Artificial Cell-Mediated Photodynamic Therapy Enhanced Anticancer Efficacy through Combination of Tumor Disruption and Immune Response Stimulation

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ABSTRACT: Recent studies have identified photodynamic therapy (PDT) as a promising approach for cancer treatment. Here, in this study, we have constructed cancer cell membrane (CCM)-coated silica nanoparticles (SIL) as an artificial cell carrier (CCM/SIL) to effectively deliver chlorin e6 (Ce6), a commonly adopted photodynamic reagent (CCM/SIL/Ce6), to achieve enhanced PDT of cancer. In addition, apart from the generally recognized cytotoxicity induced by reactive oxygen species (ROS), our study also revealed that ROS could further potentiate the loss of intercellular junctions and integrity disruption as a result of down-regulation of VE-cadherin and CD31. Consequently, dendritic cells (DCs) were more readily accumulated to the tumor tissue and became maturated, which secreted tumor necrosis factor-α and interleukin-12 (IL-12) to trigger the following immune responses. Our work not only explored the anticancer feasibility of a new system but also demonstrated the underlining mechanisms responsible for PDT-induced anticancer effects, which offers a new perspective to employ and improve the efficacy of PDT and related systems.

1. INTRODUCTION

Photodynamic therapy (PDT) is currently one of the most effective approaches for cancer therapy.1 Compared with common chemotherapy, PDT was generally recognized as a novel way to employ toxic reactive oxygen species (ROS) to kill cancer cells, which has more advanced properties such as being noninvasive and precise.2 The transfer of oxygen into ROS relies on a photodynamic agent, which is the core of PDT.3 Among the currently applied photodynamic agents, chlorin e6 (Ce6) is widely considered as the most preferable one, both in experimental and clinical applications due to its irreplaceable advantages over its counterparts.4,5

Although PDT has been widely adopted in many previous research works, for a long time, ROS was considered to be fully responsible for all therapeutic merits of PDT. However, with the advancement in understanding of biochemical mechanisms in cancer therapy, the introduction of PDT was reported to change the in situ structure of tumor tissue.6 Moreover, the significant apoptosis of cancer cells and the mass release of cancer-related proteins and nucleic acid are expected to induce potential immune responses. Therefore, the efficacy of PDT might also involve the remodeling of tumor tissue as well as immune responses, which deserves further investigations.7

The effective PDT usually requires the aid of a well-designed drug delivery system (DDS). In the past decades, the development of DDS for cancer therapy has attracted the interest of scientists from all over the world.8–10 The commonly adopted materials for construction of DDS involves both organic and inorganic ones, such as conjugated polymers,11 naturally originated polymers,12 gold,13 calcium carbonate, and so on. In particular, silica nanoparticles (SIL) as a kind of easily accessible carrier is generally considered as a promising candidate due to its multiple merits, including high biocompatibility, high drug loading, high paintability, and low cost. As a result, many SIL-based DDSs have been successfully developed and have made great progress in the treatment of different kinds of cancers.14–16

Cancer cell membrane (CCM) is the coating of cancer cells, which plays important roles in many indispensable biological processes through proteins inserted in the phospholipid bilayer.17 As a result, because of the inheritance of the full proteins in the membrane, CCM was reported to effectively target the homologous cancer cells via positive targeting.18 Moreover, the CCM shows high biocompatibility with the capability to avoid potential capture of the reticuloendothelial system (RES).19 Therefore, the CCM-coated carrier for targeted delivery of anticancer agents is becoming the hot topic of current research.20 In particular, cell mimic carriers of high biocompatibility are preferably welcomed.21

In our work, SIL was coated with CCM to construct an artificial cell-like DDS for effective delivery of Ce6 (CCM/
SIL/Ce6). The CCM/SIL/Ce6 was expected to serve as a versatile DDS to exert and stimulate immune responses for inhibition of cancer metastasis. First, the as-prepared CCM/SIL/Ce6 was injected intravenously, followed by proper light irradiation. The activated Ce6 could effectively transform the oxygen in the blood vessels into toxic ROS, which could exert PDT to kill in situ cancer cells. Afterwards, the disrupted tumor tissue was expected to increase the accumulation of other constituents such as dendritic cells (DC). The maturation of DC might be able to trigger the following immune responses through the secretion of cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-12 (IL-12).

