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REVIEW

The Current Status of Chlorin e6-Based Nanoscale Delivery Systems for Cancer Therapy

Zhengyi Li[1,2,3] and Lihua Qiu[1,2,3,*]

[1]College of Stomatology, Chongqing Medical University, Chongqing, 401147, China

[2]Chongqing Key Laboratory of Oral Diseases and Biomedical Sciences, Chongqing, 401147, China

[3]Chongqing Municipal Key Laboratory of Oral Biomedical Engineering of Higher Education, Chongqing, 401147, China

[*]Corresponding Author: Lihua Qiu. Email: 500082@hospital.cqmu.edu.cn

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ABSTRACT

Improving the effectiveness of cancer treatment has become a central concern for the public. In recent years, in order to maximize the efficiency of cancer treatment, photodynamic therapy (PDT) and sonodynamic therapy (SDT) have received widespread attention. Chlorin e6 (Ce6) is a fluorescent dye with strong optical properties and excellent photoconversion efficiency under near-infrared light irradiation, which has been widely used in PDT in recent decades due to its superior antitumor ability. Of note, Ce6 can be used as a sonosensitizer for SDT, which generates large amounts of reactive oxygen species (ROS) for tumor treatment after ultrasound activation. These strategies can selectively kill local tumors without endangering normal tissues. At present, there are more and more researches on optimizing the pharmacological properties of Ce6-based therapeutic agents. Therefore, this review focuses on the properties of Ce6 and the research progress of various nano-scale delivery strategies based on Ce6 in tumor treatment. At last, we summarized the positive impact and bright prospects of Ce6-based nanomaterials in cancer treatment applications.

KEYWORDS

Cancer; chlorin e6; photodynamic therapy; sonodynamic therapy

1 Introduction

Recently, cancer is a severe life-threaten disease for human being, causing the highest mortality in the world [1]. In the field of tumor treatment, surgical resection involving the use of radiotherapy and chemotherapy have emerged as critical therapeutic strategies. However, the conventional therapies suffer from multidrug resistance, systemic toxicity, low bioavailability and poor target specificity, resulting in unsatisfied therapeutic outcomes [2,3]. To solve these issues mentioned above, nanotechnology provides a new therapeutic strategy to enable targeted delivery of anti-tumor drugs to cancerous areas.

Photodynamic therapy (PDT) is an emerging minimally invasive treatment modality that requires specific wavelengths of laser light and photosensitizers to produce reactive oxygen species (ROS) to kill tumor cells, providing a promising option for cancer treatment [4,5]. Notably, PDT-based sonodynamic therapy (SDT) has a better prospect for application due to the deeper penetration [6]. Briefly, ultrasound can activate the sonosensitizer in the body to produce ROS, consequently killing tumor cells. Compared

with PDT, SDT is not only non-invasive and low toxic, but also has deeper penetration to reach deep tumors. Meanwhile, the energy does not decrease even during the process of ultrasonic transmission.

Altogether, sensitizers play crucial roles in PDT and SDT. As a second-generation photosensitizer, Ce6 overcomes the limitations of the first-generation photosensitizer, such as long shielding time, complex composition, weak absorbance, and poor selectivity. Moreover, Ce6 has been proven to be an effective and safe sonosensitizer in SDT, producing toxic substances and inducing apoptosis in tumor cells under low-intensity ultrasound excitation. However, the poor water solubility, short circulation time *in vivo* and low tumor uptake rate limit its clinical application.

Therefore, our review focused on the chemical modification and encapsulation of Ce6 in nanocarriers (polymers, liposomes, proteins or inorganic nanoplatforms, etc.), thereby increasing the solubility of Ce6 and prolonging the circulation time *in vivo* (Fig. 1). Meanwhile, we summarized the application of Ce6-based nanomaterials in tumor treatment (PDT and SDT) and the synergistic effects to guide clinical translation.

