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Self-assembled aptamer-hyperbranched polymer nanocarrier for targeted and photoresponsive drug delivery

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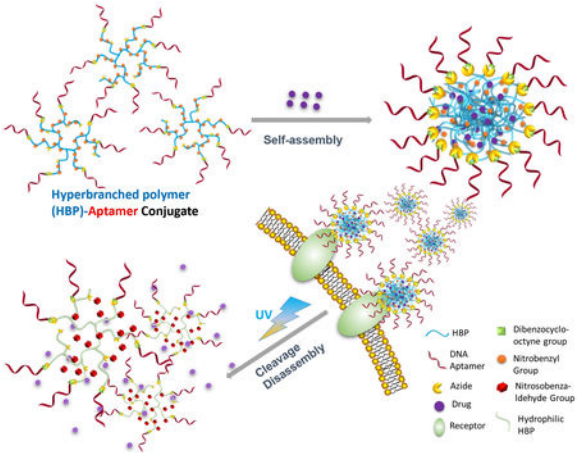
Abstract

Photoresponsive materials are emerging as ideal carriers for precise controlled drug delivery owing to their high spatiotemporal selectivity. However, drawbacks such as slow release kinetics, inherent toxicity, and lack of targeting ability hinder their translation into clinical use. We herein constructed a new DNA aptamer-grafted photoresponsive hyperbranched polymer, which can self-assemble into nanoparticles, thereby achieving biocompatibility and target specificity, as well as light-controllable release behavior. Upon UV-irradiation, rapid release induced by disassembly was observed for Nile Red-loaded nanoparticles. Further *in vitro* cell studies confirmed this delivery system's specific binding and internalization performance arising from the DNA aptamer corona. The DOX-loaded nanoassembly exhibited selective phototriggered cytotoxicity towards cancer cells, indicating its promising therapeutic effect as a "smart" drug delivery system.

Graphical Abstarct



Keywords

self-assemble; hyperbranched polymer; photoresponsive; aptamer; drug delivery

Interest in drug delivery systems (DDSs) has persisted over many decades, and considerable effort has been devoted to developing on-demand drug delivery systems able to achieve site-specific delivery with reduced side effects, such as premature release and rapid body clearance. Upon the arrival of DDSs at the target disease sites, a triggered release of the encapsulated drugs is highly desired. In view of this, the field of stimuli-responsive DDSs has flourished based on their ability to undergo dramatic chemical/physical changes that prompt release of therapeutics in response to environmental changes when crossing the interface between healthy and diseased tissues.

Various physiological stimuli, including pH, temperature, enzymatic action, and redox gradients, have been utilized to construct most stimuli-responsive DDSs.[1] However, tremendous challenges, such as achieving precisely controlled release rates, still remain due to the complexity of human physiology. As a result, previous DDSs have primarily been designed to respond to external stimuli, such as light, magnetic or electric fields, and ultrasound. In particular, light has recently attracted considerable attention to provide precise spatiotemporal control in drug release.[2] In addition, irradiation parameters, such as wavelength, luminous intensity, and irradiation time, can be easily modulated to control the drug release kinetics.

Besides precise control over drug release, it is also highly important to endow DDSs with an active targeting functionality which can induce the efficient delivery of therapeutics to specific disease sites.[3] Many previous reports by our group have demonstrated the potential utility of DNA aptamers because of their unique advantages, including flexible design, simple modification and negligible immunogenicity.[4]

Previous reports on photoresponsive carriers involving an aptamer moiety mainly focused on the utilization of metal or inorganic nanoparticles, such as silica, graphene, gold, and upconversion nanoparticles.[5] Nevertheless, the intrinsic cytotoxicity arising from these

nonbiodegradable nanoparticles remains a major concern for *in vivo* and clinical applications. As an important class of biomedical materials, polymer-derived nanoparticles are garnering considerable interest thanks to their enhanced permeation and retention effect, prolonged circulation, and low cytotoxicity.[6] Moreover, diverse chemical properties, such as biodegradability and stimuli-responsiveness, can be easily incorporated by rational design of polymer building blocks. Among different architectures of polymers, hyperbranched, or dendritic, polymers display unique structural features, such as internal cavities and a large number of functional groups in their periphery. As structure begets properties,[7] the presence of internal cavities enables encapsulation of small-molecule cargos like drugs. In addition, peripheral functional groups can serve as handles for further modifications with therapeutic molecules and/or targeting moieties.[8] Therefore, hyperbranched polymers are promising candidates for various applications in biological and biomedical fields.[9]

