Synthesis of carbon-dots@SiO$_2$@TiO$_2$ nanoplatform for photothermal imaging induced multimodal synergistic antitumor

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**HIGHLIGHTS**

- Multifunctional carbon dot@SiO$_2$@TiO$_2$ nanoplatforms (CST NPs) were firstly fabricated.
- The CST NPs possessed outstanding photodynamic ability by fluorescence resonance energy transfer effect.
- The synthesized CST NPs achieved synergetic PTT and enhanced PDT both in vitro and in vivo.
- The photothermal imaging property of CST NPs endowing real-time phototheranostics for tumor in vivo.

**GRAPHICAL ABSTRACT**

A nanoplatform could perform excellent photothermal therapy and fluorescence resonance energy transfer induced photodynamic therapy for real-time photothermal imaging guided synergistic phototherapy of cancer.

**ABSTRACT**

For facilitating theranostic nanoplatform with multimodal therapeutic ability, we develop the core-shell structured CDs@SiO$_2$@TiO$_2$ nanoplatforms (CST NPs). The designed CST NPs possess excellent photothermal effect and fluorescence resonance energy transfer (FRET) induced photodynamic property, which could achieve synergetic photothermal and photodynamic therapy. Meanwhile, the photothermal ability of CST NPs acts as a key role in the application of real-time photothermal imaging, benefitting for the diagnosis of tumor accurately. Moreover, the obtained CST NPs also exhibit outstanding sonodynamic effect with huge potential for sonodynamic therapy. Under the 650 nm laser irradiation, the synthesized CST NPs not only inhibit the growth of cancer cells in vitro, but also display precise photothermal imaging and photo-induced ablation to tumor in vivo. As a result, the prepared CST NPs may potentially serve as multifunctional nanoplatform for theranostic antitumor and pave the avenue for clinic cancer therapy.

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Introduction

Due to the massive lacks of traditional cancer therapies, the clinic treatments for cancer are facing extensive challenges which include toxic side effect, low specificity, and huge invasion [1]. Recently, phototherapies such as photothermal therapy (PTT) and photodynamic therapy (PDT) attract abundant attentions of scientists possessing the advantages of non-invasion, excellent selectivity, and low toxicity [2]. However, PTT and PDT in cancer treatment induce the cell apoptosis by the enhanced temperature and cytotoxic singlet oxygen (\( {\text{O}}_2^\cdot \)) (or ROS (reactive oxygen species)) respectively, which could not achieve effective cancer therapy individually [3]. The previous reports demonstrate the combination of PTT and PDT exhibits significant photothermally enhanced PDT efficacy by the synergistic effect, because the photothermal effect could increase intratumoral blood flow transporting more oxygen into tumor subsequently, which improve the PDT efficiency [4]. Therefore, designing novel nanoplatform with bi-function of PTT and PDT to perform the synergetic effect is the critical avenue for phototherapy of cancer.

In PDT, many photosensitizers are developed and utilized for the elimination of cancer, such as porphyrin, phthalocyanine, methylthionine chloride, hypocrellin, and titanium dioxides etc [5–9]. As a widely used photosensitizer for PDT, titanium dioxide (TiO2) could absorb ultraviolet (UV) light and blue light effectively, producing electrons and holes for the generation of \( {\text{O}}_2^\cdot \) or ROS subsequently [10]. The generated oxygen species could damage the cell membrane or DNA of cancer cells by the high reactive property at the location enriching large number of particles, which induce severe toxicity to cancer cells [11]. Despite probable outcomes for triggering apoptosis of cancer cells, the major challenge for the application of TiO2 in PDT is that the penetration ability of UV light or blue light in tissue is limited, which cause the failure of PDT for the cancer cells locating far from the surface [12]. Thus, the designing of long-wavelength light triggered TiO2 nanoparticles with better penetrating property to kill cancer cells in deep tissues is a promising way to extend the application of TiO2 nanoparticles in cancer therapy.