2. RESULTS AND DISCUSSION

2.1. Preparation and Characterization of CCM/SIL/Ce6. The preparation of CCM/SIL/Ce6 contains two independent processes. First, the SIL core was synthesized using microemulsion and then subjected to drug loading of Ce6. At the same time, CCM from MCF-7 was also isolated. Finally, the two components were integrated into one system to prepare CCM/SIL/Ce6. As shown in Figure 1A, the as-prepared CCM/SIL/Ce6 was well-dispersed nanoparticles with size narrowly distributed at around 100 nm. The morphology observation presented in Figure 1B also confirmed this conclusion. In addition, transmission electron microscope (TEM) results also found that CCM/SIL/Ce6 was spherical particles with a core–shell structure, with CCM serving as the membrane while SIL acts as cytoplasm. As a result, TEM provided preliminary evidence to prove the successful modification of CCM on the surface of SIL.

In order to double confirm the successful construction of cell-like CCM/SIL/Ce6, two marker proteins (CXCR4 and AT1R) on CCM were selected and their existence in both free CCM and CCM/SIL/Ce6 was compared. As shown in Figure 1C, CCM/SIL/Ce6 showed comparable expression of CXCR4.
and AT1R to free CCM, indicating that the CCM was actually integrated in CCM/SIL/Ce6 with full membrane components, which was beneficial to inherit the character of MCF-7 cells to bypass potential capture of RES and positively target tumor cells.

The CCM/SIL/Ce6 was incubated in both phosphate-buffered saline (PBS) and plasma for 48 h and the variation in nanoparticle size was employed to be a convenient parameter to indicate the stability of CCM/SIL/Ce6. As shown in Figure 2A, CCM/SIL/Ce6 showed merely slight fluctuation on size (less than 10%) during the whole period. Therefore, it was suggested that CCM/SIL/Ce6 could maintain preferable stability under physiological conditions, which is one favorable advantage for CCM/SIL/Ce6 to serve as a DDS in various advanced applications.

Apart from the size changes, the stability of Ce6 within CCM/SIL/Ce6 was also tested using free Ce6 as control. As displayed in Figure 2B, free Ce6 rapidly degraded under light irradiation for merely 1 day and persistently decreased to only 18.87% at the end of the test, which was consistent with the previous report that free Ce6 without protection is susceptible to light irradiation. In contrast, CCM/SIL/Ce6 offered satisfied protection to the encapsulated Ce6. In detail, the degradation of Ce6 in CCM/SIL/Ce6 was greatly retarded and only 13.21% of the Ce6 was lost at 6 days post irradiation. According to the previous report, free Ce6 is prone to absorb energy from light and transfer it to the neighboring oxygen in the solvent, which results in self-degradation and generation of singlet oxygen. However, in CCM/SIL/Ce6, the Ce6 was safely encapsulated in the matrix of SIL and kept away from the oxygen in the medium. The increased ionic concentration at low pH might weaken the interaction between Ce6 and SIL. Moreover, the increased solubility of Ce6 at lower pH might also contribute to this result. As a result, the Ce6 within CCM/SIL/Ce6 was able to remain stable in the circulation and exert its photodynamic function upon being released in the tumor tissue.

