Supramolecular Assembly of Coronene Derivatives for Drug Delivery

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Supporting Information

ABSTRACT: Possessing a small size and $C_3$-symmetrical rigid backbone, a coronene derivative was synthesized from $\beta$-cyclodextrins and hexa-cata-hexabenzocoronene, and then a water-soluble and biocompatible nanographene/polysaccharide supramolecular assembly was successfully fabricated through noncovalent interactions between adamantly grafted hyaluronic acids and $\beta$-cyclodextrin-modified hexa-cata-hexabenzocoronene. Moreover, the ternary supramolecular assembly showed not only a fluorescence imaging ability toward cancer cells but also good anticancer activity and low toxicity.

As a kind of fascinating nanomaterial, coronene, also called nanographene, has attracted increasing attention in many fields for use in electronic devices,¹ chemical sensing,² and cellular imaging.³ Given these special characteristics, one can hypothesize that incorporating nanographene into composite materials may bring a breakthrough in nanoscience and technology. In the early stages, scientists mainly focused their efforts on applications of nanographene (such as hexa-peri-hexabenzocoronene) in electronics owing to the special intrinsic properties such as hole-transport capability, charge-carrier mobilities, and $\pi$-stacking induced self-assembly abilities.⁴ Recently, more attention has been paid to applications of hexa-peri-hexabenzocoronene in biological systems, which displayed an efficacious ability compared to those of graphenes and carbon nanotubes as cell-imaging agents and drug carriers. Prasad et al.⁵ prepared a kind of water-dispersible polymeric micelle encapsulated with hexa-peri-hexabenzocoronene nanographene for cellular imaging. Müller et al.⁶ reported the assembly of peptides and hexa-peri-hexabenzocoronene modified with special functional groups for bioprobing. Xiong et al.⁷ demonstrated that HepG2 cancer cells could be selectively killed by three-dimensional nanographene. In sharp contrast with hexa-peri-hexabenzocoronene, the application of hexa-cata-hexabenzocoronene in biological areas has been rarely reported. On the other hand, cyclodextrin (CD), a class of cyclic oligosaccharides that can encapsulate various organic or biological molecules in their hydrophobic cavities, and hyaluronic acid (HA), a widely distributed glycosaminogly that can recognize the CD44/RHAMM receptors overexpressed on cancer cells,⁸ are water-soluble, biocompatible, and widely used in biological systems as drug carriers.⁹ In this work, we report a nanographene/polysaccharide supramolecular assembly, i.e., CHBC-2/HA-AD assembly (Scheme 1), constructed from hexa-cata-hexabenzocoronene,¹⁰ $\beta$-CD, and HA via a bottom-up strategy. First, CHBC-2, which possesses three $\beta$-CD cavities and a $C_3$-symmetrical rigid fluorescent nanographene core, was synthesized in satisfactory yield (52%) through a click reaction. Subsequently, the targeted polysaccharide HA was noncovalently linked to CHBC-2 through the strong binding of adamantanyl moieties grafted to HA with $\beta$-CD cavities.¹¹ There are several advantages to combining nanographene with $\beta$-CD and HA: (1) it is possible to take advantage of the intrinsic fluorescence properties of coronene derivatives (such

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as anti-photobleaching ability, strong fluorescence emission, and excitability at the β/π band in the UV/vis region, the Cβ-symmetrical nanographene could act as a fluorescence probe in cell imaging reagents; (2) the introduction of β-CD and HA units can not only efficiently increase the water solubility and biocompatibility of nanographene but also prevent the self-quenching of graphene, leading to fluorescence emission that could be readily distinguished by the naked eye; (3) encapsulation and loading efficiency of anticancer drugs could be improved by introducing the small and rigid backbone nanographene to this drug delivery system. Furthermore, the noncovalently loaded anticancer drugs could be readily delivered to cancer cells owing to the targeting ability deriving from the hyaluronated adamantane (HA-AD) chains, in which the HA skeleton can specifically recognize HA receptor-overexpressing tumor cells in cancer metastasis. Consequently, the CHBC-2/HA-AD assembly could not only act as an imaging agent with photostability and low toxicity toward cancer cells but also be utilized as a convenient platform for targeted drug delivery. To the best of our knowledge, this kind of nanographene/polysaccharide supramolecular conjugate has not been reported so far despite the advantages mentioned here.