As an efficient method to activate the photosensitizers via fluorescence resonance energy transfer (FRET) efficacy, the upconversion materials could convert the lower energy light to higher energy light, emitting the UV or short-wavelength light effectively [13]. Surprisingly, carbon dots (CDs) are novel fluorescent materials with the upconvertable ability which could convert the long-wavelength light to short-wavelength light (blue light), achieving the utilization of bio-imaging in cancer diagnosis [14]. Synthetic approaches for CDs are generally classified into two categories: “top-down” and “bottom-up” [15]. The “top-down” methods include arc-discharged soot, laser ablation, electrochemical method and so on. On the other hand, the “bottom-up” avenues are obtained from molecular precursors such as citrate, carbohydrates, and polymer–silica nanocomposites through thermal treatments, and supported synthetic and microwave synthetic routes. Owing to the special optical property, the sensitization of TiO2 nanoparticles by CDs is possible via the FRET effect in PDT. Moreover, CDs are excellent photothermal agents which are applied for ablating cancer cells successfully [16]. According to the reported work, the mesoporous silica nanoparticles were used as a coating material to encapsulate the graphene quantum dots and doxorubicin hydrochloride, forming the nanocomposites (DOX-GQD-MSNs) for synergistic photothermal and chemotherapy. Besides, we found that the FRET effect could induce fluorescence quenching of CDs, enhancing the photothermal property of the synthesized nanocomposites obviously [17]. Furthermore, the mesoporous silica based-nanosystems possess controlled size and shape can be varied widely to accommodate high payloads of disparate cargos [18]. The colloidal mesoporous silica is biodegradable and generally recognized as safe by the Food and Drug Administration. Hence, many kinds of mesoporous silica based-nanosystems have been developed for delivering drugs to anticancer. However, a new attempt of combining TiO2, CDs, and SiO2 to synthesize a

![Fig. 1. The (A) synthesis and (B) simulating response of multifunctional CST NPs.](image-url)
long-wavelength triggered nanoplatform for synergistic PDT and PTT is not explored.

For resolving the above-mentioned problems in cancer therapy, a multifunctional nanoplatform was designed by the combination of CDs, SiO2, and TiO2 with the core-shell structure (As shown in Fig. 1A). The CDs were coated with SiO2 to form the spherical CDs@SiO2 nanospheres (CS NSs), acting as the multifunctional core for photothermal therapy and photothermal imaging (PI). Then the synthesized CDs@SiO2 NSs were further wrapped by TiO2 as the shell to produce the core-shell structured CDs@SiO2@TiO2 nanoplatforms (CST NPs), realizing the PDT by FRET effect. For the comparison with other TiO2-based PDT systems, the novelty of this work includes the following. (1) CST NPs possess good bio-compatibility and uniform size, which are suitable for the application in cancer therapy. (2) The multifunctional core of CST NPs not only contributes for PTT and PI inducing apoptosis of cancer cells but could absorb long-wavelength light to achieve synergistic PTT and PDT powerfully, benefitting for the cancer therapy in deep tissues. (4) The obtained CST NPs also have response under ultrasonic stimulation with the generation of ROS, harboring available prospect in the field of sonodynamic therapy to cancer. Therefore, Fig. 1B shows that the designed CST NPs are novel multifunctional nanoplatforms which could perform multimodal cancer therapy synergistically, exhibiting significant potential in cancer therapy.

Materials and methods

Materials

Urea (CO(NH2)2), ethanol, and hydrochloric acid (36.0–38.0 wt%), were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Citric acid, sodium hydroxide, and 1, 3-diphenylisobenzofuran (DPBF) (C2O1H3O), were obtained from Sigma-Aldrich Co. LLC. Hoechst 33342, propidium iodide (PI), penicilllin, streptomycin, tetraethyl orthosilicate (TEOS), (3-Aminopropyl) triethoxysilane (APTES), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), pancreatic solution (25 wt%), high glucose medium (DMEM), polyvinyl pyrrolidone (PVP), and fetal bovine serum (FBS) were purchased from Sangon Biotech Inc. Tetraethyl titanate (TBOT), and ammonia water (25.0–28.0 wt%) were obtained from Macklin Inc. All the agents were analytical pure and used as received without further purification. The water used in the experiments was deionized (Millipore Mili-Q grade) with resistivity of 18.0 MΩ·cm.

