Development of porphyrin and titanium dioxide sonosensitizers for sonodynamic cancer therapy

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Key Words:
cancer treatment; porphyrins; sonodynamic therapy; sonosensitizers; TiO_2

From the Contents

ABSTRACT

Sonodynamic therapy for malignant tumours has gained much attention for its deep penetration effect and efficient tumour killing ability. The design, modification, and utilization of sonosensitizers are important aspects of sonodynamic therapy. As an essential factor in this process, highly effective sonosensitizers should be developed to facilitate the clinical applications of sonodynamic therapy. This review takes porphyrin- and titanium dioxide (TiO_2)-based systems as representative organic and inorganic sonosensitizers respectively, and summarizes their characteristics and biological effects as sonodynamic therapy. Upon discovery of novel sonosensitizers, sonodynamic therapy becomes an efficient means of adjuvant therapy for the treatment of malignant tumours.

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Introduction

The increasing incidence and mortality of malignant tumours is one of the most serious diseases threatening human health.[1-5] In addition to surgery, radiotherapy and chemotherapy, other adjuvant treatment schemes also show therapeutic efficacy.[6-9] Among these treatment approaches, phototherapy including photodynamic therapy (PDT) and photothermal therapy (PTT) have shown promising potential to treat many types of cancer.[10-12] PDT produces large numbers of reactive oxygen species (ROS) at the tumour site to induce cell death and also has the ability to induce immune activation.[13] Thus, it is considered one of the most promising tumour treatment options.[9, 14-19] To be an effective tumour treatment method, phototherapy must overcome many disadvantages, such as low tissue-penetration depth of light,[20-22] low target specificity of nanoparticles,[23-25] and the hypoxic microenvironment of the tumour.[26, 27] Among these limitations, the issue of target specificity can be solved by loading tumour-specific targeting ligands,[28-30] while the hypoxic local microenvironment can be overcome by the catalase characteristics of the photosensitive materials or by oxygen transportion,[31-33] and the inadequate effect of a single treatment can be solved by combining with other treatment methods.[34-36] However, poor light penetration is difficult to improve through modification of the materials themselves, meaning that the clinical use of phototherapy may only be applicable to the treatment of epidermal tumours such as skin cancer.[20, 37, 38] To address this issue, sonodynamic therapy (SDT) was proposed.

SDT refers to the strategy of stimulating sonosensitizers by ultrasound to produce cytotoxic ROS to kill tumour cells.[39-42] Currently, high-intensity ultrasound therapy is used in the clinic to treat a variety of tumours, including

glioma,[43, 44] nasopharyngeal cancer, liver cancer,[45] pancreatic cancer and ovarian cancer. Compared with phototherapy, SDT replaces the stimulus from near-infrared light with a penetration depth of only 1–3 mm with ultrasound, which has deeper penetration ability. The effective combination of low-intensity ultrasound and sonosensitizers enables this treatment method to achieve deeper tissue penetration, so it can be used in the treatment of deep solid tumours. As more and more sonosensitizers have been developed, the tumour-killing efficiency of SDT has gradually been recognized by researchers. On the other hand, it is still premature for sonosensitizers to be applied in the clinic, because the tumour-killing mechanism of SDT is not clear enough.[46-48] Therefore, this review summarizes the recent development of representative porphyrin- and titanium dioxide (TiO_2)-based sonosensitizers and their SDT effects in treating malignant tumours.

An electronic search of the Medline database for literature describing porphyrin- and TiO_2-based sonosensitizers and their SDT in cancer treatment from 2010 to 2020 was performed using the term sonodynamic therapy (SDT). The results were further screened by title and abstract to ensure they were relevant to the topic.

Generality and Classification of Sonosensitizers

Sonosensitizers can be roughly classified into two categories, organic and inorganic, as shown in **Figure 1**. The organic sonosensitizers are represented by porphyrins and their derivatives, which have excellent ultrasound conversion performance. However, they are mostly fat-soluble small molecules, which have poor water solubility and a rapid clearance rate *in vitro*, resulting in low tumour site enrichment, unsatisfactory SDT efficacy, and certain phototoxicity. Compared with organic sonosensitizers, inorganic ones such as TiO_2 nanomaterials have better chemical stability and lower phototoxicity. On account of rapid recombination of their electrons and holes (50 ± 30 ns), pure TiO_2 nanomaterials have lower quantum yield as sonosensitizers, leading to an inefficient antitumour effect. Based on these shortcomings, researchers have developed different strategies to enhance their SDT efficiency.

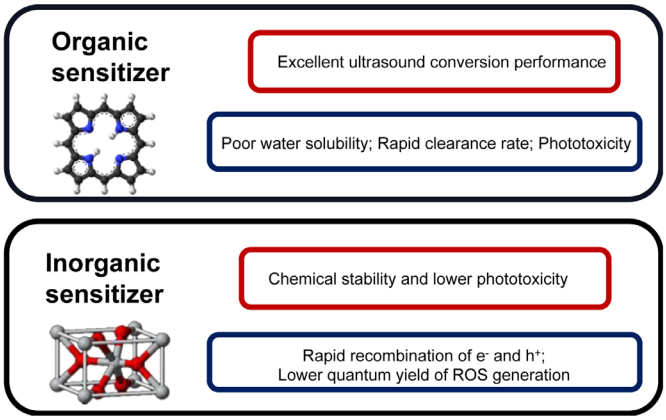

Figure 1. Classification of organic and inorganic sonosensitizers and their features. e^-: electrons; h^+: holes; ROS: reactive oxygen species.

Two main approaches–developing new sensitizers and modifying existing ones–have been employed. For example, porphyrins and their derivatives acting as photosensitizers coordinate with metal ions to form porphyrin-based metal-organic frameworks (MOFs).[29] The periodic porous structure of the MOFs effectively avoids the self-aggregation and self-quenching of photosensitizers and improves the diffusion efficiency of cytotoxic ROS. At the same time, good biocompatibility and targeting characteristics also make the MOFs suitable for PDT. Similarly, the porosity of MOFs and the large number of active sites on the surface enable them to load or couple with acoustically-sensitive molecules to construct ultrasound-sensitive systems with good biocompatibility. Moreover, MOF-based sonosensitizers can be directly used in SDT. In addition to porphyrin-based MOFs, some small molecule drugs have also been proven to possess acoustic characteristics, which will be discussed in this review.