To the best of our knowledge, a light-sensitive drug delivery platform that relies on a biocompatible DNA-grafted polymer has not yet been reported. Therefore, in this contribution, we aimed to design a novel photoresponsive drug delivery system consisting of a DNA aptamer-grafted, ultraviolet (UV)-sensitive, and hyperbranched polymer (HBP) for aptamer-targeted cancer therapy and light-mediated drug release (Scheme 1). Such DNA aptamers not only exhibit specific and high affinity to cancer cells by enhanced receptor-mediated internalization, but also serve to functionalize the HBP and serve as the hydrophilic shell to enable HBP-DNA nanoparticle (HDNP) self-assembly and enhance colloidal stability of this amphiphilic nanostructure.[10] For a UV-responsive hydrophobic core of the delivery system, a HBP containing pendent *o*-nitrobenzyl moieties was synthesized. Moreover, pi-pi stacking effect arising from nitrobenzyl rings is also believed to contribute to self-assembly. Photoredox chemistry of *o*-nitrobenzyl derivatives will occur after UV irradiation, resulting in the liberation of free carboxylic acid groups and enhanced hydrophilicity of the core.[11] Not only does this hyperbranched polymer possess numerous hydrophobic inner cavities capable of encapsulating small-molecule therapeutics, but it also has a large number of azide groups which can be conjugated with dibenzocyclooctyne group (DBCO)-modified aptamer. After encapsulation of the model drug Nile Red, drug release kinetics was examined upon UV irradiation (365nm). In the presence of UV irradiation, the hydrophobicity of the DDS core was rapidly reduced due to cleavage of *o*-nitrobenzyl moieties, causing disassembly of the nanoparticles and concomitant drug release. Moreover, specific binding and internalization of polymer-sgc8 nanoparticles in CEM cells were demonstrated, suggesting unfettered functionality of the aptamer after biorthogonal strain-promoted azide-alkyne cycloaddition (SPAAC). Notably, Doxorubicin (DOX)-loaded polymer-sgc8 nanoparticles under UV-irradiation induced significantly more cancer cell death compared to the condition without UV. These results indicate that UV-responsive, DNA-grafted hyperbranched polymers may hold substantial promise as smart and effective drug delivery platforms.

The photoresponsive HBP was synthesized by self-condensing vinyl polymerization (SCVP) (Figure 1A).[12] In the course of polymerization, 2-(2-bromoisobutyryloxy) ethyl acrylate (BIEA) served as an inimer that led to branching. *O*-nitrobenzyl acrylate (NBA) was used as a comonomer to introduce the pendent UV-sensitive functional groups. Moreover, an appropriate amount of poly(ethylene glycol) acrylate (PEGA) was added as a comonomer to

provide increased hydrophilicity, enhanced permeability and prolonged circulation of the nanostructure.[13] Upon polymerization (total monomer conversion over 99%, shown in supporting information), pendent hydroxyl functionalities were transformed to bromines through esterification with 3-bromopropionyl chloride. After that, both terminal and side-chain bromines underwent substitution with sodium azide, leading to HBP-bearing clickable azide moieties (Figure 1A). The successful bromination was verified by nuclear magnetic resonance (NMR) spectroscopy and Fourier-transform infrared spectroscopy (FTIR). According to ^{1}H-NMR spectra, a new sharp signal (peak f) at 4.2 ppm was attributed to protons from methylene adjacent to the original hydroxyl after esterification. In addition, peak g at 2.8 ppm can be assigned to the methylene protons next to the bromines derived from 3-bromopropionyl chloride (Figure S5). In the FTIR, the disappearance of hydroxyl peark over 3000 also proved highly efficient bromination (Figure S6 and S8). Azide functionalization was proven to be quantitative by ^{1}H NMR spectroscopy, which showed full diminishment of peak g after substitution (Figure S7). FTIR spectra further demonstrated the appearance of a characteristic azide absorption at 2100 cm^{-1} after reaction (Figure S8). In addition, a strong signal at 1580 cm^{-1} was assigned to N-O stretching in *o*-nitrobenzyl functionalities, suggesting fidelity of the photo-sensitive NO_2 groups upon post-polymerization modification. Notably, gel permeation chromatography (GPC) analysis revealed the apparent molecular weight (MW) of HBP-OH to be 9.5 kDa (Poly(methyl methacrylate) (PMMA) calibration), which was significantly lower than the absolute MW (56 kDa) determined from GPC-multi angle light scattering (MALLS) (Figure 1B). This well-known phenomenon can be attributed to the globular and compact architecture of hyperbranched polymers as compared to the linear calibration standards.[14] Based on the absolute MW of HBP and molar ratio of BIEA/NBA/PEGA (1:5:2.5), each hyperbranched polymer should have on average of 23 BIEA units, 58 PEG-acrylate units, and 116 *o*-nitrobenzyl acrylate units. Moreover, the degree of branching (DB) of the hydroxyl functional polymer (HBP-OH) was determined to be 0.18, according to equation 1-3 (Supporting information) and Figure S5, thus confirming the hyperbranched structure of the polymer. The MW of aptamer sgc8 (sequence information seen in Table S1) measured by GPC-MALLS is consistent with its theoretical MW (13307.9 g/mole, from Integrated DNA Technologies, Inc.)