Afterwards, hemolysis test was conducted with the aim to further explore the safety profile of CCM/SIL/Ce6. As illustrated in Figure 3A, after being incubated with 2% RCB for 1 h, the hemolysis of all concentrations of CCM/SIL/Ce6 was lower than 2%. Although hemolysis was observed to positively relate to CCM/SIL/Ce6 concentration, the hemolysis risk of CCM/SIL/Ce6 can be ruled out because the practical in vivo nanoparticle concentration is much lower than even the lowest concentration adopted in our test. Furthermore, the protein absorption potential of CCM/SIL/Ce6 was investigated with SIL/Ce6. It was well recognized by the previous report that nanoparticles with positive surface charges are more likely to interact with negatively charged proteins, which is the majority component of plasma proteins, to form large aggregates. These aggregates not only change the in vivo fate of nanoparticles but also hold the risk to embolize blood vessels to cause death. As a result, the protein absorption potential as a critical parameter of nanoparticles was worthy of investigation. In our study, SIL/Ce6 with abundant free amine groups on the surface was employed as a control and the variations in bovine serum albumin (BSA) absorption as a function of BSA concentrations are summarized in Figure 3B. Consistent with previous re-
ports, positively charged SIL/Ce6 showed severe absorption to BSA, which resulted in significant changes in OD278 with the increase of BSA concentrations. In contrast, CCM/SIL/Ce6 was inert to BSA with almost no variation in OD278. Although CCM/SIL/Ce6 could offer satisfactory protection to the loaded Ce6, whether the encapsulated Ce6 can respond to laser irritation to exert photodynamic effects remains to be explored. As a result, 1,3-diphenylisobenzofuran (DPBF) as a ROS detector was employed to determine the ROS generation capability of CCM/SIL/Ce6. According to a previous report, the decrease of UV absorbance at 418 nm is negatively related to the ROS concentration in the medium, which was an easy way to evaluate the ROS generation capability of CCM/SIL/Ce6. As shown in Figure 4A, under laser irradiation, the ROS generation of CCM/SIL/Ce6 (Ce6: 0.1 mg/mL) increased as a function of time with 79% of quench at 6 min post irradiation. Moreover, the results in Figure 4B also revealed that ROS generation of CCM/SIL/Ce6 (1 W/cm² for 60 s) was also positively related to Ce6 concentration. Both results suggested that the Ce6 in CCM/SIL/Ce6 preserved the capability to generate ROS.

The drug release behavior of CCM/SIL/Ce6 in two different pHs (7.4 and pH 5.5) was evaluated to explore the difference in physiological environment and acidic tumor cells. As displayed in Figure 5A, CCM/SIL/Ce6 showed much more slower drug release at pH 7.4 after 120 h of incubation (35.15%). However, the accumulated release percentage at pH 5.5 could increase to 79.74%. These results clearly demonstrated that CCM/SIL/Ce6 could preferably release its payload in an acidic environment than in physiological environments, which was beneficial for CCM/SIL/Ce6 to realize differential drug release between the physiological environment and malignant tumors.

2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) as the cell permeable form of DCF, is nonfluorescent until oxidization by ROS, which can exert greatly enhanced fluorescence. The intracellular ROS generation profile of CCM/SIL/Ce6 was assessed by DCFH-DA. As shown in Figure 5B, cells without Ce6 showed no observable fluorescent signal after light irradiation, suggesting that Ce6 was responsible for the generation of ROS. As expected, a weak fluorescent signal was observed in cells treated with free Ce6. Moreover, elevated ROS concentration was shown in SIL/Ce6-treated cells, inferring that the intracellular Ce6 concentrations in this group were higher than that in free Ce6. It was noted that the fluorescent intensity in cells treated with CCM/SIL/Ce6 was much more potent than other groups, indicating preferable ROS production. Confocal laser scanning microscopy (CLSM) images (Figure 5B, inset picture) also give consistent results. Therefore, CCM/SIL/Ce6 with effective delivery efficacy was suggested to be a preferable DDS for PDT of cancer.

2.2. Cellular Uptake and in Vivo Distribution. The accumulation of different formulations in MCF-7 cells (pretreated with/without CCM). (B) Mean fluorescence intensity of dissected tumors and major organs of mice treated with SLN/Ce6 and CM/SLN/Ce6 at 48 h post injection. Data are expressed as mean ± S.D. (n = 3). **P < 0.01.
noted that compared to both nanoparticles, free Ce6 showed the lowest intracellular accumulation. A previous report has demonstrated that DDSs can exert beneficial effects to increase the uptake of drugs into cells, which fully comply with our observations. Moreover, without CCM pretreatment, the CCM/SIL/Ce6 group always showed stronger Ce6 signals than SIL/Ce6 in all tested time intervals. Interestingly, the intracellular Ce6 intensity in CCM/SIL/Ce6 group suffered dramatic decline upon CCM pretreatment, whereas both free Ce6 and SIL/Ce6 groups showed almost no changes. These results suggested that the MCF-7 CCM modification could increase the internalization of CCM/SIL/Ce6 to MCF-7 cells, possibly via CCM-mediated endocytosis. According to a previous report, the DDS not only increases the solubility of drugs but is also capable of positively transporting it into cells via receptor-mediated endocytosis. As a result, free Ce6 showed much inferior uptake than the nanoparticles.