Development of Porphyrin-Based Organic Sonosensitizers

Sonosensitizers are the key ingredient in SDT for their action of transforming ultrasonic energy into radiant energy. Porphyrin and its derivatives, as typical organic sonosensitizers, are widely used in SDT. These molecules have many unique characteristics, such as the π electron conjugation and wide range of photoelectric properties.[49-51] These properties can be adjusted by the coordination of various metals in the porphyrin ring. Thus, porphyrin-based sonosensitizers have changeable features, and can be variously adjusted to meet different clinical needs. At present, the commonly-used clinical sonosensitizers

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are haematoporphyrin, photo-porphyrin and others. However, these sonosensitizers are often chemically unstable, toxic to the skin, and poorly targeted, with low water solubility and fast metabolism meaning that they are rapidly cleared from the blood circulation, seriously affecting their SDT effect and associated clinical applications.[52] Therefore, the development and improvement of sonosensitizers with good stability, low phototoxic side effects and high specificity are an important research direction in the field of SDT.

Porphyrins are a type of heterocyclic compound formed by the interconnection of four α-carbon atoms of pyrrole subunits through a methine bridge (=CH-). The porphyrin ring has 26 π electrons and is a highly conjugated system. Many porphyrins exist in nature in the form of a ring coordinated with a central metal ion, such as chlorophyll containing a coordinated magnesium ion in the centre, and haem having a coordinated iron. Having different coordinated metal ions, loading porphyrins onto other materials, or developing subcellular sensitizers are some of the main methods used to improve the antitumour effect of porphyrin systems (**Figure 2**).

In addition to the basic structure of pyrrole subunits, porphyrins can coordinate with different metal ions to form different metallated porphyrins. In order to explore the characteristics of porphyrin-based sensitizers composed of different coordinated metals, Ma et al.[53] synthesized a series of metallated porphyrin complexes. In this work, tetratolyl porphyrin (TTP) was used as the ligand to coordinate with Mn, Zn, and Ti (**Figure 3**), and the respective obtained MnTTP, ZnTTP and TiOTTP complexes were encapsulated with human serum albumin (HSA) to form nanosized sonosensitizers (MnTTP-HAS, ZnTTP-HAS, and TiOTTP-HSA). This design of conjugating HSA with organic sensitizers can improve the biocompatibility and extend the retention time of the materials within the tumour. These nano-sized sensitizers can generate a large amount of singlet oxygen ($^1O_2$) under ultrasonic radiation, and still exhibit the acoustic energy excitation response in muscle-simulating tissues up to 10 cm thick. When compared with ZnTTP-HSA and TiOTTP-HSA, MnTTP-HSA showed the strongest ROS activation effect. After injecting this type of sensitizer into tumour-bearing mice via the tail vein, low-power ultrasound was applied to the body of tumour-bearing mice to effectively stimulate the sensitizer enriched in the tumour to produce singlet oxygen, thereby inhibiting growth of the tumour. The process did not cause any obvious damage to normal tissues and organs. This nanoparticle system can also be used for deep tissue photoacoustic/magnetic resonance bimodal imaging to track the accumulation of nanoparticles in a tumour.

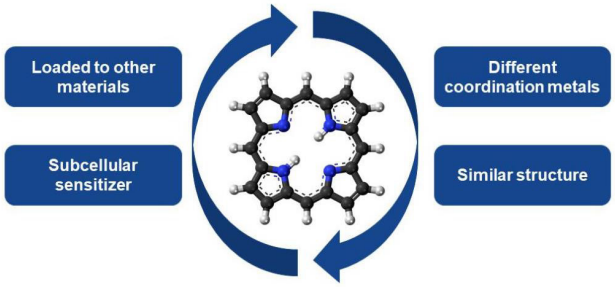

Figure 2. Methods to improve the antitumor effect of porphyrin systems.

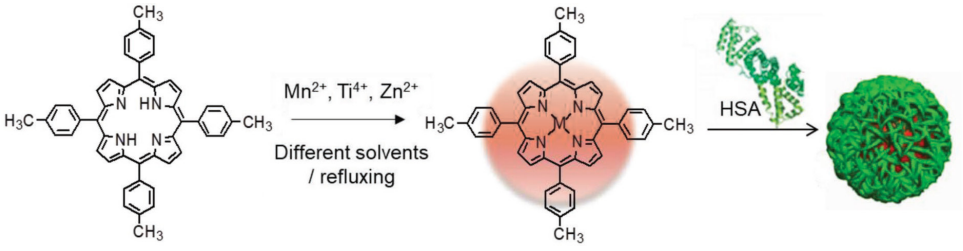

Figure 3. Schematic representation of MnTTP, ZnTTP and TiOTTP complexes synthesized using TTP as the ligand and Mn, Zn and Ti as the central metal ion, followed by HSA encapsulation to create MnTTP-HAS, ZnTTP-HAS, and TiOTTP-HAS, respectively. Reproduced with permission from Ma et al.[53] Copyright WILEY-VCH Verlag GmbH & Co. 2019. HSA: human serum albumin; TTP: tetratolyl porphyrin.

In addition to different coordination metals, the unique structure of porphyrins is also beneficial to the development of sonosensitizers. Pan et al.[39] discovered that mesoporous carbon spheres derived from porphyrin-based MOFs can be used as an efficient acoustic sensitizer for SDT. By using the present system, low accumulation in the tumour site was improved, leading to an enhanced sonodynamic treatment effect. When compared with amorphous carbon nanospheres, the superior acoustic sensitivity of mesoporous carbon spheres is closely related to the porphyrin-like macrocycle in the MOF-derived nanostructure, because the large energy gap between the highest occupied molecular orbit and the lowest unoccupied

molecular orbit promotes the production of ROS. Therefore, the SDT enhancement of mesoporous carbon spheres was confirmed to be structurally dependent.

The modification and loading of existing porphyrin sensitizers onto other materials is another effective way to achieve high efficiency. The main purpose of modifying existing porphyrin-type sensitizers is to either improve their ultrasound sensitivity or obtain the combined therapeutic effect through the loading process. Huang et al.[50] loaded a metalloporphyrin onto hollow mesoporous organic silica to achieve high SDT treatment efficiency. The specific advantages of mesoporous materials for the delivery of sensitizers are that they can protect organic sensitizers from the physiological environment, enhance biological stability, improve tumour accumulation, and afford sustained sensitizer release. In this work, mesoporous organic silica nanoparticles were chosen as the nanocarriers of sensitizers, which had a high loading capacity, easy biodegradability, and good biocompatibility. Due to the presence of a manganese ion in the metalloporphyrin, the nanoparticles could be used for magnetic resonance imaging-guided SDT. In another case, Chen and coworkers[54] reported a tumour treatment method that uses nanosensitizers to enhance SDT when combined with checkpoint-blocking immunotherapy. The major components of the nanosensitizer used in the experiments are clinically approved. Among them, the liposome acts as a carrier, which encapsulates a haematoporphyrin-based sonosensitizer and an immune adjuvant. Their experiments proved that the combined-therapy strategy of SDT and immunotherapy can trigger the antitumor response, which not only prevents the growth and proliferation of the primary tumour, but also stops its metastasis and recurrence. This strategy provided a long-term immune memory function to SDT, warranting its future clinical applications.