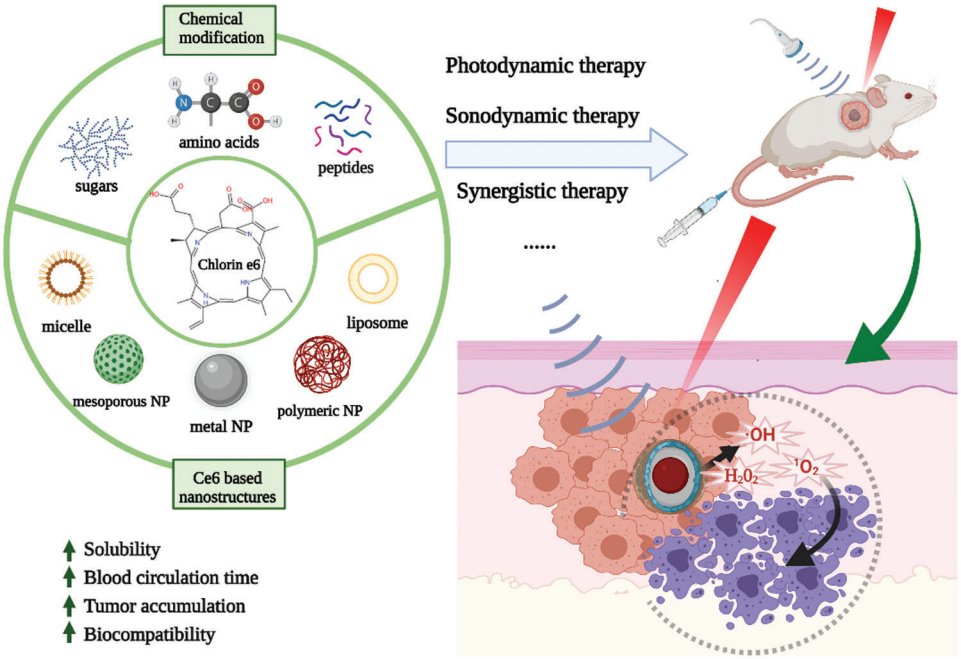

Figure 1: Schematic diagram of Ce6-based nanomaterials in cancer treatment

2 Properties and Chemical Modification

Nowadays, Ce6 has attracted a lot of attention due to its strong absorption and penetration ability in the near-infrared region. As a stable degradation product of chlorophyll a, Ce6 is an asymmetric molecule with three ionizable carboxyl groups and four pyrrole rings (Fig. 2). It has the advantages of single chemical composition and clear structure, and can be activated for PDT at a wavelength of 660 nm. In addition, the amphiphilic structure of the Ce6 molecule can help it penetrate the cell membrane and accumulate in the cell. At the same time, in an environment of pH 7–8, the high quantum yield of singlet oxygen is 0.65 [7]. As mentioned above, Ce6 can be activated by ultrasound for the treatment of diseases [8]. By co-culturing human lung adenocarcinoma cells (SPCA-1) and Ce6 under ultrasound (1.6 w/cm^2), it was found that the growth of tumor cells was inhibited ($p < 0.05$). Flow cytometry results showed that Ce6 could produce strong cytotoxicity after ultrasound activation [9].

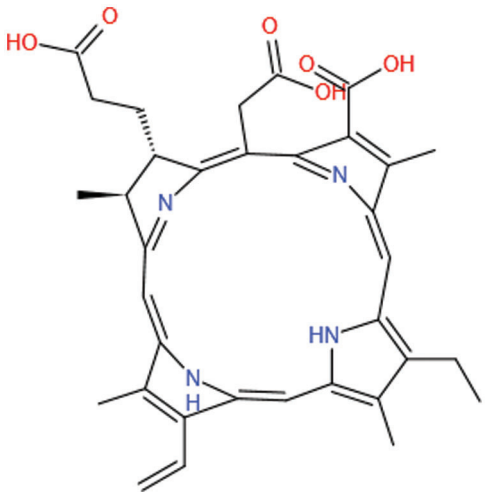

Figure 2: The structure of Ce6

However, the poor water solubility and short circulation time *in vivo* limit its clinical application. To overcome the above problems, Ce6 was reported to bind with peptides, amino acids and sugars to improve the solubility *in vivo* [10]. For instance, inserting lysine and aspartic acid residues at positions 17^3, 15^2 and 13^1 of Ce6 can give three regioisomeric [11]. It has been reported that amino acid derivatives were more likely to accumulate in HEP 2 than Ce6. In particular, the 15^{2-}lysyl derivative was taken up by cells to a significantly higher extent than the other derivatives. In contrast, Ce6 functionalized with 13^{1-}lysine residues has the strongest phototoxicity, indicating that the amino acid conjugation site on the Ce6 macrocycle is the primary determinant of the phototoxicity For the purpose of promoting the efficacy and reducing the systematic toxicity of PDT for cancer treatment, a new photosensitizer (β-M-Ce6) was synthesized as a tumor-targeting nanomedicine with enhanced PDT efficacy through the combination of β-mannose and Ce6 [12].

Because of their unique Warburg effect, tumor cells are able to use anaerobic glycolysis to produce lactate and energy, thus taking up glucose more readily than the surrounding normal cells [13]. Therefore, the new photosensitizer (β-M-Ce6) designed using this phenomenon has the specificity and selectivity of cancer cells. Compared with the clinically approved photosensitizer talaporfen sodium (TS), β-M-Ce6 accumulates more rapidly in tumor cells and is distributed in multiple organelles such as mitochondria, lysosomes and Golgi apparatus, with a therapeutic effect 1000-fold higher than that of TS. Most importantly, β-M-Ce6 is expected to be a promising photosensitizer due to its perfect stability, targeting and efficient anti-tumor ability. Covalently linking Ce6 to the Shiga-like toxin B subunit (SLTB) to obtain targeted delivery [14]. This strategy markedly reduced the cytotoxicity of Ce6 and significantly improved the photosensitization activity. The results showed that Ce6-SLTB enhanced the targeted delivery compared to free Ce6. In Vero cells, the photodynamic toxicity of Ce6-SLTB is about 12 times higher than that of free Ce6 at the same concentration. Therefore, chemical and structural modifications of Ce6 resulted in high stability, low toxicity, and high tumor selectivity.

3 Ce6 Based Nanoplatforms

In addition, various strategies have been tried for Ce6 delivery, and multiple nano-platforms based on organic, inorganic, and other nanomaterials have been studied for efficient and targeted Ce6 delivery. Here, we review various nano-platforms used to deliver Ce6, such as polymer-based platforms, lipid-based liposomes, protein-based nanoparticles, inorganics, and biomimetic nanosystems, and so on.

