Increased photoluminescence and photodynamic therapy efficiency of hydroxyapatite- β -cyclodextrin-methylene blue@carbon powders with the favor of hydrogen bonding effect

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Abstract

To meet the requirements of theranostics with diagnosis and treatment, photodynamic-based therapy is simultaneously enabled with the incorporation of methylene blue (MB) as imaging agent and photosensitizer in core-shell structured drug vehicles. Citrate-modified hydroxyapatite (HAp) powders are first grafted with β -cyclodextrin (CD), then combined with MB molecules through electrostatic interactions, and finally encapsulated with carbon shells through hydro-thermal carbonization of glucose to prepare HAp-CD-MB@C powders. Processing parameters of carbonization temperature, glucose addition, reaction time and CD addition are varied to prepare drug carriers with modulated crystallite degrees and photo-physical properties. Increased crystallite sizes of HAp are accompanied with the formation of C=O, C=C and C–OH groups in carbon shell, endowing sustainable release behaviors of MB through carbonous structures. High photoluminescence intensities are fairly related with red-shifted vibration peaks of groups in tightly combined MB molecules through hydrogen bonds. This hydrogen bonding effect is significantly increased for HAp-CD-MB@C140 with the splitting of CH₃-involved vibration peaks in infrared spectra, which causes increase in photoluminescence intensity and four-fold increase in generation ratio of singlet oxygen. The present studies shed light on preparation of core-shell structured drug carriers, modulation of aggregate states of MB molecules, enhancement of photo-physical properties and improvement of generation ratio of singlet oxygen during photodynamic-based therapy.

Keywords Drug delivery · Theranostics · Singlet oxygen · Carbonization · Aggregation state · Core-shell structure

1 Introduction

To optimize cancer therapy through suppressing side effect and multiple drug resistance [1–5], photodynamic therapy (PDT) is considered as a promising clinical noninvasive strategy to induce oxidative stress and cause damage of cancer cells by apoptosis and/or necrosis [6–10]. During PDT, electrons of photosensitizers in ground states are bumped to their excited states under light irradiation, and then transferred to triplet states through intersystem crossing process. These relaxation energies are finally decayed in a non-irradiated way through exciting oxygen molecules

in triplet states to singlet states [11]. For this type II photosensitized oxygenation reaction, PDT efficiency is closely relied on the generation ratio of singlet oxygen molecule, whose low intra-tumoral yield is a major limitation for traditional and available photosensitizers [12–14] with requirements of high absorption coefficient [4, 15] and monomer state of these lipophilic dyes without dimeric aggregation [16–18]. Many efforts are devoted to increase generation efficiencies of singlet oxygen molecules through promoting intersystem crossing processes by spin coupling of heavy atom effect [19–23], optimizing state energy levels through fine-tuned absorption wavelengths [20, 22] or employing Förster resonance energy transfer pairs with photoinduced charge transfer [24–26]. Structural modifications [22] or sulfur substitutions of carbonyl thionaphthalimides at 2- and 6-positions [20] endow remarkable fluorescence quenching and tremendous enhancement in the rate constant of intersystem crossing, due to the significant increase of spin-orbit

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coupling constant and decrease of singlet–triplet energy gap [20, 22].

