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Ligand-decorated click polypeptide derived nanoparticles for targeted drug delivery applications

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Abstract

A ligand decorated, synthetic polypeptide block copolymer platform with environment-responsive capabilities was designed. We evaluated the potential of this system to function as a polymersome for targeted-delivery of a systemic chemotherapy to tumors. Our system employed click chemistry to provide a pH-responsive polypeptide block that drives nanoparticle assembly, and a ligand (folic acid) conjugated PEG block that targets folate-receptor over-expressing cancer cells. These nanocarriers were found to encapsulate a high loading of conventional chemotherapeutics (e.g. doxorubicin at physiological pH) and release the active therapeutic at lysosomal pH upon cellular uptake. The presence of folic acid on the nanoparticle surface facilitated their active accumulation in folate-receptor-overexpressing cancer cells (KB), compared to untargeted carriers. Folate-targeted nanoparticles loaded with doxorubicin also showed enhanced tumor accumulation in folate-receptor positive KB xenografts, resulting in the suppression of tumor growth in an *in vivo* hind flank xenograft mouse model.

Key words: Poly (propargyl L-glutamate); Drug delivery; Nanocarriers; Block copolymers

Nanoscale drug delivery systems that can release their therapeutic payload to disease-sites in response to endogenous biological cues are an active area of translational medical research.[1–4] These engineered systems are capable of exhibiting unprecedented benefits in the management of non-metastatic cancer and solid tumors by enhancing the treatment efficiency of frontline anticancer agents, hence offsetting the need for the development of newer, more expensive drug entities.[5]

Abbreviations: PEG, poly (ethylene glycol); PPLG, poly (γ-propargyl L-glutamate); NCA, N-carboxyanhydride; CAC, Critical Aggregation Concentration; FR α, Folate receptor α; EPR, Enhanced Permeation Retention Effect.

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Amphiphilic block copolymers are a robust chemical platform that can be modularly designed to generate self-assembled nanoparticles in the form of micelles or polymersomes. They have been widely used for encapsulating a broad spectrum of potent anticancer agents through supramolecular interactions.[6–8] The reversibility of these interactions is particularly well suited for designing stimuli-responsive drug delivery systems. Although block copolymer-based nanocarriers have been shown to prolong the circulation half-life of small-molecule drugs and promote their accumulation in diseased tissues, the inclusion of enhanced functional attributes such as microenvironment-sensitivity, active targeting capability, and programmable destabilization within these synthetic constructs calls for further molecular engineering of the constituent blocks.[9,10] Examples of chemical modifications of structural polymers include: (a) conjugation of small molecule ligands to the macromolecular backbone that can complementarily engage over-expressed, disease-specific cellular receptors, (b) introduction of ionizable groups within the architecture thereby allowing modulation of the surface charge of the scaffold to optimize cellular interaction and cytosolic uptake, and (c) incorporation of chemical modalities within the polymer to promote self-assembly phase-transitions in response to environmental stimuli.[11] While designing these systems, it is necessary to control dose-limiting toxicities attributed to the premature bolus release of the drug from the delivery vehicle. Such off-target release can begin as early as the introduction of the drug carrier to the systemic environment, and indicates a certain level of instability of the carrier scaffold and dissociation of the encapsulated active drug, in most cases well before the carrier accumulates at the site of drug action.

To optimize colloidal stability and stimuli-responsive behavior, we previously designed and characterized poly (ethylene glycol)-b-poly (γ-propargyl L-glutamate) (PEG-b-PPLG) block copolymers and investigated their potential therapeutic application for environment-responsive drug and gene delivery, and as antimicrobial agents.[12–15] A unique feature of these novel polypeptides is their biodegradable scaffold containing pendant alkyne groups. The alpha-helical arrangement of these groups makes them readily accessible for a "click" alkyne-azide cycloaddition reaction, hence enabling the generation of a large repository of biomaterials with desired form and function. In our recent studies we have shown that PEG-b-PPLG block copolymers with pH-responsive tertiary amine side chains, show pH-dependent and reversible aggregation behavior to form polymersomes (100-150 nm diameter), that can be used as endosome-solubilizing transporters of hydrophilic drug molecules.[16] However, this system, which relied on the tumor-associated enhanced permeation and retention (EPR) effect, showed limited selectivity in targeting tumor mice xenografts. To optimize the targeting of this nanocarrier, we have designed an orthogonal synthetic pathway to covalently link a specific molecular target onto the PPLG scaffold.

