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Hydrogel-nanoparticle composites for optically modulated cancer therapeutic delivery

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ABSTRACT

A poly(N-isopropylacrylamide-co-acrylamide) (NIPAAm-co-AAm) hydrogel with near-infrared (NIR) absorbing silica–gold nanoshells was designed as a platform for pulsatile delivery of cancer therapeutics. This hydrogel was designed to have a lower critical solution temperature (LCST) above physiologic temperature, such that the material will transition from a hydrated state to a collapsed state above ~40 °C. Additionally, the silica–gold nanoshells used were designed to have a peak extinction coefficient in the NIR, where penetration of light through tissue is maximal. This heat-triggered material phase transition of the composite was found to follow exposure of NIR light, indicating the ability of the NIR absorption by the nanoshells to sufficiently drive this transition. The composite material was loaded with either doxorubicin or a DNA duplex (a model nucleic acid therapeutic), two cancer therapeutics with differing physical and chemical properties. Release of both therapeutics was dramatically enhanced by NIR light exposure, causing 2–5× increase in drug release. Drug delivery profiles were influenced by both the molecular size of the drug as well as its chemical properties. The DNA therapeutic showed slower rates of nonspecific delivery by passive diffusion due to its larger size. Additionally, only 70% of the more hydrophobic doxorubicin was released from the material, whereas the more hydrophilic DNA showed over 90% release. Further, hydrogel composites were used to deliver the doxorubicin to CT.26-WT colon carcinoma cells, eliciting a therapeutic response. This work validates the potential application for this material in site-specific cancer therapeutic delivery.

1. Introduction

Pulsatile drug release, defined as a rapid release of drug molecules following an off-release period [1], is beneficial for the treatment of a wide range of diseases. For example, in the treatment of hormone disorders and diabetes, a constant plasma drug level is not desirable. Rather, a rapid increase in drug concentration should follow in response to a biologic stimulus [1,2]. Diseases which benefit from chronotherapy, in which a drug administration timing is optimized [3], would also benefit from a pulsatile delivery system. Cancer is an area where advantages of chronotherapy have been widely studied [4].

While great improvements have been made over the past 50 years in the use of chemotherapy to treat cancer, these treatments still often fail to completely cure malignant disease [5]. This is primarily due to cancer cells becoming genetically resistant to anti-cancer drugs, as well as the adverse side effects of these treatments dictating patients' regimens [4]. Chronotherapy can be used to overcome some of these problems by optimizing the timing of drug administration with the circadian rhythms of cancer cell susceptibility and those of adverse side effects [4]. For example, in the treatment of metastatic colorectal cancer, it has been shown that administration of chemotherapeutics by chronotherapy is more efficacious than when the same treatment is administered as a constant infusion [6]. Additionally, the lessening of side effects due to chronotherapy also allows for administration of fuller, more effective doses of chemotherapeutics [4]. While these approaches show promise, implementation of such approaches is often logistically difficult, leading to the need for new methods to improve control of such delivery [3].

One approach for the creation of a pulsatile release system employs thermally responsive polymer–nanoparticle composite materials [7]. These composites couple a polymer material with physical properties that are dependent on temperature with nanoparticles that generate heat in response to external stimuli. Commonly, these materials will transition from a hydrated state at lower temperatures and undergo collapse by releasing water when the temperature is raised above a lower critical solution temperature (LCST). These types of materials have been investigated for drug delivery applications, where therapeutic molecules are absorbed into the material and subsequently released as water is expelled from the hydrogel during the phase transition [8].

This study investigates a poly[N-isopropylacrylamide-co-acrylamide] (NIPAAm-co-AAm)-gold nanoshell composite material for use in optically triggered cancer therapeutic delivery. Poly(NIPAAm) is a

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commonly investigated thermally responsive polymer for biomedical applications, as it has an LCST of 32 °C, which is near the physiologically relevant body temperature for humans [8]. In addition, by polymerizing with a more hydrophilic copolymer, such as acrylamide, the LCST of the material can be raised above 40 °C [9]. A 95:5 molar ratio of NIPAAm:AAm has been shown to result in an LCST above 37 °C, such that the material will be in a swollen state under physiologic conditions, but will collapse slightly above body temperature. This allows drug molecules to be absorbed into and constrained within the material until the material deswells due to a temperature increase above the LCST. This copolymer has been previously used in drug delivery investigations [10,11].

NIPAAm:AAm copolymers can undergo their LCST transition in response to localized heating by encapsulated nanoparticles [10,11]. Gold–silica nanoshells are a class of nanoparticles that consist of a dielectric silica core surrounded by a thin gold shell [12,13]. These particles have a tunable plasmon resonance dependent on the core size and shell thickness [12,13]. As the particles are exposed to wavelengths of light matching their plasmon resonance, an oscillation of conduction band electrons results in dissipation of this light energy as heat [14,15]. Particles that have a plasmon resonance in the near infrared (NIR) range (700–900 nm) are of particular interest for biological applications. This range is above where most biological chromophores absorb light but below wavelengths where water starts to strongly absorb; thus NIR light is easily transmitted through biological tissue resulting in relatively little attenuation or tissue damage [16]. Gold–silica nanoshells with a 120 nm silica core and 10–15 nm gold shell maximally absorb light in the NIR range at approximately 800 nm, and have been previously investigated for use in cancer diagnostics and photothermal therapy [17–21].

