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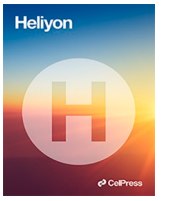

Research article

Novel near-infrared BODIPY-cyclodextrin complexes for photodynamic therapy

Bowei Lu [a,1], Xu Lu [b,1], Manman Mu [d], Shuxian Meng [a,**], Yaqing Feng [a,***], Yi Zhang [a,c,*]

[a] School of Chemical Engineering and Technology, Institute of Molecular Plus, Tianjin University, Tianjin, China
[b] Ministry of Health and Medical, General Hospital of Tianjin Medical University, Tianjin, China
[c] Haihe Laboratory of Sustainable Chemical Transformations, Tianjin, China
[d] School of Chemistry and Chemical Engineering, Tianjin University of Technology, Tianjin, China

A B S T R A C T

To meet the requirements of diagnosis and treatment, photodynamic therapy (PDT) is a promising cancer treatment with less side-effect. A series of novel BODIPY complexes (BODIPY-CDs) served as PDT agents were first reported to enhance the biocompatibility and water solubility of BODIPY matrix through the click reaction of alkynyl-containing BODIPY and azide-modified cyclodextrin (CD). BODIPY-CDs possessed superior water solubility due to the introduction of CD and their fluorescence emission apparently redshifted (>90 nm) on account of triazole units as the linkers compared to alkynyl-containing BODIPY. Moreover, all the BODIPY-CDs were no cytotoxicity toward NIH 3T3 in different drug concentrations from 12.5 to 200 μg/mL, and had a certain inhibitory effect on tumor HeLa cells. Particularly, BODIPY-β-CD exhibited high reactive oxygen species generation and excellent photodynamic therapy activity against HeLa cells compared to other complexes. The cell viability of BODIPY-β-CD was dramatically reduced up to 20% in the concentration of 100 μg/mL upon 808 nm laser irradiation. This architecture might provide a new opportunity to develop valuable photodynamic therapy agents for tumor cells.

1. Introduction

Photodynamic therapy (PDT) is a promising therapy strategy for a controllable and noninvasive cancer treatment due to low side effects, high therapeutic selectivity and efficiency [1–4]. Typically, PDT requires three primary components, that is to say photosensitizer, light and oxygen [5,6]. Several organic photosensitizers have been reported in PDT including porphyrin, phthalocyanine and cyanine-based derivatives [7–9] but the weak absorption in near-infrared region, low photo-stability and high cytotoxicity restrict their biocompatibility and applications. Moreover, these conventional photosensitizers are prepared by the complicated synthetic processes and purification procedures. Thus, the development of novel, facile and effective PDT photosensitizers with biocompatibility and broader absorption range is desirable.

Recently, boron dipyrromethene (BODIPY) derivatives are a concerned near-infrared fluorescent dye with the characteristic of high extinction coefficient, high fluorescence quantum yield, narrow absorption and emission bands, excellent photostability [10–15]. Therefore, the BODIPY derivatives have attracted immense research interests as these assemblies offer various applications in the areas like pharmaceuticals, stabilizations of organic dyes/drugs, targeted drug delivery [16–18], and, of course, as photosensitizers have also

* Corresponding author. School of Chemical Engineering and Technology, Institute of Molecular Plus, Tianjin University, Tianjin, China.
** Corresponding author.
*** Corresponding author.
E-mail addresses: msxmail@tju.edu.cn (S. Meng), yqfeng@tju.edu.cn (Y. Feng), yi.zhang@epfl.ch (Y. Zhang).
[1] These authors contributed equally to this work and should be considered co-first authors.