As a cationic photosensitizer of phenothiazinium, methylene blue (MB) can absorb visible light in the near-infrared range (absorbance peak at ~660 nm) with visible red-end to near-infrared emission. Its singlet oxygen quantum yield (Φ_{Δ}) of 0.5 [27, 28] is related to MB's excitation, intersystem crossing transition and population of excited triplet states. These processes are promoted in hybrid photosensitizers, where energy and charge transformations between quantum dots (CdTe [24] or Ag [25]) and MB are employed to significantly increase the production efficiencies of singlet oxygen [24]. Recently, MB molecules are combined in cardiolipin monolayer systems [28], C' dots of silica core [23] and cucurbituril [29] to diminish π stacking-induced self-aggregation and avoid the reductive process of MB during its delivery to cancer cells. MB molecules in the triplet excited states are also protected to live with long life inside these capsules, which results in the promotion of triplet–triplet energy transfer between MB and O_2 molecules to yield singlet oxygen, exceeding performances of single MB molecules. The intersystem crossing from singlet states (S_1) to triplet states (T_2 and T_3) is underwent due to vibronic and pure electronic spin–orbit coupling [30], whose vibration energies are sensitive with the surrounding environments of photosensitizers. Particularly, hydrogen bonds formed between photosensitizers and water would raise vertical excitation energy levels and cause the yield variations of fluorescence, semi-reduced radical and/or singlet oxygen [28, 30–32]. These studies of electronic structures are mainly performed on quantum chemical calculations to investigate the influence of surrounding polarities on photo-physical properties of photosensitizers and their productions of reactive oxygen species. These corresponding studies of hydrogen bonding effects are rarely investigated from experimental viewpoints to enhance the generation of singlet oxygen molecules and meet the requirements of careful coordination of diagnosis and therapy.

To perform theranostics with imaging-guided focal therapies, drug delivery systems are developed with combinations of imaging, biomarkers and therapeutics through tracking penetration, drug release and PDT within tumors located in the surrounding environment of immediate vicinity of 10–20 nm around PSs [33, 34]. Novel MBPB nanoplatforms are demonstrated to be only activated by H_2O_2 to generate MB molecules in tumor cells and avoid the induction of "always on" phototoxicity of photosensitizers in normal tissues before their arrivals at tumors [12, 35, 36]. Herein, MB molecules are first absorbed by citrate and/or β -cyclodextrin (CD)-modified hydroxyapatite (HAp) and then encapsulated by carbonized glucose during hydro-thermal treatment to finally modulate pH-sensitized release behaviors of MB molecules in a sustainable way. The processing parameters of

carbonization temperature, glucose addition, hydro-thermal time and CD additions are varied and purposed to investigate how they influence the crystallization behaviors of HAp and photo-physical properties of MB molecules. Energy gaps, photoluminescence yields and photochemical products of singlet oxygen molecules are estimated and discussed in detail according to the influence of hydrogen bonding effect on the shifts of vibration peaks of chemical groups. MB molecules are released from these drug vehicles with high brightness to efficiently generate singlet oxygen during PDT as shown by Scheme 1, which would shed lights on preparation of drug carrier systems containing photosensitizers with modulated aggregation states [24, 37–39], emission enhancement for chemophores with less decay in non-irradiated ways and generation improvement of singlet oxygen molecules to meet the simultaneous requirements of trace imaging, diagnosis and photodynamic-based therapy.

2 Experimental

2.1 Preparation of HAp-MB, HAp-CD-MB and HAp-CD-MB@C

Colloidal HAp was prepared by a wet-precipitated method with presence of citric acid [40, 41]. Typically, a solution of 4 wt% H_3PO_4 is added dropwise to 100 mL of suspension containing 5 wt% of $Ca(OH)_2$ and 2 wt% of citric acid under stirring. When the pH value of reaction mixture decreases to 10, the addition is ceased and the stirring is continued for another 2 h. After 12 h, precipitations are collected by centrifugation and dispersed in 200 mL of solutions with 2 wt% of NH_4Cl . The collected sediments are re-dispersed in 200 mL of de-ionized water to prepare colloidal HAp solution. 0.10 g of MB is added and stirred for 2 h to prepare colloidal HAp-MB solutions. In prior to addition of MB,

Scheme 1 Schematic illustration of photoluminescence and PDT of core-shell structured HAp-MB@C and HAp-CD-MB@C

3.66 g of CD and 0.91 g of KH_2PO_4 are added to the above-prepared colloidal HAp solution and treated hydrothermally under 140 °C for 3.5 h [42]. After that, 0.10 g of MB is added and the mixture is stirred for another 2 h to prepare colloidal HAp-CD-MB solutions.

