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A Review on Bottlebrush Prodrug: Synthesis, Characterization and Applications

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Abstract

Prodrugs are engineered so as to get activated in disease or tumour tissue rather than normal tissue. Prodrugs have high tumor suppression effectiveness good intra-tumoral permeability, and good circulation stability in tumor, all of which are helpful for the creation of next-generation nanomedicines for more effective chemotherapy. The most important characteristics of novel nanotherapeutic platforms are that they are able to avoid blood elution, enter and consolidate in deep tumoral tissues, and start selective drug release. Polymer-based nanotherapeutics can react to the tumor microenvironment and have a great therapeutic effect because they have stable or adaptable chemical structures and excellent biocompatibility. Bottlebrush polymers (BBPs) is a fascinating class of materials with a wide range of uses, including energy storage, biomedical devices, super soft elastomers, organic optoelectronics, templates for grafting one-dimensional (1D) nanomaterials, targeted drug delivery in tumor tissues, and organic optoelectronics. By utilizing modern synthetic techniques, this makes it possible to design BBPs with controlled dimensions, compositions, and architectures. This review aims to provide a comprehensive and critical summary that highlights the recent advances in bottlebrush prodrugs in terms of their controlled syntheses techniques, self-assembly, properties, characterization and applications.

Keywords: Bottlebrush Polymer, Prodrug, HCPT-Prodrug, EPR Effect

Introduction

Systems for delivering nanosized drugs have received a lot of attention in the treatment of cancer in recent times. Due to the enhanced permeability and retention (EPR) effect, nanosized medicines preferentially aggregate in tumor tissues and produce increased anti-cancer efficiency. So far, small molecule medications were physically enclosed using the physical encapsulation approach inside self-assembled nanocarriers such as liposomes, micelles, and polymersomes. Premature burst release and low drug loading content were issues with such physical encapsulation techniques. Researchers have developed polymeric prodrug techniques to address these issues. In this approach, potent anti-cancer drugs are incorporated into a polymer via stimuli-responsive linkages followed by self-assembly of the polymer into nano micelles. Two major procedures are typically used to create such polymeric prodrugs. One involves using drugs directly to create the monomers that make up the polymer backbones. The other involves creating side-chain functional polymers by conjugating a potent anti-cancer agent to the polymer backbone as side chains [1-4]. Bottlebrush polymers, which have a polymer backbone and polymeric side chains conjugated at each monomer unit, have been demonstrated to be an inventive and efficient drug delivery strategy among side-chain functional polymers. Due to the abnormal architecture of tumor blood vessels, polymeric nanoparticles have a tendency to accumulate in tumor tissue at a higher rate than in healthy tissue. Because of the inadequate lymphatic drainage in the region of the quickly expanding tumor, the deposited polymers are thus not properly removed. This type of phenomenon is known as enhanced permeability and retention (EPR) effect [5, 6]. Several forms of polymer-based prodrugs with the conjugation of anti-cancer drugs have been created to take advantage of the acidic environment around tumor. Once such polymer-based prodrugs build up in the tumor tissue as a result of the EPR effect, the weakly acidic environment around the tissue cleaves the acid-cleavable bonds, causing the drug to release at the tissue location. Anti-cancer drugs 10-hydroxycamptothecin and doxorubicin are drugs that undergo acid-based cleavage and release at the target site [7, 8, 9].

Synthesis of Bottlebrush Polymer

Bottlebrush polymers (BBPs) are a significant class of high-density side-chain-grafted polymers with a high molecular weight (MWs). Each repeating unit of a bottlebrush-shaped linear polymer backbone is attached to one or more polymeric side chains, giving the macromolecules their name (Figure 1).

Following methods have been used to produce BBPs with a range of structures, including Random (heterografted), Bottlebrush Block Copolymers (BBCPs), Cor-Shell, Janus, Brush-on-Brush, etc. [10, 11, 17]

