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Spatiotemporally photoradiation-controlled intratumoral depot for combination of brachytherapy and photodynamic therapy for solid tumor

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ABSTRACT

In an attempt to spatiotemporally control both tumor retention and the coverage of anticancer agents, we developed a photoradiation-controlled intratumoral depot (PRCITD) driven by convection enhanced delivery (CED). This intratumoral depot consists of recombinant elastin-like polypeptide (ELP) containing periodic cysteine residues and is conjugated with a photosensitizer, chlorin-e6 (Ce6) at the N-terminus of the ELP. We hypothesized that this cysteine-containing ELP (cELP) can be readily crosslinked through disulfide bonds upon exposure to oxidative agents, specifically the singlet oxygen produced during photodynamic stimulation. Upon intratumoral injection, CED drives the distribution of the soluble polypeptide freely throughout the tumor interstitium. Formation and retention of the depot was monitored using fluorescence molecular tomography imaging. When imaging shows that the polypeptide has distributed throughout the entire tumor, 660-nm light is applied externally at the tumor site. This photo-radiation wavelength excites Ce6 and generates reactive oxygen species (ROS) in the presence of oxygen. The ROS induce *in situ* disulfide crosslinking of the cysteine thiols, stabilizing the ELP biopolymer into a stable therapeutic depot. Our results demonstrate that this ELP design effectively forms a hydrogel both *in vitro* and *in vivo*. These depots exhibit high stability in subcutaneous tumor xenografts in nude mice and significantly improved intratumoral retention compared to controls without crosslinking, as seen by fluorescent imaging and iodine-125 radiotracer studies. The photodynamic therapy provided by the PRCITD was found to cause significant tumor inhibition in a Ce6 dose dependent manner. Additionally, the combination of PDT and intratumoral radionuclide therapy co-delivered by PRCITD provided a greater antitumor effect than either monotherapy alone. These results suggest that the PRCITD could provide a stable platform for delivering synergistic, anti-cancer drug depots.

1. Introduction

The intratumoral (i.t.) administration of titanium encapsulated radionuclides, brachytherapy (BT), offers several desirable features: the predictable dosimetry of the titanium 'seeds', the capability of clinical monitoring, mild side effects, and short duration. Compared to external beam radiotherapy (EBRT), BT possesses key advantages: 1) BT irradiates tumor cells in an inside-out manner and avoids pass-through injury, unlike EBRT; 2) BT enables the use of higher doses (up to145 Gy) due to reduced side effects, while EBRT is limited to 70Gy [1]; 3) BT is more efficient than EBRT due to the "cross-fire" effect [2], and 4) conjugation with carrier does not alter the therapeutic activity of radionuclides as opposed to chemotherapeutic controlled release methods. Despite these advantages, certain constraints currently limit its applications in the clinic. These include the complicated placement procedures required for seed insertion, post-treatment excision of the seed implants, and occasional brachytherapy seed migration [3]. To improve upon these issues, polymeric nanoparticles carrying

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therapeutic radionuclides have recently been developed and demonstrated some success for use in brachytherapy [4–7]. Before these materials can be translated to clinical application, though, several delivery concerns must overcome. First, the macromolecular carrier carrying the radionuclide has to overcome the high interstitial fluid pressure of the tumor to penetrate into the interstitium [8]. Second, the delivered agents must be retained selectively at the target site for a sufficient time to allow the agents to kill the tumor cells. While challenging for chemotherapeutics, prolonged tumor retention of radionuclide over its decay half-life is even more imperative.

Convection-enhanced delivery (CED) is of great interest with regards to the issue of proper payload delivery. It enables direct localization of high concentrations of therapeutics within the tumor to maximize interstitial tumor distribution [9,10]. The pressure gradient of the injected agent drives the therapeutics through the interstitial spaces of the tumor by convective flow. This results in a higher and more uniform concentration of therapeutic agents over a larger area. However, both free drug and their carriers can still be rapidly cleared from the tumor. In a related CED study, free drug concentration dropped by 25% at the injection site two hours after delivery and was decreased 10-fold at points 3 mm away [11]. In a second study, only 40% of initial dose of liposomal ^{186}Re was retained in the tumor after 4 h [12]. To address the issue of intratumoral retention, stimulus-responsive polymers have gained popularity as improved drug delivery vehicles. For example, Pluronic (an F-127RT-Gel) exhibited 49% retention of In-111 after 24 h in a mouse tumor [13]. Pronounced dose-dependent tumor growth reduction was also achieved by single dose of ^{131}I-labeled polymer poly(N-isopropyl acrylamide) in a murine xenograft model, a thermally responsive polymer that achieved a 48 day retention of 60% [14]. These materials utilized strategies that balanced intratumoral payload retention with the solubility necessary for injection. To build on these findings, we turned to another class of stimulus-responsive polymers: elastin-like polypeptides (ELPs).