This work describes the synthesis and characterization of this composite hydrogel, and then evaluates the ability to load the hydrogels with cancer therapeutics and trigger drug release upon NIR light exposure. We assessed release of two therapeutics: a chemotherapeutic (doxorubicin) and a biologic therapeutic (a DNA duplex targeting EphA2). These are both important cancer therapeutics and also represent opposite ends of the delivery spectrum in terms of molecular size and hydrophobicity. Doxorubicin is a small molecule (580 Da) chemotherapeutic indicated in a wide variety of cancers including hematopoietic malignancies; carcinomas of the breast, lung, ovary, stomach, and thyroid; and sarcomas of bone and soft tissue [22]. The primary mechanism of action is intercalation with DNA during replication, causing inhibition of topoisomerase II binding and arrest of cell replication [23]. Side effects of doxorubicin include myelosuppression, mucositis, and cardiac toxicity; furthermore, these side effects often cause patients to cease doxorubicin therapy, even if the drug is still effective against their malignancy [24].

In addition, delivery of a larger, more hydrophilic molecule was assessed. A short DNA duplex was investigated as a model nucleic acid therapeutic. This dsDNA molecule was designed to be similar in chemical structure to an siRNA therapeutic. Typical siRNAs are double-stranded with sticky ends and molecular weights of 12–15 kDa. This study employed a 21 base pair (12,850.5 kDa) oligonucleotide equivalent in sequence to an siRNA targeting the EphA2 protein (target sequence 5′-AATGACATGCCGATCTACATG-3′) [25]. EphA2 is a receptor tyrosine kinase known to be upregulated in many cancers; its functions include signaling involved in cell–cell contacts, cell migration, and angiogenesis [26]. Down regulation of EphA2 has been shown to reduce tumorigenicity in preclinical studies of several cancer types, including pancreatic and breast carcinomas [27].

2. Materials and methods

All reagents were purchased from Sigma-Aldrich and used as received, unless otherwise noted. All water used in synthesis, purification, and testing was treated by a Milli-Q system (18.2 MΩ cm resistivity, Millipore).

2.1. Gold–silica nanoshell fabrication

Gold–silica nanoshells, consisting of a silica core surrounded by a thin gold shell, were fabricated based on previous methods. Silica cores 120 nm in diameter (Precision Colloids) were surface-functionalized with amine groups *via* a reaction with aminopropyltriethoxysilane (Gelest Inc.). Colloidal gold was prepared by a reduction of chloroauric acid (Alfa Aesar) as previously described in the literature [28]. The aminated silica cores were then mixed with this gold colloid suspension to adsorb the colloidal gold onto the silica core *via* electrostatic interactions with the amine groups. These adsorbed colloids then acted as nucleation sites for growth of a continuous gold shell. In this final shell growth step, additional gold was reduced onto the adsorbed gold colloids in a reduction of $HAuCl_4$ by formaldehyde, causing the gold to coalesce to form a continuous shell of ~15 nm around the silica core. The extinction characteristics of the particles were analyzed by UV–vis spectroscopy (400–1100 nm, Cary 50 Varian). Transmission electron microscopy (TEM) (FEI Tecnai G^2 Twin) and dynamic light scattering (DLS) (Malvern Zetasizer Nano ZS) were employed to further characterize and size the resulting particles. Only particles with a polydispersity index (PDI) of <10% were used in subsequent steps.

2.2. Poly(NIPAAm-co-AAm) hydrogel synthesis

Poly(NIPAAm-co-AAm) hydrogels were synthesized by free radical polymerization. Prior to use, NIPAAm (97%, Sigma-Aldrich) was dissolving in tetrahydrofuran (THF) and recrystallized three times in *n*-hexane to remove *p*-methoxylphenol, a polymerization inhibitor present for packaging.

A 1.75 M monomer solution composed of a 95:5 molar ratio of NIPAAm:AAm and a 1:750 molar ratio of monomer:crosslinker (MBAAm) was prepared in H_2O and added to a three-neck round bottom flask (3.75 ml total volume). Argon (Ar) gas was bubbled through this solution for at least 15 min. With rapid stirring, 37.5 μl of 10% (w/v) ammonium persulfate and 7.5 μl of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine were added to initiate polymerization. Composite hydrogels were synthesized in a similar fashion, with 8×10^9 nanoshells/ml added to the monomer solution prior to adding APS/TEMED. This concentration of nanoshells was chosen since previous studies indicate that this concentration should be sufficient to cause enough heating to drive our polymer phase transition [10,11]. The polymerization solution was then quickly poured into a mold consisting of 2 glass slides separated by a 1.5 mm Teflon® spacer held together by metal clamps. The hydrogel was cured at 30 °C for 2 h under vacuum. After curing, the hydrogel slab was soaked in 95% EtOH for at least 12 h followed by MilliQ H_2O for at least 12 h to remove any unreacted monomers. Hydrogel disks of a 4 mm diameter were punched out of the hydrogel slab with a cork borer.

2.3. Thermal and photothermal behavior of hydrogel–nanoshell composites

After synthesis, the swelling behavior of the poly(NIPAAm-co-AAm) hydrogels was analyzed in response to temperature. The hydrogels were allowed to swell at room temperature (22 °C) for at least 24 h before testing. To determine the LCST of the hydrogels, the hydrogels were first weighed and placed in TRIS buffer (pH 7.4), and then incubated at various temperatures (29 °C, 33 °C, 37 °C, 41 °C, 45 °C, and 50 °C) for 10 min. Deswelling ratio (DSR) was calculated as follows: $DSR = 100 * Weight_{Temp}/Weight_{Temp\ =\ 22\ °C}$.

Next, the thermal behaviors of hydrogels with and without nanoshells were compared. The hydrogels were placed in 2 ml TRIS buffer (pH 7.4) and incubated for 30 min in a 50 °C water bath or exposed to an NIR diode laser (Coherent; Santa Clara, CA) at 808 nm, 8 W/cm^2 for

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