In order to improve the accumulation ability of therapeutic species in the tumour site, researchers have developed nanoparticles that can only convert into sonosensitizers inside the tumour to maximize the antitumour efficiency. Wang et al.[55] designed tumour-targeted core-shell nanoparticles for controlled drug release and SDT of cancer (**Figure 4**). In this study, artemisinin (ART) was found to have the ability of producing endoperoxide under ultrasound stimulation, which meant that it could be used as the SDT sensitizer in addition to its tumour-killing ability. Both ART and 5-aminolevulinic acid (5-ALA) were incorporated into a poly(lactic-co-glycolic acid) copolymer. After accumulation into the tumour, 5-ALA was released by cleavage of the pH-sensitive bond to generate protoporphyrin IX for the formation of haem. Haem can improve the SDT efficacy of subsequently-released ART in combination cancer therapy.

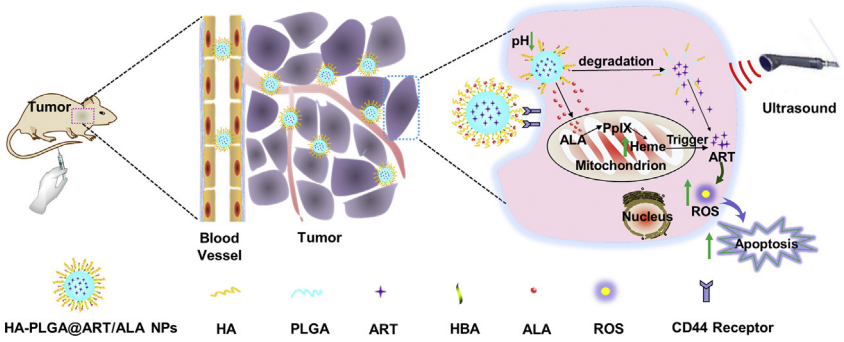

Figure 4. Tumour-targeted drug delivery system based on PLGA NPs, showing the functions of dual drug administration and SDT to maximize the synergistic effect of antitumor treatment. Reproduced with permission from Wang et al.[55] Copyright (2018) Elsevier. ALA: 5-aminolevulinic acid; ART: artemisinin; HBA: 4-hydrazinobenzoic acid; NPs: nanoparticles; PLGA: poly(lactic-co-glycolic acid); PpIX: protoporphyrin IX; ROS: reactive oxygen species; SDT: sonodynamic therapy.

To overcome the limitations of conventional delivery systems, more and more studies have focused on the development of endogenous cell or subcellular structure vectors. For example, exosomes are nanoscale species (30–150 nanometres) carrying macromolecules derived from parent cell-derived membrane vesicles, which can be used as diagnostic markers for various malignant tumours, and also for the delivery of anticancer drugs. Therefore, researchers employed exosomes to load sonosensitizers for accurate delivery.[56-59] Liu et al.[60] designed a nanoparticle system by loading a porphyrin-based sonosensitizer (DVDMS) onto exosomes derived from 4T1 cells for therapeutic and imaging applications (**Figure 5**). Because this sonosensitizer system (EXO-DVDMS) used the same type of tumour exosomes, it showed increased stability in the blood and the tumour microenvironment, and also enhanced delivery of DVDMS to primary and metastatic tumours. First, guided ultrasound was used to promote the local accumulation of EXO-DVDMS in the tumour area, and subsequently therapeutic ultrasound was applied to the tumour for SDT. EXO-DVDMS demonstrated a controlled ultrasound response to drug release and enhanced ROS generation, so that enhanced SDT treatment was achieved both *in vivo* and *in vitro*.

Integration of sonosensitizers with other functional units can enhance SDT. Li et al.[61] combined fluorinated chitosan (FCS) with a catalase–porphyrin (CAT–TCPP) assembly to facilitate the transmucosal delivery of sonosensitizers for sonodynamic treatment of bladder cancer (**Figure 6**). The CAT–TCPP/FCS nanoparticles formed after bladder infusion showed good transmucosal and intratumoural penetration ability, and were

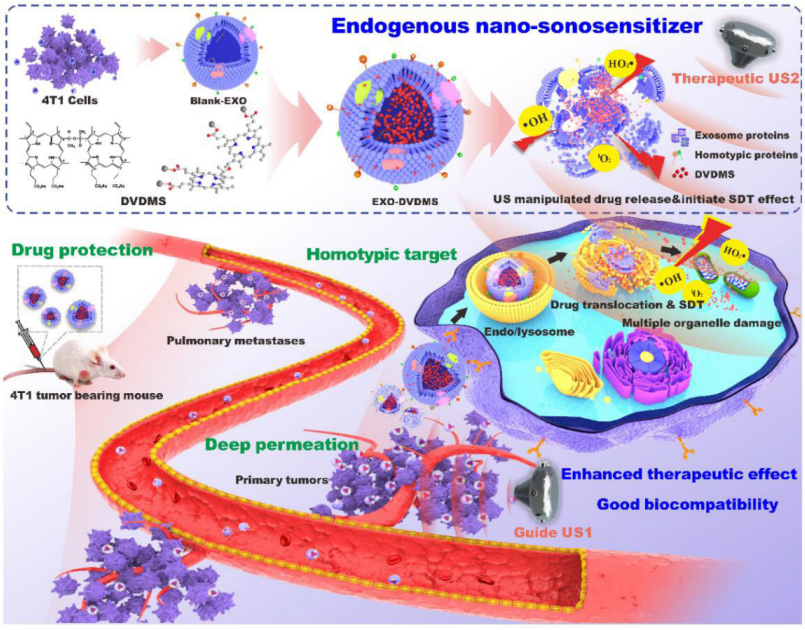

Figure 5. Preparation of an exosome-based sonosensitizer system. The sonosensitizer was loaded onto the exosomes derived from 4T1 cells. This exosome-based sonosensitizer system showed specific accumulation in the primary tumour and in metastatic lesions, achieving ultrasound-controlled drug release and effective SDT. Reproduced with permission from Liu et al.[60] Copyright (2019) Ivyspring International Publisher. $^1O_2$: singlet oxygen; DVDMS: sinoporphyrin sodium; EXO-DVDMS: a functionalized smart nanosonosensitizer created by loading sinoporphyrin sodium; SDT: sonodynamic therapy; US: ultrasound.