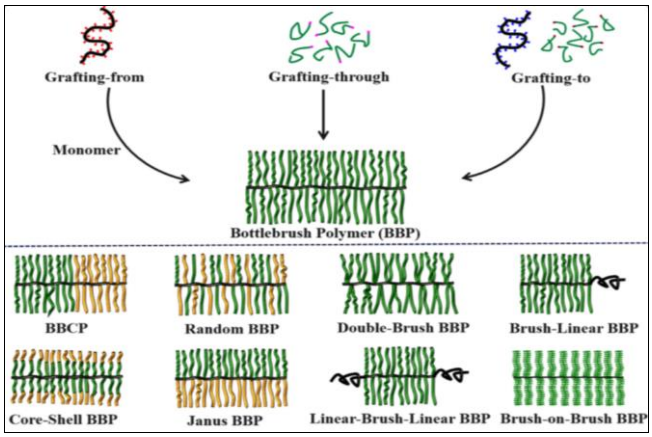

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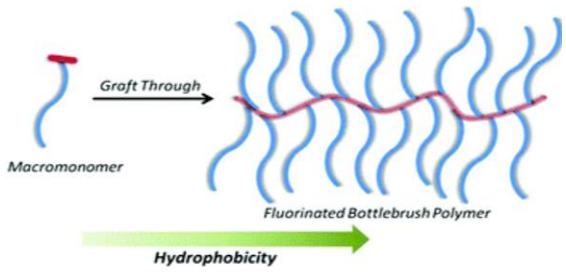
Fig 1: A representation of the primary techniques for the formation of BBPs and sophisticated BBP structures

1. Grafting-from approach:

In the "grafting-from" method, the development of the polymer brushes begins at reactive sites on the polymer backbone. The poly-initiators are introduced to polymerize the ionomers and to add the initiating moieties. The "grafting-from" method can produce high-MW side chains because the monomers can easily diffuse to the initiator sites as the polymers develop progressively from the active centers [12, 14, 15].

2. Grafting-through approach:

This method assures that the generated BBPs have a 100% grafting density by having a pre-attached side chains in each repeating unit. There, the side chains have been prepared and thoroughly described before the backbone is formed. Grafting density is a well-known factor in regulating the characteristics of BBPs [12, 13].



3. Grafting-to approach:

Minimal grafting density on the BBPs is frequently achieved using this technique because free active sites are likely present on the backbone during grafting. The "grafting-to" technique is simple, but because of the unfavorable kinetic and thermodynamic barriers, it frequently experiences the low grafting density problem [12, 14, 15].

4. Hybrid approach:

To prepare complex-structured BBPs, it integrates the "grafting-from" along with "grafting-to" methods. A strategy that combines two or three complimentary tactics can result in well-designed BBPs as well as make it easier to prepare complex BBPs simply [16, 17].

Advanced Architectures of Bottlebrush Polymers

Bottlebrush copolymers include BBCPs and core-shell BBPs, whereas homopolymer BBPs only have one type of backbone and side chain. Comb-like polymers are BBP-like polymers with low grafting density. Asymmetrically linking two distinct polymer chains to a single repeating unit along a polymer backbone allows for the creation of Janus BBPs in particular. The BBP should have a shorter side chain length (i.e., a lower degree of polymerization (DP) of the side chain) than a longer backbone length (i.e., a higher DP of the backbone). Brush polymers with secondary side chains on the primary side chains that are directly bonded to the backbone are known as brush-on-brush BBPs [17, 18, 19].

Properties of Bottlebrush Polymer

Materials' structures control their qualities, which in turn dictate their applications. Because of steric repulsions between their packed branches, BBPs have intrinsic extended 1D conformations. Additionally, by allowing BBPs to self-assemble in solution or film states, a variety of assembled conformations have been accomplished. The conformations of BBPs are hierarchically arranged and provide them with intriguing features. Furthermore, BBPs with exactly well-defined architectures have been achieved by rational design and the application of cutting-edge synthetic techniques. In other systems, it is challenging to implement all of these features. As a result, altering the chemical characteristics of BBPs can produce many of their distinctive traits. The following are some of the characteristics of bottlebrush polymers: [24]

1. Crystalline Property

The significant steric crowding of the crystalline side chains in BBPs, in contrast to linear polymers, can promote crystal nucleation but inhibit crystal development. Because of this, the size of the bottlebrush polymer may be controlled throughout production [24, 28].