Characterization

UV-Vis spectra were collected by a U-3900 UV spectrophotometer (Hitachi) ranging from 200 to 800 nm with DI water as the solvent. The fluorescent spectra were measured in aqueous dispersion by a fluorescence spectrophotometer (F-4600, Hitachi). X-ray diffraction (XRD) patterns were obtained from a X-ray diffraction (DX-2700, λ = 1.5406 Å) to investigate the phases of the products. The morphology and structure of the products were observed on scanning electron microscope (SEM, Sigma 500/VP, the accelerate voltage was 5 kV) and transmission electron microscope (TEM, JEM-100SX, the accelerate voltage was 200 kV). The photothermal effects of all samples and the IR thermal images were performed with an IR thermal camera (Fluke, Everett, WA). The laser equipment (STL650T1-1.0 W) in all experiments was obtained from Beijing Stone Boyuan Laser Technology Co., Ltd. The fluorescent images were captured by an inverted fluorescence microscope (IX83, Olympus). The sonodynamic experiments were performed with an ultrasonic cleaning machine (40 kHz, 400 W, Kunshan Ultrasonic Instrument CO., Ltd).

Preparation of fluorescence CDs

The fluorescent CDs were synthesized in accordance with the reported literature [1].

Preparation of CDs@SiO2 nanospheres (CS NSs)

Generally, CDs (100 mg) and PVP (1.0 g) were added into the mixture solution of ethanol and water with the volume ratio of 4:1 (total volume is of 50 mL), followed by the addition of APTES (100 μL) and ammonia water (2 mL, 25.0–28.0 wt%) [19]. After sonication for 30 min, TEOS was dropped into the mixed dispersion slowly with a 3 h agitation at room temperature (20 °C). The suspension was centrifuged (10000 r min⁻¹ for 10 min) to collect the blue CS NSs. Thus, the obtained CS NSs were washed firstly by pure water (30 mL) for 3 times following by ethanol (30 mL) for another 3 times with centrifugation to remove redundant PVP and unreacted agents. Finally, the precipitations were dried by freeze-drying for 24 h.

Preparation of CDs@SiO2@TiO2 nanoplatforms (CST NPs)

In brief, CS NSs (50 mg) were dispersed in ethanol (100 mL) by sonication for 30 min. After that, ammonia water (1 mL) was added into the suspension under stirring violently, followed by the careful addition of TBOT (1 mL). For a reaction of 4 h, the precipitates were obtained by the centrifugation (4000 r min⁻¹, 10 min). Finally, the precipitates were washed by pure water and ethanol for 6 times, freeze-drying subsequently for 24 h to acquire the CST NPs.

Singlet oxygen (¹O2) generation detection

DPBF was used as the probe to study the photodynamic effects of CST NPs. Generally, 1 mL of mixed-solution of alcohol and water (with the ratio of 10:90) containing DPBF (0.196 mg) and 1 mL of CST NPs dispersion (0.5 mg·mL⁻¹) were mixed evenly. Subsequently, the mixture was irradiated by a 650 nm laser for 10 min. During the irradiation, the absorption intensities of the mixed dispersion at 410 nm were collected using a spectrophotometer at predefined time points (0 min and 10 min respectively). For comparison, the photodynamic effects of CS NSs were also performed by the same process. All dark groups were treated in the same way as the control groups.

Intracellular ROS determination

HepG2 cells were cultured in the dishes (35 mm) for 24 h in an atmosphere of 5% CO2. The DMEM suspensions containing CS NSs and CST NPs (0.5 mg·mL⁻¹) were used to incubate the cells for 6 h, followed by washing cells 3 times with PBS. The control group was co-incubated without samples at the same condition. Immediately, all groups were co-incubated with 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) (10 μmol·L⁻¹) for 50 min. The cells were further washed 3 times by PBS before and irradiated under a 650 nm laser for 3 min. The DCFH could be oxidized to the oxidation of 2, 7-dichloro-fluorescein (DCF) by the ¹O2 molecules, demonstrating the generation of intracellular ROS.

Cell viability assay

To determine the cell viability with or without laser treatment condition, HepG2 cells were used to evaluate the cytotoxicity of CS
NSs and CST NPs by MTT assay [20]. HepG2 cells were seeded on 96-well plates with an initial seeding density of 5 × 10³ cells per well. After 24 h incubation, all wells were washed by PBS for 3 times. Subsequently, 100 µL of DMEM medium containing CS NSs and CST NPs with different concentrations of 0, 0.1, 0.2, 0.5, and 1.0 mg·mL⁻¹ were added into each well respectively. With further co-incubation for 10 h, the irradiated groups were taken out for 10 min irradiation under a 650 nm laser, following by 14 h co-incubation in the incubator. Then, 100 µL of freshly prepared MTT solution (0.5 mg·mL⁻¹) was added into each well for further 4 h incubation after washing every well with PBS for 3 times. Finally, 200 µL of DMSO was added into wells dissolving the fomazan crystals to measure the characteristic absorption at 490 nm by the microplate reader. The calculated cell viabilities according to formula (1):

\[
\text{Cell viability} = \frac{OD_{\text{exp}} - OD_{\text{blank}}}{OD_{\text{con}} - OD_{\text{blank}}} \times 100\%
\]

where, \(OD_{\text{blank}}\), \(OD_{\text{exp}}\), and \(OD_{\text{con}}\) are the optical densities of blank, experimental, and control groups respectively.