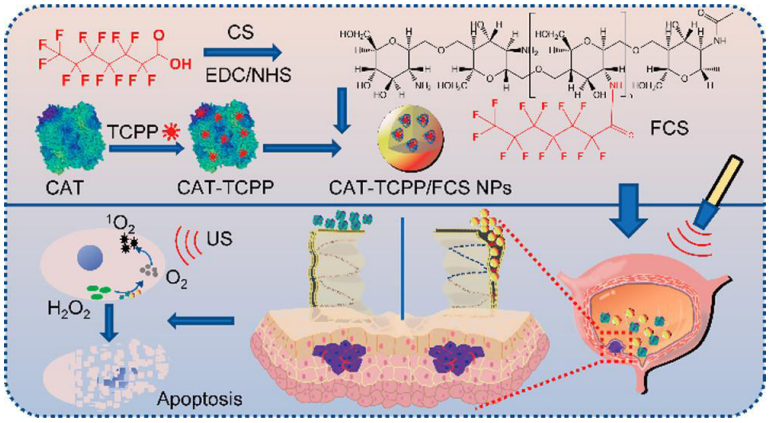

Figure 6. Formation of CAT–TCPP/FCS NPs to facilitate transmucosal delivery of sonosensitizers for enhanced SDT of bladder cancer. Reproduced with permission from Li et al.[61] Copyright (2020) American Chemical Society. $^1O_2$: singlet oxygen; CAT–TCPP: catalase–porphyrin; CS: chitosan; EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; FCS: fluorinated chitosan; H_2O_2: hydrogen peroxide; NHS: N-hydroxysuccinimide; NPs: nanoparticles; SDT: sonodynamic therapy; US: ultrasound.

able to produce O_2 by catalysing endogenous hydrogen peroxide (H_2O_2) to effectively relieve hypoxia in tumour tissues, thereby improving the efficacy of SDT in the treatment of bladder tumours under the action of ultrasound. This work proposes a sonosensitizer formulation containing FCS to enhance transmucosal transmission and intratumoural delivery, and CAT–TCPP to improve oxygenation in the tumour.

Development of TiO_2-Based Inorganic Sonosensitizers

TiO_2 is a widely-used inorganic sonosensitizer. TiO_2 nanoparticles have been proven to have high biocompatibility, stability and acoustic sensitivity, and thus they have been used in SDT.[62-66] The mechanism of their acoustic sensitivity is based on the energy band structure of TiO_2. Because it has semiconductor properties, it can facilitate the separation of electrons (e^-) and holes (h^+) under the trigger of ultrasound. Due to rapid electron–hole recombination, however, the ROS production yield of TiO_2 is low. Consequently, a lot of research efforts have been devoted to developing precious metal-modified TiO_2 nanoparticles, such as Ag-TiO_2 and Au-TiO_2. The modification of TiO_2 is an important strategy to improve its acoustic sensitivity. Some general methods employed in the development of TiO_2-based sonosensitizers are shown in **Figure 7**.

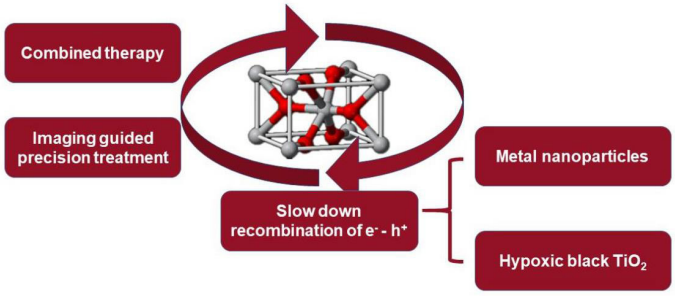

Figure 7. General methods to develop TiO_2-based sonosensitizers for tumour treatment. e^-: electrons; h^+: holes; TiO_2: titanium dioxide.

The integration of metal nanoparticles with TiO_2 is a common method of creating new sonosensitizers. For example, Gao and coworkers[67] successfully synthesized a nanostructure (Au/TiO_2) based on Au nanorods and TiO_2 with a controllable shell thickness for synergistic PTT and SDT. Since Au can suppress rapid electron–hole recombination, the obtained Au/TiO_2nanostructure showed a light-to-heat conversion efficiency of 42.05% with improved ROS generation ability.

In order to solve the rapid electron–hole recombination problem, researchers also used hypoxic black TiO_2 in the near infrared-II biological window for synergistic cancer treatment. Chen et al.[68] carried out tumour treatment research by combining SDT and PTT in the near infrared-II biological window based on a TiO_2 nanoplatform (**Figure 8**). They produced a core/shell structure (TiO_2@TiO_{2-x}) by coating TiO_{2-x} onto TiO_2. The TiO_{2-x} coating enhanced the separation of electrons (e^-) and holes (h^+) under ultrasound, leading to increased ROS generation for cancer treatment. The design idea was derived from conventional black TiO_{2-x} photocatalysis, where black TiO_{2-x} with oxygen-deficient characteristics in the crystal structure improves the separation efficiency of electrons and holes, thereby enhancing the photocatalytic ability. As compared with the TiO_2 nanoparticles, this core/shell structure showed an enhanced ultrasound-triggered SDT effect for efficient tumour treatment.

In addition to enhancing acoustic sensitivity, some metal nanoparticles also have other properties such as magnetic resonance imaging, so that they can perform dual functions of tumour treatment and imaging. Shen et al.[63] reported a Fe_3O_4-$NaYF_4$@TiO_2 nanocomposite as a magnetically-targeted drug carrier for both bioimaging and therapy. TiO_2 here acts as a sonosensitizer, and $NaYF_4$ serves as an upconversion luminescence material for bioimaging. The nanocomposite loaded with doxorubicin was used to carry out synergistic SDT and chemotherapy. This nanocomposite showed cellular uptake ability and good nuclear targeting effect in tumour cell lines. In another case, Harada and coworkers[69] encapsulated TiO_2 in polyion micelles. This preparation process effectively improved the dispersion stability of TiO_2 under physiological conditions. Under ultrasound treatment, the entrapped TiO_2 nanoparticles generated sufficient $^1O_2$ for SDT. This approach is not only able to improve the biological stability of TiO_2, but also boosts ROS generation ability, thereby enabling an enhanced sonodynamic treatment effect.