Synthetic route to the water-soluble Cβ-symmetrical nanographene derivative CHBC-2 is shown in Scheme 2. Vanillin reacted with 3-bromopropyne in the presence of K2CO3 to give compound 2. Intermediate compound 3 was synthesized by Suzuki reaction of 3,4-dialkoxyphenylboronic acid with 1,3,5-tri(bromomethyl)benzene. Although a series of Cβ-symmetrical hexa-cata-hexabenzocoronene derivatives were successfully synthesized, further derivation is still difficult. According to the reported method of Wei et al., the intermediate compound 3 reacted with compound 2 in the presence of FeCl3 and anhydrous acetic anhydride in anhydrous DCM to give the building block with three alkynyl-substituted hexa-cata-hexabenzocoronenes 4 in 23% yield. Subsequently, three β-CD-modified nanographene has been synthesized from 4 and excess 6-deoxy-6-azido-β-CD in anhydrous DMF by means of “click chemistry” in 52% yield. As shown in Figure S1, two singlet signals lying at 5.06 and 2.63 ppm were assigned to the protons of methylene and alkynyl in compound 4, respectively. A clear single peak at 8.29 ppm was assigned to the proton of the triazole ring (Figure S4), indicating that three β-CDs were grafted to the nanographene. Moreover, both compound 4 and CHBC-2 were further confirmed by MALDI-TOF experiments (Figures S3 and S6).

Subsequently, the CHBC-2/HA-AD assembly could be constructed conveniently by the association of CHBC-2 with HA-AD in aqueous solution due to the strong binding of adamantane moieties to the β-CD cavity in CHBC-2. By monitoring the UV–vis spectrum of CHBC-2/HA-AD at 470 nm, the photometric standard curve of CHBC-2/HA-AD was measured (Figures S22 and S23). The solution of CHBC-2/HA-AD at 500 μM was turbid; thus, the solution was centrifuged and filtrated. The UV–vis spectrum of the obtained clear solution was recorded, and the water solubility of supramolecular assembly was calculated as 6.98 mg/mL. The obvious Tyndall effect (Figure S15) indicated the formation of a large CHBC-2/HA-AD supramolecular assembly. Both TEM and SEM images at different magnifications (Figure S17) showed that the CHBC-2/HA-AD assembly existed as discrete spherical particles with an average diameter of ca.140 nm, accompanied by a relatively narrow particle size distribution (Figure S17i). In addition, the hydrodynamic diameter of the assembly was also measured by DLS to be ca. 234 nm. Moreover, the effect of the HA-AD/CHBC-2 ratio on the structure of supramolecular assemblies was also studied. As shown in Figure S24, the sizes of supramolecular assemblies gradually increased and then slowly decreased with increasing HA-AD/CHBC-2 ratio, and a HA-AD/CHBC-2 ratio of 3:1 was the lowest ratio to obtain soluble assembly.

ζ potential measurements gave a ζ potential of CHBC-2/HA-AD assembly as −45 mV, indicating that the assembly possessed the capability of associating cationic substrates. It is noteworthy that, when excited at the β/π band of hexa-cata-
hexabenzocoronene, CHBC-2 emitted bright green fluorescence (Figure S8), which could be readily distinguished by the naked eye. This photophysical property of CHBC-2 is quite different from that of reported graphenes that barely fluoresced or emitted in at near-infrared fluorescence.12 Furthermore, when HA-AD (0–3.0 equiv) was added to the solution of CHBC-2, the fluorescence intensity of CHBC-2 did not change (Figure S9). Significantly, the CHBC-2/HA-AD assembly also presented good luminescence properties in living cells. Figure 2

![Fluorescence confocal microscopic images of NIH3T3 (a) and MCF-7 cells (b) after 24 h incubation with CHBC-2/HA-AD.](image)

Figure 2. Fluorescence confocal microscopic images of NIH3T3 (a) and MCF-7 cells (b) after 24 h incubation with CHBC-2/HA-AD.

