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Dual-responsive polymer-coated iron oxide nanoparticles for drug delivery and imaging applications

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ABSTRACT

We reported the synthesis and characterization of dual-responsive poly(*N*-isopropylacrylamide-acrylamide-chitosan) (PAC)-coated magnetic nanoparticles (MNPs) for controlled and targeted drug delivery and imaging applications. The PAC-MNPs size was about 150 nm with 70% iron mass content and excellent superparamagnetic properties. PAC-MNPs loaded with anti-cancer drug doxorubicin showed dual-responsive drug release characteristics with the maximum release of drugs at 40 °C (~78%) than at 37 °C (~33%) and at pH of 6 (~55%) than at pH of 7.4 (~28%) after 21 days. Further, the conjugation of prostate cancer-specific R11 peptides increased the uptake of PAC-MNPs by prostate cancer PC3 cells. The dose-dependent cellular uptake of the nanoparticles was also significantly increased with the presence of 1.3 T magnetic field. The nanoparticles demonstrated cytocompatibility up to concentrations of 500 μg/ml when incubated over a period of 24 h with human dermal fibroblasts and normal prostate epithelial cells. Finally, pharmacokinetic studies indicated that doxorubicin-loaded PAC-MNPs caused significant prostate cancer cell death at 40 °C than at 37 °C, thereby confirming the temperature-dependent drug release kinetics and *in vitro* therapeutic efficacy. Future evaluation of *in vivo* therapeutic efficacy of targeted image-guided cancer therapy using R11-PAC-MNPs will reinforce a significant impact of the multifunctional PAC-MNPs on the future drug delivery systems.

1. Introduction

Temperature-sensitive polymers such as poly(*N*-isopropylacrylamide) (PNIPAAm) have been widely used in drug delivery systems to release drugs in response to the changes in surrounding temperature (Schmaljohann, 2006). Copolymerization of PNIPAAm with hydrophilic acrylamide (AAm) has shown success in increasing the lower critical solution temperature (LCST) of PNIPAAm from 32 °C to the temperatures above physiological temperature, which is more suitable for *in vivo* controlled drug release (Rahimi et al., 2010). However, the non-biodegradable nature of PNIPAAm limits its widespread *in vivo* use due to the possibility of accumulation, toxicity and inflammatory responses (Cui et al., 2011).

To overcome the non-biodegradability limitation, PNIPAAm has been copolymerized with a variety of natural polymers employing their properties of biodegradability (Dash et al., 2011). For example, PNIPAAm hydrogels have been grafted with natural polymers such as chitosan, collagen, and gelatin to form a degradable gel (Curcio et al., 2010; Fitzpatrick et al., 2010). Biocompatible chitosan is a cationic polysaccharide that enzymatically degrades *in vivo* at physiological conditions and has been extensively studied for biomedical applications (Dash et al., 2011; Yomota et al., 1990). Copolymerization of PNIPAAm with chitosan thus makes it degradable to have PNIPAAm in smaller fragments, such as dimers and trimers. Such small length PNIPAAm can be easily removed out of the body by renal clearance (Patenaude and Hoare, 2012). Chitosan also displays pH-sensitivity due to protonation–deprotonation of a large number of amino groups present (Chuang et al., 2010). Further, incorporation of superparamagnetic iron oxide magnetic nanoparticles (MNPs) adds further functionalities to the design, such as non-invasive image-guided magnetic targeting, contrast agents for magnetic resonance imaging (MRI), hyperthermia of solid tumors, providing heat and

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stimulating drug release from thermo-sensitive polymer (Rosen et al., 2012; Sun et al., 2008). Besides magnetic targeting, active targeting of nanoparticles to the tumor site has also been effective in cancer treatment due to increased specificity and bioavailability of particles at the target site (Danhier et al., 2010). A variety of targeting ligands such as antibodies, aptamers, folic acid, and RGD peptides have shown promise in prostate cancer targeting (Sethuraman and Bae, 2007). Recently, the cell-penetrating R11 peptides have been proven to have the ability to bind to prostate cells at specific sites such as the laminin receptor site and also have the potential to be useful in prostate cancer detection (Zhou et al., 2012).

Encompassing the advantages of PNIPAAm, AAm, chitosan, MNPs, and R11 peptides, our aim was to synthesize and characterize R11-conjugated, drug-loaded PNIPAAm-AAm-chitosan (PAC)-coated MNPs (PAC-MNPs) as a potential drug delivery and imaging system for prostate cancer management. PNIPAAm-chitosan nanogels have previously been synthesized without AAm, MNPs, and targeting ligands for drug delivery and tissue engineering applications (Chuang et al., 2009; Li et al., 2009). In our design, the differences with these studies are that the R11 peptides and magnetic guidance can ensure that the particles reach the site of action. Furthermore, an external magnetic field can be used to heat up the MNPs, leading to hyperthermia and temperature-responsive drug release from polymer shell. In addition, the pH-responsiveness provides an added advantage of delivering the drugs only to the tumor, owing to its extracellular acidic pH (Min et al., 2010b). Employing our PAC-MNPs in cancer therapy would facilitate a biocompatible, degradable, tumor-targeted, dual-responsive drug release and magnetic hyperthermia/imaging system. The dual-targeting (magnetic targeting and receptor-mediated targeting) capability of particles combined with dual-responsive (pH and temperature) drug release would provide a highly specific and efficient system for cancer treatment.