3.1 Polymer-Based Platforms

Polymer nanocarriers have easy-to-modify size, surface functionalization, and high-efficiency bioavailability, so they have significant advantages in drug delivery applications. Polyvinylpyrrolidone (PVP) is a non-ionic polymer compound with the general properties of a water-soluble polymer compound. The Belarusian Institute of Medicine improved the hydrophilicity of Ce6 by mixing Ce6 and polyvinylpyrrolidone (PVP) 1:1 (mass ratio). When Ce6 interacts with PVP, it is embedded in the hydrophobic region of the polymer, resulting in significant changes in its spectral and kinetic characteristics. It has a higher quantum yield of fluorescence and $^1O_2$ quantum yield. Chutoprapat et al. [15] used the chicken chorioallantoic membrane model to research the impact of the hydrophilic polymer PVP on the poorly water-soluble Ce6. Ce6 can interact with PVP through hydrophobic bonds to improve the solubility of Ce6 in an aqueous solution. *In vitro* experiments have proved that PVP can be used as a penetration enhancer to improve the solubility, diffusivity and disaggregation of poor water solubility drugs. In addition, poly (lactide-glycolic acid copolymer) (PLGA) is a linear aliphatic polyester with outstanding biodegradability and biocompatibility. Due to the high loading capacity of PLGA, it can be used as an ideal nanocarrier in cancer treatment, and a variety of hydrophobic and hydrophilic therapeutic drugs can be co-encapsulated by the double emulsion method. Recently, Chen and colleagues fabricated a nanosystem coated with the FDA-approved PLGA containing ferroferric oxide (Fe_3O_4) and Ce6 through hydrophobic interactions and chelation, which was used to combine ferroptosis-photodynamic for tumor treatment [16]. And it can also be used for near-infrared fluorescence (NIRF)/MRI bimodal image-guided cancer diagnosis. Under acidic conditions, the formed Fe_3O_4-PLGA-Ce6 NPs can be degraded to release ferric/ferrous ions and Ce6. Ferrous or ferric ions can react with excess H_2O_2 in tumor cells to trigger the Fenton reaction, producing hydroxyl radicals (˙OH) to promote tumor cell apoptosis. At the same time, a large amount of oxygen can be made through the Fenton reaction to alleviate the hypoxia in the tumor microenvironment and improve the therapeutic effect of PDT. And Ce6 can produce singlet oxygen under laser irradiation, thereby inducing tumor cell necrosis and further promoting ferroptosis. In addition, it is worth noting that iron-based NPs can be used for T2-weighted MRI for their good superparamagnetism [17]. The ratio changes of ferric to ferrous leads to the responsive MRI that can be used to monitor tumor growth [18]. Using the DCFH-DA fluorescent probe to detect the intracellular ROS level proved that the 4T1 cells were incubated with Fe_3O_4-PLGA-Ce6 NPs plus light irradiation had the highest ROS concentration [19]. The results of *in vivo* and *in vitro* experiments also strongly support the synergistic treatment of ferroptosis and PDT. Due to its sensitivity to ultrasound and good affinity to tumor tissues, Ce6 can also be used as a sonosensitizer. For example, by encapsulating the sonosensitizer Ce6, perfluoropentane (PFP) and docetaxel (DTX) into a PLGA nanoplatform with a core-shell structure, the combination of SDT and chemotherapy (CT) for tumors is realized [20]. Compared with the single use of chemotherapeutics drug DTX, the effective encapsulation of the drug can help significantly reduce the side effects of DTX, and the generated ROS can kill tumor cells. And *in vitro* studies have shown that Ce6 coated with nanoparticles can generate more ROS than Ce6 alone. This is of great significance for improving the anti-tumor effect and reducing patients' pain during treatment. And the ability to use PFP's ultrasound imaging to improve the diagnosis of tumors is also helpful to observe the anti-tumor effect. It can achieve the synergistic treatment of CT and SDT, which significantly enhances the anti-tumor outcome.

In addition to PLGA, amphiphilic block copolymers can form nanocarriers with drug loading capabilities through self-assembly, which has attracted the attention of many scholars. Ma et al. [21] used the amphiphilic block polymer Chol-PEG2000 as a stabilizer, and loads the chemotherapy drugs 10-hydroxycamptothecin (HCPT) and the photosensitizer Ce6 through a simple antisolvent precipitation method to achieve the chemical photodynamic effect. The obtained nanoparticles have excellent monodispersity, uniform rod shape and good aqueous storage stability. And HCPT/Ce6 NPs show a significantly higher uptake efficiency than free HCPT. Most importantly, under laser irradiation,

HCPT/Ce6 NPs showed significantly more potent anti-tumor efficacy *in vivo*, which was attributed to the enhanced permeability and retention (EPR) effect, but the systemic toxicity is negligible. Similarly, Kumari and his colleagues designed an amphiphilic polymer self-assembled to form polymer micelles in an aqueous environment. Connect Ce6 with monomethoxy poly (ethylene glycol)-poly (D, L-lactide) (mPEG-PLA) through disulfide bonds to obtain micellar drug mPEG-PLA-S-S-Ce6 [22]. This dramatically improves the solubility of Ce6 and its cyclic stability, and through the EPR effect, a large number of drugs can be selectively accumulated and released into cancer cells. In addition, glutathione (GSH) is a thiol-containing reducing tripeptide that can cleave nanocarriers with disulfide bonds. More importantly, the concentration of GSH in tumor cells is about 4 times that of normal tissues. Using this considerable difference can better deliver Ce6 in cancer cells and reduce adverse side effects. And DNA fragmentation and nuclear staining experiments evaluated that compared with free Ce6, mPEG-PLA-S-S-Ce6 micelle treatment caused more significant DNA damage to tumor cells and showed excellent phototoxicity.