ELPs are a class of recombinant peptide polymers that mimic the structural sequence of the naturally occurring protein tropoelastin and provide an extremely attractive material for localized drug delivery. ELPs are composed entirely of repeats of the pentapeptide sequence Val-Pro-Gly-Xaa-Gly (where Xaa can be any amino acid except Pro). Because their composition consists entirely of natural amino acids, ELPs are biocompatible, biodegradable, and non-toxic [15–17]. To date, two approaches have been taken to optimize ELP-based depots for intratumoral delivery. In the first approach, an ELP with a transition temperature ($_t$) of 21 °C was designed in order to undergo its inverse phase transition at physiological temperature. This ELP transitioned from soluble state to a viscous, insoluble state upon intratumoral injection and exhibited between 75 and 90% *in vivo* retention over a week [18,19]. The conjugation of a radionuclide such as ^{131}I provided a means to perform brachytherapy by injection of an ELP-radionuclide conjugate that undergoes its phase transition to form an insoluble coacervate within the tumor, which irradiates the tumor from the inside out. While this approach is attractive due to its simplicity, these ELP depots are not chemically crosslinked, which can ultimately limit their *in vivo* retention. In the second approach, an ELP was engineered to contain periodic cysteine residues at the Xaa position. Co-delivery of low concentrations of hydrogen peroxide (H_2O_2) with this cysteine containing ELP (cELP) induced rapid oxidative, intermolecular disulfide mediated crosslinking after subcutaneous injection [20]. Because this approach required premixing the H_2O_2 with the cELP, neither the timing nor the coverage of the crosslinked depot could be controlled. In an effort to develop a temporal method for controlling the disulfide mediated crosslinking of cELP, we turned our attention to photodynamic therapy (PDT).

Since we have previously designed and synthesized cysteine containing ELP (cELP) that can readily crosslinked *in vitro* when premixed with H_2O_2 [20], we hypothesized that the cELP crosslinking reaction could be induce by an photodynamic mechanism that could produce oxidative conditions. Specifically, the generation of the strong oxidative agent, singlet oxygen ($^1O_2$), could potentially crosslink ELP *in situ* [21]. In PDT, the molecular photosensitizer (PS) is externally excited by light of a specific wavelength, which then reacts with cellular oxygen to generate singlet oxygen ($^1O_2$) and other ROS [22]. These ROS can then oxidize the thiol moiety of cysteines to induce disulfide crosslinking. ROS can also induce tumor cell death through several indirect mechanisms, including damaging mitochondrial DNA in the cytoplasm (apoptosis), destabilizing the cell membrane (necrosis), or vascular shutdown [21]. PDT has been successfully against a spectrum of cancers and malignancies, including lung cancer, metastatic breast cancer, refractory ovarian cancer, malignancies of the esophagus and stomach [23–26]. For this study, Chlorine e6 (Ce6) was selected as the photosensitizer (PS), as it is activated by near infrared light at 660 nm while generating singlet oxygen species in high yield. This is ideal for clinical application as the activation wavelength has relatively high penetration depth through tissue. The 660 nm LED was selected as the light source for the activation of Ce6 in this polymerization system.

In this study, we created a photoradiation controlled intratumoral depot (PRCITD) that enables spatial and temporal control of the delivery of intratumoral radionuclide therapy to provide optimized coverage and retention. The cELP was chemically conjugated with the Ce6 photosensitizer at the N-terminus. We hypothesized that the CED administration would drive the conjugate to uniformly diffuse throughout the tumor interstitium which could be monitored using fluorescence molecular tomography. Upon achieving optimal tumor coverage, application of 660-nm LED light would activate the Ce6 and crosslink the cELP into a stable hydrogel depot. Once formed, the hydrogel structure would enhance the exclusive retention of the cELP within the tumor for radionuclide therapy. Moreover, excess ROS generated through the photodynamic activation of the Ce6 would provide a combinatorial treatment modality to improve the overall tumor response (Fig. 1). Once established, the PRCITD system is expected to be an attractive material for delivering combinatorial anti-cancer therapy that advances previous work in radionuclide brachytherapy [20] and could potentially be utilized in intratumoral chemotherapy strategies [27].

2. Materials and methods

2.1. cELP design and gene construction

The cysteine-containing ELP consists of 160 repeats of the pentapeptide sequence V–P–V-X-G, where X denotes an amino acid insert of either A, G or C in a ratio of 14:1:1, denoted ELP [A_{14}VC-16] or cELP. The cELP also had a C-terminal tyrosine tail (YGYGYGY) to facilitate conjugation to radioactive iodine. The recombinant ELP[A_{14}VC]$_{16}$ was synthesized as described previously [20] using the RDL method [28]. Briefly, the ELP [A_{14}VC]$_{16}$ gene was assembled by annealing sense and antisense oligonucleotide strands (Integrated DNA Technologies, Coralville, IA) to form a cassette. The gene was then subcloned into the EcoRI and HindIII sites of pUC19 (New England BioLabs, Beverly, MA) and oligomerized by RDL methods. The final oligomerized genes encoded for a 160 pentapeptide sequence, which was then excised from the pUC19 by digestion with PflMI and BglI. The gene was subcloned into the SfiI site of a modified pET-25b (+) vector (Novagen Inc, Madison, WI) and transformed into the expression host *E. coli*