2. Stimuli-Responsiveness

Smart polymeric materials adjust their functions (such as ion as well as molecule delivery, wetness, morphological and chemical changes) in response to signals from external stimuli in order to adapt to the environment. Because of the solvent molecules' Brown's law of motion, which requires

just modest energies for the movement of macromolecular segments, the polymers can easily develop stimuli-responsive behaviours in solution. Numerous developing domains, including self-healing, self-cleaning, medication delivery, biological engineering, and other systems, have examined stimuli-responsive materials in depth [29, 30].

a) Temperature-responsive bottlebrush polymers:

The most typical trigger for biocompatible responsive polymers is temperature. Implementing thermal modification is simple in both *in vivo* and *in vitro* systems. Temperature-sensitive polymers exhibit lower critical solution temperature behaviour where phase separation is induced at a certain temperature threshold. Polymers of this type undergo thermally induced reversible phase transition. They are soluble in aqueous solutions at low temperatures but become insoluble as the temperature rises above the lower critical solution temperature [24, 31].

b) Photo-responsive bottlebrush polymers:

Photochromic compounds such as azobenzene, coumarin, cinnamate, and those with o-nitrobenzyl group exhibit isomerization between the two chemical structures with different physical and chemical properties in response to light illumination. It is simple to accurately control the building and disassembly of nanoobjects with tailored architectures and properties using light of different intensities and wave lengths. These have been incorporated into the side chains to prepare photo-responsive BBPs [24, 32].

c) pH-responsive bottlebrush polymers:

By changing the pH of the surrounding environment, weak polyelectrolytes' conformations can be reversibly transformed from an extended state into a collapsed state. The molecular conjugates' steric hindrance, solubility, as well as light scattering characteristics can all be affected by this change. The morphological modifications of the individual BBPs frequently coexist with modifications within the ionization state brought on by pH fluctuation [33].

d) Mechanically responsive bottlebrush polymers:

By adjusting the molecular structure of the BBP, one may accurately control its internal tension. The intrinsic tension of the BBP can be precisely controlled by manipulating its chemical structure. The external force can dominate the conformation changes of the BBPs during their spreading on a substrate via the Langmuir Blodgett (LB) method. Atomic Force Microscopy (AFM) has been applied to trace the in-situ shape transformation in response to the force induced by the substrates. Along the spread flow direction of the drop on the substrates, the BBPs exhibited an extended worm-like morphology. Mechanochemistry induced by grinding or sonication with a destructive force mediate the BBP architectures [24, 34].

e) Other responsive bottlebrush polymers:

The BBP conformation can be altered by any stimuli that causes the polymer side chain solubility to release drug at target site. BBPs are capable of assembling themselves into cylindrical, spherical, lamellar, and other intricate designs by altering the ratio of good to bad solvents. Ionic strength of the salt is a powerful stimulant for polymer solutions. Due to electrostatic screening, an addition of monovalent salt caused a cationic polyelectrolyte to shift shape from stretched worm-like to collapsed form. Similar to cationic BBPs, anionic surfactants may cause a worm-to-sphere transition because of the development of insolubilized side chains. It has been demonstrated that adding additional stimuli-responsive moieties to BBPs modifies their exterior responsiveness without altering their conformation. Additionally, multiscale conformation changes could be controlled for multi-responsive BBPs [24].

3. Mechanical and rheological properties of bottlebrush polymers:

Viscoelastic qualities, which depend on the creation of entanglements along with structures, can be used to express both the mechanical as well as rheological features of BBPs. Therefore, by adjusting both the grafting density and length of the backbone or side chains, the mechanical and rheological properties of the BBPs can be controlled. Due to their distinct extended cylindrical geometries without intermolecular entanglement, BBPs have lower mechanical and shear moduli than linear polymers.

The Mechanical and rheological properties of bottlebrush polymers are follows:

1. **Zero Shear Viscosity:**
   Zero shear viscosity is the viscosity of a material when it is effectively at rest.
2. **Strain Hardening Factor:**
   Strain hardening is related to microscopic plastic events, which are required to maintain chain connectivity. As the stress rises with increasing strain, both the number of events and the energy dissipated per event increase.
3. **Plateau Shear Modulus:**
   Mechanical moduli of amorphous polymers above their glass transition temperature are governed by entanglements, which are temporary physical cross-links in the polymer that create a plateau modulus at time scales before the chains can unentangle and the modulus can further decrease [24, 35].

Synthesis of Bottlebrush Prodrug

Different bottlebrush prodrugs are created using a bottlebrush polymer for drug delivery. Two instances of bottlebrush prodrugs utilized in cancer therapy are listed below [1].