The azide groups of the hyperbranched polymer allowed for further conjugation with DBCO-modified DNA via SPAAC “click” chemistry in dimethyl sulfoxide (DMSO).[15] This metal-free biorthogonal conjugation can efficiently click two macromolecular components at high yield without compromising the properties of either component, in particular, the aptamer’s targeting efficiency.[16] Self-assembled nanoparticles were prepared by a nanoprecipitation method (Figure S10). According to native polyacrylamide gel electrophoresis (N-PAGE) analysis (Figure S11), a new band with low mobility, staying at the top was observed and ascribed to the polymer-DNA conjugate, indicating the successful click reaction. Excess free DNA aptamer in the crude product was removed by ultra-centrifugation (membrane size 30 kDa) (Figure S11). MW of HBP-DNA was measured as 341 kDa by GPC-MALLS (Figure 1B). Therefore the number of sgc8 on each polymer molecule was calculated to be 20 based on the MWs of aptamer sgc8, HBP and HBP-sgc8

conjugate. The negative shift on the zeta potentials of HBP and HDNP further proved the successful ligation of negative charged DNA aptamers on the neutral HBP (Figure 1C).

Self-assembly behavior of HBP-DNA conjugates was elucidated by dynamic light scattering (DLS) and transmission electron microscopy (TEM). According to DLS, HDNP displayed a hydrodynamic diameter of 40 nm with a narrow distribution (PDI = 0.17) by intensity (Figure S12). Notably, the size of the nanoparticles remained constant without aggregation after 24 h at room temperature, indicative of their excellent colloidal stability (Figure S13). The uniform size and core-shell morphology of the nanoparticles were further visualized by TEM. As shown in Figure 1D, the hydrophilic DNA-based outer layer of spherical nanoparticles could be clearly observed when negative stain was applied in sample preparation. In addition, high absolute value the HDNP zeta potential (−68.3mV) indicated the high colloidal stability attributed to out layer DNA aptamers (Figure 1C). To quantify the DNA strands on each HNDP, DNA aptamer strand was modified with tetramethylrhodamine (TAMRA) at 3’ end to provide concentration related fluorescent intensity. Number of particles was measured to be $1.54 \pm 0.2 \times 10^{11}$ /ml by Nanoparticle Tracking Analysis (NTA). According to the total concentration of DNA and HDNP (Support Information), the average DNA strands was estimated to be 1096 per HDNP. As the there were 20 DNA strands on each HBP molecule, the aggregation number of HBP-DNA conjugate forming into HDNP was about 54.

The photoresponsiveness of this drug delivery system relies on *o*-nitrobenzyl moieties in the HBP side chains, which can be rapidly cleaved under UV irradiation at 365 nm (Figure 2A). To elucidate the light-responsiveness of the HBP-DNA conjugate, a proof-of-concept experiment was designed, involving time-dependent UV treatment of HBP-OH. Since the cleaved fragments are fluorescent *o*-nitrosobenzaldehyde groups, we observed a gradual increase in fluorescence intensity (Figure 2B), especially the rapid increase within 10 min. Moreover, the decreased absorption peak at 260 nm indicates that the *o*-nitrobenzyl groups underwent photodegradation as UV irradiation time increased. The gradual increase of absorption at 320 nm is ascribed to the formation of nitrosobenzaldehyde groups in buffer (Figure 2C).