10 mL of colloidal HAp-MB or HAp-CD-MB solutions is mixed with 20 mL of glucose-contained solutions and then hydrothermally treated at 120–180 °C. After washing precipitations with 20 mL of water and 20 mL of ethanol, HAp-MB@C or HAp-CD-MB@C powders are finally prepared after dry treatment at 80 °C for 12 h. The detailed processing parameters of carbonization temperature, glucose addition, hydro-thermal time and CD additions for synthesis of these samples are shown in Table 1.

3 Results and discussion

3.1 Influence of carbonization temperature on the carbonization of HAp-MB@C

Pure phases of HAp are observed in HAp-MB@C powders because all XRD patterns are well indexed by the standard HAp phase (JCPDS No. 09-0432, Fig. 1). With increasing carbonization temperature, crystallite intensities become high with increased crystallite sizes determined by the Scherrer's equation (Tables 2, S1). Under 120 °C, spherical particles of HAp-MB@C120 are prepared (Fig. 2), which is similar with our previously prepared HAp particles with citrate additions [40, 41]. When the carbonization temperature is raised to 140 °C, rod-like HAp-MB@C140 powders are prepared with slightly increased sizes. For samples

Fig. 1 XRD patterns of HAp-MB@C120 (a), HAp-MB@C140 (b), HAp-MB@C160 (c) and HAp-MB@C180 (d) indexed by standard HAp phase as control (vertical lines, JCPDS No. 09-0432)

synthesized at 160 or 180 °C, carbonized spheres with sizes of 1–3 or 3–5 μm are formed with nano-sized HAp rods (Fig. 2c, d) assembled as skeletons in carbon spheres (TEM images of Fig. 3), which are amorphous with the broad diffraction peak centered at around 20° (Fig. 1d).

In Figs. 4a, S1 and Table S1, FTIR absorbance peaks appear at 1513, 900–500 and 1000–1500 cm^{-1} , which are assigned to bending vibrations of C=C and C–H groups, stretching vibration of C–OH groups and bending vibrations of O–H groups [43, 44], respectively. These absorbance peaks are split with the proceeding of carbonization reactions at high temperatures. A new absorbance peak appears at 1700 cm^{-1} and is assigned to stretching vibration of C=O group ($\nu_{\text{C=O}}$) as an indication of enhancement of carbonization. Through the intensities of peaks at ~603 and 563 cm^{-1} over that of valley at ~690 cm^{-1} ($(I_{603} + I_{563})/I_{590}$) [45]), the determined crystallite degrees of HAp (X_{FTIR}) increase distinctly for samples synthesized under increasing carbonization temperature (Tables 2, S1). The stretching band of absorbed water at ~3423 cm^{-1} is red-shifted for HAp-MB@C synthesized at high temperatures, revealing tight combinations of water with chemical groups in carbon shells. The absorbance peaks at ~1332, 1418 and 1600 cm^{-1} (Figs. 4a, S1 Tables 2, S2) are ascribed to bending vibration ($\delta_{\text{ring-CH}_3}$), stretching vibrations of C–N bonds ($\nu_{\text{C-N}}$) and rings (ν_{ring}) of MB molecules in HAp-MB@C [46, 47], respectively. Particularly, absorbance peaks assigned to $\nu_{\text{C-N}}$ are shifted toward high frequencies with strong absorbance intensities (Tables 2, S2). These phenomena indicate stretching vibrations of MB molecules increase greatly in HAp-MB@C with weak hydrogen bonds in relatively loose structures. These blue-shifted FTIR peaks are fairly related with their photo-physical properties, that is, photoluminescence emission peaks become weak and are red-shifted for samples synthesized under increasing carbonization temperature (Tables 2, S3). These observations indicate that molecular vibrations are non-irradiated ways for the decay of excited energies. The quantum yield is quite high for