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been applied in drug tracking, cancer diagnosis and fluorescent switch [19–21]. However, the absorption and emission wavelength of BODIPY dye are difficult to reach the near-infrared region which had more absorption disturbance in bio-imaging. Furthermore, the low water solubility and weak biocompatibility limit their application in living systems [22,23]. Aiming at these problems, the strategy by grafting hydrophilic molecule with BODIPY core is proposed to broaden the absorption wavelength and increase water solubility, which further achieve the purpose of PDT. Some hydrophilic proteins, lipidosome and PEG materials have been incorporated into hydrophobic BODIPY core but still face some obvious issues including complicated preparation procedures, high costs and undefined structures [24–26]. Based on this, cyclodextrin (CD) as a cheap hydrophilic molecule are grafted on BODIPY core to largely improve the water solubility and biocompatibility [27–30].

Herein, three novel near-infrared BODIPY-CD complexes by the click reaction of alkynyl-containing BODIPY and azide-modified CD were designed and synthesized (Scheme 1). The CD units were decorated by BODIPY core to improve the water solubility and the obtained BODIPY-γ-CD possessed the broader absorption and fluorescence emission range compared with BODIPY core. Moreover, all the BODIPY-CDs were no cytotoxicity toward NIH 3T3 and tumor HeLa cells in the dark. Finally, the photodynamic therapeutic properties of BODIPY-CD complexes were investigated against tumor HeLa cells with 808 nm laser irradiation [31–33].

