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Clickable and imageable multiblock polymer micelles with magnetically guided and PEG-switched targeting and release property for precise tumor theranosis

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ABSTRACT

Targeted delivery of therapeutics and diagnostics using nanotechnology holds great promise to minimize the side effects of conventional chemotherapy and enable specific and real-time detection of diseases. To realize this goal, we report a clickable and imageable nanovehicle assembled from multiblock polyurethanes (MPUs). The soft segments of the polymers are based on detachable poly(ethylene glycol) (PEG) and degradable poly(ε-caprolactone) (PCL), and the hard segments are constructed from lysine- and cystine-derivatives bearing reduction-responsive disulfide linkages and click-active alkynyl moieties, allowing for post-conjugation of targeting ligands *via* a click chemistry. It was found that the cleavage of PEG corona bearing a pH-sensitive benzoic-imine linkage (BPEG) could act as an on-off switch, which is capable of activating the clicked targeting ligands under extracellular acidic condition, followed by triggering the core degradation and payload release within tumor cells. In combination with superparamagnetic iron oxide nanoparticles (SPION) clustered within the micellar core, the MPUs exhibit excellent magnetic resonance imaging (MRI) contrast effects and T_2 relaxation *in vitro*, as well as magnetically guided MR imaging and multimodal targeting of therapeutics to tumor precisely, leading to significant inhibition of cancer with minimal side effect. This work provides a safe and versatile platform for the further development of smart theranostic systems for potential magnetically-targeted and imaging-guided personalized medicine.

1. Introduction

The effective treatment and early detection of cancers still remain great challenges, since the transport of therapeutic and diagnostic agents to the tumors have been seriously hampered by the complicated body environments and various physiological barriers [1]. To address these challenges, targeted delivery system based on nanotechnology holds enormous potential to enable engineered nanomedicines to travel through the body in a specific way [2]. On the one hand, drug-loaded nanoparticle systems can overcome the problems brought by conventional free drugs, including poor solubility, low stability, rapid clearance and lack of selectivity, which have exerted adverse effects on healthy cells and prevented the dose escalation necessary to eliminate malignant cells [3]. On the other hand, the encapsulation of diagnostic agents into polymeric nanovehicles can remedy the inadequate sensitivity and specificity of most commercialized contrast agents for imaging (e.g., Endorem, Resovist, etc.) [4], resulting in an improved spatial revolution and remarkable contrast against surrounding tissues by clustering these agents and localizing them at the sites of interest [5].

Relying on the so-called enhanced permeability and retention (EPR) effect ascribable to the presence of leaky vasculature and impaired lymphatic drainage in most rapidly growing tumors, nanomedicines show higher accumulation at tumor sites than the conventional drugs and contrast compounds [6]. However, EPR effect has been regarded recently as an immensely heterogeneous phenomenon, which varies with the types and stages of diseases

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and differs from person to person [7]. Therefore, the EPR-based passive targeting seems not sufficient to match the changeable and complicated tumor microenvironment. As a result, it is only effective for limited patient subpopulations [8]. The cooperation of magnetic nanoparticles is expected to offer improved specificity *via* magnetic drug targeting (MDT) [9,10]. As a physical force, the magnetic stimulus is independent of the complicated physiological conditions. Hence, MDT employing an external field gradient to manipulate magnetically responsive particles can expedite the extravasation and transport of nanoparticles into tumors, and thereby break through the physical barriers and overcome the intrinsic limitations of EPR effect [9,11]. Furthermore, magnetic nanoparticles can also act as excellent contrast agents for magnetic resonance imaging (MRI), which provides great opportunity to construct theranostic platform for specific disease detection, real-time monitoring and potential imaging-guided therapy [11,12].

Although nanomedicines can passively or magnetically target to tumor tissues, the scarcity of cell-specific interaction and insufficient uptake of nanoparticles may decrease the therapeutic efficacy and even induce drug expulsion and multiple drug resistance (MDR) [13]. To overcome this problem, nanocarriers can be equipped with a good variety of targeting ligands, such as antibodies, folic acid (FA) and peptides that can actively bind to antigens and receptors highly overexpressed by tumor cells [14]. Unfortunately, most of the actively targeted delivery systems contain ligands on their surface [15]. The exposure of active surface may promote nonspecific interactions with endothelial and other non-cancerous cells and result in opsonization-mediated clearance of nanomedicines from the body [16]. To address this dilemma, coating nanocarrier with hydrophilic polyethylene glycol (PEG) corona (known as PEGylation) can increase the circulation time by reducing interactions with serum proteins and mitigating uptake by phagocytic cells [17], whereas such a strategy in turn compromises the targeting specificity [18].