Table 1 Processing parameters for the preparation of HAp-MB@C and HAp-CD-MB@C

Sample	Temperature/°C	Glucose/g	Time/h	CD/g
HAp-MB@C120	120	1.98	4	0
HAp-MB@C140	140	1.98	4	0
HAp-MB@C160	160	1.98	4	0
HAp-MB@C180	180	1.98	4	0
HAp-MB	180	0	4	0
HAp-MB@C1	180	0.99	4	0
HAp-MB@C10	180	9.90	4	0
HAp-MB@C1h	180	1.98	1	0
HAp-MB@C2h	180	1.98	2	0
HAp-CD1-MB@C180	180	1.98	4	0.50
HAp-CD2-MB@C180	180	1.98	4	1.00
HAp-CD3-MB@C180	180	1.98	4	1.50
HAp-CD1-MB@C140	140	1.98	4	0.50
HAp-CD2-MB@C140	140	1.98	4	1.00
HAp-CD3-MB@C140	140	1.98	4	1.50

Table 2 Crystallite size (d , nm), degree (X_{FTIR}), photoluminescence properties of peak wavelength (λ , nm), intensity (Int., %) and relative quantum yield (Φ_s), absorbance frequencies (cm^{-1}) of peaks in the infrared spectra

Samples	d	X_{FTIR}	λ	Int.	Φ_s	ν_{ring}	$\nu_{\text{C-N}}$	$\delta_{\text{ring-CH}_3}$
HAp-MB@C120	6	3.61	684	398	243.41	1601	1450 1417	1298
HAp-MB@C140	16	3.43	672	26	6.87	1599	1452 1419	1302
HAp-MB@C160	17	4.96	672	10	1.18	1601	1431	1315
HAp-MB@C180	25	5.67	699	12	1.08	1613	1431	1313
HAp-MB	17	4.20	656	137	62.95	1597	1448 1420	1302
HAp-MB@C1	20	5.66	644	7	0.90	1607	1433	—
HAp-MB@C10	—	2.91	684	18	2.14	1616	1400 1450	1298
HAp-MB@C1h	14	4.74	644	22	2.77	1600	1423	1327 1308
HAp-MB@C2h	13	5.27	674	18	2.06	1608	1423	—
HAp-CD1-MB@C180	26	3.76	699	5	0.76	1612	1431	1381
HAp-CD2-MB@C180	21	—	699	5	0.60	1611	1431	1377
HAp-CD3-MB@C180	23	2.27	700	4	0.49	1612	1431	1379
HAp-CD1-MB@C140	23	7.82	650	22	3.80	1599	1417	1323
HAp-CD2-MB@C140	23	9.33	651	36	5.70	1602	1416	1317
HAp-CD3-MB@C140	26	7.70	656	20	5.21	1600	1416	1319

Fig. 2 SEM characterization of HAp-MB@C120 (a, scale bar: 500 nm), HAp-MB@C140 (b, scale bar: 500 nm), HAp-MB@C160 (c, scale bar: 5 μm) and HAp-MB@C180 (d, scale bar: 5 μm). Nanosized HAp rods as skeletons in carbon spheres are indicated by white arrows

Fig. 3 TEM characterization of HAp-MB@C140 (a) and HAp-MB@C180 (b) with thin carbon shells. The scale bars are 50 nm

samples synthesized under 120 °C and this value decreases distinctly for samples synthesized under increased carbonization temperatures. These might be caused by increased decay in non-irradiated ways for MB with decreased energy gaps (Fig. S1), which is facilitated for the intersystem crossing from singlet state to triplet states for MB with lower energy gaps [20, 22].

The compact densities of carbon shells become high for HAp-MB@C synthesized under high carbonization temperatures, which are confirmed by release behaviors of MB (Fig. S2). In phosphate-buffered saline (PBS, pH 7.2–7.4) buffer, the burst release of MB from HAp-MB@C-120 at 1 h is followed by a sustained release until 72 h, while, MB molecules are hardly diffused outward from samples carbonized under 140, 160 and 180 °C. MB molecules are released completely after 6 h from HAp-MB@C120 during incubation in simulated gastric acid (SGA, pH 1.2) buffer. The rapid releases of MB in acidic solutions are resulted from protonated citrate ions on HAp surfaces and carboxyl groups in carbon network, whose prohibition effects decrease significantly on MB's outward diffusions [37].