2. Materials and methods

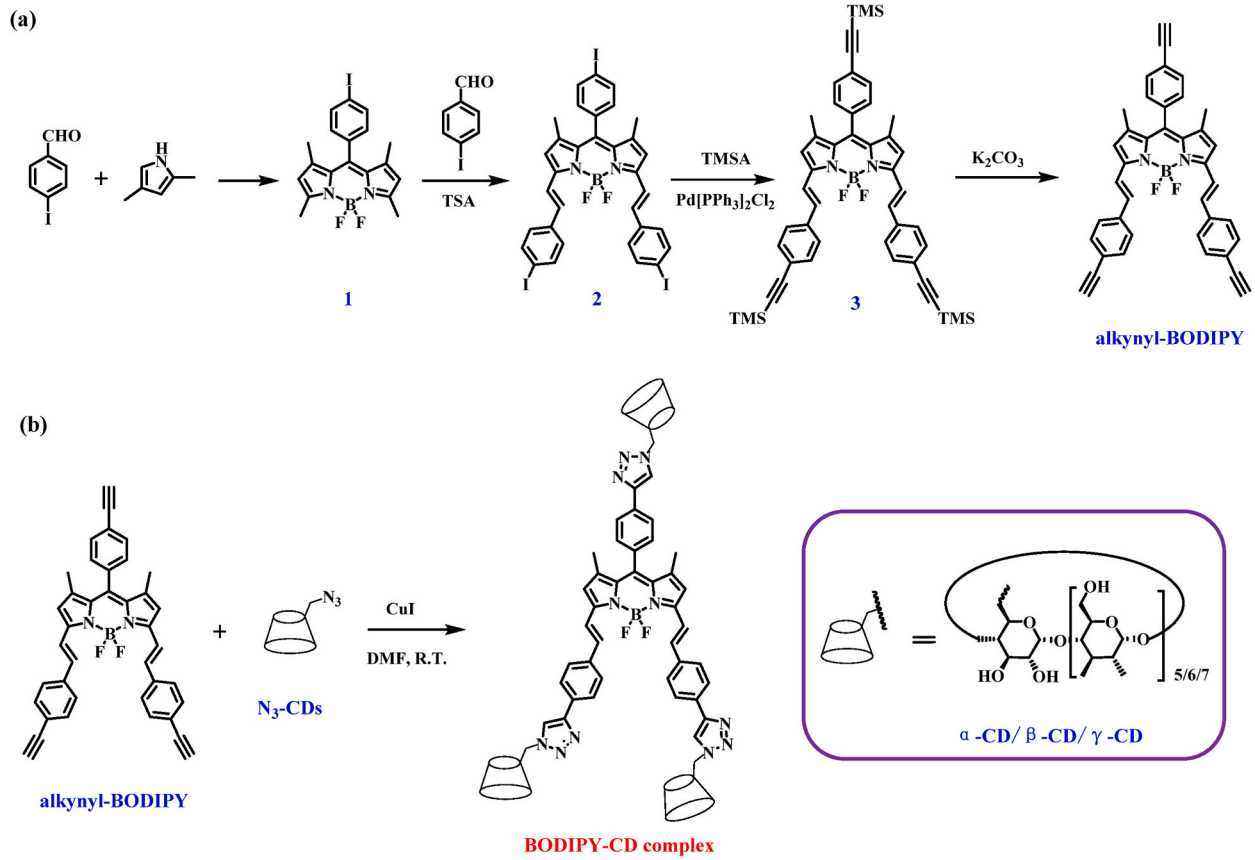
2.1. Materials

All the reagents were purchased from commercial sources and used without purification. The corresponding characterizations are located in the Supporting Information.

2.2. Preparation of BODIPY-CD complexes

2.2.1. Preparation of alkynyl-BODIPY dye (Scheme 1a)

Preparation of 4-iodophenyl BODIPY 1: 2, 4-dimethylpyrrole (0.98 mL, 9.50 mmol) and 4-iodobenzaldehyde (1g, 4.31 mmol) were dissolved in 100 mL DCM at room temperature under N_2 gas. Trifluoroacetic acid (0.066 mL, 0.862 mmol) was added dropwise and the suspension was stirred at 5 h. Then, DDQ (1.08 g, 4.73 mmol) was added and the suspension continued to be stirred at 3 h.



Scheme 1. The synthesis route of (a) alkynyl-BODIPY dye and (b) BODIPY-CD complexes.

When the mixture was cooled dawn to 0–5 °C, TEA (13.14 mL, 94.7 mmol) was added and stirred for 20 min, followed by the dropwise addition of $BF_3{\cdot}OEt_2$ (16.2 mL, 129 mmol) to the reaction for overnight. The mixture was washed with H_2O and DCM, dried with anhydrous magnesium sulfate. Finally, the solvent was evaporated followed by purification using column chromatography. (Yield: 0.729 g, 37.2%) ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.87$ (d, $J = 8.3$ Hz, 2H, ArH), 7.07 (d, $J = 8.3$ Hz, 2H, ArH), 6.01 (s, 2H, pyrrole-H), 2.57 (s, 6H, CH_3), 1.44 (s, 6H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 155.86$, 142.97, 138.38, 134.51, 131.13, 129.94, 121.48, 94.80, 47.08, 14.69, 8.73. HRMS (ESI): *m/z* calcd for $C_{19}H_{18}BF_2IN_2$ $[M+H]^+$ 450.0580; found 450.0587.

Preparation of dibromophenyl BODIPY 2: BODIPY 1 (700 mg, 1.56 mmol) was dissolved in 30 mL piperidine. Then 4-iodobenzaldehyde (1.266 g, 5.46 mmol) and TSA (0.06 g, 0.312 mmol) were added successively, and the reaction was heated at 95 °C for 2 h until the reaction color was changed from orange to dark blue. The mixture was washed with EtOH, dried overnight. (Yield: 1.15 g, 85%) ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.76$–7.69 (m, 8H, ArH), 7.37 (d, $J = 8.4$ Hz, 4H, ArH), 7.21 (dd, $J = 12.9$ Hz, 4H, CH═CH), 6.67 (s, 2H, pyrrole-H), 1.51 (s, 6H, CH_3).

