Development of Chemotherapeutic Nanoparticles for Targeted Cancer Therapy

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Introduction

There has been progressively heightened interest in the development of targeted nanoparticles (NPs) for differential delivery and controlled release of drugs. Despite nearly three decades of research, approaches to reproducibly formulate targeted NPs with the optimal biophysicochemical properties that result in a desirable biodistribution and drug release profiles, using processes that are amenable to scale-up and manufacturing have remained elusive. Here we report a potentially scalable strategy for narrowly changing the biophysicochemical properties of NPs in a reproducible manner, thereby enabling systematic screening of optimally formulated drug-encapsulated NPs. NPs were formulated by the self-assembly of an amphiphilic triblock copolymer comprised of end-to-end linkage of poly(D,L-lactide-co-glycolide) (PLGA), polyethylene glycol (PEG) and the A10 aptamer (Apt) which binds to the extracellular domain of the prostate specific membrane antigen (PSMA) on the surface of prostate cancer (PCa) cell. Fine-tuning of NP size and drug release kinetics was further accomplished by controlling the copolymer composition. Using distinct ratios of PLGA-b-PEG-b-Apt triblock copolymer with PLGA-b-PEG diblock copolymer, we developed NPs with varying Apt surface densities and identified the narrow range of Apt density for maximum PCa cell uptake in vitro. The use of this triblock copolymer approach demonstrated that the NP biophysicochemical properties may be systematically fine tuned within a narrow range requiring no additional chemistry after their self assembly. This approach may contribute to further development of targeted NPs as highly selective and effective therapeutic modalities.

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:

To engineer biomaterials that can self assemble into a targeted NP, we developed a bio-integrated block copolymer consisting of the following three polymer blocks: PLGA, PEG and the A10 2'-flouropyrimidine RNA Apt (11). The PLGA polymer block provides a biodegradable matrix for the encapsulation and sustained release of therapeutic agents while the PEG polymer acts as the antibiofouling coating on the NP surface. Both PLGA and PEG have been previously approved by the FDA for a number of clinically used products. The A10 RNA Apt (11) which binds to the extracellular domain of the prostate specific membrane antigen (PSMA) (11-15) directs the delivery and uptake of NPs in a cell-specific manner. We postulated that a nucleic acid composition of Apt would be uniquely suited for the development to our tri-block copolymer to be used for NP self assembly, as it would remain stable in organic solvents during polymer synthesis and NP formulation. The PLGA-b-PEG-b-Apt triblock copolymer was synthesized using a two step reaction (Fig 1A): first, the carboxyl capped PLGA was conjugated to the amine terminal of heterobifunctional PEG (amine-PEG-carboxyl), forming the PLGA-b-PEG diblock copolymer; second, the carboxyl end of PEG-PLGA diblock copolymer was conjugated to the amine functional group of the A10 PSMA Apt, forming the PLGA-b-PEG-b-Apt triblock copolymer. The NMR characterization of PLGA, PLGA-PEG diblock copolymer, and PLGA-b-PEG-b-Apt triblock copolymer is shown in Fig 1B. By precipitating the PLGA-b-PEG-b-Apt triblock copolymers in water, the polymer self-assembles to form PSMA-targeted polymeric NPs without post-particle conjugation steps: the hydrophobic PLGA blocks form a core to minimize their exposure to aqueous surroundings, the hydrophilic PEG blocks form a corona-like shell to stabilize the core, and the hydrophilic Apt blocks thrust into aqueous solution on NP surface as the targeting moieties (Figure 1C).