3.2 Influence of glucose addition on the carbonization of HAp-MB@C

Hydrothermal treatments are performed at 180 °C to study the carbonization process with varied processing parameters. As shown in Fig. S3 and Tables 2, S1, crystallite sizes of HAp become high with increasing

Fig. 4 FTIR (a) and photoluminescence spectra (b) of HAp-MB@C120 (i), HAp-MB@C140 (ii), HAp-MB@C160 (iii) and HAp-MB@C180 (iv)

glucose addition, indicating that glucose carbonizations are facilitated for the promotion of HAp's crystallization. This crystallization enhancement is reduced for HAp-MB@C10 with presence of an amorphous diffraction peak of carbon centered at around 20° . This variation of HAp crystallization with glucose addition is confirmed with that of crystallite degree (X_{FTIR}) in Tables 2 and S1. For more additive glucose, peaks assigned to stretching vibrations of PO_4^{3-} groups (at ~ 1043 and 1099 cm^{-1}) and H_2O (at $\sim 3371\text{ cm}^{-1}$) are shifted to high frequencies due to weakening combinations of those chemical groups in HAp-MB@C. Absorbance peaks at 1413 cm^{-1} assigned to $\nu_{\text{C-N}}$ of MB molecules are shifted and closely related with the variation of photoluminescence intensities (Fig. S4b). These stretching vibration modes of chemical groups are blue-shifted in relatively free and loose spaces, resulting in decreased quantum yield of MB after decay of excited energy in non-irradiated way (Fig. S4b, Tables 2, S3).

3.3 Influence of carbonization time on the carbonization of HAp-MB@C

As shown in Fig. S5, Tables 2 and S1, both crystallite sizes and crystallite degrees of X_{FTIR} increase for HAp-MB@C with prolonging carbonization time. The red-shifted absorbance peaks of water (at $\sim 3381\text{ cm}^{-1}$) and PO_4^{3-} (at $\sim 1093\text{ cm}^{-1}$) in Fig. S6a and Fig. S5 indicate tight bonds of water and PO_4^{3-} on samples with increased carbonization degrees. For samples synthesized under long carbonization time, blue-shifted FTIR peaks of $\nu_{\text{C-N}}$ are attributed to weak effects of hydrogen bonds formed in MB molecules, resulting in significant quenches of quantum yields of MB by these kinds of energy relaxations (Tables 2, S3). Their red-shifted emission peaks in Fig. S6b are observed with decreased energy gaps between the lowest unoccupied molecular orbital and highest occupied molecular orbital for samples synthesized with prolonged carbonization times (Fig. S5).

3.4 Influence of β -CD addition on the carbonization of HAp-CD-MB@C180

To further study the influence of hydrogen bonding effect on the carbonization process, β -CD is grafted with citrate ions through covalent bonds [42] in prior to the incorporation of MB on HAp's surfaces. With different additions of CD, HAp-CD-MB@C180 samples are prepared with large shifts of diffraction peaks (to $\sim 12^\circ$) for amorphous carbon and significant decreases in both crystallite sizes and crystallite degrees of HAp (Fig. S7, Tables 2, S1). Combinations of water on HAp-CD-MB@C are enhanced due to hydrogen bonds with evidences of broad FTIR peaks at $3600\text{--}1800\text{ cm}^{-1}$ in Fig. S8a. With planar and even ionic structures, MB molecules with hydrophobic characters tend to aggregate in these hydrophilic surroundings [48–50] and their peaks assigned to $\delta_{\text{ring-CH}_3}$ are distinctly shifted to $\sim 1380\text{ cm}^{-1}$, rather than the conventional frequency of $\sim 1332\text{ cm}^{-1}$ [46, 47]. The infrared peaks ascribed to $\nu_{\text{C-N}}$ are also blue-shifted to $\sim 1430\text{ cm}^{-1}$ as indications of MB molecules combined in a relatively free and loose surrounding, which is formed with steric hindrance of large-sized CD molecules on electrostatic interactions between MB and carboxyl groups. In comparison with that of HAp-MB@C-180, emission intensities of HAp-CD-MB@C180 decrease severely in Fig. S8b because of the self-quenching process of MB dimers, which is influenced by the di-polarity/polarizability of surroundings [51]. Significantly, the infrared peak assigned to $\delta_{\text{ring-CH}_3}$ of MB is red-shifted for HAp-CD2-MB@C with high-emission intensity, revealing that MB molecules are tightly combined in this delivery system with high quantum ratio of photoluminescence.