Preparation of TMS-modified BODIPY 3: BODIPY 2 (500 mg, 0.57 mmol), $Pd[PPh_3]_2Cl_2$ (60 mg, 0.086 mmol) and CuI (32.57 mg, 0.17 mmol) were dispersed in 8 mL THF and 4 mL TEA at room temperature under N_2/Ar gas. TMSA (0.291 mL, 2 mmol) was added and stirred for 5 h. The reaction was filtrated and the dark blue solid was washed with EtOH, dried overnight. (Yield: 383 mg, 85%)

Preparation of alkynyl-BODIPY: BODIPY 3 (300 mg, 0.354 mmol) and K_2CO_3 (0.22 g, 1.593 mmol) were added in 10 mL MeOH and 10 mL THF, and the suspension was stirred at room temperature for 2h. The reaction was filtrated and the solvent was evaporated followed by purification using column chromatography to obtain blue solid. (Yield: 162 mg, 80%) ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.75$ (s, 1H, ArH), 7.71 (s, 1H, ArH), 7.66 (d, $J = 8.0$ Hz, 2H, ArH), 7.54 (m, 8H, ArH), 7.33 (d, $J = 8.1$ Hz, 2H, CH═CH), 7.26 (s, 1H, pyrrole-H), 7.20 (s, 1H, pyrrole-H), 3.21 (d, $J = 2.6$ Hz, 2H, C≡CH), 1.57 (s, 1H, C≡CH), 1.48 (s, 6H, CH_3).^{13}C NMR (100 MHz, $CDCl_3$) $\delta =$ 152.59, 142.24, 138.18, 136.89, 135.51, 132.91, 132.56, 128.82, 128.60, 127.41, 123.12, 122.44, 120.11, 118.25, 83.70, 82.87, 78.86, 78.74, 68.17, 38.72, 28.93, 23.74, 14.91, 10.98. HRMS (ESI): *m/z* calcd for $C_{39}H_{27}BF_2N_2$ $[M+H]^+$ 572.4636; found 572.4628.

2.2.2. Preparation of N_3-α-CD, N_3-β-CD and N_3-γ-CD (Scheme S1)

Preparation of Ts-γ-CD: γ-CD (10 g, 7.7 mmol) was dissolved in 500 mL H_2O at 60 °C. After cooled to room temperature, 1-(p-toluene sulfonyl) imidazole (6.84 g, 30.8 mmol) was added and stirred for 2h. Then, sodium hydroxide (4 g, 98.44 mmol) was added and the reaction was filtrated. NH_4Cl (10.5 g, 0.2 mol) was added in the filtrate and the mixture was concentrated, washed with ice water and acetone. The white solid was collected and dried at $CaCl_2$. (Yield: 5 g).

Preparation of N_3-γ-CD: Ts-γ-CD (4 g, 2.75 mmol) was dispersed in 100 mL H_2O and heated at 80 °C. Then, NaN_3 (2.15 g, 33 mmol) was added and continued to be stirred for 5h. After cooled at room temperature, acetone was added and the mixture was filtrated to obtain the white powder. (Yield: 2.47 g).

The synthetic procedure of N_3-α-CD and N_3-β-CD were similar to that of N_3-γ-CD.

2.2.3. Preparation of BODIPY-α-CD, BODIPY-β-CD and BODIPY-γ-CD complexes (Scheme 1b)

The preparation of BODIPY-γ-CD complex was as follows: N_3-γ-CD (93.1 mg, 0.118 mmol), alkynyl BODIPY (373 mg, 0.531 mmol) and CuI (73 mg, 0.531 mmol) were dispersed in 10 mL DMF and stirred at room temperature for 2h under N_2 gas. The mixture was monitored by TLC until the alkynyl BODIPY was completely reacted. The mixture was filtrated, washed with DMF, dried overnight to obtain the final product. Similarly, the synthetic procedures of BODIPY-α-CD and BODIPY-β-CD complexes were analogous to that of BODIPY-γ-CD complex.

2.3. Cell viability assays

HeLa cells were incubated in DMEM containing 1% antibiotics and 10% fetal bovine serum at 37 °C in a humid atmosphere of 5% CO_2. The MTT assay was used to determine the cytotoxicity of BODIPY-CD complexes against NIH 3T3 and tumor HeLa cells, and the cells were seeded in 96-well plates and incubated at 37 °C and 5% CO_2 for 24 h with a density of 1×10^4 cells/well. The cells were treated with a range of test compound concentrations (0, 12.5, 25, 50, 80, 100, 150, 200 μg/mL) for 24 h.