The intracellular cytotoxicity of CS NSs and CST NPs could also be evaluated by fluorescent images using Hoechst 33,342 and PI as the imaging agents. In brief, HepG2 cells were seeded into 6-well plates at a density of 5 × 10⁴ each well and maintained in an incubator at 37 °C, 5% CO₂ for 24 h. After 3 mL of DMEM suspension containing CS NSs and CST NPs (0.5 mg·mL⁻¹) were added to each well, followed by incubating in dark. After 10 h incubation, the irradiated groups were taken out and irradiated by a 650 nm laser for 10 min following by continual incubation. After all groups were incubated for 24 h, the medium were replaced by PBS buffer. Then 0.5 mL of Hoechst 33,342 (10 µg·mL⁻¹) and 0.5 mL of PI (10 µg·mL⁻¹) were added to each well for 15 min. The final cells were washed twice with PBS and observed by an inverted fluorescence microscopy to obtain fluorescent images.

**In vivo photothermal imaging**

H22 (mouse hepatoma cell line) cells (1 × 10⁶ cells) were implanted subcutaneously into the mice [21]. When the tumor volume grew up to about 1000 mm³, 1 mL of PBS suspension containing CST NPs (2 mg·mL⁻¹) was injected into the mice intratumorally. Then, the dosed mice were irradiated by a 650 nm laser for 10 min continuously at the tumor site. The photothermal images were taken at the time points of every 1 min to evaluate the photothermal imaging ability of the obtained CST NPs. All procedures for in vivo experiments were performed in accordance with Fuyang Normal University of Science and Technology guidelines on animal care and use.

**In vivo phototherapy of tumors**

H22 tumor-bearing mice (tumor volume: ≈1000 mm³) were divided into six groups (4 mice per group) randomly, and treated with PBS, PBS + Laser, CS NSs, CS NSs + Laser, CST NPs, or CST NPs + Laser, respectively [22]. Samples were intratumorally injected into the mice with the concentrations of CS NSs and CST NPs corresponding to ca. 2 mg·mL⁻¹. After 20 min post-injection, the irradiated groups were exposed under a 650 nm laser for 10 min. The tumor size was calculated by the formulation of volume = (tumor length)×(tumor width)²/2. Body weight and tumor volume were measured every 2 days. Tumor sections were stained with hematoxylin and eosin (H&E).

**Results and discussions**

**Morphology and structure of samples**

For the morphological investigation of the CS NSs and CST NPs, the SEM and TEM images were collected. As shown in Fig. 2A, the CS NSs possess spherical structures with the uniform size, which the average diameters are about 100 nm. The formation of CS NSs is due to the addition of PVP in the reacting process, which acts as dispersant, structure-directing agent and linker [19]. Meanwhile, the CS NSs are well dispersed benefitting for the application in cancer therapy. Fig. 2B shows that the CS NSs have the similar morphology and size, which are corresponding to the results in Fig. 2A. The TEM image of CS NSs also exhibits darker area which is close to the center of the nanosphere, demonstrating that the different brightness in CS NSs is caused by the encapsulated CDs (Inset in Fig. 2B). After coating with TiO₂, the obtained CST NPs (Fig. 2C) displays the coarser surface than that of CS NSs with an enlarged diameter (ca. 150 nm). And the related TEM image in Fig. 2D clearly reveals that the synthesized CST NPs possess obvious core–shell structure with rough outer surface. The size enhancement of CST NPs is owing to the TiO₂ coating on the outer surface of CS NSs. To investigate the structure of CST NPs clearly, HRTEM image of which was obtained (Shown in Fig. 2E). The darker area in the center was the core structure combining by CDs and SiO₂, and the brighter edge closing to the center was the shell structure forming by TiO₂ with the thickness about 15 nm. Meanwhile, no crystallized structure could be found in the image demonstrating the prepared CST NPs which were combined by noncrystalline components. Furthermore, the size of the prepared CST NPs is advantageous for the enrichment in tumor sites via the enhanced permeability and retention (EPR) effect. The thermogravimetric curves of CS NSs and CST NPs were collected in Fig. 2F. Both curves displayed a slow reduction under the temperature of 150 °C, assigning to the absorbed H₂O in the samples (ca. 6.1 wt% and 7.7 wt%, respectively). The fast mass loss from 150 to 650 °C were attributed to the existence of PVP and CDs in the structure of CS NSs and CST NPs with the total contents about 33.6 wt% and 22.7 wt%, respectively. The calculated SiO₂ contents in every sample were of 60.3 wt% and 40.7 wt% individually. The redundant weight in CST NPs was about 28.9% originating from TiO₂. Thus, all the aforementioned results represent the obtained CST NPs possessing compatible morphology and size, benefitting for the application in cancer therapy by passive targeting as multifunctional nanoplatforms.