3.2 Lipid-Based Platforms

Lipid-based nanoparticles (LNP) have become one of the most researched platforms due to their biocompatibility, biodegradability, and ability to capture hydrophilic and hydrophobic therapeutic agents. At present, the four representative LNP platforms commonly used for therapeutic diagnosis mainly include stealth liposomes, porphyrins, triggered-release liposomes, and lipid-coated calcium phosphate nanoparticles (LCPs) [23]. Stealth liposomes are liposomes modified by polyethylene glycol (PEG) or polyethylene glycol linked to ligands, which have the advantages of prolonging the half-life of liposomes in blood circulation and improving their stability. This is due to the delayed recognition of the reticuloendothelial system (RES). It proved that PEG on the surface of liposomes could reduce the uptake of the mononuclear phagocyte system (MPS) and prolong the time of blood circulation [24]. In addition, to enhance the ability of nanoparticles to target tumor tissue actively, ligands such as peptides and folic acid (FA) are combined with particles so that they can specifically bind to overexpressed receptors in different types of tumors. Zhang et al. [25] constructed an FA-modified nanostructured lipid carrier loaded with paclitaxel and Ce6 (PTX@FA-NLC-PEG-Ce6), which overcomes the shortcomings of PTX and Ce6 in terms of hydrophobicity and increase the concentration of the drug at the tumor site to achieve long-term blood circulation and tumor targeting ability after intravenous administration. In another work, compared with liposome Ce6 and liposome doxorubicin (DOX) used alone, PEGylated dual-effect liposome (named PL-Dox-Ce6) has a significant therapeutic effect, realizing the combination of PDT and CT [26]. *In vitro* release studies have shown that after laser irradiation, the chemotherapeutic drug released by the dual-effect liposome is 4 to 5 times that of the other two liposomes. Furthermore, according to the kinetic data of tumor and plasma, a single dose of PL-Dox-Ce6 combined with laser irradiation can completely eradicate more than 90% of the tumors in mice, indicating that the treatment efficiency of tumor-bearing mice is improved while reducing the toxicity of the drug to the human body. Nevertheless, although medications can be delivered to the tumor site through EPR effects, the release behavior of many nanocarriers depends on the autogenous degradation of the carrier in the body, which causes an accidental release of drugs during circulation [27]. Therefore, it is necessary to explore a nanocarrier that only releases drugs at the tumor site, but not at the non-cancerous part. Triggered release liposomes came into being, an intelligent system that releases drugs for local administration in response to external stimuli. Zhou and colleagues use ultrasound to trigger the release of drugs and enhance controlled-release and storage stability [28]. They designed a particular liposomal drug delivery system that mixed lipophilic Ce6 with lipids to synthesize ultrasound-triggered liposomes (UT-L). The chemotherapy drug DOX was encapsulated in hydrophilic capsules inside the liposomes. Under the stimulation of US, Ce6 can destroy the lipid bilayer of liposomes, leading to the destruction of UT-L. In addition, to achieve active targeting of tumor sites, they inserted NGR peptides into liposomes, allowing

NGR/UT-L to accumulate the tumor site to exert the targeting effect of NGR. Then due to the sonodynamic impact, it is destroyed by ultrasound irradiation to release the drug. On the other hand, Zhang et al. [29] developed an enteric-coated granule that can respond to pH/ultrasound, with targeting and controlled release capabilities, for use in combined sonodynamic-chemotherapy. The mesoporous silicon-coated gold nanoparticles loaded with Ce6 and DOX are covered with phospholipid modified by folic acid (SLB-FA) and coated with a layer of carboxymethyl chitosan (CMC). Due to the existence of CMC, the stability of the nanoparticles in the robust acid environment of the stomach and the safety of the gastrointestinal tract can be guaranteed, so they can be administered orally, which overcomes the shortcomings of traditional intravenous injection. In addition, the gold nanoparticles in the granule have the function of Computed Tomography (CT) imaging, which can be used for tumor diagnosis. At the same time, the FA-modified nanoparticles can be actively targeted to the colorectal tumor, and the particles can be destroyed and released the loaded Ce6 and DOX under the solid penetrating power of ultrasound. These new nano-platforms show better-controlled release and tumor targeting capabilities *in vivo* and *in vitro* experiments, which have broad prospects in the early diagnosis and early treatment of cancer in the future.

3.3 Protein-Based Platforms

As the leading carrier of life activities, protein has become a multifunctional carrier for various biological applications in the past few decades due to its non-toxic, non-immunogenic, excellent biocompatibility and biodegradability [30]. Among them, albumin has been widely used clinically, and the FDA has also approved the albumin-based nano-drug Abraxane for the treatment of metastatic breast cancer [31]. Others including human serum albumin (HSA) [32], bovine serum albumin (BSA) [33], and hemoglobin (HB) [34] have also been used in preclinical research. Phuong et al. [35] used β-carotene cross-linker agent to assemble Ce6 and BSA into nanoparticles through nanoparticle albumin binding technology for PDT. After laser irradiation, the tumor growth of the mice in the Ce6-BSA-BC-NPs group was significantly inhibited, and the tumor volume was 5 times smaller than the control group (using only Ce6). Through improved tumor targeting ability and prolonged circulation time, effective Ce6 (from Ce6-BSA-BC-NP) can accumulate at the tumor site. As we all know, due to the uncontrolled proliferation of cancer cells, low tissue oxygen concentration and insufficient blood flow, a hallmark feature of the tumor microenvironment is hypoxia [36]. Therefore, it is necessary to develop hypoxia-responsive nanoplatforms to respond to hypoxia at the tumor site. Yang et al. designed a unique HSA nanoparticle (HCHOA) to respond to hypoxia [37]. The photosensitizer Ce6 and the oxaliplatin prodrug each bind to HSA by forming an amide bond to form ultra-small HC and HO nanoparticles, both of which are less than 10 nm in diameter. Then the HC and HO are connected by the hypoxia-sensitive linker azobenzene to form the HCHOA assembly. When entering the hypoxic tumor microenvironment, the azobenzene part of the HCHOA structure is cleaved by many reductases, which causes the nanosystem to be decomposed into ultra-small nanoparticles with a diameter of less than 10 nm, thereby enhancing their permeability. Compared with the control group, HCHOA nanoparticles with a controllable size showed stable blood circulation and effective tumor accumulation and significantly increased intratumor penetration. In addition, to overcome the unsatisfactory effect of PDT due to hypoxia in the tumor microenvironment and continuous oxygen consumption in the PDT process, people have integrated manganese dioxide (MnO_2) nanoparticles and albumin through biomineralization strategies. The use of MnO_2 can trigger the decomposition of endogenous H_2O_2 to produce oxygen to improve the tumor hypoxic microenvironment, thereby greatly improving the anti-tumor effect [38,39]. This, the use of protein as a carrier to design and develop nano-platforms for oncotherapy has shown effective therapeutic capabilities, which indicates that they have great potential in future clinical applications.