The rapid development of graphene has had a significant impact on SDT research. Dai et al.[70] reported the integration of graphene oxide with a TiO_2-based sonosensitizer to improve the efficiency of sonodynamic treatment (**Figure 9**). Firstly, based on the good conductivity of graphene oxide nanosheets, its presence can effectively separate electron and hole pairs generated through the cavitation effect induced by ultrasonic radiation. Secondly, the photothermal conversion ability of graphene can significantly enhance the efficiency of synergistic SDT/PTT treatment. Thirdly, MnOx nanoparticles integrated on the surface of TiO_2-graphene nanocomposites can be used as magnetic resonance T1-weighted contrast agents and can be used for magnetic resonance imaging applications. Therefore, this work provides a method of enhancing the efficiency of TiO_2-based sonosensitizers for improved sonodynamic tumour treatment.

The approaches used for modification of TiO_2 nanoparticles also include combination with other treatment options. For example, Shen et al.[64] developed magnetic Fe_3O_4 nanoparticles coated with TiO_2 and loaded with doxorubicin to achieve targeted co-delivery of sonosensitizers and anticancer drugs for combined treatment of cancer. The obtained nanocomposites exhibited high drug-loading capacity and pH-sensitive drug release ability. Under ultrasonic waves, the nanocomposites efficiently produced ROS. In addition, under the action of an external magnetic field, the nanocomposites exhibited a tumour-targeting effect after intravenous injection. The researchers concluded that the combined chemo-SDT provided by the nanocomposites showed an obvious synergistic effect.

Other Types of Sonosensitizers

In addition to these typical porphyrin- and TiO_2-based sonosensitizers, development of new types of sonosensitizers is a meaningful research direction in the field. Pan and coworkers[71] prepared double-layer hollow manganese silicate nanoparticles (DHMS), which showed an efficient ROS production ability under ultrasound for multimodal imaging-guided SDT. DHMS were prepared by in-situ growth of Mn^{2+} using zeolitic imidazolate frameworks (ZIF-8) as a template (**Figure 10**). The Mn species in DHMS can be oxidized by holes under ultrasonic radiation, thereby improving the efficiency of the ROS generation. DHMS were able to generate

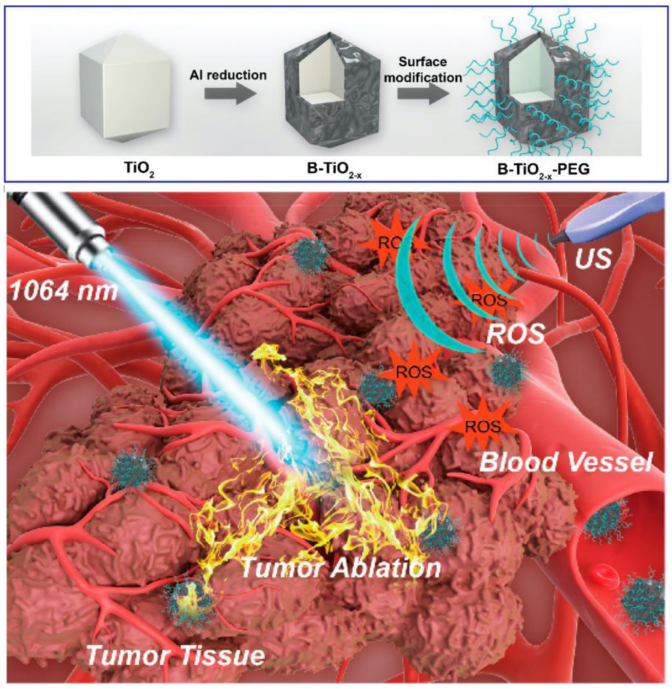

Figure 8. Preparation of TiO_2@TiO_{2-x} core/shell nanostructure for synergistic cancer therapy. Reproduced with permission from Han et al.[68] Copyright (2018) American Chemical Society. B-TiO_{2-x}: black TiO_{2-x}; PEG: polyethylene glycol; ROS: reactive oxygen species; TiO_2: titanium dioxide; US: ultrasound.

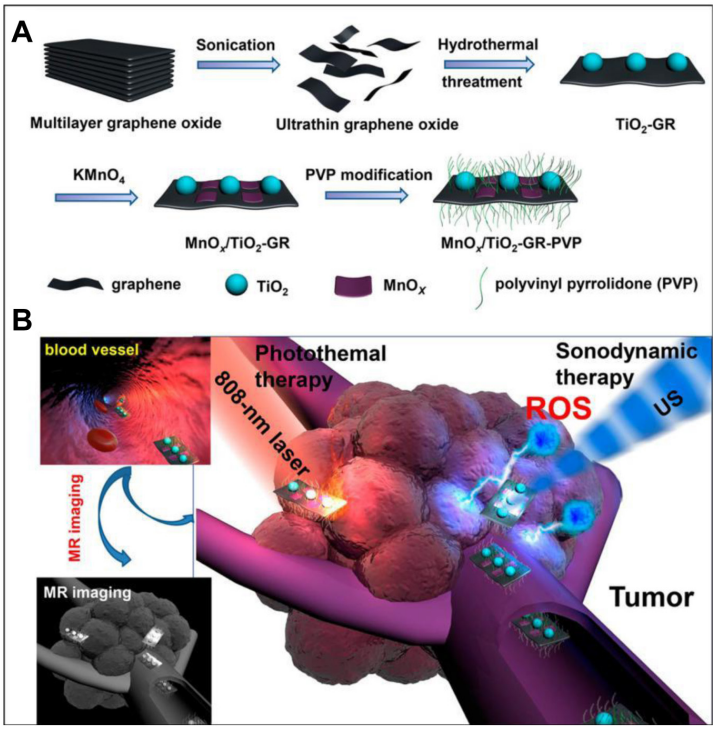

Figure 9. (A) Synthesis of nanocomposites (MnO_x/TiO_2-GR-PVP) containing MnOx, TiO_2, reduced graphene oxide, and PVP. (B) Theranostic ability of MnO_x/TiO_2-GR-PVP nanocomposites for MR imaging-guided SDT/PTT against cancer. Reproduced with permission from Dai et al.[70] Copyright (2017) American Chemical Society. GR: graphene; MR: magnetic resonance; PVP: polyvinylpyrrolidone; ROS: reactive oxygen species; TiO_2: titanium dioxide; US: ultrasound.

large numbers of $^1O_2$ and hydroxyl radicals (•OH) under ultrasonic radiation. The study demonstrated that DHMS with ultrasound sensitivity and oxygen-generating ability can overcome the shortcomings of O_2-dependent SDT, achieving effective cancer treatment.