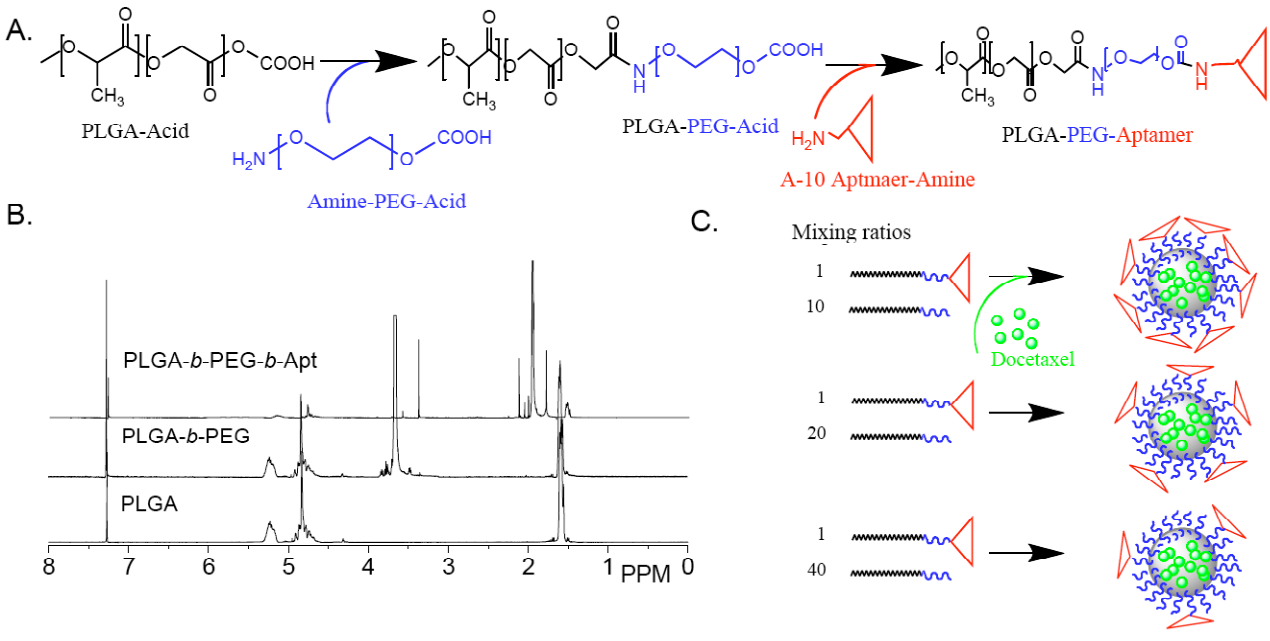

Figure 1. Development of PSMA targeted NPs using PLGA-b-PEG-b-Apt TCP. (A) The PLGA-b-PEG-b-Apt biointegrated TCP was synthesized in two steps: 1) synthesis of PLGA-b-PEG by conjugating carboxyl capped PLGA (PLGA-acid) to the amine terminals of heterobifunctional PEG (amine-PEG-acid); and 2) formation of PLGA-b-PEG-b-Apt by conjugating the carboxyl ends of PLGA-b-PEG-acid to the amine ends of A10 PSMA Apt. (B) 1H NMR characterization of PLGA-b-PEG, and PLGA-b-PEG-b-Apt. (C). By titration in water, the PLGA-b-PEG-aptamer TCPs self-assemble and form PSMA targeted NP-Apt bioconjugates. Using distinct ratios of PLGA-b-PEG-b-Apt TCP with PLGA-b-PEG DCP lacking the A10 Apt during NP formulation, the Apt surface density can be precisely and reproducibly changed.