Since our purpose was to utilize this photoresponsiveness to control the release of drug molecules, we further examined loading efficiency (Support Information) and light-induced release behavior of a hydrophobic model drug, Nile Red (NR), based on HDNP. The fluorescence of NR is present only in an organic or hydrophobic environment and is fully quenched in aqueous phase.[17]. As HDNPs disassembled in response to UV stimulus, NR was released into the buffer solution, resulting in quenching of the fluorescent signal. Especially, in the first 15 min, the rapidly decreasing fluorescent signal implied instantaneous disassembly of HDNP and rapid release of NR (Figure 2D). This observation is consistent with the fluorescence enhancement of nitrosobenzaldehyde (Figure 2B). Based on these results, we concluded that HDNPs rapidly degraded to a large extent within 20 min upon irradiation. Importantly, HDNP exhibited good stability in PBS buffer with serum at 37 °C. Constant sizes at 10 and 24 h were observed by DLS (Figure S13), and no obvious leakage of loaded NR occurred after 15 h in the dark (Figure 2E). To demonstrate the ability of our DDS to remotely and precisely control the release, HDNP solutions were irradiated

for three “on-off” cycles (Figure 2F). As expected, pulsatile release was achieved, as evidenced by a significantly faster rate when the light was on. Such release kinetics results unequivocally credit the liberation of NR to the UV trigger. Because light can be operated easily and precisely, we envision that an HDNP-based DDS is quite promising to deliver and release therapeutics with control of site, time, and dose of UV irradiation.

Sgc8 is an aptamer selected through cell-systematic evolution of ligands by exponential enrichment (SELEX). It features selective binding affinity to cells, such as CCRF-CEM, with high expression of protein tyrosine kinase 7 (PTK7).[18] However, other cell lines lacking PTK 7, such as Ramos cells, cannot be targeted by sgc8. Library DNA was applied as a negative control in our study (Table S1). This mixture of random sequence DNA strands with a number of bases equal to sgc8 showed no binding affinity to either CCRF-CEM or Ramos cells. Sgc8 and Library labeled with TAMRA were applied for flow cytometry and confocal microscopic detection. As shown by flow cytometry, CEM cells treated with HDNP-sgc8 resulted in a much larger shift compared to CEM cells treated with HDNP-Library. Furthermore, only a negligible shift was observed in the case of Ramos cells treated with either HDNP-sgc8 or HDNP-Library (Figure 3A). In a further cellular uptake study, highly efficient cell internalization of HDNP-sgc8 was observed in CEM cells, but not Ramos cells, under confocal microscopy. These results clearly demonstrated the exceptional targeting specificity and cellular uptake of sgc8-functionalized nanoparticles (Figure 3B).

Prior to the cytotoxicity study with HDNP-sgc8, the maximum exposure time of CEM cells under UV light was determined to ensure a safe threshold of irradiation. As shown in Figure S15, no reduction of cell viability was observed in the first 20 min (Figure S19). On the other hand, UV-triggered release of NR was instantaneous, resulting in the release of more than 70% of Nile Red within 20 min (Figure 2D). Therefore, the UV irradiation time was set to 15 min to achieve greater nanoparticle disassembly and significant drug release without cell damage. To verify the material’s intrinsic safety and biocompatibility, CEM cells were cultured with medium containing different concentrations of HDNP-sgc8 followed by 15 min of UV irradiation. As shown in Figure S20, no toxicity was observed, even at a high dose of 200 μg/mL.

Based on the established method of loading NR into HDNP, the cancer therapy drug doxorubicin (DOX) was encapsulated into HDNP for selective and light-triggered therapy study. Pretreated hydrophobic DOX was added at 20% weight of HBP-DNA conjugate, leading to high drug-loading content (DLC) and efficiency (DLE), which were determined to be 32.35 ± 1.17 μg/mg and 16.28% ± 0.67%, respectively by equation 4 and 5 (Supporting Information) The stability of DOX-loaded HDNP was illustrated by unchanged particle size at around 50 nm for 24 hours in PBS with 10% serum (Figure S21). In the following investigation of *in-vitro* therapeutic effect (Figure 3C), DOX-loaded HDNP-sgc8 and DOX-loaded HDNP Library were separately incubated with CEM cells. As expected, DOX-loaded HDNP-sgc8 showed significantly higher cytotoxicity after UV irradiation compared to that of HDNP-sgc8 without UV light, indicating that more DOX was released when UV stimulus was applied. However, in the case of HDNP-Library, less cytotoxicity was observed compared to the sgc8-based systems, irrespective of UV exposure. The obvious cytotoxicity difference among these four groups demonstrated that HDNP is an effective drug delivery