2. Experimental

2.1. Materials

All the materials were purchased from Sigma–Aldrich (St. Louis, MO), if not mentioned. Ferric oxide magnetic nanoparticles (MNPs, Meliorum Technologies, Rochester, NY), doxorubicin (Dox, Tocris Bioscience, Ellisville, MO), and R11 peptides (Anaspec, Fremont, CA) were purchased and used without further purification. Roswell Park Memorial Institute medium (RPMI), neonatal calf serum (NCS), penicillin–streptomycin, and Trypsin were purchased from Invitrogen, Carlsbad, CA. Clonetics® Prostate Endothelial Basal Medium (PrEBM™) and SingleQuots® Kit Supplements & Growth Factors (PrEGM™) were purchased from Lonza, Walkersville, MD. Whereas, human dermal fibroblasts (HDFs), normal prostate epithelial cells (PZ-HPV-7), and prostate cancer cells (PC3) were purchased from ATCC, Manassas, VA.

2.2. Synthesis of PAC nanoparticles, PAC-MNPs, and R11-conjugated PAC-MNPs

PAC nanoparticles were prepared by free radical graft copolymerization. Briefly, 0.25 g chitosan was added to 5% acetic acid solution. After complete dissolution, 0.886 g *N*-isopropylacrylamide (NIPAAm), 0.1137 g AAm and 0.01 g *N,N*-methylenebisacrylamide (BIS) were added and the reaction was heated to 80 °C. Polymerization was initiated by adding 1.44 ml tert-butyl hydroperoxide (TBHP); the reaction was purged with N_2 and stirred for 3 h. As-prepared PAC nanoparticles were purified by dialyzing against deionized (DI) water overnight using 100 kDa cut-off dialysis membranes (Spectrum Laboratories Inc., Rancho Dominguez, CA).

For PAC-MNPs, silane-functionalized MNPs were prepared as reported previously (Wadajkar et al., 2012b). Briefly, 0.74 g MNPs were dispersed in 100 ml of 99% ethanol solution. Then, 3 ml acetic acid was added and sonication was continued for another 10 min. The reaction was transferred to a magnetic stirring plate; 0.49 ml vinyltrimethoxysilane (VTMS) was added and stirred for 24 h. The silane-functionalized MNPs were collected using a magnet and washed several times with 99% ethanol solution. These particles were then used as a template to form core-shell PAC-MNPs following the same protocol as PAC nanoparticles, as described earlier. Briefly, 0.028 g silane-functionalized MNPs were suspended in 5% acetic acid solution by sonication, after which chitosan, NIPAAm, AAm, BIS, and TBHP were added. The reaction was carried in the same way as mentioned above, and PAC-MNPs were washed and collected using a magnet.

Further, R11 peptides specific to prostate cells were conjugated to PAC-MNPs using carbodiimide chemistry. Briefly, 1.75 μl R11 peptide solution (10 μM) was added to 5 mg *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimidehydrochloride (EDC) in 0.5 ml 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer. After 30 min of shaking, 5 mg *N*-Hydroxysuccinimide (NHS) was added and left for another 30 min for shaking. Separately, 1 mg PAC-MNPs were suspended in 0.5 ml MES buffer by sonication; added to above solution and incubated for 12 h at room temperature on a shaker. The R11-conjugated PAC-MNPs (R11-PAC-MNPs) were washed several times with DI water and collected using a magnet.

2.3. Characterization

Scanning electron microscope (SEM, S-3000N, Hitachi, Pleasanton, CA) and transmission electron microscope (TEM, FEI Technai G2 Spirit BioTWIN, Hillsboro, OR) were used to analyze the particle size, shape and morphology. Dynamic light scattering (DLS, ZetaPals, Brookhaven Instruments, Holtsville, NY) was used to measure the particle size and polydispersity index. Fourier transform infrared spectroscopy (FTIR, Nicolet 6700, Thermo Scientific, Madison, WI) was performed to confirm the presence of the characteristic peaks of the bonds in the PAC. The LCST of the particles was evaluated by spectrophotometry (Smartspec™ plus, Bio-Rad Laboratories, Philadelphia, PA). Briefly, absorbance of the particle solution at 500 nm was measured from 25 °C to 50 °C with an increment of 1 °C. The graph of temperature *vs*. transmittance was plotted and the temperature at 50% transmittance was noted as the LCST of the polymer (Li et al., 2011).

Iron content of PAC-MNPs was quantified by iron assays (Gupta and Gupta, 2005). Briefly, 100 μl particle solution (1 mg/ml) was incubated with 100 μl of 50% hydrochloric acid solution at 50 °C for 2 h, followed by addition of 100 μl ammonium persulfate solution (1 mg/ml). After shaking for 15 min, 100 μl potassium thiocyanate solution (0.1 M) was added and incubated for another 15 min. The plate then read at 478 nm using UV–vis spectrophotometer (Infinite M200, Tecan, Durham, NC). In addition, the magnetic properties of PAC-MNPs were analyzed using vibrating sample magnetometer (VSM, KLA-Tencor EV7, San Jose, CA) and compared to those of bare MNPs. The samples consisting of equal amounts of iron oxide were embedded in wax and hysteresis loops were obtained by varying the magnetic fields at room temperature.

2.4. Drug loading and release

Dox was chosen as an anticancer drug model for encapsulating in PAC-MNPs. For drug loading, 12 mg PAC-MNPs and 2.5 mg Dox were suspended in 6 ml DI water and incubated at 4 °C for 3 days on a shaker to allow drug absorption in polymer shell. Dox-loaded PAC-MNPs were then collected by using a magnet and the supernatant containing un-loaded Dox was analyzed to quantify