We next demonstrated that by combinatorially varying the individual components of the triblock copolymer we can systematically change of the following NP biophysiochemical properties: 1) NP size, 2) drug release kinetics, and 3) differential targeting. The optimization of the NP size was performed by varying the composition of the triblock copolymer. A series of targeted PLGA-b-PEG-b-Apt NPs were formulated using combinations of various molecular weights of PLGA and PEG (Fig 2A). Previous work with diblock copolymers of PLGA-PEG showed that NP size was dependent on size of the PEG polymer (16, 17). We sought to determine if varying the length of the PEG segment in the triblock copolymer could control the NP-Apt bioconjugate size. We found that, indeed, the molecular weight of PEG but not PLGA was a key factor in controlling the NP size for the triblock system. By shortening the PEG segment on the triblock copolymer from 10,000 Da to 5,000 kDa, the diameter of NP reduced from 291±5.2 nm to 160±3.7 nm (Fig 2A). Using docetaxel (Dtxl) as a model drug we varied the intrinsic PLGA viscosity to control drug release kinetics. We determined that increasing the length of the PLGA segment of the PLGA-b-PEG-b-Apt triblock copolymer prolonged the rate of drug release in vitro. The length of PEG chain had a minimal effect on the drug release properties of NP-Apt bioconjugates. Using PLGA with an intrinsic viscosity of greater than 0.67 g/dL in hexafluoroisopropanol (approximately 50,000 g/mol in molecular weight), Dtxl was released at a sustained rate for 3 days (Fig 2B).

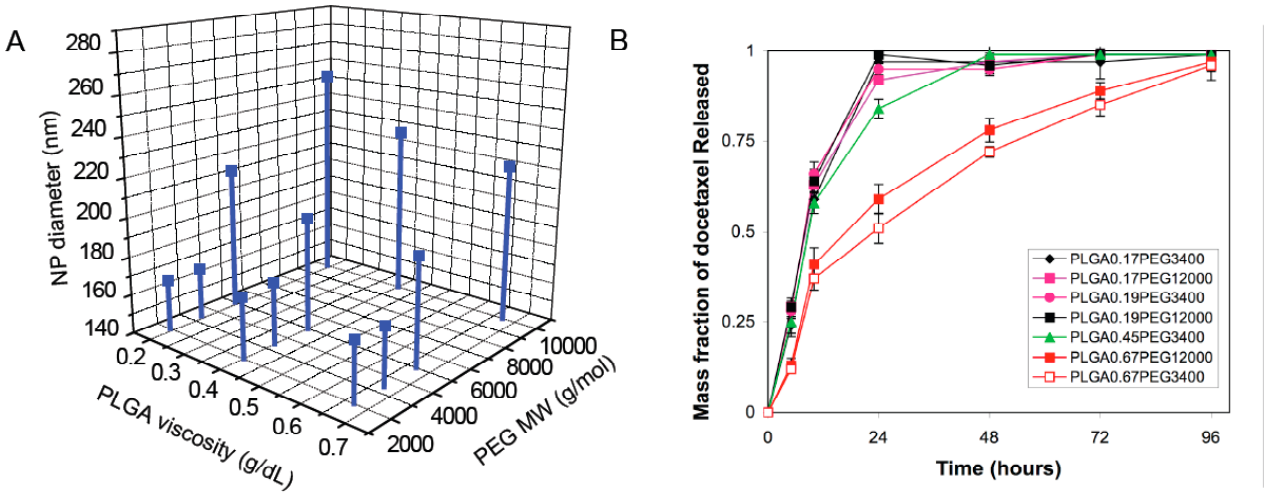

Figure 2. Fine-tuning the nanoparticle physiochemical properties using PLGA-b-PEG-b-Apt TCPs. (A) Effect of TCP composition on NP size. Synthesis of TCP containing different molecular weight segments of PLGA and PEG. Targeted NPs were formulated by TCP self-assembly. The size of each NP formulation was measured by dynamic light scattering. (B) NP drug release properties as a function of PLGA-b-PEG-b-Apt TCP composition. Dtxl was encapsulated into various NPs at a mass loading of 1.5 w/w%.