system able to perform target specificity, due to the aptamer on the outer shell, and controllable drug release, depending on external UV stimulus.

In conclusion, we have, for the first time, developed a selective targeting and light-controlled drug delivery nanostructure formed by self-assembly of a DNA-grafted polymer. A new photoresponsive and hyperbranched polymer was designed and prepared by straightforward one-step SCVP. An aptamer was efficiently conjugated with the hyperbranched polymer by bioorthogonal click chemistry, allowing unfettered functionality of both aptamer and polymer. Self-assembled nanoparticles sustained colloidal stability and homogeneous distribution owing to the negatively charged DNA shell. Moreover, efficient loading, externally-triggered controllable release, and rapid release kinetics were observed for the model drug, NR. *In vitro* cell study demonstrated excellent binding to target cancer cells and cellular uptake behavior. DOX-loaded HDNPs significantly inhibited tumor cell proliferation, but only in the presence of aptamer sgc8-grafted HDNP and only when external UV stimulus was imposed. By achieving targeted drug delivery and remote pulsatile drug release, this photoresponsive DNA aptamer-hyperbranched polymer nanoassembly could act as an efficient drug delivery platform, highlighting its immense potential in cancer therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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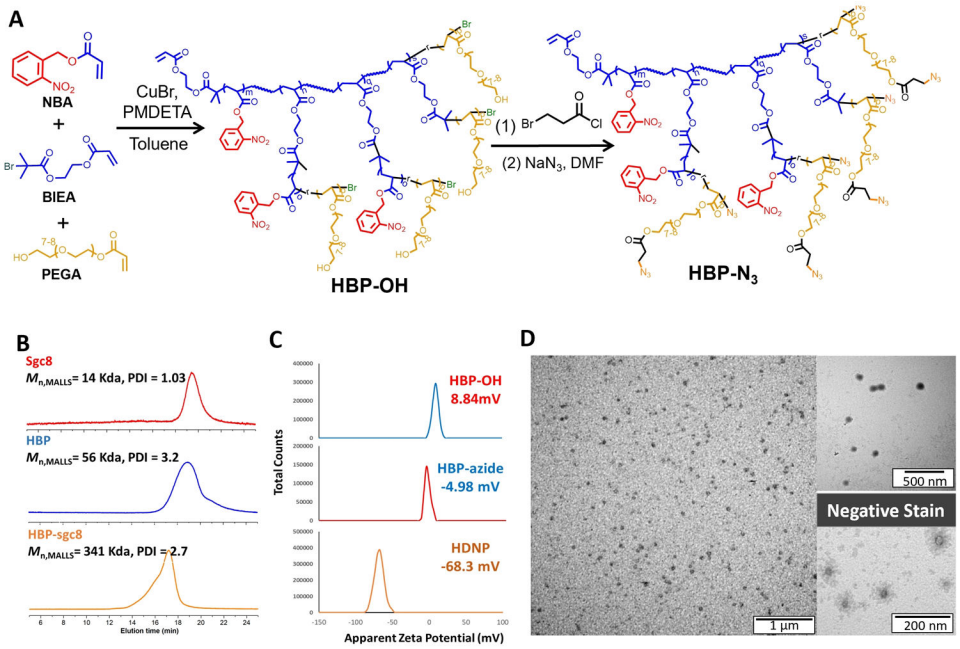


Figure 1.

(A) HBP synthesis pathway. (B) GPC trace of aptamer sgc8, HBP and HBP-sgc8 conjugate. (C) Zeta potential of HBP-OH, HBP-N_3 and HDNP. The highly negative potential on HDNP indicates the successful ligation of DNA on polymer and stable colloidal stability of HDNP. (D) TEM images of polymer-DNA nanoparticles. Inset: Magnified image of negatively stained (phosphotungstic acid) polymer-DNA nanoparticles. The high-magnification HDNP example shows an outer layer on the polymer core, which we attribute to the hydrophilic aptamer.