Interestingly, some traditional Chinese medicines and drug molecules can also be used as sonosensitizers, such as rose bengal,[72] 5-ALA,[73] curcumin,[74] chlorophyll,[75] and ART.[76] As

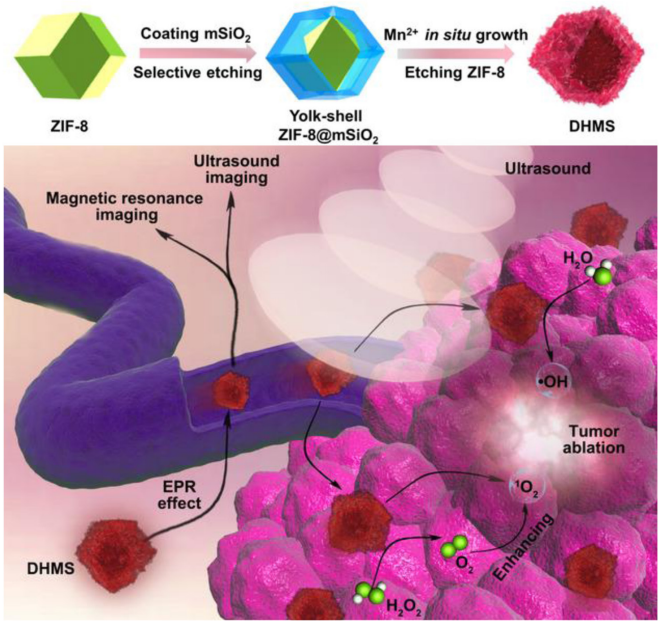

Figure 10. Preparation of DHMS capable of generating ROS under ultrasound for multimodal imaging-guided SDT. Reproduced with permission from Pan et al.[71] Copyright Wiley-VCH Verlag GmbH & Co. 2020. •OH: hydroxyl radical; $^1O_2$: singlet oxygen; DHMS: double-layer hollow manganese silicate nanoparticles; EPR: electron paramagnetic resonance; H_2O_2: hydrogen peroxide; $mSiO_2$: monodispersed mesoporous silica; ROS: reactive oxygen species; SDT: sonodynamic therapy.

some of these already have antitumour effects, the combination of their antitumour and sonosensitizer characteristics may produce a promising potential for future clinical applications.

Synergistic Effect of Sonodynamic Therapy with Other Treatment Methods

Similar to other treatment methods, single SDT often cannot achieve complete tumour ablation, and may carry risks of local recurrence and distant metastasis. Therefore, combined therapy with other treatment methods is essential. In addition, the possible immune activation induced by SDT is also one way to achieve an antitumour effect when integrated with other methods. Zhang et al.[42] proposed that the lysate produced by SDT is similar to the lysate produced by PDT, which can trigger an immune response against tumour cells from the same source, and the establishment of systemic immune memory may be related to the number of SDT cycles. Therefore, they studied the induction of systemic immune response in mice using SDT within 4 or 6 cycles to determine the killing effect on tumour cells and the immunogenicity in tumour-bearing mice. The results indicated that SDT could not only kill tumour cells, but also promote the expression of calreticulin on the cell surface to trigger an immune response. At the same time, they observed that SDT induced a functional antitumour vaccine in tumour-bearing mice. Thus, this strategy conferred mice with an immune memory based on SDT, which prevented tumour recurrence after eliminating the initial tumour. This work indicates that SDT plays an important role in the immune response.

Previous research has shown that moderate thermal effects (42°C) can improve the efficacy of PDT by increasing the rate of photosensitivity and improving tumour hypoxic conditions.[77] Since PDT is a treatment approach similar to SDT, researchers proposed that SDT with moderate heat (42°C) would achieve a synergistic treatment effect on brain glial tumors.[78] In this study, they prepared manganese ion (Mn^{2+})-chelated HSA-chloramphenicol e6 nanocomposites as targeted imaging and therapeutic agents. In the mouse model, the nanocomposites exhibited both imaging ability and moderate thermal (42°C) effect as SDT for glioma. Unlike hyperthermia or PTT, this composite system was constructed by integrating magnetic resonance imaging-based temperature monitoring and ultrasound-based SDT, showing unique advantages. For example, the nanocomposites actively targeted glioma under precise imaging guidance to enable visualization of the tumour location and the ultrasound focus. Real-time temperature monitoring realized by magnetic resonance technology also ensured the safety and effectiveness of SDT.

In another case, Liang and coworkers[79] directly combined SDT with PTT. They synthesized a Pt-CuS material composed of hollow semiconductor CuS and precious metal Pt. The hollow cavity of CuS was used to load the sonosensitizer molecules to achieve SDT. In addition, the deposition of Pt on CuS not only enhances its photothermal properties, but also introduces nanozyme activity. Pt-CuS can catalyse the decomposition of endogenous overexpressed H_2O_2 inside a tumour to produce O_2, relieving the hypoxic tumour microenvironment and thus increasing SDT-induced production of highly-toxic ROS to achieve effective apoptosis of cancer cells. More importantly, the heat generated by Pt-CuS can enhance the catalytic activity of Pt under 808 nm laser irradiation, increasing its O_2-producing ability and promoting the therapeutic efficacy of SDT. The enhanced SDT efficiency and high photothermal

effect of this Pt-CuS system led to effective tumour inhibition without obvious recurrence.

In addition to synergistic treatment with immunotherapy and chemotherapy, researchers also used anti-metabolic drugs in combination with SDT to obtain the best possible therapeutic effect. McEwan et al.[80] combined SDT with antimetabolite therapy and used oxygen-carrying microbubbles (OxyMB) as a means of delivery to improve the therapeutic effect of pancreatic cancer (**Figure 11**). The surface of OxyMB was then connected with either rose bengal sonosensitizer (OxyMB-RB) or 5-fluorouracil antimetabolite (OxyMB-5-FU). Compared with single treatment alone, three different pancreatic cancer cell lines (BxPc-3, MIA PaCa-2, and PANC-1) showed maximized death rates when they were simultaneously treated with SDT and antimetabolites. These results not only illustrate the potential of SDT/antimetabolite combination therapy, but also show that OxyMB has the ability to transport O_2 to the tumour microenvironment thus achieving enhanced tumour-treatment efficacy.