2.4. ROS generation of BODIPY-CD complexes in solution

1,3-Diphenylisobenzofuran (DPBF) was used to quantify the ROS generation of BODIPY-CD complexes under 650 nm (200 mW/cm^2) laser irradiation for 5 min. UV–Vis absorption spectrometer was used to monitor the residual amount of DPBF.

2.5. Photodynamic therapy (PDT) studies

Similarly, the MTT assay was used to evaluate the therapeutic effect of PDT against tumor HeLa cells. The cells were seeded in 96-well plates with a density of 1×10^4 cells/well and treated with various concentrations of BODIPY-CD complexes (0, 12.5, 25, 50, 100 μg/mL) at 37 °C and 5% CO_2 for 24 h. Then, cells were exposed to laser (808 nm, 1 W/cm^2) for 5 min respectively. After laser irradiation, MTT solution was added into per well and incubated for 4 h to calculate cell viability.

3. Results

The synthetic route of novel near-infrared BODIPY-CD complexes was listed in Scheme 1. Briefly, alkynyl-BODIPY dye was

designed and synthesized via four steps, including two-step condensation, following sonogashira coupling and de-protection reaction, respectively. All the intermediates were obtained with precise structure (Figs. S1–S2) in satisfactory yields. Simultaneously, the chemical structure of alkynyl modified BODIPY was confirmed by 1H NMR, 13C NMR and MALDI-TOF-MS. In the 1H NMR specturm (Fig. S3), the double peaks appeared at 3.21 ppm and single peak appeared at 1.57 ppm indicated the successful substitution of alkynyl group at the iodine atom of intermediate 2, which was also observed in the ^{13}C NMR spectrum (Fig. S4). The MALDI-TOF-MS spectrum also verified relative molecular mass of alkynyl BODIPY which was similar to the calculation result. Then, N_3-CDs were prepared by the toluene-sulfonylation of γ-CD (α-CD or β-CD) followed with azide reaction. Finally, three novel BODIPY complexes (BODIPY-CD) were first constructed through the facile click reaction of alkynyl-containing BODIPY and N_3-CDs to enhance the biocompatibility and water solubility of BODIPY matrix. The conjugation of CD substituent in BODIPY core not only expended the range of absorption and fluorescence emission spectrum, but also reduced in vitro cytotoxicity and promoted photodynamic therapeutic property.

The optical properties of the synthesized alkynyl-BODIPY dye and BODIPY-γ-CD complex were investigated by UV–vis absorption and fluorescence spectrum measurements in aqueous solution containing DMF. As displayed in Fig. 1, the maximum absorption for alkynyl-BODIPY was 661 nm which showed a red-shift for BODIPY core at 649 nm previously reported. This phenomenon was ascribed that alkynyl group served as electron acceptor to conjugated with BODIPY core, thus leading to a corresponding red shift. Furthermore, the maximum absorbance peak for all the BODIPY-CD complexes presented a further red-shift of 49 nm as compared to alkynyl-BODIPY. This was owing to the increased conjugation degree after CD units were incorporated by the triazole linkages. Thus, BODIPY-γ-CD complex exhibited a broader light absorption capacity compared to the BODIPY matrix. Moreover, compared to BODIPY-α-CD and BODIPY-β-CD, BODIPY-γ-CD complex exhibited stronger absorption intensity.