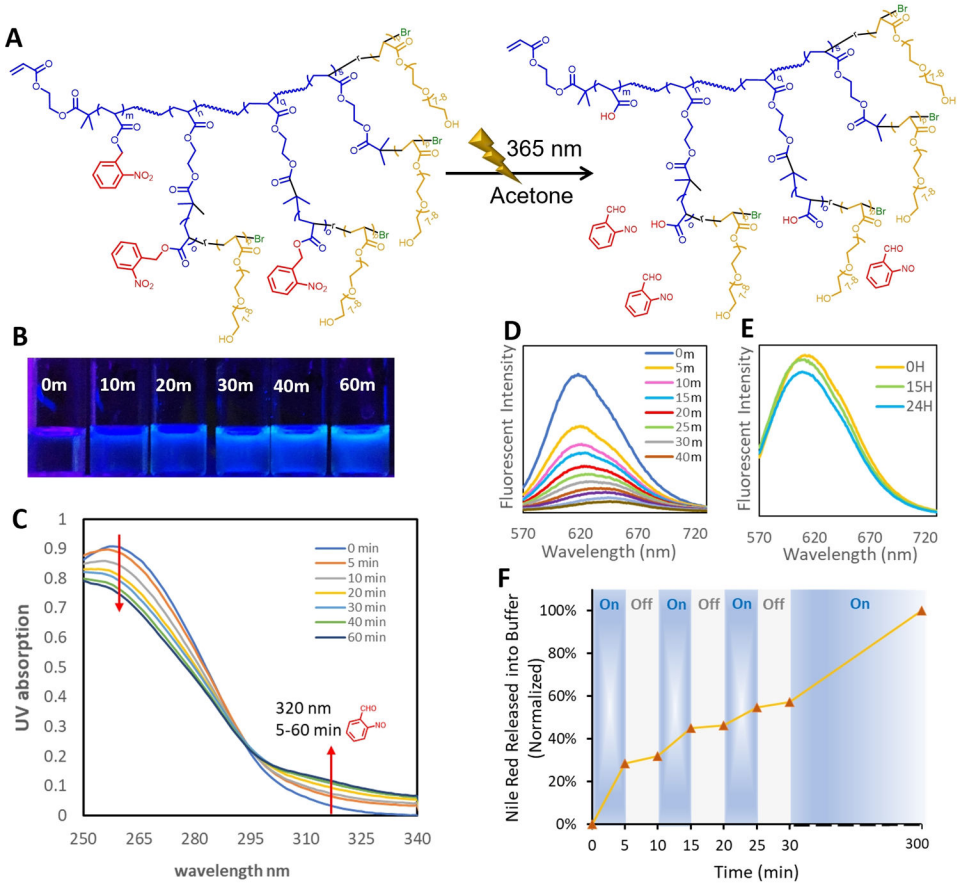


Figure 2.

(A) Schematic illustration of UV-promoted degradation of HBP-OH; (B) Photos of fluorescent HBP solutions in acetone under UV irradiation at different times; (C) UV absorption spectra of HDNP solutions after UV treatment for varying times; (D) Fluorescence emission spectra (excited at 430 nm) of NR-loaded polymer-DNA nanoparticles under UV irradiation for varying times; (E) Fluorescence spectra of NR-loaded HDNP in PBS+10% serum for different times in the absence of UV light; (F) On-demand release of NR release from NR-loaded nanoparticles can be triggered multiple times to provide time specific release.