SDT has shown synergistic effects when combined with other therapeutic drugs, including some natural products, as antitumour medication. For example, Prescott et al.[81] tested natural compounds, namely alkaloid Sanguinarine and ginger root extract, and found that Sanguinaria canadensis had antitumour activity both *in vivo* and *in vitro*. Importantly, the cytotoxic effects of these two compounds were due to their ability to produce ROS, thus disrupting cellular mitochondria. Since the generation of ROS provides a common sonochemical effect for cavitation microbubble collapse, and is one of the main mechanisms of SDT treatment, they confirmed that these compounds could be viable sonosensitizers for the SDT treatment of pancreatic cancer, showing a significant synergistic effect in combination with ultrasound treatment.

Targeted Sonodynamic Therapy

Modifying sonosensitizers with specific tumour-targeting proteins may confer tumour-targeting capabilities on the obtained sonosensitizers. Since the tumour-killing effect of ROS is location-dependent, targeting tumour cells is an easy method of improving the efficacy of SDT. In addition to some less specific tumour-targeting species such as folic acid and hyaluronic acid, there are also some unique antigens specific for certain types of tumours. For instance, Ninomiya and co-workers[62] used a protein that recognizes the liver cancer cell HepG2 human hepatitis B virus pre-S1/S2 protein. After complexing with TiO_2, they found that the protein-modified TiO_2 nanoparticles exhibited improved tumour cell uptake capacity and exerted a more powerful tumour-killing effect under the action of ultrasound radiation. Then, they modified TiO_2 nanoparticles with avidin[82] in order to achieve tumour-targeting functions and distinguish healthy cells from cancer cells. After activation by external ultrasonic radiation, hydroxyl radicals generated from TiO_2 nanoparticles exhibited an SDT effect. They found that more than 80% of breast cancer cells (MCF-7 cells) showed uptake of avidin-modified TiO_2 nanoparticles.

Compared with cell-targeting approaches, subcellular-targeted SDT has more significant antitumour effects and greater

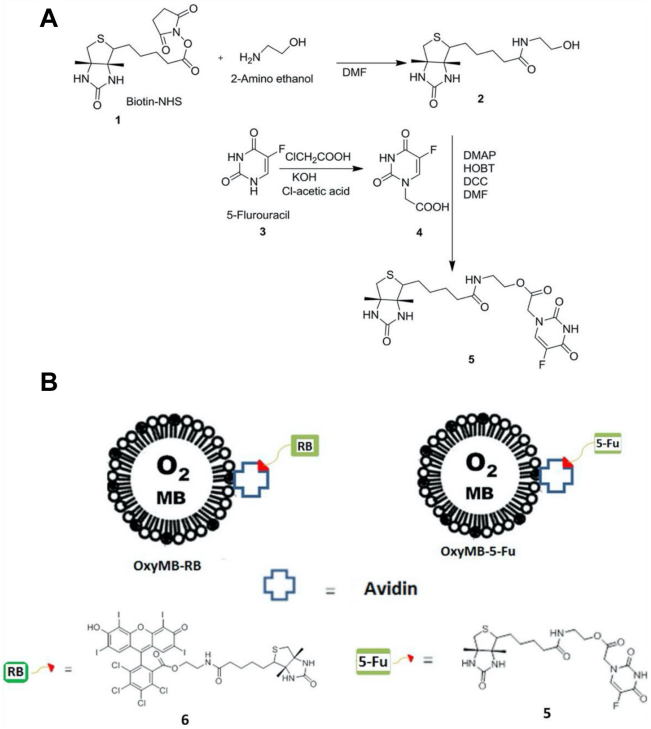

Figure 11. (A) Synthetic scheme of modified 5-fluorouracil antimetabolite (5-Fu). (B) Schematic structures of OxyMB-RB and OxyMB-5-FU conjugates. Reproduced with permission from McEwan et al.[80] Copyright (2016) Elsevier. DCC: N,N′-dicyclohexylcarbodiimide; DMAP: 4-dimethylaminopyridine; DMF: N,N-dimethylformamide; HOBT: hydroxybenzotriazole; OxyMB (O_2 MB): oxygen-carrying microbubbles; RB: rose bengal.

potential for clinical application. Using this method, Zhang et al.[83] reported use of mitochondria-targeted nanodroplets with an ultrasonic response function to enhance sonodynamic cancer treatment (**Figure 12**). In this study, IR780 dye-based nanodroplets (IR780-ND) were demonstrated to have an ultrasound-sensitive effect and mitochondria-targeting function. In the presence of ultrasound treatment, acoustic droplet vaporization greatly aided the transport of IR780-ND from the circulatory system to the tumour area, and ultrasound also increased its penetration depth within the tumour tissue. In addition, IR780-ND showed mitochondria-targeting ability, thereby improving the efficacy of the SDT treatment. Their studies indicated that mitochondria-targeted IR780-ND produced a large amount of ROS under ultrasound treatment for ROS-induced apoptosis of cancer cells. In addition, IR780-ND was also good for photoacoustic and fluorescence imaging, providing the possibility of imaging-guided SDT.

In addition to the mitochondria-targeted SDT, subcellular structure-targeted studies have also been carried out. For example, porphyrin derivatives as sonosensitizers can easily interact with oligonucleotides. Chemical coupling of porphyrin derivatives onto oligonucleotides significantly improves the biological properties of the resulting conjugates. Moreover, the selective and specific integration into the target sequence of oligonucleotides can strengthen the theranostic effect of the porphyrin derivatives, presenting unique tumour cell DNA sequence-targeting properties.

Sonodynamic Therapy-Induced Changes in Cell Physiological Functions

Similar to PDT, SDT has the function of causing immune death of tumour cells by regulating the body's immune system. Peng et al.[84] found that SDT can enhance the antitumour immune effect by increasing the infiltration of $CD8^+$ T cells inside the tumour and changing the tumour blood vessels in the B16F10 melanoma xenograft mouse model. In this study, 5-ALA as a sonosensitizer combined with ultrasound were used to treat B16F10 melanoma xenografts in mice. The SDT treatment increased the numbers of $CD45^+$, $CD8^+$ and $CD68^+$ cells, and upregulated the expression of CD80 in tumour tissues. The endothelial cells in the tumour centre were damaged, while the luminal area of the tumour peripheral blood vessels increased. These results indicated that SDT improved the therapeutic outcome of melanoma-bearing mice by influencing the transendothelial migration of immune cells and the antitumour immune response.