The fluorescence spectrum of alkynyl-BODIPY dye and BODIPY-CD complexes were shown in Fig. 2. The maximum emission peaks for alkynyl-BODIPY dye and BODIPY-CD complex appeared at 783 nm and 874 nm. Obviously, so a broad red-shift (>90 nm) of the emission peak was observed after the incorporation of CD which was strongly influenced by the conjugation effect. This conclusion was consistent with that of the corresponding absorption spectrum. Similarly, BODIPY-γ-CD had stronger fluorescence intensity than BODIPY-α-CD and BODIPY-β-CD. Moreover, the Stokes shift for BODIPY-γ-CD complex was up to 164 nm, thus having an excellent fluorescence imaging capability. In addition, the fluorescence intensity of BODIPY-γ-CD complex increased compared to BODIPY core, indicating that the fluorescence ability of complex was enhanced when the CD unit was incorporated at the BODIPY core. The reason was that the increased water solubility of complex inhibited the aggregation of BODIPY core, improved light transmittance and resulted in fluorescence enhancement.

Subsequently, the influence of the concentration and pH value for BODIPY-γ-CD complex were investigated and the corresponding fluorescence spectra were displayed in Figs. S5–S6. The fluorescence intensity was 1624 when the concentration of the complex was 5 μM, suggesting its high sensitivity (Fig. S5). With the increase of the concentration, its corresponding fluorescence intensity also enhanced. Then, 20 μM BODIPY-γ-CD complex was added into the solution with different pH value from 3.41 to 10.64 and their fluorescence spectra could be observed in Fig. S6 that pH value of solution had a litter influence on fluorescence intensity but all the intensities became weaken in the acidic or basic solution and the maximum fluorescence intensity appeared in neutral solution. Then, the recognition effect of BODIPY-γ-CD complex on metal ions was also studied and it could be seen from Fig. S7 that in the presence of 2 equiv. metal ions, their fluorescence intensities were significantly enhanced and blue-shift phenomenon occurred. The ligand and metal energy transfer (LMCT) occur in the metal coordination, thus increasing light absorption and leading to a fluorescence enhancement. The BODIPY-γ-CD complex therefore was benefit to capture metal ions which inhibited electron transfer and destroyed the fluorophore structure to promote the blue-shift of fluorescence.

The cytotoxicity studies of BODIPY-α-CD, BODIPY-β-CD and BODIPY-γ-CD complexes were performed in vitro towards NIH 3T3 cells by quantification of surviving cells after the treatment with various concentrations from 12.5 to 200 μg/mL. As shown in Fig. 3a–c, three BODIPY-CD complexes did not affect the cellular viability in the entire concentration intervals as compared with the

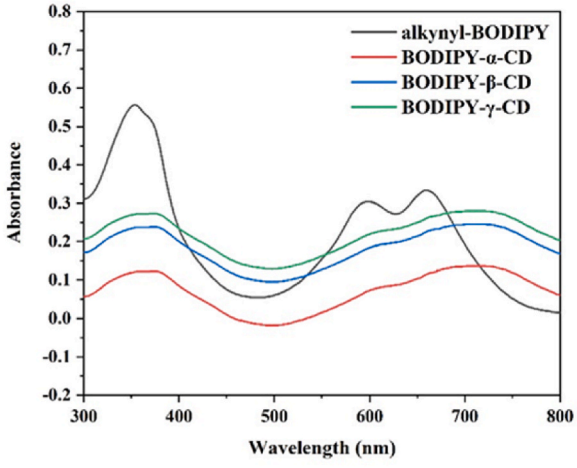


Fig. 1. UV–vis absorption spectrum of alkynyl-BODIPY and BODIPY-CD complexes in DMF-water.

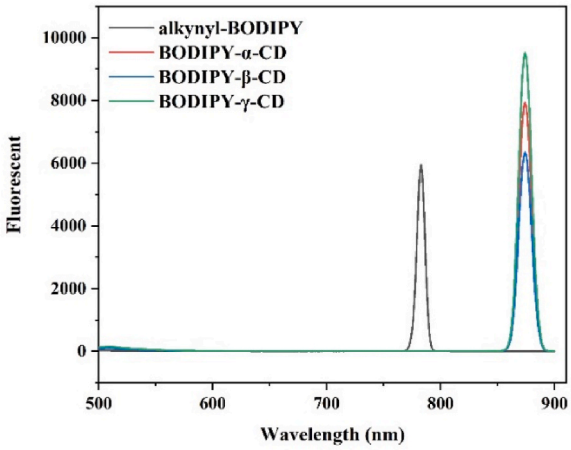


Fig. 2. Fluorescence spectra of alkynyl-BODIPY and BODIPY-CD complexes in DMF-water.

control cells. This demonstrated that three BODIPY-CD complexes had no cytotoxicity towards NIH 3T3, which was expected to apply for the bio-imaging technology in the living cell system.

