactivity inside the cells after loading the nanoparticles with 14C paclitaxel. The blue dotted line represents the amount of 14C paclitaxel endocytosed in the cells from non-targeted nanoparticles. (n=5).

We examined the effect PLGA-PEG nanoparticle composition on the rate of docetaxel release (Figure 4). Docetaxel was released at a much slower rate from nanoparticles containing high molecular weight PLGA than those with lower molecular weight. It was found that docetaxel can be released from nanoparticles at a sustained rate for over three days by using PLGA with intrinsic viscosity of over 0.4 dL/g.

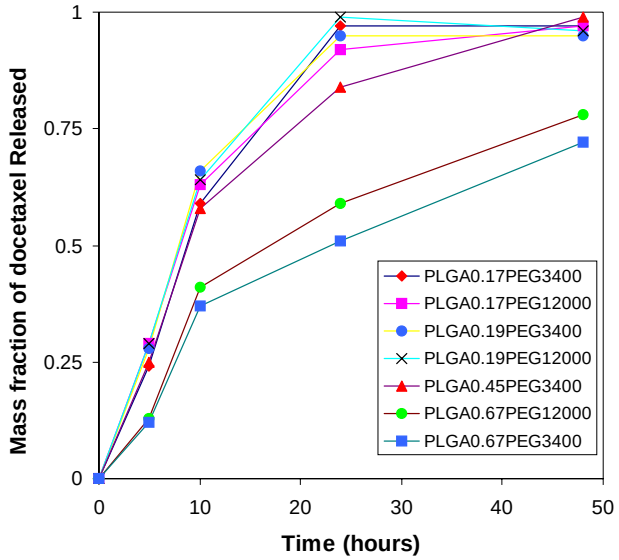


Figure 4. Release kinetics of docetaxel from targeted nanoparticles. The release of docetaxel from nanoparticles was measured in PBS over a period of 3 days and quantified by HPLC.

**Conclusion**:

Both in vitro cell binding and docetaxel release results demonstrated cancer cell specific targeting with sustained docetaxel drug release using the aptamer-PEG-PLGA nanoparticles. The formulation of such polymer can be further developed as a platform technology for cancer therapeutic and diagnostic applications.

**References**:

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