HCPT-Prodrug Bottlebrush Polymer:

- Biocompatible mPEG chains within a polyester backbone and the anticancer medication HCPT (10-Hydroxycamptothecin) were combined to create a novel bottlebrush polymer-HCPT prodrug through the thiol-ene reaction.
- It was established that the bottlebrush polymer-HCPT prodrug had a comparatively high HCPT drug loading content.
- Due to the EPR effect, the bottlebrush polymer-HCPT prodrug may assemble themselves into spherical nanosized micelles in aqueous solution, improving its potential to target tumor.
- Polyester, mPEG-SH, and Boc-HCPT-SH were combined to create a bottlebrush polymer-HCPT prodrug through a thiol-ene reaction.
- When exposed to esterase, the carbonate ester links that were used to conjugate the anticancer medication HCPT were quickly broken down.

- Esterase overexpression within the tumor microenvironment was well established. The produced polymeric prodrug micelles become intelligent stimuli-responsive nanomedicines due to their esterase-responsiveness [1].

Manufacturing Steps Involved: [1, 24, 25, 26, 27]

1. **Synthesis of polyester (Backbone of structure):**
   Maleic anhydride and ethylene glycol were bulk polymerized in presence of phenol to create polyester.

2. **Synthesis of mPEG-SH (Polymeric side Chain):**
   When making mPEG-AC for the first time, CH_2Cl_2 was used to dissolve mPEG in Et_3N. The mixture solution was then added dropwise with acryloyl chloride in CH_2Cl_2 at 0°C, followed by stirring for an overnight period at room temperature. Diethyl ether was used to precipitate the pure products from CH_2Cl_2. The next step was the synthesis of mPEG-SH, which involved dissolving mPEG-AC, EDT, and Et_3N in the CH_2Cl_2 solution and stirring the mixture for an overnight period at room temperature. Following reaction, the mixture was adjusted to neutral, poured into a specific volume of deionized water, and then washed with hexane. By freeze-drying, the pure mPEG-SH was produced.

3. **Synthesis of Boc-HCPT-SH:**
   1. Synthesis of Boc-HCPT:
   Boc-HCPT was created by combining di-tert-butyl decarbonatewith HCPTin CH_2Cl_2. As a catalystEt_3N was utilized.
   2. Synthesis of Boc-HCPT-SH:
   EDC HCl and mercapto acetic acid were combined at room temperature in CH_2Cl_2. Boc-HCPT and 4-dimethylaminopyridine were put into the mixture after two hours of reaction, and the mixture was then stirred at room temperature. After that, deionized water, hydrochloric acid solution, and a saturated sodium carbonate solution were used to wash the resulting mixture. The final outcome was produced by vacuum-removing CH_2Cl_2.

4. **Preparation of bottlebrush polymer-HCPT prodrug:**
   Using Et_3N as a catalyst, MPEG-SH, polyester, and Boc-HCPT-SH were dissolved in CH_2Cl_2. Et_3N was removed by washing with hydrochloric acid solution after being stirred for an entire night at room temperature. The solution was subsequently dried with anhydrous $MgSO_4$. TFA was introduced to the filtered clear solution and agitated to deprotect Boc- groups. TFA was removed with deionized water. The polymer's final product was obtained when CH_2Cl_2 was removed under vacuum.

5. **Preparation of bottlebrush polymer-HCPT prodrug micelles (Aqueous solution):**
   The nanoprecipitation technique was used to create the bottlebrush polymer micelles.

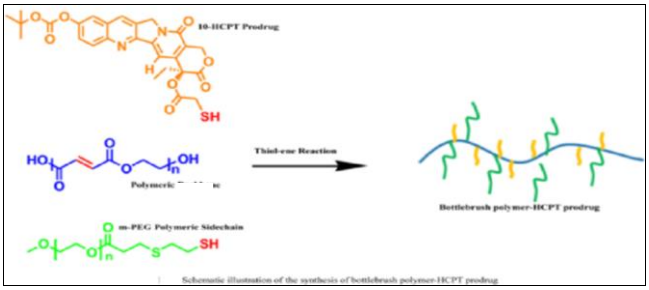


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Fig 2: Illustration of the bottlebrush polymer-HCPT prodrug production in schematic form

DOM@DOX Prodrug Bottlebrush Polymer

- Dextran (DEX) polysaccharide, which binds with a hydrophilic polyethylene glycol chain by atom transfer radical polymerization, functions as the basis for the bottlebrush architecture of acidic environment active supramolecular nano-prodrugs (DOM@DOX), which further conjugate with the anticancer drug doxorubicin (DOX) at the backbone of the copolymer via an acidity-responsive hydrazine bond.
- The prodrug DOM@DOX has a high drug loading for DOX and uses self-assembly to maintain a stable nano-sized spherical form in aqueous solution.
- The prodrug's hydrazine bond breaks in the acidic environment of tumor cells, releasing DOX from parental micelles [6, 12, 17].