DPBF was applied to evaluate the ROS generation performance of BODIPY-α-CD, BODIPY-β-CD and BODIPY-γ-CD complexes and the corresponding degradation rates were shown in Fig. 4. Under laser irradiation, the concentration of DPBF was almost unchanged without additives while BODIPY-CD complexes could efficiently degrade DPBF. Particularly, the degradation efficiency of BODIPY-β-CD was higher than that of BODIPY-α-CD and BODIPY-γ-CD, which indicated that BODIPY-β-CD had a quicker ROS formation rate compared to BODIPY-α-CD and BODIPY-γ-CD. Finally, BODIPY-β-CD exhibited more ROS formation and excellent generation ability.

The in vitro phototoxicity experiments of BODIPY-α-CD, BODIPY-β-CD and BODIPY-γ-CD complexes as potential photodynamic therapy agents were performed towards tumor HeLa cells by an MTT assay. As depicted in Fig. 5a–c, three BPDIPY-CD complexes presented the weak influence on the cell viability in the dark. On the contrary, the cell viability became weaken with the increasing concentration of BODIPY-CD complexes with the laser irradiation. Wherein, BODIPY-α-CD and BODIPY-γ-CD complex had moderate cell inhibition efficiency and the cell viabilities were about 50% in the concentration of 100 μg/mL. Nevertheless, the cell viability of BODIPY-β-CD complex was dramatically reduced up to 20% in the concentration of 100 μg/mL upon 808 nm laser irradiation, suggesting its excellent PDT activity against HeLa cells. This was ascribed to the more singlet oxygen generation from the combined influence of BODIPY-β-CD complex and irradiation. The above conclusion reflected the therapeutic potential of BODIPY-CD complex for photodynamic therapy.

4. Conclusions

In summary, we have developed a simple and reliable strategy for producing water-soluble and biocompatibility BODIPY-CD complex by the click reaction of alkynyl-containing BODIPY and azide-modified CD. Due to stronger conjugation effect, the fluorescence emission wavelength apparently red-shifted after the incorporation of triazole and CD units as compared with alkynyl-containing BODIPY. Moreover, all the BODIPY-CDs were no cytotoxicity toward NIH 3T3 and tumor HeLa cells in the dark. Particularly, BODIPY-β-CD presented excellent PDT activity against HeLa cells and the cell viability was dramatically reduced up to 20% in the concentration of 100 μg/mL with laser irradiation. This work provided a possibility of the rational integration of BODIPY and CD materials for photodynamic therapy for treatment of prostate cancer.