3.4 Inorganic Platforms

As compared to other nanoparticles, inorganic nanoparticles have unique physical/chemical properties, facile preparation, good biocompatibility, and ease functionalization [40]. Therefore, these inorganic nanoparticles, such as gold nanoparticles, magnetic nanoparticles, mesoporous silica nanoparticles, and hollow manganese dioxide nanoparticles, have received extensive attention in potential applications in cancer treatment and diagnosis [41]. Among inorganic nanoparticles, silica-based nanoparticles exist in various forms, such as solid, hollow, and porous. This controllable characteristic makes it have a large surface area and pore volume and a sustained drug release ability, which has broad application prospects in cancer treatment and diagnosis [42]. Mesoporous silica nanoparticles (MSN) have abundant silanol groups on the surface, which can react with other functional groups to change their physical or morphology. In addition, it has been proven that MSN can effectively produce sonodynamic effects for the treatment of HEP-2 and Lewis carcinomas in mice [43]. Xu et al. [44] designed mesoporous silica nanoparticles loaded with DOX and sonosensitizer Ce6 to explore its anti-tumor effect under ultrasound treatment. Interestingly, the inhibitory efficiency of MSN-DOX-Ce6 on tumor cells under the US is not much higher than that of other treatment groups *in vitro*. However, the anti-tumor effect of MSN-DOX-Ce6 + US on xenograft tumor-bearing mice was markedly better than that of DOX + Ce6 + US or DOX alone *in vitro*. This is because nanoparticles (MSN-DOX-Ce6) in the blood circulation can be passively targeted and enriched to the tumor site through the EPR effect, and then release DOX and Ce6 to increase the drug concentration around the tumor cells. Similarly, MnO_2 nanoparticles with a hollow structure are also designed as functional Ce6-based inorganic nano-platforms for drug delivery. And MnO_2 can catalyze the decomposition of hydrogen peroxide to improve the hypoxic tumor microenvironment, and the Mn^{2+} produced simultaneously can be used for MRI imaging. Zhu et al. [45] designed multifunctional manganese dioxide nanoparticles (Ce6@MnO_2-PEG) whose surface is modified by PEG and loaded with Ce6, which can be used to enhance tumor-specific PDT. Compared with free Ce6, Ce6@MnO_2-PEG nanoparticles show excellent PDT therapeutic effects even in low-oxygen environments, and can significantly inhibit tumors at a greatly reduced dose. According to the *in vivo* experimental data, the therapeutic effect achieved by intravenous injection of Ce6@MnO_2-PEG seems to be much better than the effect obtained by using 3.5 times the dose of free Ce6. Interestingly, MnO_2 can be converted into Mn^{2+} in the weakly acidic tumor microenvironment, providing strong T1 MR contrast, which has great potential in the early and accurate diagnosis of tumors. In addition, Ce6@MnO_2-PEG can be effectively retained at the tumor site after systemic intravenous injection, and MnO_2 nanoparticles are decomposed into Mn^{2+} that are easy to excrete so that the kidneys can quickly filter them.

Different from other morphologically controllable nanoparticles, nanostructures composed of precious metals such as gold also have unique advantages. Gold nanostructures are widely used in cancer diagnosis and treatment due to their unique optical properties, multifunctional surface, easy processing and excellent biocompatibility. What's more, Au NP has the ability of surface plasmon resonance (SPR), which generates a solid electromagnetic field on the surface of the particles, thereby enhancing all the radiative (absorption and scattering) and non-radiative (absorbed light into heat). By combining the photosensitizer Ce6 with gold nanorods (Au NR), Zhang et al. [46] demonstrated that reduced plasma quenching can enhance the generation of $^1O_2$. After 660 nm laser irradiation, it can be found that the intensity of the $^1O_2$ signal is 7 times higher than that of the control group. This conclusion is also confirmed by the fluorescence of the singlet oxygen sensor green (SOSG) of the $^1O_2$ probe and the oxidation of the antioxidant sodium ascorbate. In addition, their research shows that the plasmon resonance energy transfer (PRET) effect is more effective than the traditional local field enhancement effect in increasing the output of $^1O_2$. Likewise, Liu et al. [47] adsorbed Ce6 to gold nanoparticles connected with molybdenum disulfide (MoS_2) nanosheets to form a multifunctional metal anti-tumor nanocomposite, which enhances the imaging performance of CT/NIRF. Under 808 nm laser irradiation, the PEG-MoS2-Au mixture can

enhance the photothermal therapy (PTT) effect and improve CT imaging. The surface of AuNPs has SPR capability, which can keep Ce6 in a quenched state. Therefore, Ce6 released from the particle surface due to the PTT effect can be unquenched and elevated to a high-energy state. Then the energy is transferred to adjacent molecular oxygen under 660 nm laser irradiation to generate ROS. Thus, PTT can remotely induce Ce6 release and ROS generation, realizing the synergistic effect of PTT and PDT in anti-tumor therapy and NIRF dual-modal imaging. This dual-modal nanosystem achieves accurate cancer diagnosis and treatment at the same time.