Manufacturing Steps Involved: [12, 17]

1. Preparation of a DEX–Br polymer
   1. Synthesis of a DEX-POEGMA polymer
   2. Preparation of DEX-POEGMA-b-PMGMA (DOM)
2. Synthesis of the DOM@DOX prodrug
3. Preparation of DOM@DOX micelles

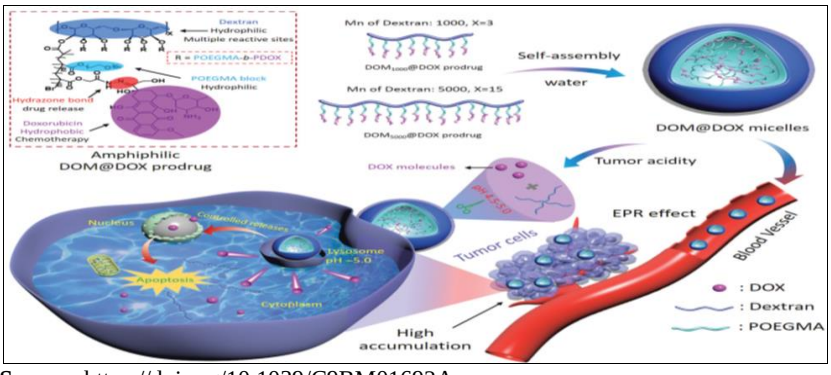

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Fig 3: Schematic representation of the production process for DOM@DOX micelles, the EPR effect's role in drug accumulation, the internalization of drugs by cells, and the pH-responsive drug release mechanism

Characterization of Bottlebrush Prodrug

1. Structural or Morphological Identification:

1.) 1H NMR spectroscopy:

It is used to determine the identity and structure of molecules. The generated structure of the bottlebrush polymer prodrug was confirmed using the 1H NMR characterization approach. A bottlebrush polymer prodrug's 1H NMR spectrum is compared to the polyester backbone to characterize it [1].

2.) Transmission Electron Microscopy (TEM):

The shape of bottlebrush polymer prodrug micelles has been verified by TEM. It is used to measure particles, size distribution, and morphology quantitatively [1].

3.) Laser-scanning Confocal Microscopy (LSCM):

LSCM involves the automated collecting of 3D data, the imaging of multiple labelled specimens, the measurement of physiological events in living cells, and the imaging of the spatial distribution of macromolecules in living cells. LSCM is used to examine the localisation and cellular uptake of bottlebrush polymer prodrug micelles in living cells [1].

4.) Atomic Force Microscopy (AFM):

The mechanical characteristics of cells, contact forces between cells, how cells and biomolecules interact, as well as viruses, membranes, cellular compartments, cellular processes (such as exocytosis), and DNA and how other biomolecules interact with DNA have all been imaged using atomic force microscopy (AFM) [1].

2. Functional Group identification:

1.) FT-IR spectrophotometry:

This technique focuses on the infrared light absorption that results in molecular vibrations. While the frequencies of these vibrations give qualitative information, such as oxidation, changes in the fluidity of cell membranes, and alterations to protein and DNA, the intensities of these vibrations provide quantitative information. Utilizing this technique, produced bottlebrush polymer prodrugs can be characterized [1].

3. Size Identification:

1.) Gel permeation chromatography:

The distribution of molecular weights and the relative molecular weight of polymer samples are both determined using gel permeation chromatography.

2.) Dynamic Light Scattering (DLS):

DLS was the first to describe the bottlebrush polymer prodrug micelles that had formed. Additionally, it evaluates how well bottlebrush polymer prodrug micelles are stable. Dynamic light scattering can be utilized to establish precise shelf life as well as the longevity and mechanical properties of products [1].

4. Drug entrapment to Polymer side chain:

1.) UV spectrophotometer:

It is used to identify, characterize, and quantify molecular compounds complexed with bottletlebrush polymer.

2.) FT-IR spectrometer (Functional group of drug):

This technique focuses on the infrared light absorption that results in molecular vibrations. Utilizing this technique, produced bottlebrush polymer prodrugs are characterized [1].