Another approach to targeting is to take advantage of the special microenvironments of tumors to achieve on-demand delivery, which in principle allow for tailored release profiles with excellent temporal, spatial and dosage control [19]. It is known that most solid tumors develop unique microenvironments, such as lowered interstitial pH, elevated glutathione (GSH) concentration and increased level of certain enzymes [20]. In particular, the GSH concentration in some tumors is several times higher than that in normal tissues, and the intracellular level of GSH (1–11 mM in the cytoplasm) is 2–3 orders higher than that outside the cells (2–20 μM) [21,22]. Therefore, bio-responsive formulations containing disulfide functionality can facilitate tumor-specific and intracellular transport of therapeutics by the cleavage of disulfide bond through thiol-disulfide exchange reactions triggered by GSH [23]. Nonetheless, recent *in vivo* studies have shown that disulfide could be potentially cleaved under circulation due to the presence of reducing species in the blood [24], thus decreasing the specificity of redox-responsive delivery systems [25]. On the other hand, the current studied reduction-responsive systems are commonly prepared by incorporating non-native segments containing disulfide linkages, which may raise a safety concern [21,26].

As mentioned above, although the conventional targeting and responsive systems taking advantages of EPR effect, targeting ligands and tumor microenvironments allow for specific delivery of theranostics, each of these approaches is still suffering from some limitations. Hence, the development of versatile nanomedicines to overcome all the limitations and realize multimodal targeting and precise therapy is highly desirable. To fulfill this need, multiblock polyurethane (MPU) appears as a promising platform due to their good biocompatibility and high molecular tunability [27,28]. MPUs could be molecularly engineered to integrate various desired functions into a single macromolecule in a smart and coordinated way, as demonstrated recently by our group [29]. However, the role of MPUs in the field of imaging and personalized nanomedicine remains largely unexplored [30]. To date, the theranostic property of MPUs have yet to be demonstrated. In addition, to make MPUs more clinically approvable, a further improvement of PU versatility and optimization of polymer structure is clearly warranted [27].

In this study, we report a clickable and imageable multiblock polyurethane (MPU) system with switchable tumor targeting and triggered drug release properties for precise tumor therapy and specific MR imaging (Fig. 1). The soft segments of MPUs comprise PEG and PCL, and the hard segments contain L-lysine ethyl ester diisocyanate (LDI) (Fig. 1A). To eliminate the need of multiple chain extenders and complicated synthetic procedures for the construction of multifunctional MPUs [29], an L-cysteine-derived versatile chain extender (Cys-PA) was incorporated to endow polymers with a number of reduction-cleavable disulfide linkages in the backbone and clickable alkyne sites on the side chains (Fig. 1). Subsequently, as a proof-of-concept, a post-conjugation of targeting ligand was performed using a facile click chemistry after the formation of polymer micelles (Fig. 1C). We found lately that FA-clicked polymer micelles exhibit relatively lower targeting efficacy compared with other FA-based delivery systems [31]. Herein, it is interesting to note that the cleavage of PEG segment bearing a pH-sensitive benzoic-imine linkage (BPEG) could act as a switch, which is capable of activating cell targeting under extracellular condition, followed by triggering the cleavage of disulfide and accelerating the release of payloads within tumor cells (Fig. 1E). Furthermore, superparamagnetic iron oxide nanoparticles (SPIONs) and doxorubicin (DOX) as model contrast and anticancer agents were efficiently co-encapsulated into the micellar core to enable magnetophoretic enhance of targeting and theranostic capability of MPUs. The multiple stimuli-responsive property and multimodal targeting effect of MPUs were systematically demonstrated, and the magnetically guided cancer therapy and MR imaging were also evaluated *in vivo*.

2. Materials and methods

2.1. Materials

N,N-Dimethylacetamide (DMAc) was purchased from Adamas Reagent Co., Ltd., China, and dried over CaH_2 overnight and vacuum distilled. Methoxyl-poly(ethylene glycol) (MPEG, MW 1900, Alfa Aesar) and PCL (MW 2000, Dow Chemical) were dehydrated under reduced pressure at 90 °C for 2 h before use. LDI, BPEG, Cys-PA, and azido modified FA (AzFA) were synthesized according to previous reports [31,32]. Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was purchased from Tianjin Damao Chemical Co., Ltd., China. $CuSO_4.5H_2O$ was attained from Tianjin Bodi Chemical Co., Ltd., China. Sodium ascorbate was purchased from Aladdin Chemical Co. Ltd., China. Iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), ammonium hydroxide solution ($NH_3 \cdot H_2O$), oleic acid, propanediol (PDO, ≥98.0%) and dimethyl sulfoxide (DMSO) were supplied by Chengdu Kelong Chemical Co., Ltd., China. Doxorubicin hydrochloride (DOX·HCl) was obtained from Tecoland Co., USA. 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich, USA. 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) was obtained from Roche Diagnostics, Germany. Other reagents were purchased from commercial suppliers and used without further purification.

2.2. Characterization

Proton nuclear magnetic resonance (^{1}H NMR, 400 MHz)