3.5 Self-Assembled Nanoparticles

In addition to loading nano-carrier materials to form nanoparticles, some anti-tumor drugs (e.g., 10-hydroxycamptothecin, gambogic acid, sorafenib, doxorubicin, and paclitaxel, etc.) and sensitizers can self-assemble into nanoparticles without a carrier. At present, although nano-drug delivery system (NDDS) based combination therapy is a popular and effective strategy, there are still some potential problems, such as complex manufacturing processes (mainly through complex multi-step preparation processes and, or the use of toxic organic solvents), low drug loading (usually less than 10%), low cost-effectiveness, carrier-caused toxicity and damage to the kidneys and other organs [48]. Therefore, the carrier-free pure drug self-delivery nanosystem has become a potential tumor treatment strategy due to its economy, simple preparation process, higher drug loading rate, effective pharmacodynamics, and low side effects. Zhang et al. [49] self-assembled Ce6 and DOX into nanoparticles with an average particle size of 70 nm and a surface charge of −20 mV through electrostatic, π–π stacking, and hydrophobic interactions, which have high drug encapsulation efficiency and excellent colloidal stability. The self-assembly allows for better biodegradability and safety of the nanosystems due to the absence of other materials or functional organic molecules involved in the nanomaterials. In recent years, researchers have been inspired by the ability to target biomolecules precisely. By using ligands that can specifically bind to overexpressed receptors on the tumor cell surface, they can be used in carrier-free pure drug self-delivery nanosystems to enhance active targeting. Lan et al. [50] has developed a new type of carrier-free nanomedicine (GA-Ce6-FA NPs) to achieve synergistic treatment of CT combined with PDT. Through intermolecular static electricity, hydrophobicity, and π–π stacking, gambogic acid (GA), Ce6 and FA are self-assembled into carrier-free nanoparticles. In vivo fluorescence imaging shows that the nanoparticles have the targeting ability of FA and can accumulate specifically at the cancer site. And the circulation time is greatly increased, and the intracellular Ce6 uptake is significantly enhanced. As a natural chemotherapeutic drug, GA can destroy cell redox homeostasis, leading to the increase of ROS and the consumption of intracellular antioxidants (such as GSH), which significantly improve the efficiency of PDT to treat cancer. In another work, assemble the hydrophobic dye Ce6 and paclitaxel with the hydrophilic dye IR783 through the hydrophilic-hydrophobic self-assembly technology [51]. IR780 has a specific tumor targeting ability, which can increase tumor accumulation. And the use of its excellent fluorescence imaging capabilities can assist in the optimal time of precise ultrasound irradiation. This achievement provides a new idea for designing low-cost and effective nano-platforms for tumor diagnosis and treatment.

3.6 Bionic Nanoplatforms

Biomimetic nanotechnology using cell membrane-encapsulated nanoparticles with immune escape and excellent targeting ability plays an important role in the drug delivery system. In recent decades, with the rapid advancement of cell membrane camouflage nanotechnology, various types of cell membranes such as red blood cells, cancer cells, platelets, and bacteria have been used to achieve long-term circulation and targeted tumor therapy. The erythrocyte membrane was initially used to wrap synthetic nanoparticles to extend circulation in the body. The cyclic half-life of polymer nanoparticles coated with the red blood cell (RBC) membrane (39.6 h) is significantly longer than that of PEG-modified nanoparticles (15.8 h)

[52]. Inspired by the critical role of nanomedicine and RBC membranes, Zhang et al. [53] developed PLGA-based nanoparticles loaded with the photosensitizer Ce6, the surface of which is wrapped by an FA-modified RBC membrane. This way improves the long-term blood circulation and enhances tumor site accumulation, thereby improving the combined treatment effect of image-guided tumors. In addition, using cancer cell membranes (CCM) with the same targeting ability, coated nanoparticles (CCM@NPs) can actively move to tumors or metastatic diseased tissues. Yang's group used the CCM derived from SGC7901 cells to decorate silica nanoparticles (SLN). It used its good drug-carrying ability to load the photosensitizer Ce6, successfully constructed a biomimetic targeting nanosystem suitable for the treatment of gastric cancer [54]. Experiments have proved that due to CCM modification, nanoparticle CCM/SLN/Ce6 can specifically target homologous SGC7901 cells. Further *in vivo* experiments showed that CCM/SLN/Ce6 successfully inhibited the growth of tumors. At the same time, the bodyweight of the mice did not change significantly, indicating that CCM/SLN/Ce6 can improve curative effects while reducing adverse toxic effects. What is exciting is that with the continuous advancement of bionic technology, a hybrid cell membrane (HMC) is formed by fusing cell membranes from different sources, which can integrate different membrane functions into a bionic platform. For instance, Xie et al. [55] fuses the RBCm and the CCM into a mixed cell membrane to assemble a liposome-based nanoparticle (LSCMR NPs) containing the photosensitizer Ce6 and the chemotherapeutic drug chikusetsusaponin IVa methyl ester. According to SEM and DLS analysis, the zeta potential and average diameter of LSCMR NPs increased slightly. And performing protein electrophoresis further verified the successful fusion of the cell membrane. *In vitro* cell uptake and immune evasion ability experiments show that Ce6-loaded LSCMR NPs have lower Ce6 fluorescence intensity than control LSC NPs, attributed to hybrid cell membranes' inherent immune evasion characteristics. Since the characteristics of red blood cells are still retained in the hybrid membrane, the cyclic half-life of LSCMR NPs is about 3 times longer than that of Ce6 alone and shows apparent cytotoxicity under laser irradiation. On the other hand, *in vivo* experiments found that after 6 h of intravenous injection of nanoparticles, Ce6 and LC hardly aggregated at the tumor site, but LSCMR showed a strong fluorescence signal at the tumor site. These results prove that the obtained biomimetic nanoparticles have good biocompatibility and excellent chemical/photodynamic therapy effects on breast cancer.

Altogether, the hybrid cell membrane bionic technology has massive potential for clinical application in the future.