In order to further verify the tumor-homing capability of CM/SIL/ICG to the isogenous MCF-7 cells, the fluorescence distribution of Ce6 in both tumor and major organs was obtained by ex vivo imaging. As shown in Figure 6B, because of its poor tumor targetability, the SIL/Ce6 was largely trapped in many organs, especially RES (liver and spleen). In contrast, the untargeted distribution was significantly alleviated in CM/SIL/ICG as the Ce6 signal in tumor tissue was much higher than that in other organs.

2.3. In Vitro Anticancer Effect. After detailed characterization of CCM/SIL/Ce6, its in vitro anticancer effect was investigated on MCF-7 cells using the methyl thiazolyl tetrazolium (MTT) assay. First, a drug-free carrier was incubated with cells to explore the biocompatibility. As shown in Figure 7A, no evident decrease in cell viability after MCF-7 was incubated with CCM/SIL at the concentration of 100 μg/mL for 48 h. These results indicated that CCM/SIL was highly biocompatible, which was suitable to be adopted as a drug carrier for drug delivery.

Afterwards, the anticancer effect of drug-loaded CCM/SIL/Ce6 was assessed using free Ce6 and SIL/Ce6 as controls. Based on results in Figure 7B, it was inferred that the PDT effect was positively related to the given concentration of Ce6. Moreover, unlike DDSs, free Ce6 showed much more inferior anticancer effects, suggesting that DDS could exert beneficial effects on drug delivery. Most importantly, compared with SIL/Ce6, CCM/SIL/Ce6 showed much elevated benefits with cell viability lower than 10% at the Ce6 concentration of 5 μg/mL, which was the best among all tested formulations.

In order to evaluate the in vitro anticancer effect from another point of view, the Western blot assay was conducted to determine the in vitro changes of three apoptosis-related proteins. Previous studies have identified Bcl-2 as the suppressor of apoptosis, which exerts beneficial effects on the survival of cells. Cytochrome c is another protein responsible for the initiation of apoptosis; the upregulation of Cytochrome c usually indicates a rise of apoptosis, which is usually accompanied with the activation of following pathways. As a result, Caspase-3 was selected as the downstream protein to double verify the initiation of apoptosis in cells. Results in Figure 7B demonstrated that PDT of Ce6 resulted in decrease
of intracellular Bcl-2 level. The decrease in Bcl-2 was the reason for Cytochrome \(c\) elevation, which is further responsible for the increase of cleaved Caspase-3. It was noted that CCM/SLI/Ce6 showed the lowest Bcl-2 level as well as the highest Cytochrome \(c\) and Caspase-3 levels among three groups, indicating that CCM/SLI/Ce6-treated cells were undergoing severer apoptosis as compared to other groups.

Multicellular tumor spheroid (MCTS) composed of fibroblast and tumor cells represents the two basic components of solid tumors. As a result, MCTS was widely adopted to mimic solid tumors for in vitro studies. As shown in Figure 8A, the volume of MCTS without any treatment continuously increased to grow to nearly 3-fold of its original size with evident boundary, which was similar to the progression of solid tumors. After being subjected to PDT, the volume of MCTS showed significant decrease as compared to the saline group. Most importantly, consistent with the MTT assay, CCM/SLI/Ce6 showed the best anticancer effect with the smallest MCTS volume, which reversed the growth of MCTS to 0.72-fold of its original size. This conclusion was also supported by the optical images of MCTS shown in Figure 8B.

2.4. In Vivo Anticancer Study. The in vivo anticancer study was also conducted using MCF-7 tumor-bearing mice to confirm the above conclusions. The mice were randomly assigned to different groups containing six subjects. Afterwards, the mice were administered with different formulations with the same amount of Ce6 (5 mg/kg) in parallel, using saline as the control. The administration was repeated every two days and the changes in tumor volume and body weight were recorded until the final administration. As shown in Figure 9A, PDT could exert beneficial effects on the subjects as the tumor volume in all formulations showed significant decrease in tumor growth. Among them, CCM/SLI/Ce6 showed the most potent efficacy (52 ± 9 mm\(^3\)). Additionally, the variation in body weight of the subjects are summarized in Figure 9B and reveal interesting results. In consistency with results obtained in the MT assay, there was no evident decline of body weight in the CCM/SLI/Ce6 group, confirming its preferable biocompatibility. However, it was noted that mice in the SIL/Ce6 group showed steady decrease in body weight as a function of time. Considering the untargeted distribution of SIL/Ce6 in major organs as well as its positive surface charge, it was inferred that SIL/Ce6 might induced pathological changes on these organs through disruption of membrane integrity. As a result, it was concluded that the tumor homing and shielding property of CCM on the surface of CCM/SLI/Ce6 not only increase its anticancer efficacy but also reduce the undesired side effects. Finally, HE assay was performed to verify the apoptosis of tumor tissues (Figure 9C). Compared to the insignificant apoptosis in the saline group, increased apoptosis was observed in all Ce6-containing groups with the most serious one observed in the CCM/SLI/Ce6 group, which was in line with results obtained in the above assays. In a word, CCM/SLI/Ce6 holds great potential to be a preferable tumor-targeting DDS for effective PDT of cancer.