To further study the elemental components and distribution of CST NPs, the elemental mappings (Fig. 3) of CST NPs were obtained. The elements of carbon (blue), silicon (violet), oxygen (yellow), nitrogen (green), and titanium (orange) are co-existence and uniformly distribute in CST NPs. Besides, the brightness of silicon, carbon, and nitrogen are concentrated in the center, and the brightness of oxygen and titanium are symmetrically distributed, which is corresponding to the merged image (Fig. 3F) of CST NPs. However, because the TEM-mapping was performed on a carbon fiber membrane, the region of carbon was full of blue. The above-mentioned outcomes prove that the CST NPs are core-shell structured nanoplatforms with the CDs@SiO₂ core and TiO₂ shell.

For promoting confirmation of the surface elemental ingredient, XPS survey spectrum of CST NPs was applied (Fig. 4A). The characteristic peaks are observed evidently attributing to C 1s, Ti 2p, N 1s, Si 2p, and O 1s respectively, which verify the CST NPs are combined by CDs, SiO₂, and TiO₂. Further evidences come from the high-resolution spectra of C 1s, Ti 2p, N 1s, Si 2p, and O 1s, particularly. The peaks in Fig. 4B around 284.6, 285.0, 285.5, 286.4, and 287.8 eV are corresponding to the formations of C−H, C−C, C−N, C−C, and...
C=O bonds individually, which demonstrate the existence of CDs and PVP in the prepared CST NPs [23]. Meanwhile, the characteristic electronic states of N 1s at 399.5, 399.9, and 401.5 eV are observed clearly (Shown in Fig. 4C), which are related to N-H bond, pyrrolic N and graphitic N respectively [23]. The aforesaid indications all illustrate that the elements of C and N mainly originate from CDs integrating in the CST NPs. Fig. 4D shows the four typical peaks of Si 2p at the position of 101.5, 102.1, 102.8, and 103.4 eV, which are referred to Si(-O)1, Si(-O)2, Si(-O)3, and Si(-O)4 abbreviation systems [24]. The component of Si indicates the successful combination of CDs and SiO2 forming CS NSs as the core of CST NPs. Moreover, the binding energy values of 458.4 and 464.1 eV related to the spin orbit pairs of Ti 2p3/2 and Ti 2p1/2 respectively (Fig. 4E), also proving TiO2 are shell materials for the encapsulation to fabricate CST NPs [25]. Besides, more data coming from Fig. 4F display that the peaks at 529.9, 531.9, 531.2, 533.9, and 532.5 eV are attributed to the bonds of Ti–O–Ti, Ti–OH, Si–O–Si, Si–OH, and C–O particularly, which further confirm the designed CST NPs are formed by CDs, SiO2, and TiO2 [25–27]. All the results above verify that the prepared CST NPs are composed by the useful nanomaterials constructing multifunctional nanoplatforms.

In order to understand the combination of the designed samples, XRD patterns of CDs, CS NSs, and CST NPs are shown in Fig. 5A, individually. The broad peak at around 25.5° confirms the amorphous structure of the CDs, illustrating the low graphitization of CDs (Fig. 5A-a) [1]. Fig. 5A-b shows a wide peak at 2θ of ca. 23° which is assigned to the amorphous SiO2 in the CS NSs [28]. Meanwhile, the peak of CDs disappears also verifying the encapsulation of CDs by SiO2. In Fig. 5A-c, no obvious characteristic peak could be observed, which is due to that the amorphous TiO2 packages the CS NSs as the shell forming the designed CST NPs [12]. All these results in XRD patterns of all samples prove the formation of CST NPs with core-shell structure.

