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A boronate-linked linear-hyperbranched polymeric nanovehicle for pH-dependent tumor-targeted drug delivery

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ABSTRACT

Advanced drug delivery systems, which possess post-functionalization feasibility to achieve targetability and traceability, favorable pharmacokinetics with dynamic but controllable stability, and preferable tumor accumulation with prolonged drug residence in disease sites, represent ideal nanomedicine paradigm for tumor therapy. To address this challenge, here we reported a dynamic module-assembly strategy based on reversible boronic acid/1,3-diol bioorthogonality. As a prototype, metastable hybrid nanoself-assembly between hydrophobic hyperbranched diol-enriched polycarbonate (HP-OH) and hydrophilic linear PEG terminated with phenylboronic acid (mPEG-PBA) is demonstrated *in vitro* and *in vivo*. The nanoconstruction maintained excellent stability with little leakage of loaded drugs under the simulated physiological conditions. Such a stable nanostructure enabled the effective *in vivo* tumor accumulation in tumor site as revealed by NIR imaging technique. More importantly, this nanoconstruction presented a pH-labile destruction profile in response to acidic microenvironment and simultaneously the fast liberation of loaded drugs. Accordingly at the cellular level, the intracellular structural dissociation was also proved in terms of the strong acidity in late endosome/lysosome, thus favoring the prolonged retention of remaining drug-loaded HP-OH aggregates within tumor cells. Hence, our delicate design open up a dynamical module-assembly path to develop site and time dual-controlled nanotherapeutics for tumor chemotherapy, allowing enhanced tumor selectivity through prolonged retention of delivery system in tumor cells followed by a timely drug release pattern.

1. Introduction

Despite gradual advance of nanovehicle-assisted chemotherapy in tumor treatments [1,2], the drug efficacies still suffer from limited drug bioavailability inside tumor cells due to the inefficient drug release and/or the insufficient intracellular residence of the nanotherapeutics [3,4]. To address the problem, of great interest is the programmed drug release in a tumor dependent fashion that is preferred to be biologically triggered upon tumor localization [5–7]. Therefore, chemically labile linkages that respond to specific stimuli associated with tumor microenvironments while being otherwise stable have been actively exploited for the construction of tumor-specific drug vehicles, though the preparations frequently involve complicated chemistries. As a different yet rarely investigated approach, physically prolonging the in situ duration of tumor-oriented nanotherapeutics in disease sites is reasonably another promising solution. Taking advantage of reversible boronic acid/1,3-diol bioorthogonality, the present work described a dynamic module-assembly strategy for nanovehicle construction in hopes of merging those two modalities for the achievement of site and time dual-controlled drug release inside tumor cells.

It is well documented that phenylboronate bond is thermodynamically stable at neutral condition but susceptible to acidity-induced cleavage [8,9]. Given the substantial pH decline in tumor tissue/cells [10,11], the reversible covalent feature of boronic acid chemistry inspired us to establish a pH-dependent metastable nanoconstruction for tumor-specific chemotherapy, which was built on the dynamic self-assembly between hydrophobic 1,3-diol-rich

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hyperbranched polycarbonate (HP-OH) and hydrophilic polyethyleneglycol terminated with one phenylboronic acid (mPEG-PBA). The spontaneous formation of boronate linkage allows facile attachment of mPEG-PBA chains onto central HP-OH core, achieving a linear-hyperbranched nanoconstruction with a multiarm star architecture. The tumor-specific feature of this hybrid nanoconstruction is expected from the following aspects as illustrated in Scheme 1. Firstly, covalent boronate linkage with strong B–O bond strength would stabilize nanoconstruction in physiological condition with a protective PEG shell to prolong the circulating half-life in blood pool, thus facilitating passive tumor accumulation through the well-established enhanced permeability and retention (EPR) effect [12,13]. Meanwhile, supramolecular HP-OH core offers the capability of loading poorly water-soluble cargos, *e.g.* antitumor drugs or imaging agents via the hydrophobic interaction and "dendritic box" effect [14]. Secondly, the unique acidity–lability of boronate linkage imparted dynamic reversibility to the amphiphilic core–shell nanoconstruction. Upon tumor localization and subsequently being endocytosed into more acidic lysosome compartments, the nanoconstruction would readily experience a structural dissociation following the PEG detachment apart from the HP-OH core, owing to the acidity-induced cleavage of boronate linkages. In principle, this transition would make drug-loaded HP-OH residue intend to aggregate so as hard to reenter the blood stream because of its inherently hydrophobic nature, which consequently led to a locally long-term entrapment of drug-vehicle system within neighboring tumor cells [15–17]. Attractively, as demonstrated in this study, the structural dissociation simultaneously induced a selectively fast drug release, favoring the enhanced drug efficacy inside tumor cells. As such, our design provides great chances to achieve the site and time dual-controlled drug delivery for tumor chemotherapy. Last but not least, further surface decoration of nanoparticles with versatile functionalities can be easily accomplished by introducing other functional modules, *e.g.* targeting moieties and/or imaging probes via the same module-assembly pathway. As the proof-of-concept, phenylboronic acid-bearing fluorescent marker was incorporated into the nanoassemblies. It therefore offers an excellent opportunity to track intracellular fates of nanoconstruction with regard to acidity-triggered variation inside cells. This aim is hardly and rarely achievable for most the pH-sensitive nanovehicles developed to date due to the requirement of complicate chemistries.