Efficacy Study of Bottlebrush Prodrug

Efficacy study of bottlebrush prodrug can be done by using in-vitro as well as in-vivo techniques

1. In-vitro: [1]

1.) Cytotoxicity assay:

By using the MTT assay, the cytotoxicity of the bottlebrush polymer prodrug was evaluated. The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity.

2.) Cellar uptake experiments:

Live cells are used in cellular uptake assay to study how substances affect receptor or transporter systems. A culture of developing cells or organisms is given a radioactively marked molecule and left to incubate, allowing the cells to absorb the molecule. Confocal laser scanning microscopy was used to look at how the bottlebrush polymer prodrug micelles were taken up and localized in living cells.

3.) Deep penetration study in multicellular spheroids:

Multicellular spheroids (MCS) of cancerous cells were cultivated to form a solid tumor in order to assess the deep penetration capability of bottlebrush prodrug.

2. In-vivo: [1]

1.) *In vivo* fluorescence imaging:

Firstly, bottlebrush prodrug was labelled with fluorescent material. Then, free fluorescent material-labelled bottlebrush prodrug was injected into the nude mice bearing cancerous tumors. After 24 h, the optical and fluorescence images of mice and the anatomical tumor, heart, liver, spleen, lung and kidney were obtained by using an NIR imaging system.

2.) Blood routine study:

KM female mice were used for routine blood tests. Specifically, the KM mice were divided into groups, and each group was injected with bottlebrush prodrug through tail veins. Peripheral blood from the orbit of each mouse was collected. Then, the blood indicators including the white blood cell count (WBC), mean corpuscular haemoglobin concentration (MCHC), lymphocyte ratio (Lyphm), red blood cell count (RBC), haemoglobin concentration (HGB), variation coefficient of red blood cell distribution width (RDW), platelets (PLT), haematocrit (HCT) and mean platelet volume (MPV) were analysed using a haematology analyser.

3.) Pharmacokinetic study:

In the plasma pharmacokinetic study, SD mice were randomly divided in groups and injected with bottlebrush prodrug through tail veins. At predetermined time intervals blood samples were collected from the orbit of each mouse. The plasma samples were obtained by centrifugation and purified by protein precipitation using methanol. The supernatant was collected by centrifugation and the fluorescence intensity of anticancer drug in the supernatant of each group was measured with the Tecan Spark-10 plate reader.

Application of Bottlebrush Polymer [1, 12, 17]

- Bottlebrush Polymers having variety of potential applications, including templates, elastomers, organic

optoelectronic materials, and energy storage, can be achieved by carefully designing BBPs with well-designed structures and chemical components.
- Bottlebrush polymers are used in the delivery of various types of medications, films, coatings, lubricants, nanolithography, chromatography media, photonics, and stimuli-responsive materials.
- Improved effectiveness of cancer treatment while minimizing its negative effects.

Application of Bottlebrush Prodrug [1, 12, 17]
- The Bottlebrush prodrugs could achieve self-controlled drug release in the tumor environment for cancer therapy by self-assembling to produce nano sized micelles in aqueous solution. The odds of immature burst are less likely to be noticed and focused drug distribution is accomplished as a result of the complicated structure of Bottlebrush prodrugs and controlled micelle production.
- The non-toxic, water-soluble carrier substance and the small size of the prodrug micelles contributed to the Bottlebrush prodrug's good biocompatibility, good circulation stability, and tumor permeability.
- The prodrug has high tumor suppression efficacy, strong intra-tumoral permeability, and good circulation stability, all of which are advantageous for the creation of next-generation nanomedicine for improved chemotherapy.
- A prodrug technique can ensures that drug is only active at a specific time or location to optimize the killing of cancer cells and decrease off-target damage.

Conclusion
The non-toxic, water-soluble carrier material and the tiny size of the prodrug micelles contributed to the prodrug's good biocompatibility, good circulation stability, and tumor permeability. The nano-system significantly improved the drug load's efficacy in treating tumor while posing no systemic risk to healthy tissues. Prodrug made of a pH-responsive amphiphilic polymer that could self-assemble into nanosized micelles in aqueous solution to enable self-controlled drug release in the acidic environment of tumor for the treatment of cancer. Polymer-based nanotherapeutics can react to the tumor microenvironment and have a great therapeutic effect because they have stable or adaptable chemical structures and superior biocompatibility.

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