4 Ce6-Based Nanomaterials for Cancer Therapy

With the continuous progress of nanotechnology, various cancer diagnosis and treatment technologies developed based on new nanomaterials have aroused the interest of many scholars. PDT and SDT have achieved significant development due to their excellent targeting, extremely low toxicity, and the advantages of minimally invasive treatment [56]. Moreover, more and more natural products suitable for PDT and SDT have been discovered. As a fluorescent dye, Ce6, which is often used as a photosensitizer, has also been developed for acoustic therapy. Here, we outline some treatment strategies based on Ce6 nanomaterials commonly used in cancer treatment, and the synergy between different treatment modalities.

4.1 PDT

Photodynamic therapy, as a promising binary cancer therapy, uses the interaction between a specific wavelength of laser, photosensitizer and O_2 to generate high concentrations of ROS, thereby inducing vascular damage and destroying cancer cells [57]. Generally speaking, ROS is an oxygen-containing chemically reactive chemical substance, which usually exists in the body or the natural environment, and has an irreplaceable position in regulating various biological functions [58]. At present, PDT has a wide

range of applications in the clinical treatment of tumors due to its high treatment efficiency, good targeting and high safety. With the continuous advancement of nanotechnology, various nanomedicines that can produce ROS have been studied for tumor treatment. But most photosensitizers have poorly water-soluble and are easy to be removed by the body due to their polycyclic chemical structure. Son and colleagues combined different amounts of Ce6 with gelatin biopolymers to overcome these limitations to obtain two conjugates: gelatin-Ce6-2 and gelatin-Ce6-8, which have better water solubility and stability than free Ce6 [59]. And unlike free Ce6 and gelatin-Ce6-8, gelatin-Ce6-2 completely inhibits tumor growth without affecting normal tissues. It is worth noting that in the process of PDT to treat cancer, sufficient oxygen plays a vital function in ROS production. Yet, the typical sign of the tumor microenvironment is hypoxia. The rupture of blood vessels reduces the supply of oxygen to tumor tissues, and PDT needs to consume oxygen, which further deepens the hypoxia of tumor tissues, thereby inhibiting the efficiency of PDT [60]. Therefore, various methods have been developed to solve this problem, such as (1) O_2 supplement strategy, which uses high dissolved oxygen carriers to deliver oxygen to tumor tissues; (2) increases oxygen through materials such as catalase or MnO_2; and (3) system that combines PDT with hypoxia-activated chemotherapy or O_2-independent PTT and immunotherapy [61]. Perfluorocarbon (PFC) is widely used as an oxygen carrier for continuous oxygen supply to tumor tissue due to its high oxygen solubility and good biocompatibility [62]. Wang et al. [63] designed a new self-assembled amphiphilic block copolymer (Ce6-PFOC-PEI-M). Perfluoroalkyl groups are added to alleviate tumor tissue hypoxia and obtain oxygen-carrying capacity similar to that of PFC. Compared with micelles lacking perfluoroalkyl groups (Ce6-OC-PEI-M), fluorinated polymer micelles (Ce6-PFOC-PEI-M) have higher toxicity to tumor cells. In addition, *in vivo* experiments have shown that the micelles also have an excellent inhibitory effect on tumor growth.

4.2 SDT

Sonodynamic therapy is a new ROS generation therapy derived from PDT, which overcomes the laser's problem of penetrating deep tissues [64]. Due to the complexity of SDT treatment principles, the exact mechanism by which SDT kills tumor cells is still unclear. Based on the mechanism of cell apoptosis or death, it is generally believed that the ultrasound triggers the production of ROS, cavitation and thermal damage by activating sonosensitizers, which play an essential role in inducing the death of cancer cells [65]. Although compared with PDT, SDT has a deeper tissue penetration, but like PDT, tumor hypoxia will reduce the rate of ROS generation and affect the efficacy. Therefore, using some nanomaterials to improve tumor hypoxia and intensify the anti-tumor effect of SDT has been extensively developed. An et al. [66] used biodegradable hollow polydopamine particles as a carrier, embedded platinum (Pt) particles with catalase-like catalytic properties, and loaded DOX and Ce6. The mitochondrial targeting molecule triphenylphosphine (TPP) modification improves its targeting ability. The obtained nanoparticles cause the endogenous H_2O_2 of the tumor to be decomposed to produce O_2, alleviate the hypoxia of the tumor site, and carry the drug to target the mitochondria through the TPP and release the drug in response to the weakly acidic environment. The DOX release rate reached $63.91 \pm 1.67\%$. However, unlike normal cells, most tumor cells have a particular microenvironment that overexpresses GSH, which plays a crucial role in anti-cancer drug resistance through spontaneous reaction. And GSH as a reducing agent can directly react with ROS, thereby reducing the therapeutic effect of ROS-based therapies [67]. At present, all kinds of strategies have been explored to reduce the content of GSH in tumor cells to improve the anti-cancer effect. Zhang et al. [68] have created a new type of Ce6-loaded copper metal-organic framework nanoplatform that can respond to the hypoxic environments for chemodynamic therapy and sonodynamic therapy (CDT/SDT). The resulting nanoparticles can rapidly degrade and release Cu^{2+} in the hypoxic tumor microenvironment (TME), and then react with local GSH and reduce Cu^{2+} to Cu^{+}. Next, Cu^{+} can react with endogenous hydrogen peroxide in a Fenton-like reaction to produce cytotoxic hydroxyl radicals. Due to the consumption of GSH, the synergy of SDT/CDT is

significantly enhanced. Therefore, due to the unique advantages of SDT, such as high penetration and high targeting, it has excellent application prospects and provides new options for tumor treatment.