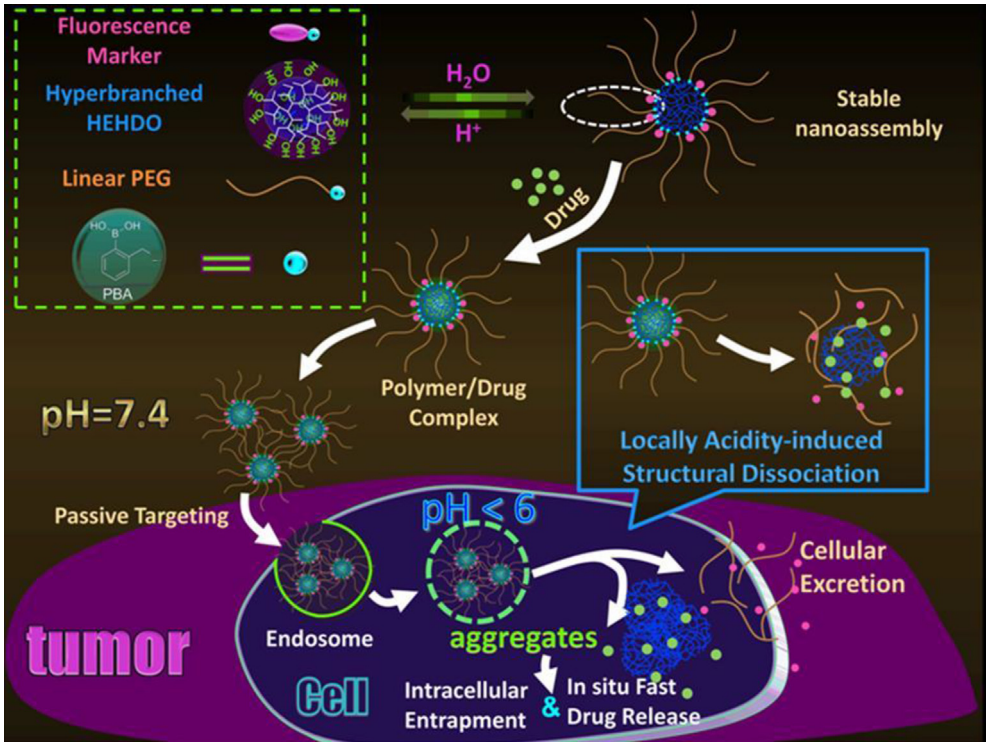
2. Materials and methods

2.1. Materials

1,1,1-Tris(hydroxymethyl) propane, diphenyl carbonate and stannous 2-ethyl hexanoate ($SnOct_2$) was purchased from Shanghai Chemical Reagent Co. (China) and used after distillation under reduced pressure. Toluene were dried by refluxing over Na/K, and distilled before using. Molecular sieve was dried under 400 °C for 6 h. 2-(Bromomethyl)phenylboronic acid was purchased from Aldrich. Polyethyleneglycol monomethyl ether (mPEG-OH) with the molecular weight of 1900 Da was purchased from Aladdin. 5-Ethyl-5-hydroxymethyl-1,3-dioxan-2-oxo (EHDO) and amine-functionalized PEG ($mPEG\text{-}NH_2$) were prepared staring from mPEG-OH according to our previous work [36,18]. 2-[4-Chloro-7-(1-ethyl-3,3-dimethyl(indolin-2-ylidene)]-3,5-(propane-1,3-diyl)-1,3,5-heptatrien-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium (Cy.7.Cl) as a NIR fluorophore was kindly offered by other laboratory. *N*,*N*-Dimethyl formamide (DMF) was used after distillation under reduced pressure. Doxorubicin (DOX) was purchased from Zhejiang Hisun Pharmaceutical Co. (China). Hoechst 33258, Dulbecco's Modified Eagle's Medium (DMEM), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT), fetal bovine serum (FBS), Dulbecco's phosphate buffered saline (PBS) were purchased from Invitrogen Corp.

2.2. Synthesis of the phenylboronic acid functionalized PEG (mPEG-PBA)

Phenylboronic acid functionalized mPEG-PBA was synthesized through the route as shown in Fig. S1. $mPEG\text{-}NH_2$ (0.41 g) and 2-(bromomethyl) phenylboronic acid (0.40 g) in 25 mL methanol was stirred in a 70 °C oil bath for 24 h. The reaction solution was concentrated and poured into a great amount of diethyl ether to precipitate the polymer product. The dissolution–precipitation treatment was repeated thrice and the product was dried under high vacuum to give 0.48 g of light yellow powder in 60% yield [37].



Scheme 1. Illustration of dynamically stable mPEG-PBA/HEHDO nanoconstruction for tumor-specific drug delivery. The stable nanoconstruction enable the low leakage of drug cargo and enhanced passive tumor-targeting; subsequently the lysosome-induced structural dissociation would lead to the prolonged intracellular entrapment of the HEHDO residues and in situ fast drug release within tumor sites.