Our data suggested that PLGA0.67-b-PEG3400-b-Apt TCP had the most desirable combination of small particle size, sustained drug release kinetics, and differential accumulation in LNCaP cells. Next, LNCaP and PC3 cells were used to examine if the self assembled NP-Apt bioconjugates were differentially endocytosed by LNCaP cells. NP-Apt bioconjugates were visualized by encapsulating a green fluorescent dye, NBD-cholesterol. Using z-axis scanning fluorescence microscopy and colocalization staining, both early and late endosomal markers were colocalized with NPs in LNCaP cells confirming a relatively rapid endocytosis of NP-Apt bioconjugates by LNCaP cells (Fig 3). In comparison, the NP-Apt bioconjugates seeded onto PC3 cells showed minimal cellular uptake consistent with the lack of PSMA expression in these cells. A detailed intracellular trafficking of NP-Apt bioconjugates resulting from the PLGA-b-PEG-b-Apt TCP is a subject of future investigation.h 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.

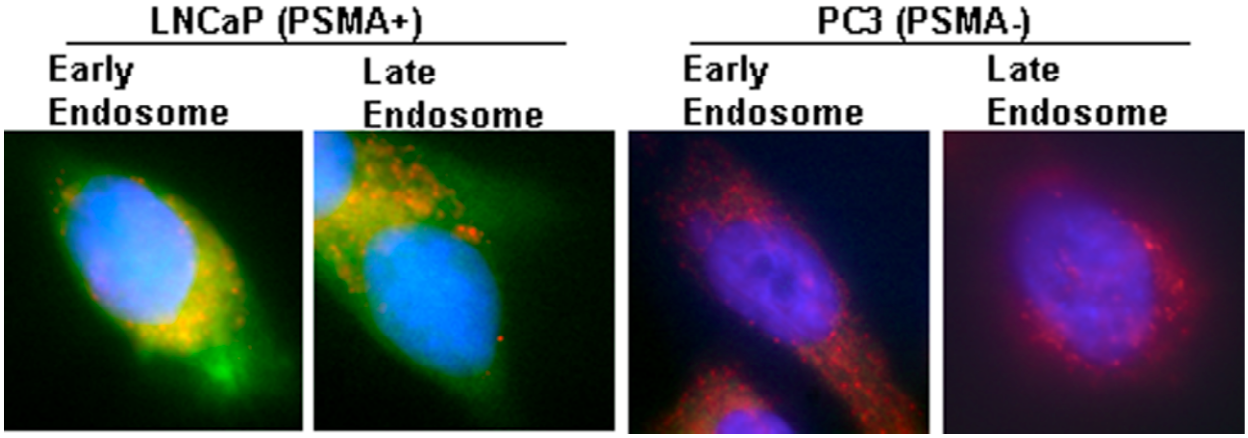

Figure 3. NP-Apt cellular uptake. The NBD dyes (green) were formulated into PLGA-PEG-aptamer triblock nanoparticles by nanoprecipitation. LNCaP (PSMA+) and PC3 (PSMA-) cells were incubated with 50 μg of NBD-encapsulated PLGA-PEG-aptamer nanoparticles for 30 minutes. The early and late endosomal markers were visualized in red. The cell nuclei were stained by DAPI (blue).

Conclusion:

We demonstrated that a biointegrated TCP consisting of PLGA, PEG and the A10 Apt could be used for the self-assembly of targeted NPs for PCa targeting. NPs with distinct biophysicochemical properties can be generated by mixing PLGA-b-PEG-b-Apt TCPs with a desired amount of PLGA-b-PEG DCPs lacking the A10 Apt and having the desired PLGA and PEG characteristics. The use of a PLGA-b-PEG-b-Apt triblock copolymer approach demonstrated the ability to systematically fine-tune Apt ligand density to maintain tumor targeting while minimizing the NP immunogenicity. The use of this triblock copolymer approach can be used as a technology platform for developing large-scale synthesis processes of pre-functionalized targeted NPs while minimizing production times and NP batch-to-batch variations. This technology may have significance in designing delivery vehicles that target cancer cells and may contribute to the development of the next generation of nano-scale diagnostic and therapeutic modalities.

References:

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