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Perspective

Enzyme-responsive multistage vector for drug delivery to tumor tissue

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ABSTRACT

Various nanodelivery systems have been designed to release therapeutic agents upon contact with specific enzymes. However, enzyme-triggered release typically takes place in the tissue interstitium, thereby resulting in the extracellular delivery of drugs. Here, we have designed an enzyme-stimulated multistage vector (ESMSV), which enables stimulus-triggered release of drug-encapsulated nanoparticles from a microparticle. Specifically, polymeric nanoparticles with a surface matrix metalloproteinase-2 (MMP2) peptide substrate were conjugated to the surface of porous silicon microparticles. In the presence of MMP2, the polymeric nanoparticles were released into the tumor interstitium. This platform can be used to attain triggered drug release, while simultaneously facilitating the cellular internalization of drugs. The results indicate that nanoparticle release was MMP2-specific and resulted in improved intracellular uptake of hydrophobic agents in the presence of MMP2. Furthermore, in a mouse model of melanoma lung metastasis, systemic delivery of ESMSVs caused a substantial increase in intracellular accumulation of agents in cancer cells in comparison to delivery with non-stimulus-responsive particles.

1. Introduction

Nanotechnology has been used in medicine to improve the biodistribution of drugs, thereby increasing therapeutic efficacy and reducing adverse side effects. In particular, nanodelivery systems can prolong the blood circulation of drugs, overcome transport barriers within the body, and preferentially accumulate in pathological tissue [1–5]. Nevertheless, in the case of all nanodrugs in current clinical use, drug release is non-specific [6,7]. Furthermore, the premature release of drugs from a nanocarrier in the circulation can increase off-target effects, while fast release in the cancer microenvironment rather than directly in target cells may reduce therapeutic efficacy. To address these issues, stimuli-responsive delivery systems that enable spatiotemporal control of drug release have been developed. Such nanocarriers respond to environments with unique properties such as increased acidity, oxidative stress, or hypoxia, which are common characteristics of tumor tissue [8–14]. For instance, polymeric nanoparticles for nucleic acid delivery have been designed to release an outer polyethylene glycol (PEG) layer when exposed to hypoxic [15] or acidic [16] environments. The removal of this stealth layer promotes the internalization of the polymeric nanoparticles, resulting in increased exposure of tumor cells to nucleic acids. Another example of stimuli-responsive carriers is nanoparticles designed to release drugs in response to reactive oxygen species (ROS). Such nanodelivery systems have utilized polymers that degrade when subjected to ROS [17] or incorporated catalase in the aqueous core of nanoparticles, consequently causing oxygen production and nanoparticle rupture in the presence of H_2O_2 [18].

Enzyme activity can also be used as a stimulus to trigger drug release, since tumor tissue usually displays increased levels of specific enzymes. For instance, cancer proliferation, metastasis, and angiogenesis often result in overexpression of matrix metalloproteinases (MMPs), which are a family of zinc and calcium-dependent proteolytic enzymes [19,20]. MMPs digest several components of the extracellular matrix (ECM), including collagen, laminin, fibronectin, vitronectin, elastin, and proteoglycans [21,22]. Especially MMP2 and MMP9 play important roles in tumorigenesis, cell

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migration, invasion, and metastasis [23–25]. Indeed, nanocarriers responding to MMPs have been designed for delivery of cancer therapeutics [26]. Typically, such nanocarriers are functionalized with a peptide sequence that can be cleaved by MMPs. For example, Dorresteijn et al. synthesized polylactide (PLLA)-peptide-PLLA triblock copolymer nanoparticles that displayed controlled drug release *in vitro* in response to MMP2 cleavage of the peptide [27]. However, since MMP-driven dissolution of nanoparticles takes place in the ECM, the released drugs may display low levels of intracellular uptake when disassociated from nanocarriers. Furthermore, once the drugs are released in the ECM, they may aggregate and degrade in this environment prior to establishing contact with cancer cells.