3.5 HAp-CD-MB@C synthesized under carbonization at 140°C

During hydro-thermal treatment at 180°C , HAp-MB particles are coated by carbon shells with high compaction densities, causing difficulties in outward diffusions for MB

molecules in PBS and SGA buffers (Fig. S2). And thus, HAp-CD-MB@C140 samples are prepared with reduced carbonization degrees to investigate the release behaviors and photo-physical properties of MB molecules. Infrared peaks at $\sim 3300\text{ cm}^{-1}$ assigned to ν_{OH} of water in HAp-CD-MB@C140 become relatively narrow and low as a result of significant decrease of hydrogen bonding effects (Fig. S9a). In comparison with those of HAp-MB@C140, crystallite sizes and crystallite degrees (X_{FTIR}) of HAp-CD-MB@C140

decreases to 58% of that in HAp-MB@C140, indicating the variation of combination mechanisms of MB in these two vehicles. These are contributed to different release behaviors from HAp-MB@C140 or HAp-CD-MB@C140 with varied carbon shells.

After 24 h of incubation in SGA buffer, MB molecules are released to the supernatant and the absorbance values of $A(\lambda)$ at 664 nm ($A(664)$) are used to determine monomer concentrations (C_M) through a non-linear regression analysis [52–54]:

$$A(664) = \varepsilon_D(664) \cdot \left(\frac{C}{2} - \frac{-K_D \pm \sqrt{K_D^2 + 8K_D \cdot C}}{8} \right) + \varepsilon_M(664) \cdot \frac{-K_D \pm \sqrt{K_D^2 + 8K_D \cdot C}}{4}, \quad (1)$$

are slightly high (Fig. S10, Tables 2, S1), which is similar with the enhanced crystallization of HAp (images in Fig. 5). The intensities of photoluminescence peaks of HAp-CD-MB@C140 are sensitized with CD addition and higher than those of HAp-CD-MB@C180. Relative to HAp-MB@C140, HAp-CD2-MB@C140 is slightly bright in emission intensity and its FTIR peaks of $\nu_{\text{C-N}}$ and $\delta_{\text{ring-CH}_3}$ are red-shifted as indications of increased hydrogen bonding effect between MB and drug carriers, which blocks the non-irradiated way of energy decay through vibration relaxation.

3.6 Photodynamic therapy efficiency of MB released from HAp-MB@C140 and HAp-CD2-MB@C140

In PBS buffer, MB molecules are hardly released from HAp-MB@C140 (Fig. S2b, loading capacities of MB as 1.36 mg/g) or HAp-CD2-MB@C140 (Fig. S11, loading capacities of MB as 0.69 mg/g). During incubation of HAp-MB@C140 in SGA buffer, 83% of MB is initially released at 1 h and the release of MB is completed at 24 h. For HAp-CD2-MB@C140, the initial burst release of 77% is observed at 1 h and the release is still continued during 48 h of incubation, exhibiting a sustainable release behavior (Fig. S11). The loading capacity of MB in HAp-CD2-MB@C140