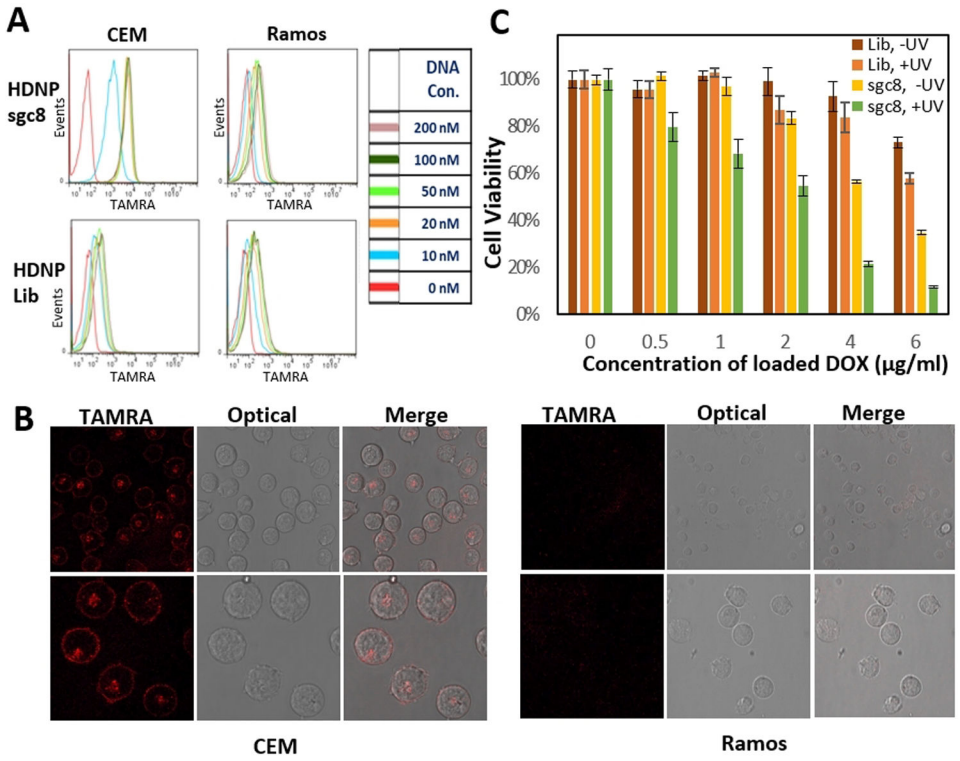
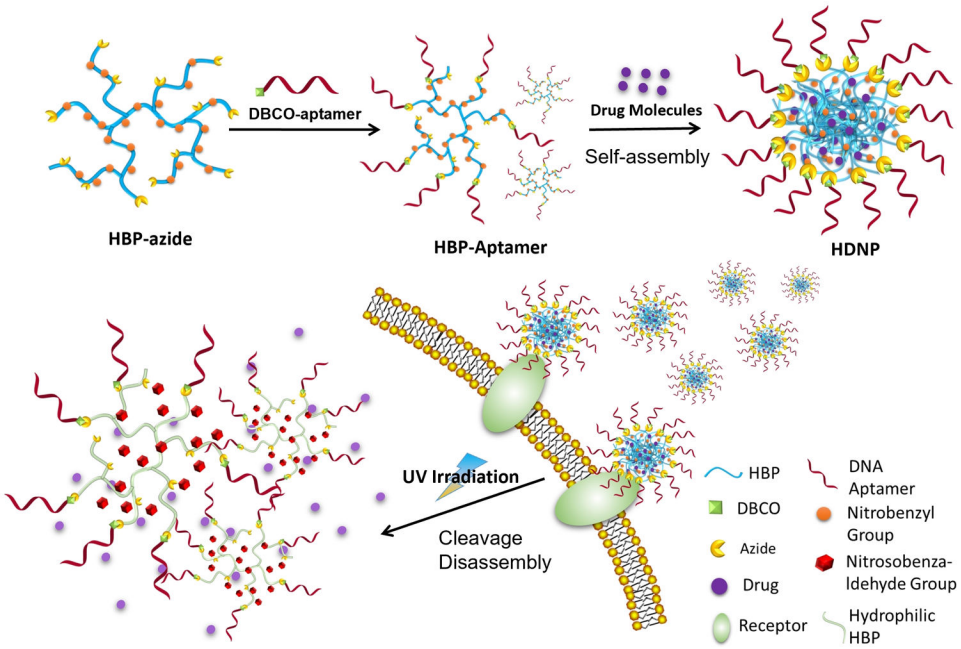


Figure 3.

(A) Cell-binding test of HDNP by flow cytometry; (B) Cell internalization test of HDNP by Confocal microscopy; (C) Cytotoxicity of DOX-loaded HDNP-sgc8/Library with and w/o UV light.



Scheme1.

Design and construction of targeted and photoresponsive drug delivery system based on aptamer-grafted hyperbranched polymer. On-demand and controlled release can be achieved when UV irradiation is applied.