Ultrasound causes different physiological responses in different cell types, and possible signal pathway and molecular activation may also occur. In type II diabetes, overload of glucose and lipids may promote oxidative stress and inflammation, resulting in β-cell failure, and SDT can be used to treat damaged β-cells. Guo et al.[85] found that SDT had a specific effect on mitochondria and could transiently induce large amounts of mitochondrial ROS production in β-cells. SDT also improved the morphology and function of abnormal mitochondria, suppressing the inflammatory response and reducing β-cell dysfunction. In addition, SDT rescued the transcription of PINK1 mRNA blocked by palmitic acid treatment. When cyclosporin A inhibited mitochondria, the protective effect of SDT was blocked. Therefore, SDT effectively relieved lipotoxicity-induced β-cell failure through PINK1/Parkin-dependent mitochondria.

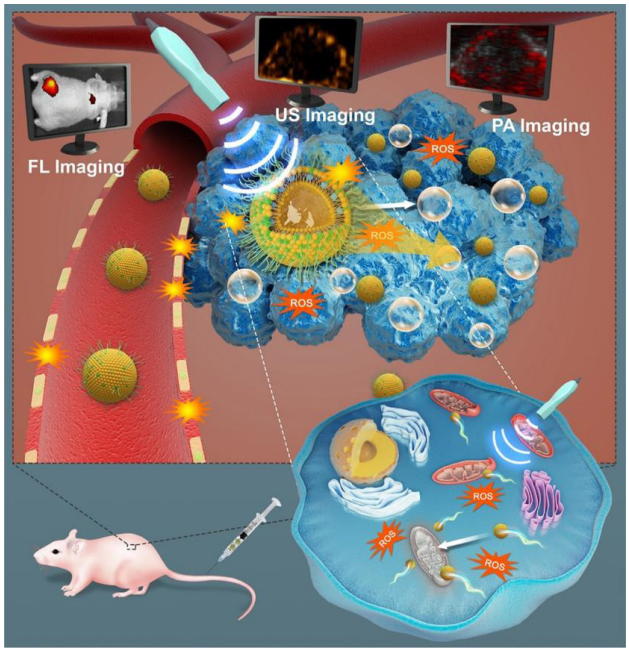

Figure 12. Schematic diagram of mitochondria-targeted SDT using US-responsive IR780-based nanodroplets. Reproduced with permission from Zhang et al.[83] Copyright (2019) American Chemical Society. FL: fluorescence imaging; IR780: an ultrasound-activated sonosensitizer; PA: photoacoustic; ROS: reactive oxygen species; SDT: sonodynamic therapy; US: ultrasound.

The reduction in the autophagy ability of macrophages is accompanied by the development of atherosclerosis, leading to a decrease in lipid loading and degradation efficiency. Kou et al.[86] discovered that berberine-mediated SDT (BBR-SDT) can be used to induce autophagy and cholesterol outflow in THP-1 macrophages and derived foam cells. After BBR-SDT treatment, autophagy in macrophages increased, autophagy resistance in foam cells was prevented, and cholesterol was induced to flow out. The first two effects were blocked by the reactive oxygen scavenger N-acetylcysteine. BBR-SDT also reduced the phosphorylation of two key molecules (AKT and mammalian target of rapamycin (mTOR)) in the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR signalling pathway. Accordingly, the autophagy-inducing effect of BBR-SDT was eliminated by the autophagy inhibitor 3-methyladenine or the PI3K inhibitor LY294002. These results indicated that BBR-SDT effectively promoted cholesterol efflux by increasing the generation of ROS, and subsequently induced autophagy in macrophages via the PI3K/AKT/mTOR signalling pathway. Wang and coworkers[87] also explored the biological effects of SDT on an atherosclerosis model. They studied the potential mechanism of 5-ALA-based SDT on atherosclerosis *in vivo*. Their results showed that 5-ALA-based SDT activated the peroxisome proliferator-activated receptor γ-liver X receptor α-ATP binding cassette subfamily A member 1/ATP binding cassette subfamily G member 1 pathway of macrophages, enhanced the endocytosis and cholesterol efflux, and induced anti-inflammatory responses, eventually improving the atherosclerosis.

Summary and Outlook

As a relatively new type of tumour treatment technology, SDT uses ultrasound to activate acoustically-sensitive species accumulated in the tumour site to destroy tumour tissues. SDT normally requires three main components, i.e., sonosensitizers, molecular oxygen and ultrasound. This review summarized recent developments of two representative types of sonosensitizers, namely, porphyrins and TiO_2, in SDT of cancer.

An ideal sonosensitizer needs to have high-efficiency sonodynamic response characteristics, excellent biocompatibility and tumour-targeting ability. It should also be relatively stable and easy to store. Inorganic nanomaterials as sonosensitizers have the advantages of good stability, circulation ability, and tumour accumulation, while organic sonosensitizers may have good biocompatibility and powerful ROS generation capability. On the other hand, the biodegradability of these nanomaterials in the body is often unknown, and their long-term accumulation may cause tissue damage, limiting their potential for clinical translation. Thus, studies on the biodegradability of sonosensitizer nanomaterials and their effective clearance from the body are an important direction for future research.

How to effectively improve the sonodynamic efficiency of sonosensitizers is another important research topic. Although it is generally believed that nanomaterials can provide the cavitation nucleation sites and promote energy transfer, specific mechanisms of acoustic dynamics still need to be fully explored. At the same time, the influence of ultrasonic radiation parameters on the acoustic dynamics should also be investigated.

Inherent characteristics of the microenvironment (such as hypoxia) of tumours may affect the efficacy of ROS-based SDT. Increasing SDT efficiency by improving the tumour microenvironment is also a research focus in this field. This review mainly discussed improving SDT efficiency from the perspective of nanomaterials. In addition to developing nanomaterials that can catalyse hydrogen dioxide inside the tumour site, the transportation of oxygen and reduction of energy consumption by tumours are other means to enhance the therapeutic effect of SDT. Thus, this review is expected to inspire further development of acoustically-sensitive materials for SDT of cancer toward future clinical uses.

Author contributions

XD conceived the idea, collected the background information, conducted literature search, and drafted the manuscript. ZS and YZ edited the manuscript. All authors have read and approved the content of the manuscript.

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Conflicts of interest statement

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