Further explorations were studied by the UV-Vis spectra in Fig. 5B to investigate the optical properties of CDs, CS NSs, and CST NPs. Cure a shows the high-energy UV absorption at around 234 and 340 nm, which are assigned to \( \pi-\pi^* \) transitions of sp2 hybridized carbon and \( \pi-\pi^* \) transitions at the edge of the carbon lattice, respectively [29]. Especially, the CDs possess a typical absorption peak at 650 nm attributing to the generation of pyridine structure in CDs, which is corresponded to the conclusions of the XPS analysis [30]. Besides, the characteristic peaks are found.
in Curve b and c too, which demonstrates the existence of CDs in the obtained CS NSs and CST NPs. The intensities of absorption bands from 200 to 300 nm and 400–550 nm are enhanced in Curve b and c, causing by the encapsulation of SiO2 and TiO2 individually. Interestingly, the absorption peak at 650 nm is assigned to the first near infra-red (NIR) window region with the wavelength range of 650–950 nm, which harbors high tissue penetration benefitting for PTT and PDT without damaging biological specimens and surrounding living tissues [30]. Thus, the synthesized CST NPs could absorb NIR light at 650 nm effectively to perform potential PTT and PDT.

The FRET effects of CS NSs and CST NPs are researched according to the fluorescence spectra in Fig. 5C. The CS NSs exhibits a strong fluorescence emission under the excitation at 340 nm which is attributed to characteristic fluorescent peak of CDs at 440 nm. The up-convertible property of CS NSs is investigated by the fluorescence up-conversion spectrum excited at 650 nm with the same fluorescence emission peak at 440 nm. The emitted fluorescence by up-conversion could be absorbed by CST NPs efficiently, corresponding to the absorption band in Fig. 5B-c. Moreover, CST NPs show invisible fluorescence emission under the excitation at 340 nm, which illustrates the occurrence of FRET effect between CDs and CST NPs. The abovementioned outcomes reveal existed FRET effect could induce the photodynamic efficacy of CST NPs.

To interpret the in vitro photothermal effect of CDs, CS NSs, and CST NPs, photothermal curves of which were performed under the same condition, using pure water as control (Fig. 5D). The temperature changes of water are ignored after a 10 min laser irradiation, demonstrating laser illumination could not induce impressive photothermal effect. Irradiating with 650 nm laser for 10 min later, the temperature enhancement of CDs (1 mg·mL⁻¹) increases about 6 °C, indicating the well photothermal effect of CDs. However, the temperature enhancement of CDs is insufficient to kill cancer cells effectually [2]. Moreover, the irradiated dispersions of CS NSs and CST NPs (1 mg·mL⁻¹) emerge remarkable temperature evolutions of ca. 19 and 21 °C respectively, which are significantly higher than that of CDs advantaging for PTT in cancer therapy. The reason is that the coated SiO2 and TiO2 increase the local concentration of CDs, enhancing the photothermal effects of CS NSs and CST NPs. The above evidences demonstrate the designed CST NPs possess excellent photothermal performance, which is novel photothermal agent for phototherma imaging and PTT.

For further investigation of the photodynamic efficacy of CST NPs and CS NSs via FRET effect, the DPBF was used as probe to detect the generation of ¹O₂ or ROS in the presence of CST NPs and CS NSs under the irradiation of the 650 nm laser. In Fig. 6A, the characteristic peak at 410 nm of DPBF decreases obviously after laser illumination for 10 min comparing to the initial curve at 0 min. As a contrast, the peak intensity of the group in dark displays negligible diminishment with the same laser irradiating. Meanwhile, the CS NSs dispersions were measured at the similar condition (Fig. 6B). The measurements show that the groups with laser or in dark both exhibit neglected decrease of the absorption intensity. The aforementioned results prove that the designed CST NPs are available photosensitizer for PDT application, inducing by the FRET effect between the encapsulated CDs and the coated TiO2.