2.5. Tumor Disruption and Immune Responses. In order to illuminate the other underlying mechanisms responsible for effective PDT, the integrality of tumor structure was investigated by studying the expressions of ECs junction-related markers, VE-cadherin and CD31, at different time intervals after PDT. As shown in Figure 10, tumors after PDT treatment suffered from significant decline of both VE-cadherin and CD31, which was also positively related to time. It is worth mentioning that the CCM/SLI/Ce6 group showed the most potent downregulation of both markers, suggesting that not only the tumor cells were killed, but also...
tumor integrity was damaged after the CCM/SIL/Ce6 treatment. The disrupted tumor tissue was expected to increase the transportation of oxygen and other constituents such as DC, which led to following immune responses.

Furthermore, two representative cytokines (TNF-α and IL-12) secreted by DC cells were selected and their concentrations in plasma were determined using an ELISA kit. TNF-α is known to play important anticancer roles, whereas IL-12 is critical for the activation of CD8+ T lymphocytes and cytotoxic activity of natural killer cells. As shown in Figure 11, at 24, 48, and 72 h after the different treatments, the plasma levels of both TNF-α and IL-12 gradually increased as a function of time in PDT-treated groups, whereas insignificant changes were observed in the control group. Most importantly, the CCM/SIL/Ce6 group showed the highest cytokine levels, indicating that CCM/SIL/Ce6 could significantly induce the immune response of the organism.

3. CONCLUSIONS

In our work, SIL was coated with CCM to construct an artificial cell-like DDS for effective delivery of Ce6 (CCM/SIL/Ce6). The CCM/SIL/Ce6 was demonstrated to be well-dispersed nanoparticles with preferable stability. In addition, CCM/SIL/Ce6 showed pH-responsive drug release as well as efficient ROS generation capacity. The CCM modification could positively guide CCM/SIL/Ce6 to target isogenous MCF-7 cells. Both in vitro and in vivo anticancer assays demonstrated that CCM/SIL/Ce6 exerted superior PDT benefits than other counterparts, which might be accompanied with tumor disruption and immune responses.

4. MATERIALS AND METHODS

4.1. Materials. DPBF, MTT, N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AEAPS), DCFC-D4A, tetraethyl orthosilicate (TEOS), Ce6, Triton X-100, and paraformaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA). The otherwise stated chemicals were all from Aladdin Co., Ltd. (Shanghai, China) in analytically pure forms.

4.2. Preparation of Artificial Cell Carrier. The preparation of the artificial cell carrier was composed of two steps. The SIL core was first synthesized according to a previous report. In brief, a water-in-oil microemulsion was prepared, to which TEOS, AEAPS, and NH$_4$OH were successively added to initiate the reaction. After being proceeded at room temperature for 24 h, the product was collected by centrifugation (3000g, 10 min).

The isolation of CCM was conducted in parallel using a previously reported protocol. In brief, MCF-7 cells were suspended in PBS and then homogenized in proper amount of extracting buffer on ice. The obtained solution was first centrifuged at 10000g (4 °C) for 10 min to remove large cellular debris. Afterwards, the obtained supernatant was further centrifuged at 10000g (4 °C) for 1 h to collect CCM. The obtained CCM was resuspended in preserving buffer and stored at −80 °C until usage. The protein concentration of purified PM was quantified using a BCA kit (Beyotime, Shanghai, China) according to the manufacturer’s instructions.

To finally construct the artificial cell carrier, SIL was well dispersed in PBS to achieve a final concentration of 1 mg/mL with the assistance of sonication, the CCM (containing 50 μg of protein) was then added dropwise into the solution under vortex. Afterwards, the mixture was subjected to probe-type sonication (100 W, 3 min). The mixture was further centrifuged (10000g, 10 min) to obtain CCM/SIL.