To address the aforementioned problems, a multistage system was utilized for drug delivery. Namely, a first-stage carrier (microparticle) was used to transport second-stage carriers (nanoparticles) to tumor tissue. Previous studies have indicated that the multistage vector system serves as a safe and effective vehicle for drug delivery [28–40]. In this study, the microparticle component consisted of a porous silicon disk (2.6 μm × 0.7 μm), while the nanoparticle component comprised poly(lactic-co-glycolic acid) (PLGA)-PEG particles. The multistage delivery system was assembled through conjugation of the polymeric nanoparticles to the surface of the silicon microdisk. Moreover, the nanoparticles were loaded with coumarin 6, a fluorescent small molecule, which served as a model for hydrophobic drugs. This multistage platform was designed to release nanoparticles from the microparticle upon contact with MMPs, as the surface of the PLGA-PEG particles was modified with a MMP2 substrate peptide. This strategy enabled intact nanocarriers to be internalized by cancer cells, thus increasing intracellular uptake and preventing extracellular aggregation/degradation of drugs. Moreover, it has been shown that nanoparticle-mediated drug uptake can decrease drug resistance by reducing the expulsion of drugs from multi-drug resistance efflux pumps [41]. The major difference between the enzyme-stimulated multistage vector (ESMSV) and the majority of previously reported enzyme-responsive drug delivery platforms is the location of drug release. While the ESMSV increases tumor-specific exposure to drugs, the drug release process does not take place in the extracellular environment, as MMPs target the bond between nanoparticles and microparticles. Previously, it has been shown that intravenously administered silicon microdisks display high accumulation in lung tissue due to geometrical features [40,42,43]. Indeed, the small capillaries of the lungs promote interactions between discoidal particles and endothelial cells [42]. Compared to spherical particles, microdisks have the ability to interact with endothelial cells through a much larger contact area [40]. These interactions promote particle adhesion to the vascular wall. In particular, discoidal microparticles have been shown to accumulate to a greater extent in tumor-bearing lungs compared to healthy lung tissue [41]. The main reason for enhanced particle accumulation in tumors is abnormal blood flow patterns that lead to reduced shear rates [40]. Consequently, discoid particles are able to adhere to cancer blood vessels, while they are dislodged from the endothelial wall of healthy vasculature. Based on this natural tropism of microdisks for tumor-bearing lungs, the performance of the ESMSV was evaluated in a mouse model of A375 melanoma lung metastasis.

2. Experimental section

2.1. Materials

PLGA-PEG-COOH was prepared as previously reported [44]. The MMP2 substrate with a peptide sequence of AGFSGPLGMWSAGSFG was purchased from Peptide 2.0 (Chantilly, VA, USA). Sulfo-NHS (N-hydroxysulfosuccinimide) was acquired from Thermo Fisher Scientific, Inc. Phosphate buffered saline (PBS), fetal bovine serum (FBS), Medium 200, Low Serum Growth Supplement (LSGS), Dulbecco's Modified Eagle Medium (DMEM), trypsin, and penicillin/streptomycin solution were purchased from GE Healthcare Life Sciences (Pittsburgh, PA, USA). All other chemicals were acquired from Sigma-Aldrich.

2.2. Preparation and characterization of the ESMSV

The ESMSV was prepared by conjugating MMP2 substrate-modified PLGA-PEG nanoparticles to 3-aminopropyltriethoxysilane (APTES)-modified silicon microdisks. Photolithography and electrochemical etching were used to produce the porous silicon microdisks (2.6 μm × 0.7 μm, 50–60 nm pores) as previously described [45]. Particles were then oxidized in a 30% solution of hydrogen peroxide for 2 h at 95 °C and aminated in 2% APTES in isopropyl alcohol for 48 h at 65 °C.

Coumarin 6-loaded PLGA-PEG-COOH nanoparticles were prepared using the nanoprecipitation method [46,47]. The nanoparticles were then modified with the MMP2 substrate according to a previously described procedure [48]. Briefly, 1 mg of nanoparticles was dissolved in 10 mL PBS. Next, 3 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 2.4 mg sulfo-NHS were added to the solution to activate the functional groups on the nanoparticle surface. The MMP2 substrate was then added to the solution at a concentration of 0.2 mg/mL and the reaction was left to proceed for 3 h. Finally, the MMP2-conjugated PLGA-PEG nanoparticles were washed with water and centrifuged three times at 10000 rpm.

To prepare the ESMSVs, 20 mg of MMP2 conjugated PLGA-PEG nanoparticles were dissolved in PBS and activated with EDC (10 mg) and sulfo-NHS (8 mg) for 30 min. APTES-modified silicon microdisks (0.2 billion) were added to the solution (final volume: 10 mL) and stirred for 3 h. After the reaction, the ESMSVs were washed with water and centrifuged three times at 4500 rpm.

The size and zeta potential of the particles were measured using the Zetasizer (Zetasizer Nano, Malvern). For each sample, three measurements were taken with ten runs each. Scanning electron microscopy (SEM) characterization was performed using an ultra-high resolution microscope (SEM 230, NovaNano). A drop of sample solution was added to a silicon wafer, vacuum dried, and coated with 5–10 nm of platinum.

2.3. Enzyme-stimulated release

Coumarin 6-loaded PLGA-PEG nanoparticles and peptide-PLGA-PEG nanoparticles were centrifuged at 1500 rpm for 5 min to discard larger particles and the supernatant containing the nanoparticles was collected. The nanoparticles were then conjugated to silicon microparticles. The micro/nano particles were centrifuged at 1500 rpm for 5 min and the pellet was collected. The micro/nano particles were then dispersed in 200 μL MMP2 reaction buffer (50 mM HEPES with 10 mM CaCl2) with or without MMP2 enzymes at the concentration of 2 μg/mL. The solutions were then placed on an orbital shaker (120 rpm, 37 °C). At designated time intervals, the solutions were centrifuged at 1500 rpm for 5 min and the supernatant was collected for analysis. Subsequently, the pellet was resuspended in fresh buffer and the drug release study was continued. The collected supernatants were added in black 96-well plates and the fluorescent intensity of coumarin 6 (Ex 430 nm/Em 485 nm) was measured using a microplate reader (Genios, Tecan, Switzerland). The experiments were performed in triplicate.