Moreover, TiO2 is widely used as an effective sonosensitizer, which could generate ROS under sonication [31]. Thus, the synthesized CST NPs possess promising sonodynamic effect under the ultrasonic treatment (Shown in Fig. 6C and D). After sonification for 30 min, the DPBF mixed dispersion with CST NPs exhibits the obvious reduction of the typical peak intensity at 410 nm, comparing to the group without sonication treatment. The outcome illustrates the CST NPs have outstanding sonodynamic ability. As control, the CS NSs dispersions display ignored diminishment with the same process, proving that the sonodynamic property of CST NPs originates from the coated TiO2 shell. The above results demonstrate the designed CST NPs are potential sonosensitizers for sonodynamic therapy of cancer.

In vitro phototherapy

In order to study the intracellular ROS generation, DCFH-DA was used to estimate intracellular ROS production for the co-incubated HepG2 cells with different samples (100 µg·mL⁻¹, 6 h)
The groups of control, CS NSs, and CST NPs without irradiation present all cells grow well and emit negligible DCF fluorescence, as shown in Fig. 7A-a2, 7A-b2, and 7A-c2 respectively, illustrating both CS NSs and CST NPs are biocompatible. The irradiated groups of control and CS NSs also exhibit the invisible DCF fluorescence and well growth (Fig. 7A-d2 and 7A-e2), confirming only laser irradiation or with CS NSs could not engender intracellular ROS effectively. However, the irradiated group of CST NPs displays the evident DCF green fluorescence in Fig. 7A-f2, suggests the effective creation of ROS in HepG2 cells. The previous results demonstrate that CST NPs are high performance photodynamic agents, inducing the generation of intracellular ROS for PDT in cancer.

More evidences coming from the MTT assay of CS NSs and CST NPs in Fig. 7B. The dark and irradiated groups are treated by CS NSs and CST NPs with different concentrations (0, 0.1, 0.2, 0.5, and 1.0 mg·mL⁻¹) [1]. Control groups (0 mg·mL⁻¹) with or without laser irradiation exhibit equivalent cell viabilities similarly, revealing laser irradiation could not affect the growth of HepG2 cells. The dark groups of CS NSs and CST NPs (0.1, 0.2, 0.5, and 1.0 mg·mL⁻¹) present the cell viabilities more than 90%, which indicate that both CS NSs and CST NPs possessing favorable biocompatibility. However, the cell viabilities of HepG2 cells are about 69.3%, 58.7%, 47.8%, and 39.0% in the irradiated groups of CS NSs respectively, evidencing the powerful photothermal abilities to kill cancer cells. Moreover, the irradiated groups of CST NPs display lower cell viabilities (ca. 49.6%, 38.1%, 29.7%, and 20.9%, individually) than those of CS NSs, illustrating the enhanced cell apoptosis is caused by the synergistic PTT and PDT of CST NPs. The aforementioned results prove that the designed CST NPs are available phototherapeutic agents for synergetic PTT and PDT in cancer therapy.

For further investigation of the synergistic PTT and PDT of the samples, HepG2 cells incubating with control, CS NSs and CST NPs were stained by Hoechst 33,342 (blue) and PI (red), respectively. In Fig. 7C, all dark groups show obvious blue fluorescence (a1-c1) and indiscernible red fluorescence (a2-c2), demonstrating all cells grow well with negligible cell apoptosis. Meantime, the...
Fig. 5. (A) XRD patterns of (a) CDs, (b) CS NSs, and (c) CST NPs; (B) UV–Vis spectra of (a) CDs, (b) CS NSs, and (c) CST NPs; (C) Fluorescence spectra of (a) CS NSs, (b) up-conversion of CS NSs, and (c) CST NPs; (D) The photothermal curves of water and the dispersion of CDs, CS NSs, and CST NPs.

Fig. 6. Photodynamic effects of (A) CST NPs and (B) CS NSs; Sonodynamic effects of (C) CST NPs and (D) CS NSs.
irradiated group of control displays blue fluorescence (d1) and invisible red fluorescence (d2), which illustrate that the mere laser irradiating could not induce massive apoptosis of cancer cells. However, the group co-incubating with CS NSs exhibits blue fluorescence (e1) and partial red fluorescence (e2) after laser irradiation, reveals that the photothermal effect of CS NSs could kill HepG2 cells efficiently. Moreover, the irradiated group of CST NPs presents an intense red fluorescence in the merged image (f3), suggesting that the CST NPs cause severe cell apoptosis by the synergetic PTT and PDT [1]. Thus, the designed CST NPs possess excellent photoinduced therapeutic abilities for synergistic cancer therapy.