It is also noteworthy that, even after the loading of DOX, the CHBC-2/HA-AD assembly still maintained a negative surface charge, which would favor its biocompatibility and prolong its circulation time in vivo. These results jointly indicate that the anticancer drug DOX was efficiently loaded onto the CHBC-2/HA-AD assembly to form biocompatible DOX@CHBC-2/HA-AD conjugates. By monitoring the UV–vis spectrum of DOX at 490 nm, the photometric standard curve of DOX was recorded (see Figures S11 and S12). Accordingly, the encapsulation and loading efficiency of DOX were calculated to be 81.4% and 11.7%, respectively (Figure S13), which was much higher than the previously reported drug delivery system.9 In this assembly, the rigid backbone nanogranophere played an important role in enhancing the encapsulation and loading efficiency. TEM and SEM images at different magnifications (Figure 1 and Figure S17) showed that, after DOX loading, the CHBC-2/HA-AD assembly remained its original morphology, accompanied by a relatively narrow particle size distribution (Figure S17) but its average diameter contracted to ca. 90 nm (hydrodynamic diameter contracted to ca. 221 nm measured by DLS), and this size would facilitate the endocytosis of DOX@CHBC-2/HA-AD into cancer cells.13 On the other hand, the release behavior of DOX@CHBC-2/HA-AD was also examined in physiological environments (0.01 M phosphate buffer solution, pH = 5.7 or 7.2, 37 °C). The DOX@CHBC-2/HA-AD displayed the slow and controlled release of drug in either an acidic or a neutral environment (Figure S14), and the release efficiency of DOX from DOX@CHBC-2/HA-AD at pH 5.7 (the endosomal pH of a cancer cell) was 1.4 times higher than that at pH 7.2 (physiological pH). This pH-responsive release of drug in the cancer cell environment will not only improve its cytotoxic efficacy against tumor cells but also reduce the toxicity of drug to normal tissues. It is also important to examine the in vitro cytotoxicity of DOX@CHBC-2/HA-AD. As shown in Figure S19, after a 24 h incubation, DOX@CHBC-2/HA-AD displayed a higher anticancer activity (relative cellular viability 42.0%) than free DOX (relative cellular viability 57.3%) toward MCF-7 cancer cells. The half-maximal inhibitory concentration (IC50) of DOX@CHBC-2/HA-AD was calculated to be 1.1 μg/mL (Figure S21). This result was 60 times lower than the corresponding value of our previously drug delivery assembly.14 A possible reason may be that the specific association between HA units in DOX@CHBC-2/HA-AD and HA receptors on cancer cell surfaces facilitated the incorporation and uptake of DOX@CHBC-2/HA-AD into MCF-7 cancer cells through the receptor-mediated endocytosis. Furthermore, when an excess amount of HA was added, the relative cellular viability of MCF-7 increased to 47.0% after treatment with DOX@CHBC-2/HA-AD, attributed to the fact that HA receptors on MCF-7 cell surfaces were blocked by an excess amount of HA.15 In addition, DOX@CHBC-2/HA-AD gave a higher cellular viability (79.4%) toward the NIH3T3 cells than free DOX (71.6%) after 24 h, indicating the low toxicity of DOX@CHBC-2/HA-AD toward normal cells. In control experiments, the CHBC-2/HA-AD assembly displayed nearly no cytotoxicity toward both MCF-7 and NIH3T3 cells. These phenomena jointly indicated that the CHBC-2/HA-AD supramolecular assembly could be utilized as a safe and promising targeted drug delivery platform. Similar results were also observed in the case of 48 h of incubation.
In summary, a water-soluble C$_x$-symmetrical rigid backbone nanographene, which has application potential as a cell imaging agent, was successfully synthesized and characterized by NMR, MALDI-TOF MS, UV/vis, and fluorescence. Subsequently, a multifunctional supramolecular platform was successfully constructed by the supramolecular assembly of β-CD-modified hexa-cata-hexabenzocoronene with the adamantyl-grafted HA. As two typical examples of possible applications, it could selectively image cancer cells over normal cells, and its conjugate with the anticancer drug DOX displayed a higher antitumor activity and a lower toxicity than the free DOX. Considering the good water solubility and biocompatibility of both β-CD-modified hexa-cata-hexabenzocoronene and adamantyl-grafted HA, this achievement would not only provide new access to associating nanographene with functional substrates but also extend the possible application of nanographene in many fields of pharmaceutical chemistry and biological technology.

ASSOCIATED CONTENT

Supporting Information
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Experimental details (PDF)

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Notes
The authors declare no competing financial interest.

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