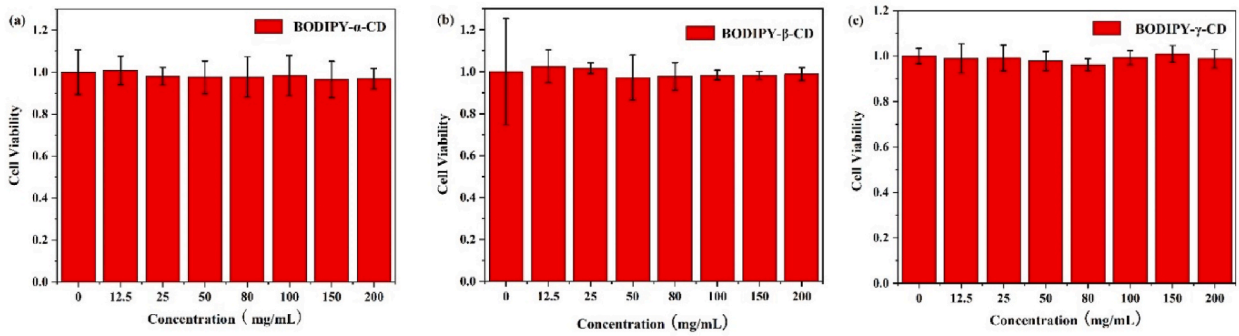


Fig. 3. The cytotoxicity of (a) BODIPY-α-CD, (b) BODIPY-β-CD, (c) BODIPY-γ-CD complex toward NIH 3T3.

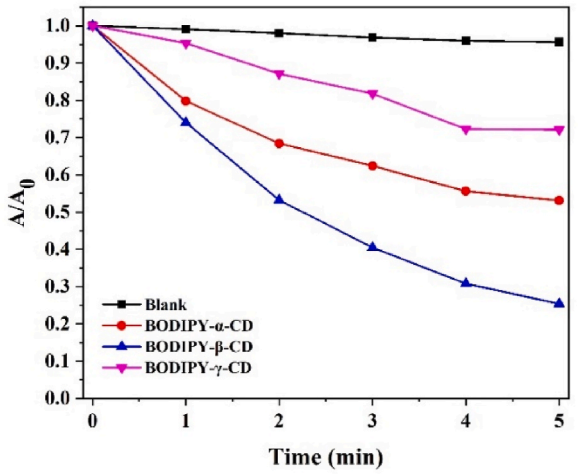


Fig. 4. The degradation rate of DPBF under laser (650 nm, 200 mW/cm^2) irradiation with BODIPY-α-CD, BODIPY-β-CD and BODIPY-γ-CD complexes.

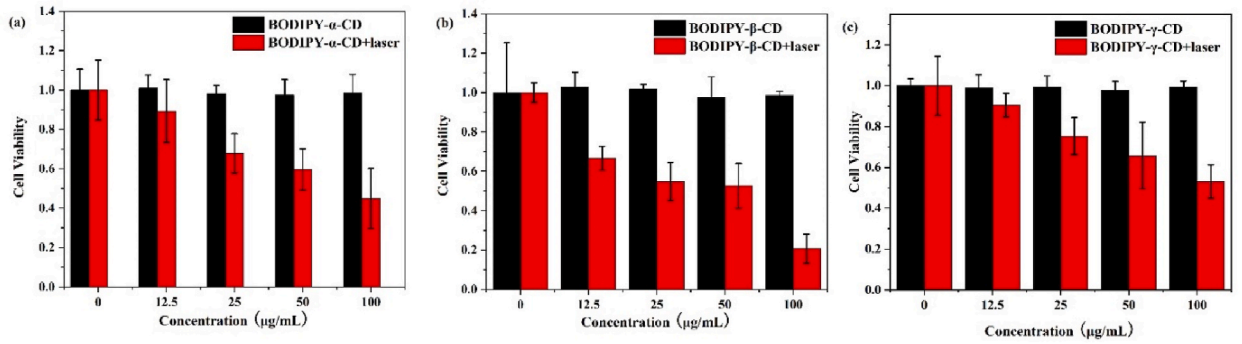


Fig. 5. Cell viability of HeLa cells after 24h of incubation with (a) BODIPY-α-CD, (b) BODIPY-β-CD, (c) BODIPY-γ-CD complex at different concentrations in the dark or 808 nm laser irradiation.

CRediT authorship contribution statement

Bowei Lu: Writing – original draft, Methodology, Data curation. **Xu Lu:** Methodology, Investigation, Data curation. **Manman Mu:** Validation, Investigation. **Shuxian Meng:** Writing – review & editing, Visualization, Validation, Supervision. **Yaqing Feng:** Writing – review & editing, Visualization, Supervision, Funding acquisition. **Yi Zhang:** Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26907.

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