**In vivo photothermal imaging**

To evaluate the photothermal effect of CST NPs in vivo, tumor-bearing mice were intratumorally injected with PBS, CS NSs, and CST NPs following by the treatment of laser irradiation or not [33]. From Fig. 8A, we could found that all dark groups showed a rapid increased tumor volume during 14 days, while irradiated groups displayed obvious tumor growth inhibition thermal images are obtained by an IR thermal mapping camera to monitor the photothermal conversion under irradiation. The mean temperature at tumor site raises nearly to 56 °C under laser irradiation for 10 min, much higher than that of the initial temperature (37 °C), revealing the excellent photothermal effect of CST NPs in vivo. The outstanding photothermal property of CST NPs not only benefits for photothermal therapy in vivo, but also advantages for real-time thermal imaging induced diagnosis and treatment of cancer.

**In vivo phototherapy**

In order to assess the antitumor properties of all samples in vivo, tumor-bearing mice were intratumorally injected with PBS, CS NSs, and CST NPs following by the treatment of laser irradiation or not [33]. From Fig. 9A, we could found that all dark groups showed a rapid increased tumor volume during 14 days, while irradiated groups displayed obvious tumor growth inhibition.

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**Fig. 7.** (A) Intracellular ROS detections of (a, d) control groups, (b, e) CS NSs groups, and (c, f) CST NPs groups without or with laser irradiation; (B) *In vitro* MTT assay of CS NSs and CST NPs with or without laser irradiation; (C) Fluorescence imaging of (a, d) control groups, (b, e) CS NSs groups, and (c, f) CST NPs groups without or with laser irradiation. All groups were co-incubated with HepG2 cells.

**Fig. 8.** *In vivo* IR thermal images of intratumoral CST NPs injected mice under constant irradiation.
except the saline group. This result indicates that the synthesized CS NSs and CST NPs possess excellent biocompatibility in vivo, and laser irradiation could activate their antitumor ability. Particularly, CST NPs + L group has smaller tumor size than that of CS NSs + L group (Fig. 9B), which is attributed to the significant synergy effects of CST NPs by PTT and PDT, and single photothermal property of CS NSs could not efficiently ablate tumor. Furthermore, the histological analyses are performed, cell nuclei are stained blue, and intracellular and extracellular proteins are stained pink [33]. As shown in Fig. 9C, obvious tumor cells damages with membrane shrinkage and nuclear condensation appear in the group of CS NSs + L (Fig. 9C-d), suggest the typical cell apoptosis. Besides, CST NPs + L group has more severe cell loss or necrosis (Fig. 9C-e), and this result further proved that the treatment of synergistic PTT and PDT is better than that of single PTT. Finally, the survival rates and body weight changes are calculated, and the results are presented in Fig. 9D and E. There is no obvious body weight loss as well as the survival rate remained at 80% for CST NPs, which demonstrate that the designed CST NPs not only have excellent phototherapeutic ability for solid tumors, but also have great biosafety in vivo. Therefore, the multifunctional CST NPs are considered as a promising nanoplatform for real-time thermal imaging induced diagnosis and combined PTT-PDT treatment in vivo.

Conclusion

In summary, we developed the novel multifunctional nanoplatforms with representative core-shell structures combining by CDs, SiO$_2$, and TiO$_2$. The designed CST NPs harbor several advantageous properties as following: (1) Suitable size and good biocompatibility are favorable for cancer therapy; (2) Excellent photothermal ability and FRET induced photodynamic property could achieve real-time thermal imaging induced diagnosis and synergetic PTT-PDT for antitumor; (3) Outstanding sonodynamic capacity endows the CST NPs with potential sonodynamic therapy to cancer. The most important is that the synthesized CST NPs exhibit effective antitumor performance both in vitro and in vivo, owning huge prospect in clinic cancer therapy. Thus, the CST NPs are novel theranostic nanoplatforms having associative phototherapeutic ability with real-time imaging capacity, paving a new way for designing and synthesis of other multifunctional materials.

Compliance with ethics requirements

This study was conducted in strict accordance with the recommendations in the Regulations on the Management of Laboratory Animals in China promulgated in 1988. Kunming white mice that were used as a model in the present examination were purchased from the Experiment Animal Center of Anhui Medical University (Certification of quality # 34000200000077, 34000200000078). All animal studies were approved by the Institutional Animal Care and Use Committee at Fuyang Normal University (Approval No. 2013007).

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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