4.3 Synergistic Therapy

Although the current traditional cancer treatment methods have made major achievements, the relatively single treatment method is not effective enough to treat metastatic cancer due to its limitations. With the continuous development of biomimetic nanotechnology, many nanotechnology-based therapies, such as PDT, PTT, SDT and immunotherapy, have been combined with traditional treatments to achieve synergistic treatment of tumors. Unlike traditional monotherapy, the synergy between several types of treatment strategies can help improve the treatment effect of tumors and can achieve the effect of "1 + 1 > 2" [69]. Phototherapy is a light induction method, including PDT and PTT, which has attracted great attention for its non-invasive and good therapeutic effect [70]. For example, using the π–π interaction between polydopamine (PDA) and the photosensitizer Ce6, PDA nanoparticles (NPs) were loaded with hydrophobic Ce6 to synthesize an auspicious PDT/PTT therapeutic agent (PDA–Ce6 NPs) [71]. PDA-Ce6 NPs can maintain excellent colloidal stability until the 5th-day photosensitizer Ce6 is still ineffective release. And compared with the use of PTT or PDT alone, the combination of PDT + PTT from PDA-Ce6 was used to increase cell killing. In addition, the current promising SDT-based cancer treatment is gradually transformed into a multi-modal treatment, which is combined with other strategies, such as PDT, PTT, CDT, starvation therapy, and immunotherapy [72]. As mentioned before, Ce6 can be used as a photosensitizer or a sonosensitizer for PDT and SDT for anti-tumor therapy. It can also be combined with other treatment methods to achieve better therapeutic effects. To overcome the shortcomings of poor light penetration in the treatment of melanoma, Huang et al. [73] used SDT instead of PDT in combination with PD-L1 antibody (aPD-L1) immunotherapy, which can not only activate local anti-tumor immunity but also produce ROS to kill tumor cells. Therefore, a lipid-based micellar nanosystem containing the sonosensitizers Ce6 and aPD-L1 was designed, with a PEG coating that can be detached from the tumor microenvironment with low pH. Combined SDT with aPD-L1-mediated immunotherapy inhibits tumor growth and effectively promotes tumor cell apoptosis and cytotoxic T cell activation. In addition, PH-sensitive nanoparticles with PEG can be targeted for tumor delivery, thereby inducing local tumor immune responses without disrupting the immune balance of the PD-L1/PD-1 signaling pathway under normal conditions. Thus, this strategy can greatly reduce the occurrence of immune-related adverse reactions (irAEs). Starvation therapy has been an emerging tumor treatment method in recent years. The purpose of "starving" tumor cells is mainly achieved by inhibiting angiogenesis, cutting off tumor nutrition and energy supply, etc. Interestingly, tumor cells prefer low-efficiency glucose glycolysis to produce energy even under conditions of sufficient oxygen. Therefore, glucose plays a vital role in providing tumor growth and metabolism nutrition. Cancer can be "starved" by inhibiting the uptake of glucose [74]. Since glucose oxidase (GOx) can convert glucose and oxygen into gluconic acid and H_2O_2, GOx-mediated starvation can be combined with other treatment methods to treat cancer synergistically [75]. For example, load GOx and Ce6 into PLGA/metal-organic framework (MOF) core-shell nano-components and deposit hyaluronic acid (HA) on the surface to stabilize MnO_2 to build the target nano-platform [76]. GOx can inhibit the nutrient supply of tumor cells by catalyzing the decomposition of glucose. The produced H_2O_2 and endogenous glutathione are also consumed by manganese dioxide, which inhibits tumor antioxidant defense. In addition, the designed nanoparticles can selectively target tumor cells through surface HA. Starvation treatment can be achieved through this series of cascade reactions while improving the hypoxic environment at the tumor site. Thence, the combined use of Ce6-based nano-platforms with other treatment modalities can be widely used in clinical practice.

5 Conclusion and Outlook

In this review, we mainly discuss the current application status of Ce6-based nano-scale delivery systems in cancer treatment. Since 2002, Ce6 has been used in PDT due to its preferable biological activity and has become one of the most widely studied photosensitizers. Studies have shown that Ce6 exhibits good accumulation in tumors, rapid clearance from the body and has a higher molar extinction coefficient and higher active oxygen quantum yield [77]. Recently, Ce6 has gained considerable attention in acoustic therapy applications as a sonosensitizer. Due to its excellent anti-tumor photo/acoustic treatment efficiency, Ce6 can be activated as a photosensitizer or sonosensitizer to produce harmful substances, induce vascular damage, kill tumor tissues, and achieve good therapeutic effects.

However, the low accumulation of Ce6 at tumor sites remains an important challenge that needs to be addressed. We can obtain passive and active targeting ability by chemical modifications, such as Radachlorin, a derivative of Ce6, which accumulates mainly in mitochondria, lysosomes and endoplasmic reticulum [78], while sugar-conjugated chlorins accumulate selectively in cancer cells through the Warburg effect [79]. In addition, encapsulating Ce6 into nanocarriers can also improve its biodistribution and bioavailability. First of all, due to the EPR effect, Ce6-based nanocarriers exhibit long-term blood circulation and effective accumulation in tumor tissues. Secondly, compared with free Ce6, the Ce6 toxic and side effects delivered by nanoparticles are lower. In addition, by analyzing the data obtained from experiments, it is known that most of the various Ce6-based nano-platforms show excellent tumor selectivity and high bioavailability. Most importantly, Ce6 has unique properties that can be combined with other therapeutic drugs or nano-platforms to maximize the therapeutic effect and produce a "1 + 1 > 2" effect.

Overall, although some Ce6-based therapeutic agents have achieved good clinical outcomes, there are still some limitations remains. We still need to explore how to modify Ce6 to improve its targeting and permeability to achieve high efficiency and low toxicity. Optimizing the size and structure of nanoparticles that can easily penetrate and ablate solid tumors has become a critical issue to be addressed. Finally, the larger sample sizes and more time-point clinical trials are needed to confirm the role of Ce6 in oncology treatment.

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