4.3. Drug Loading. The prepared SIL was suspended in proper amount of ethanol under gentle agitation. Afterwards, Ce6 in DMSO was dropwise added into SIL under agitation. After being agitated at room temperature for 24 h, the mixture was subjected to centrifugation (3000g, 10 min) to obtain the drug-loaded SIL/Ce6. The SIL/Ce6 was then subjected to CCM modification to finally obtain CCM/SIL/Ce6. In order to determine the drug-loading content (DLC) of CCM/SIL/Ce6, nanoparticles were immersed in DMSO for 48 h to fully extract the loaded Ce6. Afterwards, the solution was loaded into a high-performance liquid chromatography system to determine the DLC using the following conditions: A Thermo-Fisher UltiMate 3000 was loaded with LC-18-SUPELCO C$_18$ column (250 mm × 4.6 mm, 5 μm) and the mobile phase consisted of mixed methanol–ammonium acetate aqueous buffer (0.05 M, pH was adjusted to 3.0 by acetic acid); determined wavelength was 405 nm with flow rate at 1 mL/min under 30 °C.

4.4. Characterization. The size distribution of CCM/SIL/Ce6 was measured by a size analyzer (ZS90, Malvern, UK). The morphology was observed using TEM (Hitachi-1700, Hitachi, Japan).

The 2% red blood cells (RBC, from New Zealand rabbit, BioChannel, Nanjing, China) were incubated with different concentrations of CCM/SIL/Ce6 at 37 °C for 1 h. Afterwards, the RBCs were isolated using centrifugation and the absorbance at 545 nm in the supernatant was determined by

Figure 11. The levels of (A) TNF-α and (B) IL-12 in sera from mice isolated at 24, 48, and 72 h after different treatments. Data are shown as mean ± S.D. (n = 3). **P < 0.01 vs CCM/SIL/Ce6.
using an ultraviolet spectrophotometer (UV-1200, MAPADA, Shanghai, China).

The BSA adsorption assay was conducted according to a previous report. In brief, SIL/Ce6 and CCM/SIL/Ce6 were incubated with BSA aqueous solution (1 mg/mL) at the final ACC concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL for 2 h at 37 °C. At the end of the incubation, samples were first centrifuged at 20,000 rpm for 30 min and then filtrated through a Millipore Syringe (0.22 μm). The variation of absorbance at 278 nm was recorded and plotted against concentration. BSA aqueous solutions subjected to the same procedures were employed as blank control.

The protein was extracted by the RIPA Lysis buffer (Thermo Fisher, USA), followed by quantification by the BCA kit (Thermo Fisher). Different samples with the same amount of proteins were loaded into the SDS-PAGE gel and subjected to electrophoresis (90 V, 200 min). The isolated proteins were transferred onto a poly(vinylidene difluoride) membrane and stained with specific first antibodies at 4 °C overnight. Finally, the IRDyeR680CW-labeled second antibody was applied and the blots were observed by using a densitometer (ChemStudio touch, Analytik Jena AG, Germany).

The TNF-α and IL-12 levels in the serum supernatant were assayed using an ELISA kit (Thermo Fisher) following the manufacturer’s instructions.

4.5. Stability Assay. The size changes of CCM/SIL/Ce6 in PBS and mouse plasma were monitored for 48 h to test its colloidal stability. Moreover, the comparative fluorescence quenching between CCM/SIL/Ce6 and free Ce6 was also determined by using a fluorescence spectrophotometer (F95, Ayuc Instrument, Shanghai, China) for 6 days.

4.6. In Vitro Release and in Vitro ROS Generation. The drug release from CCM/SIL/Ce6 was performed according to a previous protocol. In brief, CCM/SIL/Ce6 in a dialysis bag was immersed in PBS containing 0.1% Tween (v/v) and placed in a thermostatic shaker (DHG-9053A; Marit, Wuxi, China). The drug concentration at different time points was determined using the protocols described above.

In order to test the ROS generation capacity, 20 μL of DPBF (10 mM) was added to the CCM/SIL/Ce6 solution. The mixture was irradiated with laser (680 nm, 1 W/cm²) for 5 min in all Ce6-containing groups. The administration and other protocols follow the previous report.

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Notes

The authors declare no competing financial interest.

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