The role and molecular understanding of folic acid (FA) as a targeting modality for small molecule drug delivery systems has been extensively studied[17–20] Owing to the high affinity of FA (K_d ~ 0.1 nM)[19] toward glycosylphosphatidylinositol (GPI) anchored cell-surface alpha folate receptors (FRα), as well as the over-expression of these receptors in many human tumors, including tumors of the ovary,[21] uterus,[22] endometrium,[23] brain,[24] kidney,[25] head and neck,[26] and mesothelium[27] with limited expression on normal cells,[28,29] many researchers have shown that the addition of folate groups to the exterior surfaces of nanocarriers enhances intracellular uptake in tumor cells both in vitro and *in vivo*, as a function of surface density balanced against PEG steric resistance.[29,30] Example of using folates as targeting moieties has been illustrated for drug conjugates,[31,32] imaging agents,[33,34] immunotherapies,[35,36] liposomal assemblies[37,38] and polymeric nanoparticles.[28,39] Attachment of FA to a drug conjugate has been reported to trigger a selective uptake pathway for the drug via folate receptor mediated endocytosis and subsequent release of the conjugate from the endosome upon receptor recycling.[40,41] Bae et al elegantly showed that adding folic acid in a mixed micellar system composed of poly(ethylene glycol)-b-poly (histidine) and poly (ethylene glycol)-b-poly (L-lactic acid) block copolymer forms pH-responsive nanoparticles, and when such mixed micellar systems were immobilized with folate, the nanoparticles demonstrated selective enhancement of nanoparticle entry into doxorubicin resistant MCF-7 cell lines.[42,43] Herein we describe the synthesis of pH-responsive PEG-b-PPLG block copolymers through a rational and facile chemical approach in which PPLG side chains are quantitatively substituted with pH-responsive tertiary alkyl amines and the PEG block is end-functionalized with folic acid. Unlike previously reported mixed micellar systems such as those prepared by Bae et al, the PEG-b-PPLG block copolymers form stable vesicles and are pH-responsive via reversible protonation of tertiary amine side chains. These newly developed block copolymers can: (i) self-assemble into nanoparticles with surface presentation of folates, (ii) encapsulate therapeutic cargo within the nanoparticle, and (iii) release the active molecule in response to a change in pH within the endosomes of folate receptor over-expressing cancer cell-lines. We investigated these folate-targeted PEG-b-PPLG nanoparticles for their dynamic self-assembly, drug encapsulation and release behavior, as well as in vitro cytotoxicity and selective cellular uptake in folate receptor over-expressing KB cell lines.[36] We also present a mechanistic deconvolution of the intracellular trafficking mechanism of these targeted PPLG-derived system. *In vivo* experiments show that the particles, when administered in KB hind flank xenografts intravenously, show prolonged systemic stability, and when loaded with doxorubicin, we observe significantly improved tumor accumulation and therapeutic efficacy of the system.

Methods

Synthesis and fabrication of folate containing block copolymer vesicle.

PEG-b-PPLG block copolymers terminated with tert- (butyl oxycarbonyl, BOC) **(3)** and substituted with diethyl amine side chains were synthesized as previously described (cf. supporting information).[12–16] Folate functionalized PEG-b-PPLG block copolymers with diethylamine side chain were self-assembled with unfunctionalized block copolymer at 1:10 weight ratio by