$$K_D = \frac{C_M^2}{C_D}, \quad (2)$$

where K_D of 1.68×10^{-4} , $\varepsilon_M(664)$ of $6.57 \times 10^4\text{ dm}^3\text{ mol}^{-1}\text{ cm}^{-1}$ and $\varepsilon_D(664)$ of $1.02 \times 10^2\text{ dm}^3\text{ mol}^{-1}\text{ cm}^{-1}$ are herein determined and used as the dimer dissociation constant of MB, molar absorption coefficients of monomer and dimer species in SGA buffer [55], respectively. The absorbance decays of uric acid (A/A_0 at 292 nm, Fig. 6) are simulated by a two-parameter decay equation as:

$$A/A_0 = C + B_1 e^{-k_1 \cdot t} + B_2 e^{-k_2 \cdot t}, \quad (3)$$

where C , B_1 and B_2 are the fitting kinetics parameters, the photo-degradation rate constant of k_1 is related with the generation efficiency of singlet oxygen and the value of k_2 is the degradation rate constant of uric acid's intermediated production during photochemical reactions [56]. It is found in Table 3 that the k_1 value increases with the supernatant addition of MB used for the photochemical process. The value of k_1/C_M is determined to compare the generation efficiency for single MB molecule released from HAp-MB@C140 and

Fig. 5 SEM (a) and TEM (b) images of HAp-CD-MB@C140

Fig. 6 Decay of absorbance of uric acid in reaction solutions containing 3 mL or 5 mL of MB supernatant released from HAp-MB@C-140 or HAp-CD2-MB @C-140 in SGA buffer

Table 3 Photodynamic therapy efficiency of MB released from HAp-MB@C140 and HAp-MB-CD2@C140

Sample	$C_M/\mu\text{M}$	$C/\%$	$B_1/\%$	K_1/min^{-1}	$B_2/\times 10^{-3}$	$K_2/\times 10^{-3} \text{ min}^{-1}$	$K_1/C_M/\times 10^6 \text{ min}^{-1} \text{ M}^{-1}$
HAp-MB@C140 (3 mL)	0.36	82	18	0.11	– 4	– 62	0.31
HAp-CD2-MB@C140 (3 mL)	0.13	57	5	0.19	381	3	1.46
HAp-MB@C140 (5 mL)	0.60	53	19	0.43	282	43	0.72
HAp-CD2-MB@C140 (5 mL)	0.22	77	9	0.38	141	33	1.73

HAp-CD2-MB@C140. In comparison with that of HAp-MB@C140, about four-fold increase in PDT efficiency is observed for MB released from HAp-CD2-MB@C140, producing singlet oxygen molecules in an efficient way, relative to other different nanoplateforms [47, 55, 57–59] (Table S4). When increasing the supernatant addition of MB, PDT efficiency increases slightly for HAp-CD2-MB@C140 due to the formation of MB aggregations, causing reduction in the generation ratio of singlet oxygen.

4 Conclusion

Citrate-modified HAp was first prepared by a conventional wet-precipitated method, followed by covalent graft with CD molecules before subsequent addition of MB, and finally encapsulated by carbon shells to prepare core-shell structured delivery system. The processing parameters of carbonization temperature, glucose addition, reaction time and CD addition were varied to modulate HAp's crystallization, shell structures and combination states of MB in drug vehicles. The crystallization of HAp was enhanced with proceeding carbonization reactions to modulate aggregation states of MB with the influence of hydrogen bonding effect on blue-shifted vibration peaks of chemical groups in MB molecules. Outward diffusions of MB from drug vehicles with carbon shells were efficiently prevented in PBS buffers, while the release behaviors of MB were sustained in acidic SGA solutions. The increased photoluminescence emission and high generation ratio of singlet oxygen were observed for carbonized powders with presence of CD molecules, which could meet the requirements of multimodality platforms with diagnosis, imaging and photodynamic-based therapy.

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Declarations

Conflict of interest There are no conflicts to declare.

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